Pharmacogenetics in Statin Use

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To my dear mother who sacrificed her all to make this achievement possible.

To my grandparents and siblings who always gave me strength in this journey.

To my dear fiancé, Boaz, who was present in the best and hardest of times.

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Abstract

The *SLCO1B1* c.521T>C (rs4149056) genetic polymorphism is implicated in decreased *SLCO1B1* function which leads to reduced uptake of simvastatin by the liver and higher simvastatin concentrations in the blood. The resulting elevated plasma simvastatin concentrations may increase the risk of simvastatin-induced myopathy (SIM).

The aim of the research was to investigate the pharmacogenetic implications of simvastatin. The objectives were to: (i) identify presence of the *SLCO1B1* c.521T>C genetic polymorphism in a cohort of cardiac patients on simvastatin and (ii) explore the correlation between genotype results and myopathy risk.

Patients on simvastatin were recruited by convenience sampling from the Cardiac Catheterisation Suite at Mater Dei Hospital after ethics approval. A peripheral blood sample was obtained from each patient and genomic DNA was extracted using the QIAamp® DNA Blood Mini kit. Real-time polymerase chain reaction *SLCO1B1* c.521T>C rs4149056 genotyping was performed with the Sacace® Biotechnology kits and Rotor-Gene™ 6000/Q. Patients were classified into 3 genotypes (phenotypes): 1. TT (normal *SLCO1B1* function), 2. TC (intermediate *SLCO1B1* function) or 3. CC (low *SLCO1B1* function). TC and CC patients were communicated to the consultant cardiologists with the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline recommendations for *SLCO1B1* and SIM. The patients were followed up 6 months post-recruitment for documented and self-reported muscle symptoms.

The 148 patients recruited (all Caucasian, 90 male, mean age 65 years) were genotyped as TT (83.1%, n=123), TC (14.9%, n=22) and CC (2%, n=3). Fifteen of the 25 TC and CC patients were prescribed simvastatin 40mg daily. At follow-up, 15 patients (12 TT, 2 TC, 1 CC) self-reported muscle symptoms; stiffness (n=6; 5 TT, 1 TC), cramps (n=4; all TT), pain (n=4; 3 TT, 1 CC) and weakness (n=1; TC).

According to the CPIC guidelines, patients genotyped as TC (15%) have mild myopathy risk and CC patients (2%) have high myopathy risk. Fifteen of the 25 TC and CC patients were prescribed simvastatin 40mg and the CPIC guidelines recommend a lower simvastatin dose (20mg/day) or consideration of another statin (rosuvastatin) in these patients. One of the 3 CC patients had documented myalgia at follow-up. The observed findings from this study are exploratory and warrant further investigation.

Keywords: Patient safety – Personalisation of statin therapy – Simvastatin – Simvastatin-induced myopathy – *SLCO1B1* rs4149056 genotyping

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List of Abbreviations

(*)	Haplotype or star allele name
ACE-I	Angiotensin converting enzyme inhibitor
ADR	Adverse Drug Reaction
CCCU	Cardiac Critical Care Unit
CCS	Cardiac Catheterisation Suite
CMW	Cardiac Medical Ward
CPIC	Clinical Pharmacogenetics Implementation Consortium
CSW	Cardiac Surgical Ward
CVD	Cardiovascular Disease
CVIS	Cardiovascular Information Management System
CK	Creatine Kinase
CKD	Chronic Kidney Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
EMA	European Medicines Agency
EU	European Union
EVDAS	EudraVigilance Data Analysis System
FDA	Food and Drug Administration
GFL	Government Formulary List
HMG-CoA	hydroxy-methylglutaryl-coenzyme-A
LDL-C	Low-Density Lipoprotein Cholesterol
MDH	Mater Dei Hospital
OATP1B1	Organic Anion Transporter Polypeptide 1B1
SAMS	Statin-Associated Muscle Symptoms

SIM	Simvastatin-Induced Myopathy
SLCO1B1	Solute Carrier Organic Anion Transporter Family Member 1B1 gene
SmPC	Summary of Product Characteristics
SNP	Single Nucleotide Polymorphism
UK	United Kingdom
ULN	Upper Limit of Normal

Chapter One

Introduction

1.1. Clinical use of statins

Statins act primarily by inhibiting 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase activity, leading to reduction in the synthesis of cholesterol and augmentation of the uptake of low-density lipoprotein cholesterol (LDL-C) from the blood, ultimately decreasing LDL-C plasma concentrations (Grundy, 2002; Grundy et al, 2004; Catapano et al, 2016).

Statins are first-line drugs in the treatment of dyslipidaemia and in cardiovascular disease (CVD) prevention and are extensively prescribed (Baigent et al, 2010; Stone et al, 2014; Collins et al, 2016). Statin therapy has proven significant benefits in promoting the regression of coronary atherosclerosis, hence reducing the incidence and risk of acute coronary syndrome and stroke, independent of age (Goldstein & Brown, 2015; Catapano et al, 2016; Ference et al, 2017; Fulcher et al, 2019).

LDL-C reduction is an important treatment goal in the management of diabetes mellitus. Statins have proven significant benefits in patients with concomitant type 2 diabetes mellitus and CVD, resulting in risk reduction of CVD events compared to non-diabetic patients (Baigent et al, 2010; Catapano et al, 2016). Statins have reported benefits in decreasing mortality rate in patients with chronic kidney disease (CKD), by lowering the risk of CVD and stroke events (Barylski et al, 2013; Kaysen, 2017). However, patients suffering from stage 3-5 CKD should be monitored and dose adjustments are usually required due to the risk of higher serum statin concentrations in renal impairment (Tonelli et al, 2014; Catapano et al, 2016).

Several studies have reported independent statin LDL-C lowering effects, known as pleiotropic effects, including anti-inflammatory and antioxidant effects, modulation of immune actions, modifications involving cholesterol intermediate signals, stabilising atherosclerotic plaques and antiplatelet effects (Liao et al, 2005; Kavalipati et al, 2015). The pleiotropic effects of statin have been linked to a number of disease states, including chronic obstructive pulmonary disease, rheumatoid arthritis, inflammatory bowel diseases, metabolic syndrome, multiple sclerosis, venous thromboembolism, systemic lupus erythematosus, cancer, stroke, Alzheimer's disease, Parkinson's disease, Human Immunodeficiency Virus, polycystic ovary syndrome and bacterial infections (Deedwanian et al, 2006; Fedson, 2006; Bifulco et al, 2008; Singh et al, 2008; Agarwal et al, 2010; Kavalipati et al, 2015).

1.2. Classification of statins and LDL-C reduction potential

Statins are classified into three categories according to potency and effectiveness with respect to LDL-C reduction (Maji, 2017; Grundy et al, 2019). The first category of statins are the low-intensity statins, including pravastatin, lovastatin and fluvastatin. These statins were introduced between 1980 and 1990 and have the lowest potency for LDL-C reduction of less than 30% with once daily dosing (pravastatin 10mg, 20mg; lovastatin 20mg; fluvastatin 20mg, 40mg). Pravastatin is the most studied of the low-intensity statins and has demonstrated effectiveness in reducing CV risk in secondary prevention of CVD and in symptomatic coronary artery disease. The benefit of fluvastatin and lovastatin in decreasing CV risk has also been documented (Kapur & Musunuru, 2008; Duncan et al, 2009; Grundy et al, 2019).

The second category of statins are the medium-intensity statins, including simvastatin and atorvastatin, which have greater efficacy in decreasing plasma LDL-C levels compared to low-intensity statins (30-45% LDL-C reduction). The 20mg and 40mg doses of simvastatin and the 10mg and 20mg doses of atorvastatin have moderate intensity when administered once daily. A 10mg daily of dose of simvastatin has a low intensity in decreasing LDL-C (Arnadottir et al, 1993; Stringer et al, 2013; Rabar et al, 2014; Grundy et al, 2019).

The third category of statins are the high-intensity statins. Rosuvastatin and high-dose atorvastatin (40mg, 80mg) are classified as high-intensity since they reduce LDL-C levels by ≥50%. Rosuvastatin has significant potency and efficacy owing to a fluorinated phenyl group, a hydrophilic methane sulphonamide group and strong binding capacity with the HMG-CoA reductase enzyme. The doses of rosuvastatin indicated as high intensity are 20mg and 40mg. A 10mg daily dose of rosuvastatin is classified as moderate-intensity, as are higher doses of pravastatin, lovastatin and fluvastatin (Duncan et al, 2009; Grundy et al, 2019).

The ‘indiVidual patient meta-analysis Of statin therapY in At risk Groups: Effects of Rosuvastatin, atorvastatin and simvastatin (VOYAGER)’ tested the different doses of simvastatin, rosuvastatin and atorvastatin with their effect on reducing LDL-C levels relative to dose. Rosuvastatin showed greater efficacy in decreasing LDL-C levels compared to similar doses of simvastatin and atorvastatin (Weng et al, 2010; Catapano et al, 2016; Karlson et al, 2016). Table 1.1 represents the percentage LDL-C reduction

for the different statins and doses available in the Maltese Government Formulary List (GFL).[1]

Table 1.1: LDL-C reduction for different statins and doses

% LDL Reduction	Simvastatin	Atorvastatin	Rosuvastatin
25-32%	10mg		
31-39%	20mg	10mg	
37-45%	40mg	20mg	
48-52%		40mg	
55-60%		80mg	20mg
60-63%			40mg

Reproduced from: Ministry of Health. DH Circular 54/2018-Deletion of Fluvastatin and Changes in Statin Entitlement. Malta: Ministry of Health; July 2018.

The effectiveness of statins in decreasing LDL-C plasma levels has been proven. However, patients on statin therapy need to be monitored closely due to the risk of adverse drug reactions (ADRs), particularly muscle symptoms (Ramachandran & Wierzbicki, 2017).

[1]Ministry of Health. DH Circular 54/2018-Deletion of Fluvastatin and Changes in Statin Entitlement [Internet]. Malta: Ministry of Health; July 2018 [cited 2019 Jun 10]. Available from: https://deputyprimeminister.gov.mt/en/pharmaceutical/Documents/Circulars/2018/circular_54_2018.pdf

1.3. Statin-associated muscle symptoms

Statins are reported to have a good safety profile with a lower risk of side-effects compared to the clinical benefits. Yet, the possibility of adverse events is important to consider when prescribing and monitoring statin treatment as part of a personalised treatment strategy (Baigent et al, 2010; Naci et al, 2013).

The most described and clinically-relevant side-effects of statins are muscle symptoms, which are the most frequent reported reason for statin discontinuation and non-adherence (Thompson et al, 2003; Catapano et al, 2016; Ramachandran & Wierzbicki, 2017). Statin discontinuation and non-adherence are reported to increase the risk of CV events (Zhang et al, 2013; Ramsey et al, 2014; Marrs & Kostoff, 2016). Statin-associated muscle symptoms (SAMS) documented in clinical studies are myalgia, myopathy and rhadbomyolysis and those typically reported from patient experience are muscle aches, tenderness, stiffness, cramps and weakness (Bruckert et al, 2005; Stroes et al, 2015; Zhou et al, 2017).

The incidence of SAMS varies from 5% to 29% (Maji et al, 2013). Myalgia and myopathy are considered milder symptoms and occur more frequently. Myalgia is defined by the American College of Cardiology/American Heart Association/National Heart, Lung and Blood Institute as muscle aches or weakness without creatine kinase (CK) elevation (Pasternak et al, 2002). Myopathy is defined as a diffuse muscle disease that causes an elevation in CK levels up to 10 times the upper limit of normal (ULN) (Link et al, 2008; Maji et al, 2013; Stewart, 2013). Rhabdomyolysis is a more serious side-effect, but is less common, occurring at an incidence of 1 in 10,000 patients yearly.

Rhabdomyolysis is characterised by severe muscular pain, muscle necrosis and myoglobinuria, potentially leading to renal failure and death (Bruckert et al, 2005; Collins & Altman, 2012; Mancini et al, 2013; Zhang et al, 2013; Keltz et al, 2014; Rosenson et al, 2014; Stroes et al, 2015; Torres et al, 2015). Literature reports three degrees of diagnosis of SAMS according to CK, namely: (i) incipient myopathy (CK between 3-10 times ULN), (ii) myopathy (CK >10-50 times ULN) and rhabdomyolysis (CK >50 times the ULN) (Mckenney et al, 2006; Link et al, 2008; Abd et al, 2011; Mombelli & Pavanello, 2013).

The occurrence of SAMS varies among the different statins and doses (Bruckert et al, 2005; Law & Rudnicka, 2006; Morival et al, 2018). Muscular symptoms occur more frequently with higher doses, for example with simvastatin 80mg daily, and the risk increases with concurrent use of certain drugs such as ciclosporin, which may decrease the metabolism of statins (Thompson et al, 2003; Law & Rudnicka, 2006; Link et al, 2008). Lipophilic statins, including simvastatin, atorvastatin and lovastatin, have a higher incidence of SAMS than hydrophilic statins, such as rosuvastatin, fluvastatin and pravastatin, and the rate of SAMS increases with synthetic statins (fluvastatin and atorvastatin) and higher statin doses (Abd & Jacobson, 2011; Maji et al, 2017). SAMS are frequently limited to a specific area and affect large muscle groups, including proximal and distal muscles, particularly the thighs, buttocks and calves (Sathasivam, 2012).

The diagnosis of SAMS continues to be challenging due to the lack of gold standard definitions, classifications and guidelines (Stewart, 2013). The 2015 European

Atherosclerosis Society Consensus statement advises three possibilities to diagnose SAMS namely; (i) timely association of symptoms and/or high levels of CK above the ULN on starting statin treatment, (ii) symptom resolvement following withdrawal of the statin, and (iii) re-appearance of SAMS when re-challenged with another statin (Stroes et al, 2015). CK levels are however not considered the most reliable indicator of SAMS, since myopathy with statins has been diagnosed with CK levels within the normal range (22-198 U/L), since SAMS could be caused by structural damage of muscle fibers (Mohaupt et al, 2009).

1.4. Adverse drug reaction reports of statins in Eudravigilance

The scope of pharmacovigilance has evolved into reporting ADRs, medication errors, falsification or substandard medicines, shortage of efficacy, and drug-drug interactions. Patient safety, treatment follow-up and surveillance of medicines has been the main focus of pharmacovigilance when a medicine is introduced on the market (Olsson & Harrison-Woolrych, 2018).

EudraVigilance Data Analysis System (EVDAS) is one of the greatest spontaneous ADR reporting systems worldwide, and is responsible for collecting, managing and analysing suspected ADRs of authorised drugs in the European Economic Area. It is operated by the European Medicines Agency (EMA) since 2001 (Postigo et al, 2018).

ADR reports of myopathy, myalgia and rhabdomyolysis with simvastatin, atorvastatin, and rosuvastatin for a five-year period (2014-2018) from the 28 European Union (EU) countries were collated from the EVDAS database and analysed according to age,

gender, statin dose and ADR seriousness. A total of 4,164 ADR reports were analysed and 78.3% of ADR reports were identified for myalgia, 15.4% for rhabdomyolysis and 6.3% for myopathy. The distribution of ADR reports according to statin were; simvastatin (33.2%), atorvastatin (47.3%) and rosuvastatin (19.5%).[2]

A higher number of ADR reports of myalgia with atorvastatin (47.1%) compared to myalgia with simvastatin (32.4%) and rosuvastatin (20.6%) were identified. Similarly, a higher number of ADR reports of rhabdomyolysis with atorvastatin (48.8%) compared to rhabdomyolysis with simvastatin (37.2%) and rosuvastatin (14.5%) were identified. A higher number of ADR reports for myopathy with atorvastatin (47.5%) compared to simvastatin (33.7%) and rosuvastatin (18.8%) were identified (Table 1.2).

Table 1.2: ADR reports of myalgia, myopathy and rhabdomyolysis with statins (28 EU countries; 2014-2018) (N=4,164)

ADR / **Statin**	**Myalgia (n=3,260)**	**Myopathy (n=261)**	**Rhabdomyolysis (n=643)**
Simvastatin (n=1,382)	1055 (32.4%)	88 (33.7%)	239 (37.2%)
Atorvastatin (n=1970)	1535 (47.1%)	124 (47.5%)	311 (48.4%)
Rosuvastatin (n=812)	670 (20.6%)	49 (18.8%)	93 (14.5%)

p=0.007

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

[2] European Medicines Agency (EMA). EudraVigilance Data Analysis System [Internet]. UK: EMA; 2019 [cited 2019 Feb 17]. Available from: https://www.ema.europa.eu/en/human-regulatory/research-development/pharmacovigilance/eudravigilance

The ADR reports were analysed according to age; which was divided into two categories (≤60 and >60 years); 445 ADR reports did not document age. A higher number of ADR reports of myalgia, myopathy and rhabdomyolysis with all the three statins were identified from patients over 60 years compared to patients ≤60 years (Table 1.3).

Table 1.3: Distribution of ADR reports of myalgia, myopathy and rhabdomyolysis with statins according to age (n=3,716)

ADR	**Myalgia (n=2,870)**		**Myopathy (n=233)**		**Rhabdomyolysis (n=613)**	
Statin	**≤60 years (n=1,174)**	**>60 years (n=1696)**	**≤60 years (n=88)**	**>60 years (n=145)**	**≤60 years (n=159)**	**>60 years (n=454)**
Simvastatin (n=1,191)	369 (41.2%)	526 (58.8%)	31 (40.8%)	45 (59.2%)	51 (23.2%)	169 (76.8%)
Atorvastatin (n=1,784)	554 (40.6%)	812 (59.4%)	41 (36.3%)	72 (63.7%)	86 (28.2%)	219 (71.8%)
Rosuvastatin (n=741)	251 (41.2%)	358 (58.8%)	16 (36.4%)	28 (63.6%)	22 (25.0%)	66 (75.0%)

p>0.05

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

When analysing the ADR reports by gender, no significant difference between gender for myalgia, myopathy and rhabdomyolysis with all the three statins was observed (55 reports did not include gender) (Table 1.4).

Table 1.4: Distribution of ADR reports of myalgia, myopathy and rhabdomyolysis with statins according to gender (n=4,109)

ADR	Myalgia (n=3,227)		Myopathy (n=255)		Rhabdomyolysis (n=627)	
Statin	**Male (n=1,689)**	**Female (n=1,538)**	**Male (n=148)**	**Female (n=107)**	**Male (n=388)**	**Female (n=239)**
Simvastatin (n=1,363)	526 (50.4%)	518 (49.6%)	46 (54.1%)	39 (45.9%)	147 (62.8%)	87 (37.2%)
Atorvastatin (n=1,943)	818 (53.9%)	701 (46.1%)	71 (58.7%)	50 (41.3%)	201 (66.3%)	102 (33.7%)
Rosuvastatin (n=803)	345 (52%)	319 (48.0%)	31 (63.3%)	18 (36.7%)	40 (44.4%)	50 (55.6%)

p>0.05 (myalgia, myopathy); p=0.001 (rhabdomyolysis)

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

The ADR reports were analysed by statin and dose. A higher number of ADR reports for myalgia and myopathy were identified for simvastatin 20mg and 40mg and atorvastatin 10mg and 20mg (Table 1.5).

Table 1.5: Distribution of ADR reports of myalgia, myopathy and rhabdomyolysis with statins according to dose (N=4,164)

Statin/ ADR	Simvastatin (n=1382)				Atorvastatin (n=1970)				Rosuvastatin (n=812)			
	10mg	20mg	40mg	80mg	10mg	20mg	40mg	80mg	5mg	10mg	20mg	40mg
Myalgia (n=3,260)	130 (12.3%)	517 (49.0%)	384 (36.4%)	24 (2.3%)	461 (30.1%)	557 (36.2%)	377 (24.6%)	140 (9.1%)	230 (34.3%)	254 (37.9%)	150 (22.4%)	36 (5.4%)
Myopathy (n=261)	14 (15.9%)	29 (33.0%)	34 (38.6%)	11 (12.5%)	37 (30.1%)	31 (14.4%)	35 (28.5%)	21 (17.1%)	16 (32.7%)	16 (32.7%)	10 (20.4%)	7 (14.3%)
Rhabdomyolysis (n=643)	26 (10.5%)	70 (29.3%)	126 (53.1%)	17 (7.1%)	44 (14.1%)	57 (18.3%)	87 (27.8%)	123 (39.9%)	13 (14.0%)	19 (20.4%)	35 (37.6%)	26 (28.0%)

$p<0.001$

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

The ADR reports were analysed by seriousness and type of seriousness. The higher number of reports for myalgia and myopathy were classified as 'No' seriousness, and for rhabdomyolysis there were a higher number of reports classified as 'Yes' seriousness (579 reports did not document seriousness) (Table 1.6).

Table 1.6: Distribution of ADR reports of myalgia, myopathy and rhabdomyolysis with statins according to ADR seriousness (n=3,585)

ADR / Statin	Myalgia (n=2,858)		Myopathy (n=204)		Rhabdomyolysis (n=523)	
	No (n=2,618)	Yes (n=240)	No (n=136)	Yes (n=68)	No (n=13)	Yes (n=510)
Simvastatin (n=1,199)	843 (90.0%)	94 (10.0%)	45 (63.4%)	26 (36.6%)	4 (2.1%)	187 (97.9%)
Atorvastatin (n=1,675)	1249 (94.1%)	78 (5.9%)	62 (68.1%)	29 (31.9%)	8 (3.1%)	249 (96.9%)
Rosuvastatin (n=711)	526 (88.6%)	68 (11.4%)	29 (69.0%)	13 (31.0%)	1 (1.3%)	74 (98.7%)

$p>0.05$ (myalgia, rhabdomyolysis); $p<0.001$ (myopathy)

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

For the analysis of type of seriousness, the ADR reports that documented type of seriousness as 'Other' were excluded (88). For myalgia, the higher number of reports documented 'Hospitalisation' as type of seriousness (Table 1.7).

Table 1.7: Distribution of ADR reports of myalgia according to type of seriousness (n=155)

Statin	Type of Seriousness		
	Hospitalisation (n=125)	**Disabling (n=27)**	**Life-threatening (n=2)**
Simvastatin (n=94)	66 (70.2%)	27 (28.7%)	1 (1.1%)
Atorvastatin (n=78)	61 (78.2%)	16 (20.5%)	1 (1.3%)
Rosuvastatin (n=68)	43 63.2%	25 (36.8%)	0

p>0.05

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

For myopathy, the higher number of reports documented 'Hospitalisation' as type of seriousness (Table 1.8).

Table 1.8: Distribution of ADR reports of myopathy according to type of seriousness (n=65)

Statin	Type of Seriousness		
	Hospitalisation (n=59)	**Disabling (n=3)**	**Life-threatening (n=3)**
Simvastatin (n=26)	26 (100%)	0	0
Atorvastatin (n=29)	23 (79.3%)	3 (10.3%)	3 (10.3%)
Rosuvastatin (n=13)	10 (76.9%)	0	0

p=0.036

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

For rhabdomyolysis, the higher number of reports documented 'Hospitalisation' as type of seriousness (Table 1.9).

Table 1.9: Distribution of ADR reports of rhabdomyolysis according to type of ADR seriousness (n=510)

Statin	Type of Seriousness		
	Hospitalisation (n=478)	**Disabling (n=4)**	**Life-threatening (n=28)**
Simvastatin (n=187)	180 (96.3%)	1 (0.5%)	6 (3.2%)
Atorvastatin (n=249)	226 (90.8%)	1 (0.4%)	22 (8.8%)
Rosuvastatin (n=74)	72 (97.3%)	2 (2.7%)	0

p=0.004

Data extracted from EudraVigilance Data Analysis System, January 2019[2]

1.5. Genetic variation in relation to statins: The Organic Anion Transporter Polypeptide 1B1

The underlying pathogenesis of SAMS is still uncertain but seems to be associated with high statin levels in the circulation (Link et al, 2008). Potential contributing risk factors of SAMS include drug pharmacokinetics, concomitant interacting medications, individual patient characteristics and comorbidities, as well as genetic factors (Chatzizisis et al, 2010).

Genetic variation is reported to have an impact on the safety of statin therapy. The genes implicated include those encoding the cytochrome P450 enzymes (*CYP2D6*, *CYP3A4*, *CYP3A5*), the mitochondrial enzyme glycine amidinotransferase (*GATM*), the cell influx transporter Organic Anion Transporter Polypeptide 1B1 (OATP1B1), and cell efflux transporters (*ABCB1*, *ABCG2*) (Lamba et al, 2002; Iwai et al, 2004; Fiegenbaum et al, 2005; Oh et al, 2007; Keskitalo et al, 2008; Seithel et al, 2008; Oshiro et al, 2010; Canestaro et al, 2014). The focus of this research is on the OATP1B1 transporter and the effect of the Solute Carrier Organic Anion Transporter Family Member 1B1 (*SLCO1B1)* c.521T<C (rs4149056) gene on statin therapy. The association between *SLCO1B1* rs4149056 and statins is the most studied *in vivo* and is reported to be clinically-relevant (Link et al, 2008; Voora et al, 2009; Ghatak et al, 2010; Mastaglia, 2010; Brunham et al, 2012; Donelly et al, 2011; Carr et al, 2013; Needham & Mastaglia, 2014).

OATP1B1 is a transporter protein composed of 691 amino acids located in the hepatocelullar membrane. The common c.521T>C variant rs4149056 produces a p.V174A substitution which is contained in *SLCO1B1*5*, **15* and **17* variant alleles (Abe et al, 1999; Hsiang et al, 1999; Konig et al, 2000a; Konig et al, 2000b; Seithel et al, 2008). OATP1B1 is responsible for the uptake of statins, including simvastatin, by the liver from the bloodstream (Rodriges et al, 2009; Niemi et al, 2011). The OATP1B1 transporter is encoded by the *SLCO1B1* gene and activity changes of the OATP1B1 transporter due to genetic variation influences the risk of occurrence of SAMS. *SLCO1B1* c.521T>C (p.Val174Ala; rs4149056) is the single nucleotide polymorphism

(SNP) reported to be associated with more than doubling of the risk of myopathy with statin therapy (Niemi, 2007; Romaine et al, 2010).

OATP1B1 is the main protein transporter for the uptake of statins, however, other OATPs transporters including OATP1B3 and OATP2B1 are also involved in statin uptake. Data indicates that the rs4149056 polymorphism is primarily associated with simvastatin-induced myopathy (SIM) and the association with atorvastatin-induced myopathy is less strong. The underlying mechanism resulting in this difference may be explained by a varying degree of contribution of other OATPs (Hagenbuch & Meier, 2004; Kameyama et al, 2005; Morimoto et al, 2005; Kalliokoski & Niemi, 2009).

The most clinically relevant c.521T>C haplotypes are the reduced function C alleles; *SLCO1B1 *5, *15* and **17* (Tirona et al, 2001; Nishizato et al, 2003; Niemi et al, 2004; Kameyama et al, 2005; Nozawa et al, 2005). Allele frequencies vary in different populations; the c.521T>C is fairly prevalent in Europeans and Asians (~10-20%), and uncommon in Sub-Saharan Africans (~2%) (Pasanen et al, 2008; Kalliokoski & Niemi, 2009). Studies have investigated the prevalence of the *SLCO1B1* C allele in different ethnicities such as Caucasians, Asians, African-American and Brazilians (Santos et al, 2011; Grapci et al, 2015; Sychev et al, 2016; Kitzmiller et al, 2017).

Presence of one (TC – heterozygous) or two (CC – homozygous) reduced function C alleles leads to decreased hepatic uptake of simvastatin and increased plasma concentrations, which may enhance susceptibility to SIM due to higher muscle exposure (Link et al, 2008).

1.6. Association between *SLCO1B1* and myopathy with statins

A study by Mulder et al, (2001) investigated the association between genes encoding OATPs and SIM, where no significant association was observed. Other studies demonstrated that plasma concentrations of statins, including simvastatin, pitavastatin, atorvastatin and rosuvastatin, were higher in *SLCO1B1* rs4149056 CC homozygotes compared to non-carriers. However, these findings were not statistically significant (Pasanen et al, 2006a; Pasanen et al, 2007; Deng et al, 2008). The *SLCO1B1* c.521T>C SNP also showed no significant effect on the pharmacokinetics of fluvastatin (Niemi et al, 2006a).

The first study which reported a statistically significant association between *SLCO1B1* rs4149056 and SIM was the 'Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)' randomised trial carried out in the United Kingdom (UK), where 85 patients diagnosed with incipient myopathy and definite myopathy were genotyped for *SLCO1B1* rs4149056. In addition, more than 60% of myopathy cases were strongly associated with the *SLCO1B1* reduced function C allele (Link et al, 2008). This study reported that the risk of SIM increases in carriers of the C allele on simvastatin 80mg. Another study showed that patients with TC or CC genotype treated with a dose of 40mg or higher demonstrated statin intolerance, resulting in discontinuation of statin treatment or a change of statin (Donelly et al, 2011).

A study by Voora et al, (2009) in the UK reported an occurrence of myopathy with simvastatin in 27% (31 of 115) of heterozygous (TC) patients and in 50% (4 of 8) of homozygous variant (CC) patients. This study established that carriers of *SLCO1B1*5*

treated with simvastatin had a higher risk of myopathy compared to patients taking pravastatin. The cases of myopathy in carriers of *SLCO1B1*5* reported in this study did not exhibit CK elevation (Voora et al, 2009).

Carriers of the *SLCO1B1* rs4149056 gene having elevated risk of myopathy was reported to be statin-specific according to a study by Brunham et al (2012). In this study, the clinical records of 9000 patients from two lipid clinics in the Netherlands were assessed. Overall, this study did not show a statistical association between the rs4149056 variant and statin-associated myopathy, however, when grouped by statin type, the *SLCO1B1* C allele was documented to be significantly associated with myopathy for patients on simvastatin, with no significance for atorvastatin, pravastatin and rosuvastatin. The risk of myopathy was highest with the use of simvastatin (51 of 108, 48%) and atorvastatin (45 of 108, 42%), and lowest with the use of pravastatin (10 of 108, 9%) and rosuvastatin (2 of 108, 1%) (Brunham et al, 2012).

Similarly, a study by Carr et al, (2013) in the UK documented 77 myopathy and severe myopathy cases. Out of these, 44 patients were carriers of 1 or 2 C alleles. A stronger association was reported with simvastatin use (30 of 56, 54%) compared to atorvastatin (4 of 11, 36%), supporting the evidence by Voora et al, (2009) and Brunham et al, (2012). There was a significant association between *SLCO1B1* c.521T>C with simvastatin use for doses ≥40mg daily. Atorvastatin showed no significant association between carriers of *SLCO1B1* c.521T>C and SIM, however atorvastatin demonstrated a higher association compared to rosuvastatin (Carr et al, 2013). Meanwhile, pharmacokinetic studies have demonstrated that the area under the plasma

concentration-time curve of active simvastatin and atorvastatin was 221% and 144% greater in CC homozygous variants compared to non-carriers (Pasanen et al, 2006b; Pasanen et al, 2007; Moßhammer et al, 2014).

In Italy, a case-control study of 66 patients studied the association between rs4149056 SNP and SIM. Patients were classified according to *SLCO1B1* rs4149056 genotype and there were 14 patients genotyped as CC, out of which 12 had an elevated plasma CK level and incipient myopathy. This study showed possible correlation between SIM and *SLCO1B1* rs4149056 (Ferrari et al, 2014).

Puccetti et al, (2010) reported a significant association between atorvastatin and the rs4149056 genetic polymorphism. Santos et al, (2012) carried out a study with a sample population of 143 Brazilians where patients were treated with atorvastatin 80mg and followed up for 1 year. From this sample, 14 patients developed myalgia and 16 developed incipient myopathy, documented as an increase in CK 3 times the ULN. The 143 patients were genotyped and 37 were identified as carriers of 1 or 2 C alelles. The study concluded no correlation between atorvastatin with *SLCO1B1* rs4149056 (Santos et al, 2012).

A study by Hubacek et al (2015) undertaken in the Czech Republic, reported no association between patients who were carriers of the rs4149056 polymorphism with statin-induced myopathy for atorvastatin 20mg and simvastatin 10mg.

A study by Danik et al, (2013) in 4404 Europeans at risk of CV events demonstrated no association between carriers of *SLCO1B1* c.521C>T and myopathy in patients on rosuvastatin. Liu et al, (2017) carried out a study in China with patients treated with atorvastatin, simvastatin, fluvastatin and pravastatin and reported a significant association between the *SLCO1B1* C allele with rosuvastatin. Rosuvastatin plasma concentrations in Asians is reported to be higher compared to Caucasians (Birmingham et al, 2015), hence the findings by Liu et al, (2017) may not be generalised to other ethnicities.

A recent meta-analysis published in 2018 on the association between *SLCO1B1* T521C polymorphism and the risk of statin-induced myopathy, which collated and analysed the studies discussed previously, concluded that the *SLCO1B1* T521C polymorphism was associated with a significantly increased risk of statin-induced myopathy, particularly for simvastatin and rosuvastatin. This meta-analysis recommended further studies to be conducted to assess the association between *SLCO1B1* T521C polymorphism with myopathy induced by the different statins (Xiang et al, 2018).

1.7. Clinical Pharmacogenetics Implementation Consortium Guideline for *SLCO1B1* and simvastatin-induced myopathy

The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *SLCO1B1* and SIM was first published in 2012 with the aim to guide dosing and routine monitoring of simvastatin according to *SLCO1B1* rs4149056. The guideline was updated in 2014 including information about other statins and indicating the resources needed for the clinical implementation of pharmacogenetics for statin therapy. The

guideline was established when the evidence of the association of SIM with *SLCO1B1* rs4149056 was considered of high quality following randomised trials and clinical practice-based cohort studies (Wilke et al, 2012; Ramsey et al, 2014).

Assignment of *SLCO1B1* phenotype and genotype in the CPIC guideline is described in Table 1.10.

Table 1.10: Assignment of *SLCO1B1* phenotype according to genotype

Phenotype (prevalence)	Genotype at rs4149056	Diplotypes observed
Homozygous wild-type or normal-high *SLCO1B1* activity (55–88%)	Individual carrying two functional T alleles (TT)	**1a/*1a, *1a/*1b, *1b/*1b*
Heterozygous or *SLCO1B1* intermediate activity (11–36%)	Individual carrying one functional allele plus one reduced C function allele (TC)	**1a/*5, *1a/*15, *1a/*17, 1b/*5, *1b/*15, *1b/*17*
Homozygous variant or *SLCO1B1* low activity (0–6%)	Individual carrying two reduced-function C allele (CC)	**5/*5, *5/*15, *5/*17, *15/*15, *15/*17, *17/*17*

Adapted from: Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for *SLCO1B1* and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther. 2014;96:423-428.

The development of the CPIC dosing recommendations for simvastatin were prompted by product-label changes by the Food and Drug Administration (FDA). These dosing recommendations may be used as guidance to inform healthcare professionals about the risk of SIM in carriers of the C allele. It is recommended that carriers of the C allele are prescribed a lower simvastatin dose (20mg) or to consider an alternative statin, such as

rosuvastatin, with CK monitoring and follow-up. Other prescribing considerations include LDL-C target level, comorbidities such as diabetes mellitus or CKD, and concomitant medications (Ramsey et al, 2014) (Table 1.11).

Table 1.11: Dosing recommendations for simvastatin according to *SLCO1B1* genotype

Genotype at rs4149056	Implications for simvastatin	Dosing recommendations for simvastatin	Classification of the recommendations
TT	Normal myopathy risk	Prescribe desired starting dose and adjust doses of simvastatin based on disease-specific guidelines	Strong
TC	Intermediate myopathy risk	Consider a lower simvastatin dose; if suboptimal efficacy, consider an alternative statin (e.g. pravastatin or rosuvastatin); consider routine CK surveillance	
CC	High myopathy risk		

Adapted from: Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for *SLCO1B1* and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther. 2014;96:423-428.

The CPIC guideline reviews and verifies information from a combination of strong pre-clinical and clinical data. The aspects taken into consideration include *in vivo* clinical results and pharmacokinetic data for statins in individuals who differ by *SLCO1B1* rs4149056 genotype. In *vitro* pharmacodynamics and pharmacokinetic data for statin therapy are also considered (Ramsey et al, 2014).

1.8. Pharmacogenetic information in official product labelling for statins

The Summary of Product Characteristics (SmPC) of simvastatin, approved by the EMA and updated in January 2019, reports the association of *SLCO1B1* c.521T>C with myopathy and rhabdomyolysis. Section 4.4 'Special warnings and precautions for use' reports that the *SLCO1B1* rs4149056 decreased function allele encoding the OATP1B1 protein transport of the liver, which is responsible for the uptake of simvastatin, leads to higher muscle exposure of simvastatin, which may increase the risk of myopathy and rhabdomyolysis. Section 5.2 'Pharmacokinetic properties' under 'Special populations', states the mean exposure of simvastatin as 120% in heterozygotes TC and 221% in homozygotes CC compared to non-carriers TT.[3]

The SmPC of rosuvastatin, approved by the EMA and updated in April 2019, reports in section 5.2 'Pharmacokinetic properties' under 'Genetic polymorphisms', the pharmacokinetics of rosuvastatin and accounts for genetic polymorphisms, including *SLCO1B1* rs4149056. The SmPC of rosuvastatin states that *SLCO1B1* c.521T>C is not established in clinical practice[4], while in the SmPC of simvastatin, the association between *SLCO1B1* rs4149056 and myopathy is supported by clinical data.[2]

The United States FDA drug label of simvastatin, updated in April 2019, mentions the OATP1B1 protein in the pharmacokinetics section. The drug label states that inhibition

[3] Electronic Medicines Compendium (eMC). Simvastatin 40mg Tablets - Summary of Product Characteristics (SmPC) [Internet]. UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4591/smpc

[4] Electronic Medicines Compendium (eMC). Rosuvastatin 20mg Tablets - Summary of Product Characteristics (SmPC) [Internet]. UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4366/smpc

of this protein results in increased plasma concentrations of simvastatin and consequently an increased risk of myopathy.[5] The FDA drug label of rosuvastatin includes a section which states that increased exposure to rosuvastatin is observed in patients with the genetic polymorphism for the OATP1B1 protein. The drug label information states that the effect of the *SLCO1B1* decreased function allele on rosuvastatin has not been clearly established.[6]

With regards to patient safety, the EMA SmPC of atorvastatin, last updated in August 2012, reports in section 5.2 'Pharmacokinetic properties' under 'Special populations' about the *SLCO1B1* polymorphism involving the OATP1B1 transporter. The SmPC states the risk of rhabdomyolysis in carriers of the c.521T>C polymorphism.[7] The FDA drug label of atorvastatin does not indicate any pharmacogenetic information about the *SLCO1B1* rs4149056 polymorphism.[8]

[5] DailyMed. Simvastatin - Drug label information [Internet]. US: FDA; 2019 [cited 2019 Jun 17]. Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=5c1c694c-4b08-469e-b538-08e69df06146

[6] DailyMed. Rosuvastatin – Drug label information [Internet]. US: FDA; 2019 [cited 2019 Jun 17 Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=bd26b8b9-baad-d988-79ba-3f908a8eaab6

[7] Electronic Medicines Compendium (eMC). Atorvastatin 10mg Tablets - Summary of Product Characteristics (SmPC) [Internet] UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4109/smpc

[8] DailyMed. Atorvastatin – Drug label information [Internet]. US: FDA;2019 [cited 2019 Jun 17]. Available from: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=cda119f2-54c8-4a08-b266-a0dbd214d2ce

1.9. Rationale of the study

Numerous studies have described the association between *SLCO1B1* rs4149056 gene and SIM, however the association is not consistent and there is still need for further investigation to assess this association and to confirm the evidence (Xiang et al, 2018). The area of pharmacogenetics is advancing in clinical practice at a slow pace due to limited clinical studies supporting evidence for its implementation. In order to promote the use of pharmacogenetics in the personalisation of statin treatment to increase patient safety, further studies and cooperation of the multi-disciplinary team are needed.

1.10. Current statin prescribing protocols in Malta

To-date (June 2019), the statins approved in Malta and provided by the Government are simvastatin, atorvastatin and rosuvastatin. The doses approved on the GFL are: Simvastatin 10mg, 20mg, 40mg; Atorvastatin 10mg, 20mg, 40mg, 80mg; and Rosuvastatin 20mg, 40mg.[9] On 11 July 2018, fluvastatin was deleted from the GFL and atorvastatin became first-line along with simvastatin. Rosuvastatin is available as second-line to be used when the target LDL-C level is not achieved with the maximum tolerated dose of atorvastatin for a minimum of 3 months.

[9] Directorate for Pharmaceutical Affairs (DPA). Hospital formulary list [Internet]. Malta: DPA; 2019 [cited 2019 April 20]. Available from: https://deputyprimeminister.gov.mt/en/pharmaceutical/Documents/GFL/hosp_gfl_mar_2019.pdf

1.11. Aim and objectives of the study

The aim of the research was to investigate the pharmacogenetic implications of simvastatin.

The objectives were to:

- Classify a cohort of cardiac patients on simvastatin according to *SLCO1B1* genotype and phenotype and explore the correlation with myopathy risk
- Discuss treatment recommendations of statins with the consultant cardiologists in relation to genetically-predisposed risk of SIM
- Follow-up patients for simvastatin-related muscle symptoms

1.12. Study setting

The study was carried out at Mater Dei Hospital (MDH). Patient recruitment was undertaken at the Department of Cardiology, from the Cardiac Catheterisation Suite (CCS), Cardiac Critical Care Unit (CCCU), Cardiac Medical Ward (CMW) and Cardiac Surgical Ward (CSW).

Genomic DNA extraction and Real-Time PCR genotyping was performed at the Molecular Diagnostics Unit of the Department of Pathology at MDH.

Chapter Two

Methodology

2.1. Research Design

This was a prospective cohort study to investigate the pharmacogenetic implications of simvastatin therapy in cardiac patients. The recruited patients, according to pre-determined inclusion and exclusion criteria, were classified according to *SLCO1B1* genotype after genomic DNA extraction and *SLCO1B1* rs4149056 (521T>C) genotyping, and matched with possible associated muscle symptoms. Patients were followed-up for simvastatin-associated muscle symptoms over 6 months (Figure 2.1).

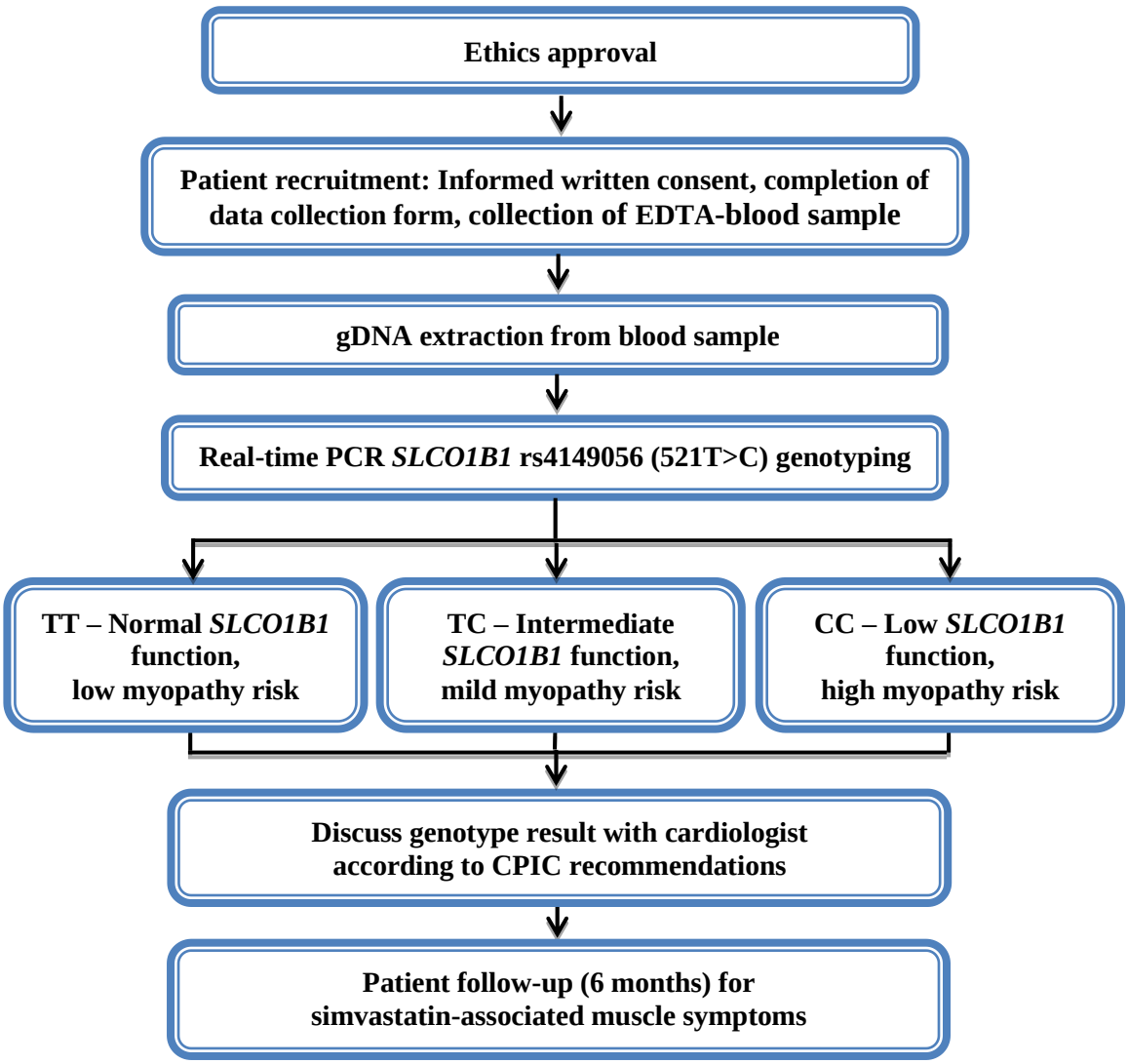


Figure 2.1: Methodology flowchart

2.2. Development and validation of patient data collection form

A patient data collection form was developed. The data collection form consisted of six sections (Table 2.1).

The data collection form was completed as follows:

- Section 1, 2, 3 and 5 were completed via patient interview/information from the patient hospital file.

- Section 4 was completed from the iSoft clinical manager software used at MDH to assist healthcare professionals to document patient laboratory investigations such as lipid, renal and liver profile.

- Section 6 was completed after genotyping was performed to document the genotype results obtained.

The data collection form was validated by two pharmacists in academia at the Department of Pharmacy, University of Malta and two consultant cardiologists, including the Chair of the Department of Cardiology at MDH (Appendix 1).

Table 2.1: Sections of the developed data collection form

Section	Title	Description
1	**Patient information**	Patient details including age, gender, ethnicity, date of recruitment, caring cardiologist, cardiac procedure undertaken
2	**Cardiac risk factors and social history**	Family history of hypercholesterolaemia, recent lipid profile, previous MI, PCI/CABG, hypertension, diabetes mellitus, BMI, waist circumference, smoking history, alcohol consumption
3	**Other comorbidities**	List of other relevant comorbidities other than hypertension and diabetes mellitus
4	**Current Medications**	Generic name, dose, dosage regimen and start date
5	**Investigations**	Liver function test, renal profile, skeletal muscle marker (CK)
6	***SLCO1B1* Genotyping results and communication ofrecommendations to cardiologists**	Homozygous wild-type TT, Heterozygous TC, homozygous variant CC; *SLCO1B1* function (Normal, Intermediate or Low), Myopathy risk (Normal, Intermediate or High), CPIC recommendations to cardiologist according to myopathy risk

MI: Myocardial Infarction; **PCI:** Percutaneous Coronary Intervention; **CABG**: Coronary Artery Bypass Graft surgery; **BMI**: Body Mass Index

2.3. Study approvals

Chair of the Department of Cardiology and Consultant Cardiologists, MDH

Chair of the Department of Pathology, MDH

Chief Executive Officer and Data Protection Officer, MDH

University Research Ethics Committee approval was granted (Appendix 2).

2.4. Patient recruitment and data collection

Patients were recruited by convenience sampling from the Cardiac Catheterisation Suite (CCS), Cardiac Critical Care Unit (CCCU), Cardiac Medical Ward (CMW) and Cardiac Surgical Ward (CSW) at the Department of Cardiology, according to pre-established inclusion and exclusion criteria. Inclusion criteria were patients ≥18 years and on simvastatin therapy. Exclusion criteria were patients with severe renal impairment (eGFR ≤15 mL/min/1.73m^2), liver impairment, and patients on holiday or non-residents in Malta.

Details of the study were explained to each patient by the investigator (JC). Information was provided verbally and via a patient information sheet which was available in both English and Maltese language (Appendix 3). Patients who agreed to participate in the study were asked to provide written, informed consent by signing a patient consent form, which was also available in English and Maltese language (Appendix 4). Each patient recruited was assigned a sequential, unique identification number for the

purpose of the study which was used solely by the investigator and research team to ensure patient confidentiality.

The data collection form was completed by the investigator for each patient at the time of recruitment via patient interview and with information obtained from patient hospital records. Peripheral blood (5ml) were collected from each patient by a physician or nurse in a purple-top ethylenediaminetetraacetic acid (EDTA) vacutainer, labelled with patient number, and stored between 2 and 8°C at the Molecular Diagnostics Unit before proceeding with genomic DNA extraction.

2.5. Genomic DNA extraction

Genomic DNA was extracted from 200 µL of the collected EDTA-anticoagulated whole blood sample using the QIAamp® DNA Blood Mini kit (*Qiagen*®) on the automated QIAcube® robotic workstation for fully automated genomic DNA extraction with these extraction kits. Training with respect to genomic DNA extraction involved two observation sessions (two-hours) and two hands-on training sessions (two-hours) with a medical laboratory scientist at the Molecular Diagnostics Unit.

Genomic DNA was extracted using spin columns, 2ml microcentrifuge collection tubes, buffer AL and ethanol for lysis, proteinase K for lysis and binding, buffer AW1 and buffer AW2 concentrate for washing, and buffer AE for elution of the genomic DNA. A maximum of 12 samples were processed in each extraction run, taking approximately 90 minutes for preparation and extraction. Typically, a 200 µL whole blood sample

yields 3 to 12 µg of genomic DNA (average 6 µg). Advantages of the automated QIAcube® are that sample-to-sample cross-contamination is avoided and it enables safe handling of potentially infectious samples. The extracted genomic DNA was stored in a freezer at -20°C prior to *SLCO1B1* (rs4149056) genotyping.

2.6. *SLCO1B1* rs4149056 genotyping

SLCO1B1 genotyping (521T>C, Val174Ala, rs4149056) was performed with the Sacace® Biotechnology kits and using real-time polymerase chain reaction (PCR) for fluorescence detection with the Rotor-Gene™ 6000/Q (Corbett Research, Qiagen®)[10] between February 2018 and February 2019. The test kits were stored between 2 and 8°C in their original box. Four integrated controls including C+ Homozygous Wild Type TT (allele 1-1), C+ Heterozygous TC (allele 1-2), C+ Homozygous Mutant CC (allele 2-2) and Negative Control C- were supplied with the genotyping test kits. In Real Time PCR the amplified product is detected using fluorescent dyes which are linked to oligonucleotide probes that bind specifically to the amplified product. Table 2.2 shows the reference SNP code, details of the polymorphisms and fluorescence channel.

[10] The centrifugal rotator of the Rotor-Gene™ makes it a precise and versatile real-time PCR cycler. Each tube spins in a chamber of moving air, keeping all samples at precisely the same temperature during rapid thermal cycling. When each tube aligns with the detection optics, the sample is illuminated and the fluorescent signal is rapidly collected from a single and short optical pathway. It has 6 channels spanning Ultra-Violet (UV) to infrared wavelengths. Rotor-Gene™ 6000/Q is operated and analysed by Q-Rex Software to interface and streamline qPCR workflow.

Table 2.2: Details of Sacace® *SLCO1B1* Biotechnology kits

Code	Gene	Polymorphism details	Fluorescence Channel: Allele
T01303	*SLCO1B1*	Val 174 Ala GTG 521 GCG rs4149056	HEX: Val(T) – allele 1
			FAM: Ala (C) – allele 2

Each PCR run was set up manually using a 36-well PCR optical plate labelled with assay name and plate identifier according to the manufacturer's instructions, and control samples including C+ Homozygous Wild Type TT (allele 1-1), C+ Heterozygous TC (allele 1-2), C+ Homozygous Mutant CC (allele 2-2) and Negative Control C- were used in each run. Each well consisted of 25µl reaction volume, including 5µl of genomic DNA and 5µl of Taq Polymerase enzyme (Table 2.3).

Table 2.3: Components of PCR reaction mixture

Reaction component	Volume / Well (µl)
Sacace® PCR Mix	15
Taq Polymerase	5
Genomic DNA	5
Total	25

Table 2.4 shows the temperatures, the time for each cycle and the number of cycles for Rotor-Gene™ 6000/Q programmed for real-time PCR.

Table 2.4: Thermocycling conditions

Step	Temperature (ºC)	Time	Cycles
Hold	80	2 min	1
Hold	95	3 min	1
Cycling	95	10 s	40
	60	40 s fluorescence detection	

Table 2.5 shows the two channels for optical detection, green and yellow with excitation and fluorophores detected. The data was analysed with the detection of fluorescent signals detected in the two channels and associated with variant type.

Table 2.5: Characteristics of the real-time PCR channels

Channel	Excitation (nm)	Detection (nm)	Fluorophores detected
Green	470 ± 10	510 ± 5	FAM (Allele 1 – Wild-type)
Yellow	350 ± 5	557 ± 5	HEX (Allele 2 – Variant)

Results were interpreted as: (1) Signal only in allele 1 (Yellow) - Homozygous wild type; (2) Signal only in allele 2 (Green) - Homozygous variant, and (3) Signal in both allele 1 and allele 2 - Heterozygous. Real-time PCR processes were always supervised by a medical laboratory scientist.

2.7. Classification of patients according to genotype results and discussion with cardiologists

After genotyping, patients were classified into three groups according to genotype, phenotype and myopathy risk namely: (1) TT homozygous wild type, normal *SLCO1B1* function, low myopathy risk, (2) TC heterozygous, intermediate *SLCO1B1* function, mild myopathy risk, and (3) CC homozygous variant, low *SLCO1B1* function, high myopathy risk.

Details of the patients who were genotyped as carriers of the *SLCO1B1* C allele (TC and CC patients) were discussed with the responsible consultant cardiologist. Informative letters with the genotype result were given to seven cardiologists completed with patient details, therapy recommendations for statins related to genotype and myopathy risk according to the CPIC guideline for *SLCO1B1* and SIM (Ramsey et al, 2014) (Appendix 5), and questions regarding if the recommendation was considered (Yes/No) and if any action was taken post-genotyping for the patients (Decrease dose; change statin; CK monitoring; follow-up for muscle side- effects). Any action taken regarding change in simvastatin dose or change in statin was documented in section 6 of the data collection form in which *SLCO1B1* function and genotype was also documented.

2.8. Patient follow-up

The patients were followed-up 6 months after recruitment using ISoft clinical manager software for relevant laboratory investigations such as CK level, the Cardiovascular Information Management System (CVIS) to check outpatient reports for changes in statin therapy and documented muscle symptoms, and telephone contact for any self-reported muscle symptoms and changes to statin therapy. The association between *SLCO1B1* polymorphisms and SIM was explored.

2.9. Data Analysis

Descriptive statistics were carried out with IBM SPSS® Statistics version 22.0. Categorical variables are presented as number (%) and continuous data are presented as mean (± 95% CI) or Standard Deviation (SD).

Population proportion hypothesis testing was used to compare the proportion of the *SLCO1B1* rs4149056 genotypes TT, TC and CC in the present study with the genotype proportions in other populations. Having the sample size and proportions of both populations, the p-value at 95% CI was calculated. A p-value less than 0.05 indicates that the proportions differed significantly, while a p-value greater than 0.05 indicates that the proportions did not differ significantly.

Chapter Three

Results

One hundred and forty-eight patients (N) were recruited, genotyped for the *SLCO1B1* c.521T>C (**5, *15, *17)* variant alleles and followed-up. Patient demographics, cardiac procedures, cardiac risk factors, comorbidities, investigations, medications prescribed, *SLCO1B1* genotyping results, and patient follow-up results are described.

3.1 Patient demographics

The mean age of the patients was 65 (±10.73) years, ranging from 31 to 86 years. One hundred and eight (72.9%) patients were male and 40 (27.0%) were female. With respect to ethnicity and nationality, all patients (n=148, 100%) were Caucasian and 144 patients (97.3%) were Maltese.

3.2 Cardiac risk factors and social history

Positive family history of hypercholesterolaemia was a risk factor in 85 patients (57.4%), either one parent (n=61, 41%) or one sibling (n=24, 16%).

For the 106 (71.6%) patients who had their weight recorded, mean weight was 82.5 kg (±17.29), with a range from 46 to 138 kg. Out of 106 (71.6%) patients who had their body mass index (BMI) recorded, 55 (37.2%) patients had a BMI $\geq$ 30 kg/m^2 classified as 'Obesity Class I, II or III' (Table 3.1).

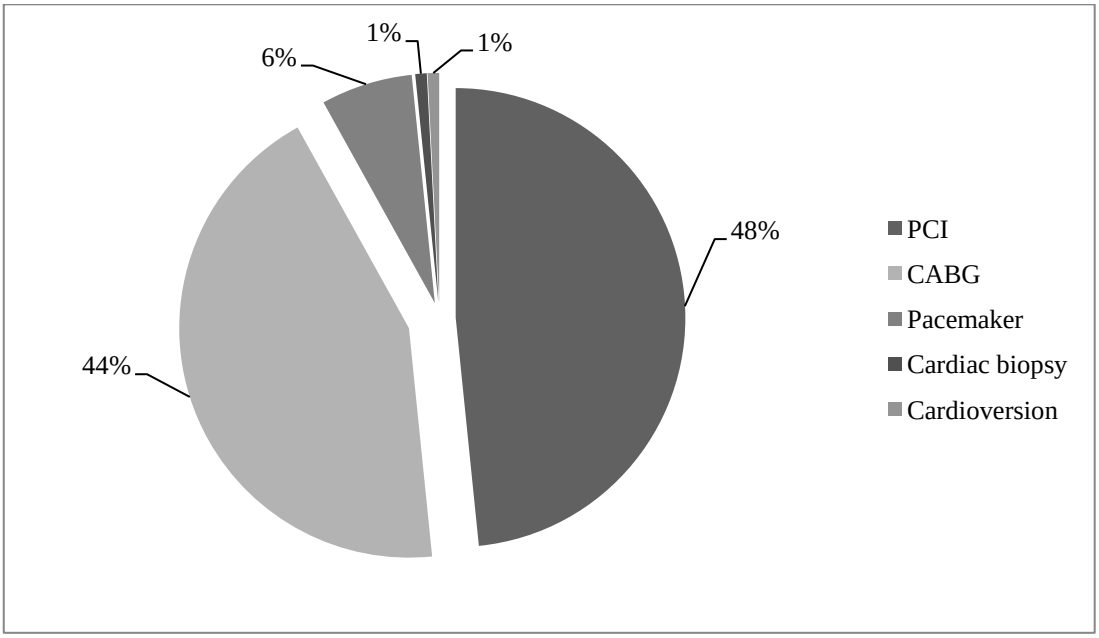
Table 3.1: BMI classification (n=106)

BMI classification (kg/m^2)	Number of patients	Percentage %
Underweight (<18.5)	1	0.9
Normal weight (18.5-24.99)	9	8.5
Pre-obesity (25-29.99)	41	38.7
Obesity Class I (30-34.99)	32	30.1
Obesity Class II (35-39.99)	16	15.1
Obesity Class III (>40)	7	6.6

Smoking was a common risk factor in the study population (n=66, 59.2%), where 34 (30.9%) patients were active smokers, 32 (29.1%) patients were past smokers and 44 patients (40.0%) never smoked.

With regards to alcohol consumption, 39 (26.3%) patients claimed to never consume alcohol. Ten (6.8%) patients consume alcohol regularly on a daily basis, 58 (39.2%) patients consume alcohol socially during the weekend, and 41 (27.7%) patients consume alcohol occasionally. Of the 58 patients who consume alcohol regularly/daily, 44 (75.8%) patients consume 1 to 5 units of alcohol, mostly wine, and 14 (24.2%) patients consume between 6 and 10 units.

One hundred and twenty-four (83.8%) patients were undergoing a cardiac procedure at time of recuirtment, mostly PCI (n=60, 40.3%) (Figure 3.1).

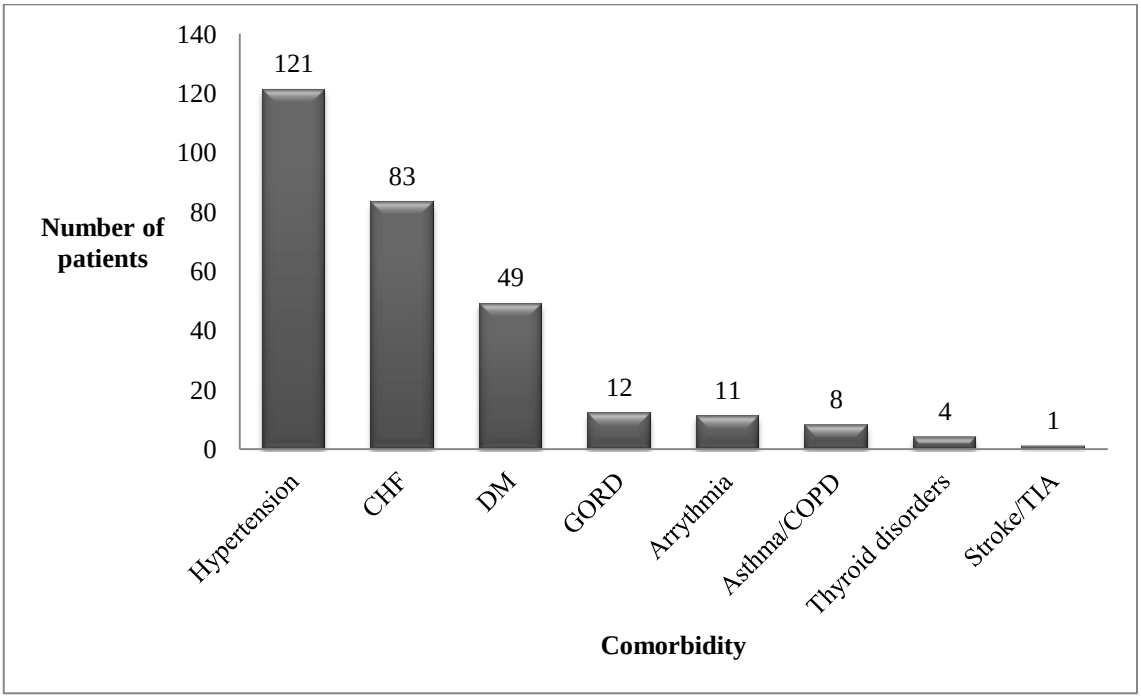


PCI: Percutaneous coronary intervention; **CABG:** Coronary artery bypass graft surgery

Figure 3.1: Distribution of cardiac procedures at time of recruitment (n=124)

3.3 Comorbidities

Hypertension, congestive heart failure and diabetes mellitus, which are all risk factors for dyslipidaemia, were the most common comorbidities with frequencies of 121 (81.75%), 83 (56.1%) and 41 (27.7%) respectively (Figure 3.2).



CHF: Congestive Heart Failure; **DM**: Diabetes Mellitus; **GORD**: Gastro-Oesophageal Reflux Disease; **COPD**: Chronic Obstructive Pulmonary Disease; **TIA**: Transient Ischemic Attack

Figure 3.2: Comorbidities (N=148)

3.4. Laboratory investigations

All patients had their lipid profile, liver function testsand renal function profile recorded. With respect to CK levels, only 12 patients had documented CK levels. The mean levels with SD are tabulated in Table 3.2. The parameters are all within range, except for LDL-C, which is higher than 2.0 mmol/L.

Table 3.2: Laboratory investigations

Parameters (reference range)	Mean ±SD
TC (2.0-5.0 mmol/L)	4.37 ±1.23
LDL-C (> 2.0 mmol/L)	2.41 ±1.04
HDL-C (0.9-1.45 mmol/L)	1.26 ±0.44
TG (0.1-2.26 mmol/L)	1.60 ±0.82
Bilirubin (0-17.1 µmol/L)	11.67 ±13.25
AP (40-129 U/L)	72.20 ±22.46
GGT (8-61 U/L)	47.58 ±48.78
ALT (10-50 U/L)	27.16 ±18.14
Serum Cr (59-104 µmol/L)	85.41 ±22.75
Urea (1.7 – 8.3 mmol/L)	6.82 ±2.88
eGFR (> 60ml/min/1.73m^2)	84.41 ±22.45
CK (22-198 U/L)	86 ±25.38

TC: Total cholesterol; **LDL-C:** Low-density lipoprotein cholesterol; **HDL-C**: High-density lipoprotein cholesterol; **AP**: Alkaline phosphatase; **GGT**: Gamma-Glutamyl Transferase; **ALT**: Alanine aminotransferase; **Cr:** Creatinine; **eGFR**: Estimated glomerular filtration rate; **CK:** Creatine kinase

3.5. Chronic Medications

All 148 patients were prescribed simvastatin. 62.4% (92) of the patients were prescribed 40mg daily (Figure 3.3).

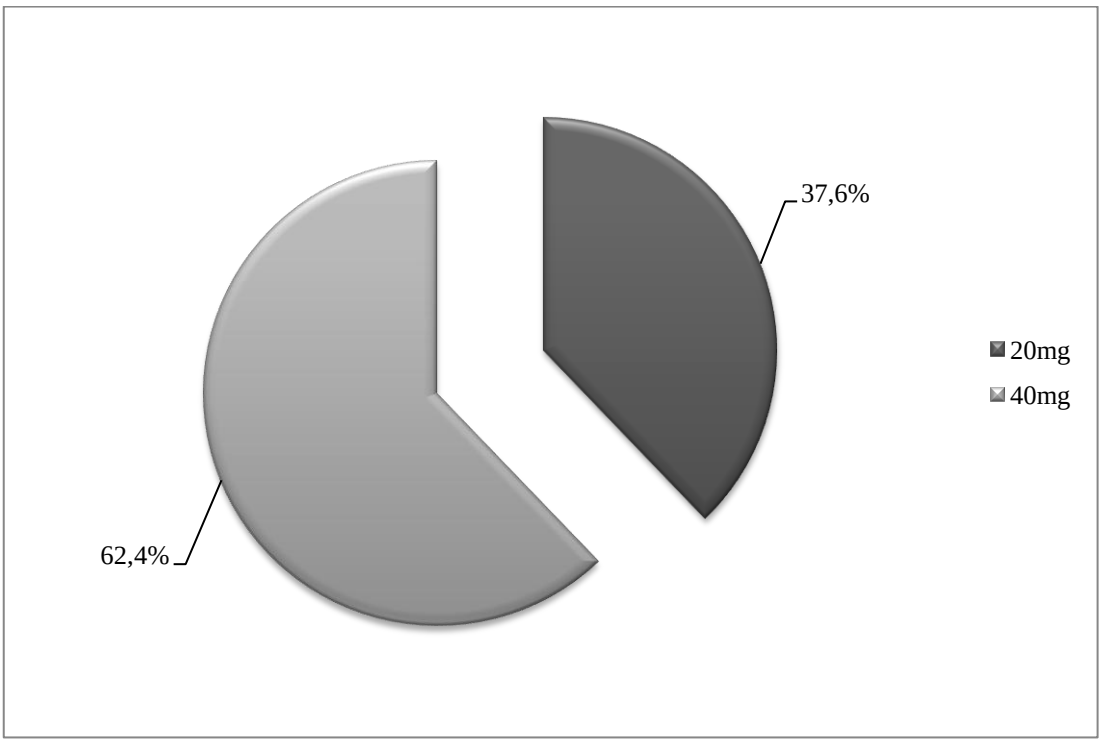
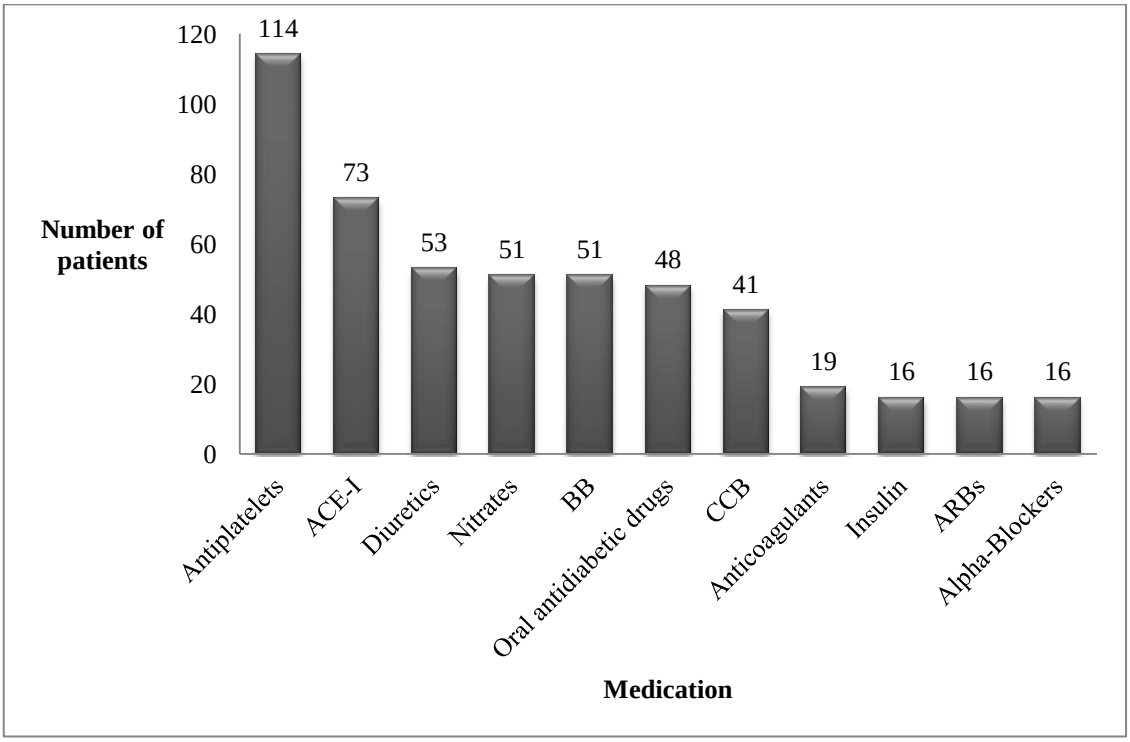


Figure 3.3: Distribution of patients according to simvastatin dose (N=148)

After simvastatin, the most frequently prescribed chronic medications in the study population were antiplatelets (n=114, 77%) and ACE inhibitors (n=73, 49.3%) (Figure 3.4).



ACE-I: Angiotensin Converting Enzyme Inhibitor; **BB**: Beta-Blocker; **CCB**: Calcium Channel Blocker; **ARB**: Angiotensin II Receptor Blocker

Figure 3.4: Chronic medications prescribed, excluding simvastatin (N=148)

Only 42 (28.4%) patients were aware of the start date of simvastatin or had it documented in hospital records; 19 patients (45.2%) started simvastatin within the previous 1-6 months from recruitment, 2 patients (4.8%) >6-12 months, 7 patients (16.7%) >1-5 years and 15 patients (35.7%) were on simvastatin for more than 5 years.

3.6. *SLCO1B1* SNP genotype results

With regards to *SLCO1B1* genotype distribution, the majority of patients (83.1%) were non-carriers of the *SLCO1B1* *5, **15* and **17* variant alleles. These patients were homozygous *1/1* wild type (TT) with normal *SLCO1B1* activity and normal myopathy risk. 14.86% were carriers of one *SLCO1B1* *5, **15* and *17 variant allele. These patients were heterozygous (TC) **1/5**, **1/*15* or **1/*17* with intermediate *SLCO1B1* activity and myopathy risk. 2.03% were carriers of two *SLCO1B1 *5, *15,* and **17* variant alleles. These patients were homozygous variant (CC) **5/*5, *5/*15, *5/*17, *15/*15, *15/*17*, or *17/*17, with low *SLCO1B1* function and high myopathy risk (Table 3.3).

Table 3.3: *SLCO1B1* genotype, *SLCO1B1* function and myopathy risk (N=148)

***SLCO1B1* rs4149056 genotype**	**Number of patients (%)**	***SLCO1B1* function - Phenotype**	**Myopathy risk**
TT	123 (83.11 %)	Normal	Normal
TC	22 (14.86 %)	Intermediate	Intermediate
CC	3 (2.03 %)	Low	High

The prevalence of *SLCO1B1* rs4149056 genotypes in this study was in accordance with the prevalence reported in the CPIC guidelines (Ramsey et al, 2014) for all three genotypes (Table 3.4)

Table 3.4: Comparison of SLCO1B1 genotype prevalence: Present study versus range reported in CPIC guideline

***SLCO1B1* rs4149056 genotype**	**Prevalence range** *(Ramsey et al, 2014)*	**Prevalence in this study**
TT	55-88%	83%
TC	11-36%	15%
CC	0-6%	2%

3.7. *SLCO1B1* rs4149056 genotype distribution in Maltese patients in present study compared to other populatations

Out of the total 148 patients, 144 patients were Maltese. With regards to *SLCO1B1* rs4149056 genotype distribution, the majority of patients (n=119, 82.6%) were non-carriers of the *SLCO1B1* C allele (TT), followed by heterozygous TC (n=22, 15.3%) or homozygous variant (n=3, 2.1%).

SLCO1B1 c521T>C genotype frequencies in Maltese patients were compared to 7 populations. Prevalence of the TT genotype in the Maltese population was only comparable with Japan and Germany ($p>0.05$). Prevalence of the TC genotype in the Maltese population was comparable only with Germany ($p>0.05$). Prevalence of the CC

genotype in the Maltese population was comparable to Brazil, China, France, Germany, Finland and Japan (p>0.05) (Table 3.5).

Table 3.5: Comparison of *SLCO1B1* genotype prevalence: Maltese patients versus other populations

Population (number of patients)	*SLCO1B1* rs4149056 genotype			References
	TT % (p-value)	TC % (p-value)	CC % (p-value)	
Malta (N=144)	83%	15%	2%	Present study
Japan (N=64)	73% (0.095)	27% (0.039)*	0% (0.254)	Tachibana-limori et al, 2004
Finland (N=468)	64% (<0.001)*	32% (<0.001)*	4% (0.254)	Pasanen et al, 2006a
Germany (N=250)	79% (0.337)	19% (0.312)	2% (1.000)	Rohrbacher et al, 2006
France (N=724)	72% (0.005)*	26.5% (0.003)*	1.5% (0.660)	Couvert et al, 2008
China (N=363)	71% (0.005)*	26% (0.007)*	3% (0.535)	Fu et al, 2010
Brazil (N=216)	71% (0.008)*	27% (0.007)*	2% (1.000)	Sortica et al, 2012
Russia (N=1,071)	62% (<0.001)*	32% (<0.001)*	6% (0.049)*	Sychev et al, 2015

*p<0.05 – statistically different

3.8. Outcome of discussion with cardiologists

Twenty-five patients were genotyped as carriers of *SLCO1B1* C allele (TC – 22; CC – 3) and details of the patients were communicated and discussed with the responsible consultant cardiologists. The cardiologists were given an informative letter with genotype results (Appendix 5), along with genotype-guided therapy recommendations according to the CPIC guidelines for *SLCO1B1* rs4149056 and SIM (Ramsey et al, 2014). Twenty-five informative letters were given to 7 consultant cardiologists and 8 letters were considered by 2 cardiologists. Two patients were changed to atorvastatin since rosuvastatin is not available on the GFL as first-line and patients refused to purchase it, and 6 patients were considered for CK monitoring and follow-up for muscle side-effects.

3.9. Patient follow-up results

All 148 patients were followed up 6 months post-recruitment. Fifteen out of 148 (10.1%) patients reported muscle symptoms at 6 months of follow up. Muscle stiffness was reported by 6 patients (5 TT; 1 TC), muscle cramps were reported by 4 TT patients, muscle pain was reported by 4 patients (3 TT; 1 CC) and muscle weakness was reported by 1 TC patient. Twelve TT patients reported muscle symptoms, 4 of these patients were on 20mg daily and 8 patients were taking 40mg daily. The 2 patients genotyped TC were on 20mg daily and 40mg daily respectively. The patient genotyped CC was on 40mg daily (Table 3.6).

Table 3.6: Muscle symptoms reported at 6-months follow-up (N=15)

Muscle symptom	TT (n=123)		TC (n=22)		CC (n=3)
	20mg	40mg	20mg	40mg	40mg
Stiffness	1	4	1	0	0
Cramps	2	2	0	0	0
Pain	1	2	0	0	1
Weakness	0	0	0	1	0
N (%)	12 (9.76%)		2 (9%)		1 (33.3%)

The pairwise comparison between TT versus TC + CC percentage differences are not significant since the p-values exceeded the 0.05 level of significance. Although one of the percentages (33.3%) is large compared to the other percentages (9.76% and 9%), this percentage was only computed on a small sample size (n=3) hence it has a small impact on the p-value.

Chapter Four

Discussion

4.1. *SLCO1B1* genotyping for personalisation of statin therapy

Muscle symptoms are the most common side-effects of statins and prevention and diagnosis are challenging (Catapano et al, 2016; Ramachandran & Wierzbicki, 2017). In 2008, the SEARCH study identified a genetic variant, the *SLCO1B1* rs4149056 gene, to be associated with simvastatin-induced myopathy (Link et al, 2008). In this study, the percentage of patients who were carriers of the *SLCO1B1* rs4149056 polymorphism in a cohort of cardiac patients on simvastatin was determined and the association between *SLCO1B1* rs4149056 and simvastatin-associated muscle symptoms was explored. Simvastatin is the statin reported to be the most influenced by the *SLCO1B1* rs4149056 genetic polymorphism (Brunham et al, 2012; Carr et al, 2013; Ramsey et al, 2014).

The majority of patients in this study population (83%) were non-carriers of the *SLCO1B1* rs4149056 SNP and were genotyped as homozygous wild-type TT, with "normal" *SLCO1B1* function and "normal" myopathy risk, which means that the function of the OATP1B1 transporter is not decreased and implies that myopathy risk is not dependent on *SLCO1B1* genetic variation. In this study, 15% of the patients were carriers of one decreased function C allele, heterozygous TC, with *SLCO1B1* "intermediate" function. This implies that the function of OATP1B1 is decreased and the myopathy risk is "intermediate" compared to the TT genotype, implying a higher susceptibility to myopathy compared to non-carriers.

Three patients (2%) were genotyped as carriers of two decreased function C alleles, classified as homozygous variant, with "low" *SLCO1B1* function and "high" myopathy risk compared to patients genotyped as TT and TC. These findings are in line with the

percentage ranges stated in the CPIC guideline and are in accordance with the incidence reported for Caucasians (Ramsey et al, 2014). The frequency of the *SLCO1B1* C allele in this study is comparable to other studies in different populations (Tachibana-limori et al, 2004; Couvert et al, 2008; Fu et al, 2010; Sortica et al, 2012; Sychev et al, 2015)

The CPIC guideline was updated in 2014 and states the importance of the correlation between *SLCO1B1* c.521T>C and SIM and lower clinical importance for the other statins. In the case of TC and CC patients, the guideline recommends prescribing simvastatin at a lower daily dose (20mg) or to consider an alternative statin, such as pravastatin or rosuvastatin, with routine CK monitoring to personalise statin therapy (Ramsey et al, 2014).

The population studied in this research were taking a 20mg or 40mg daily dose of simvastatin. The SEARCH study sample population were on 20mg and 80mg daily (Link et al, 2008), and the Heart Protection Study sample were taking 40mg daily (Collins et al, 2002). Brunham et al (2011) reported a mean dose of 30mg in their study population and the findings confirmed the association between *SLCO1B1* rs4149056 with simvastatin even at lower doses.

The 17% of patients genotyped as carriers of one or two *SLCO1B1* C alleles in the present study have mild and high risk of myopathy respectively. Moreover, 15 out of the 25 patients genotyped as TC or CC were reported to be prescribed a simvastatin daily dose of 40mg, which is a dose higher than that recommended by the CPIC guideline (20mg).

The current situation in Malta is that simvastatin is available on the GFL as 10mg, 20mg or 40mg tablets.[11] Atorvastatin and rosuvastatin are the alternatives available on the GFL. Simvastatin and atorvastatin are first-line and rosuvastatin is considered second-line if intolerability to or ineffectiveness with simvastatin and atorvastatin are demonstrated. Thus, when performing a medication evaluation according to *SLCO1B1* genotype, switch from simvastatin to rosuvastatin in carriers of the C allele is presently not an option since rosuvastatin has to be purchased out-of-pocket by the patients and patients were reluctant to do so. Hence, if pharmacogenetic testing for statin therapy were to be applied locally, a change in statin prescribing protocols would be required. For this study, the cost of the Sacace® Biotechnology kits used was €551.46 for 60 tests (December 2018), of which 4 are used for controls. The estimated cost for an individual *SLCO1B1* rs4149056 genotyping test is €13.23 (€9.39 + €3.84 for genomic DNA extraction).

Although the cardiologists were responsive to the letters of recommendation based on patient genotype and CPIC guideline, the action taken was not entirely in accordance with the CPIC guideline, mainly due to the unavailability of rosuvastatin on the Maltese GFL. The genotype was recorded by one cardiologist in the electronic patient record to be referred to when monitoring the patient for side-effects during outpatient visits. This finding indicates the first step, although small, in the personalisation of statin therapy locally since cardiologists were made aware of this pharmacogenetic testing and its implications.

[11] Directorate for Pharmaceutical Affairs. Hospital formulary list [Internet]. Malta: Ministry for Health; 2019 [cited 2019 April 1] Available from: https://deputyprimeminister.gov.mt/en/pharmaceutical/Documents/GFL/hosp_gfl_mar_2019.pdf

The implementation of pharmacogenetics into the clinical scenario is occurring at a slow pace due to various barriers including lack of test availability, evidence-based recommendations, guidelines for prescribing, integration of genotype results into health records, lack of health care professional awareness and training and cost limitations (Haga et al, 2012; Stanek et al, 2012). Pharmacogenetics education for physicians was shown to require more input to enhance the use pharmacogenetic results as part of patient management and prescribing practices (Patel et al, 2014; Taber & Dickinson, 2014; Unertl et al, 2015; St Sauver et al, 2016), and the main challenge stated by physicians was time limitations to attend courses and other educational activities on pharmacogenetics (Grimshaw et al, 2002; Selkirk et al, 2013).

The integration of pharmacogenetics services into clinical practice and education of healthcare professionals may result in improvement in patient outcomes, in the case of statin therapy to enhance patient safety and increase treatment adherence (Li et al, 2014; Haga & Lapointe, 2013).

Medication adherence may be increased by patient education of their condition, reducing concerns about medication and shared decision-making between the patient and the physician. Statin treatment adherence starts with an interaction between the patient and the consultant with an understanding of the benefits and risks of statin therapy. The patients for whom the *SLCO1B1* genotype was known had alternative statins prescribed, rousuvastatin or pravastatin, and were willing to adhere to their treatment according to Li et al, (2014). *SLCO1B1* gene information may be influential

in predicting potential side-effects of statins and in guiding dosing strength as a goal towards personalisation of statin treatment (Patel et al, 2014).

The application of pharmacogenomic information requires an understanding of molecular pathways and how genetic variation influences the pharmacokinetic and pharmacodynamic properties of a drug in specific diseases and patient populations (Haidar et al, 2015). Pharmacists may aid to increase the understanding of clinicians regarding pharmacogenetics practice and implement personalised therapy according to individual genetic implications to develop safer and more effective approaches to patient care, since pharmacists are experts on drug therapy and are able to support the clinical implementation of pharmacogenomics (McCullough et al, 2011). In order to implement pharmacogenetics into the clinical setting, pharmacists need to understand the emerging science of pharmacogenomics. Pharmacists, as drug therapy educators, should empower their roles in pharmacogenomics for the collaboration between healthcare professionals so as to promote safe, effective and cost-efficient therapy (Farrugia & Weinshilboum, 2013; Formea et al, 2013; Haidar et al, 2015; Formea et al, 2018).

One out of the three homozygous variant CC patients had documented muscle pain at six months follow-up. This patient was taking 40mg simvastatin. In this case the cardiologist did not take up the SLCO1B1 genotype result and did not reduce the dose to 20mg, hence more awareness and convincing is required. The sample size of TC and CC patients experiencing muscle side-effects is small, however this research showed a signal which warrants further investigation.

4.2. Monitoring of statin-associated muscle symptoms to promote patient safety

SLCO1B1 has been strongly associated with statin-associated muscle symptoms, however there are other patient characteristics which require monitoring. The mechanism for statin-associated muscle symptoms remains unknown, and appears to be influenced by a combination of various patient and statin characteristics, including age, gender, statin type and dose (Link et al, 2008; Voora et al, 2009; Iwere & Hewitt, 2015; Zhou et al, 2017).

The Prediction of Muscular risk in Observational conditions (PRIMO) study reported that patients taking simvastatin and atorvastatin were at higher risk of developing statin-associated muscle symptoms (simvastatin 18.2%, atorvastatin 14.9%, pravastatin 10.9%, fluvastatin 5.1%). In the PRIMO study, patients were taking high doses of simvastatin (40mg, 80mg) and atorvastatin (40mg, 80mg) (Bruckert et al, 2005). In 2010 health regulatory agencies, including the US FDA[12,13], the EMA and the UK Medicines and Healthcare Products Regulatory Agency (MHRA), followed by Health Canada in 2012[14], issued warnings against the use of high-dose 80mg simvastatin and issued treatment recommendations regarding dose-related ADRs caused by high doses

[12] Food and Drug Administration (FDA). FDA warns about increased risk of muscle injury with Zocor [Internet]. US: FDA; 2010. [cited 2019 May 20]. Available from: http://www.fda.gov/ NewsEvents/Newsroom/PressAnnouncements/ucm205215.htm.

[13] Food and Drug Administration (FDA). FDA announces new safety recommendations for high-dose simvastatin [Internet]. US: FDA; 2010. [cited 2019 May 20]. Available from: http://www. fda.gov/NewsEvents/Newsroom/PressAnnouncements/ ucm258338.htm.

[14] Health Canada. Health Canada endorsed important safety information on Zocor (simvastatin) [Internet]. Canada: Health Canada; 2012 [cited 2019 May 20]. Available from: http://www. healthycanadians.gc.ca/recall-alert-rappel-avis/hc-sc/2012/15826a-eng.php?_ga=1.181258983.15809296.1441990120.

of simvastatin (Anand et al, 2017). The latest version of SmPCs approved by EMA report the association between *SLCO1B1* rs4149056 and statin-induced myopathy with simvastatin, rosuvastatin, and atorvastatin. However, it is stated that the clinical evidence is highest for simvastatin.[15,16,17]

The risk of myalgia, myopathy and rhabdomyolysis is dependent on statin dose. High doses of statins are reported to increase the incidence of statin-associated muscle symptoms and this finding is reported multiple trials (Collins et al, 2002; Bruckert et al, 2005; Link et al, 2008; Ward et al, 2019).

The PRIMO study reported that gender is not a risk factor for developing statin-associated muscle symptoms (Bruckert et al, 2005). However, there are several studies that state female gender as a risk to develop statin-associated muscle symptoms (Bitzur et al, 2013; Mancini et al, 2013; Banach et al, 2015; Mancini et al, 2016). Age is also considered one of the risk factors predisposing the appearance of SAMS due to the presence of multiple comorbidities and polypharmacy with advancing age (Mancini et al, 2016; Ward et al, 2019). It was reported that patients ≥75 years have higher risk to develop SAMS (Catapano et al, 2016; Toth et al, 2018).

[15] Electronic Medicines Compendium (eMC). Simvastatin 40mg Tablets - Summary of Product Characteristics (SmPC) [Internet]. UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4591/smpc

[16] Electronic Medicines Compendium (eMC). Rosuvastatin 20mg Tablets - Summary of Product Characteristics (SmPC) [Internet]. UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4366/smpc

[17] Electronic Medicines Compendium (eMC). Atorvastatin 10mg Tablets - Summary of Product Characteristics (SmPC) [Internet] UK: eMC; 2019 [cited 2019 Jun 17]. Available from: https://www.medicines.org.uk/emc/product/4109/smpc

The results of the studies regarding statin-associated muscle symptoms are controversial and there is a need to carry out further investigation to support the evidence of the risk to develop myalgia, myopathy and rhabdomyolysis, incorporating genetic factors and patient characteristics.

4.3. Study limitations

The following study limitations were identified. The patients were recruited by convenience sampling and not consecutively or by random sampling. Muscle symptoms are not easy to diagnose since there are no standardised definitions and no diagnosis classification. Patient self-reported muscle symptoms could be subjective and muscle symptoms may be caused by other factors, hence they should be cautiously interpreted. Lack of documentation of muscle symptoms in hospital records, even when self-reported by patients, was observed in this study. Only 12 patients had documented CK levels in hospital records and the start date of simvastatin was not easily attainable from hospital records nor from patient self-report, and was only recorded for 42 patients.

4.4. Recommendations for further study

All the studies assessing the association between *SLCO1B1* rs4149056 and SIM (section 1.6) recommend future studies to be conducted to further explore the association of *SLCO1B1* c.521T>C with simvastatin. It is recommended for the patients recruited to be followed up for a longer period than 6 months and to conduct prospective studies with a larger patient cohort to investigate the association between the *SLCO1B1* c.521T>C genotype and myopathy for personalisation of statin therapy.

The association between *SLCO1B1* rs4149056 and rosuvastatin-induced myopathy is not clearly established, and further investigation to assess the effect of the *SLCO1B1* C allele on the safety of rosuvastatin is warranted since the CPIC guidelines recommend to switch from simvastatin to rosuvastatin in carriers of C allele. Exploring the association between *SLCO1B1* rs4149056 and rosuvastatin-induced myopathy is also in line with the pharmacogenetic recommendations in the EMA SmPC for rosuvastatin.

A study carried by De Vera et al, (2014) reported that the main reason for discontinuation and non-adherence to statin therapy is the occurrence of muscle symptoms, which may impact on the CVD benefits of statins. Further study to assess statin adherence and to investigate whether muscle symptoms impact adherence to statin therapy is recommended.

Ramsey et al, (2014) documented the effect of *SLCO1B1* rs4149056 on statins effectiveness with respect to LDL-C reduction. The gene is reported to decrease the effectiveness of statins leading to higher plasma levels of LDL-C. The *LDLR* gene is also reported to decrease the effectiveness of statins vis-a-vis LDL-C reduction. Further study to investigate the effect of these genes on the effectiveness of statin therapy is recommended.

CK levels are reported to not be a reliable indicator of statin-associated muscle symptoms, since myopathy and other muscle symptoms have been reported without CK elevations. However, CK is the only predictor to diagnose myopathy to-date. The correlation between myopathy and CK levels is recommended to be explored.

The clinical presentation of statin-associated muscle symptoms is highly heterogeneous, usually with normal or slightly elevated CK levels, and there is no standardised definition and classification of statin-associated muscle symptoms. A definitive diagnosis of statin-associated muscle symptoms is challenging since symptoms are subjective, there is no gold standard diagnostic test and no validated muscle symptom questionnaire (McKenney et al, 2006; Sewright et al. 2007; Keen et al, 2014; Stroes et al, 2015; Selva-O'Callaghan et al, 2018; Ward et al, 2019). Recommendations for further study would be to review definitions and terminologies for statin-associated muscle symptoms and to develop and validate a muscle symptom questionnaire for diagnosis and classification of statin-associated muscle symptoms.

4.5. Contribution of the study to practice

Statins are one of the most extensively prescribed drug classes. The incidence of statin-associated muscle symptoms is a main cause of discontinuation and non-adherence to statin therapy. *SLCO1B1* genotyping has the potential to identify patients predisposed to simvastatin-induced myopathy, and by decreasing the simvastatin dose or changing the statin in accordance with genotype and CPIC recommendations, the risk of SIM may be mitigated. This study is an example of personalisation of therapy to achieve precision medicine in patients on statin therapy in the interest of patient safety and to improve adherence. Findings from this study also contribute to existing studies documenting the prevalence of *SLCO1B1* genotypes in different populations.

The pharmacogenetic implications of simvastatin therapy were explored in this research in a cardiac setting and *SLCO1B1* rs4149056 may be the right indicator for the

prevention of simvastatin-induced myopathy together with consideration of other patient characteristics. However further study is warranted.

4.6. Conclusion

This study identified 15% patients as carriers of one decreased function C allele and 2% of patients as carriers of two decreased function C alelles, corresponding to mild myopathy risk and high myopathy risk respectively according to the CPIC guideline for *SLCO1B1* and simvastatin-induced myopathy. Fifteen of the 25 TC and CC patients were prescribed simvastatin 40mg and the CPIC guideline recommends a lower simvastatin dose (20mg/day) or consideration of another statin (rosuvastatin) in these patients.

One of the 3 CC patients had documented myalgia at follow-up. The CC patient reported muscle pain at 40mg which it may be a signal for further follow-up and assessment for statin therapy and safety. The findings in this study are exploratory and the signals observed warrant further investigation.

Dissemination of study findings

Cerdá Iñesta J, Wirth F, Zahra G, Xuereb RG, Barbara C, Serracino-Inglott A. SLCO1B1 genetic polymorphisms in cardiac patients on simvastatin (Poster Presentation, 78th FIP World Congress of Pharmacy and Pharmaceutical Sciences, 2-6 September 2018, Glasgow, Scotland)

Cerdá Iñesta J, Wirth F, Zahra G, Xuereb RG, Barbara C, Serracino-Inglott A. Pharmacogenetic testing as a tool in precision medicine for statin therapy.
(Oral Presentation, Maltese Cardiac Society Conference, October 2018, Malta)

Cerdá Iñesta J, Wirth F, Zahra G, Xuereb RG, Barbara C, Serracino-Inglott A. Pharmacogenetic Testing in Precision Medicine for Statin Use. Malta Medical Journal 2018; 30 (Suppl): 84.
(Oral Presentation, 10th Malta Medical School Conference, 2018, Malta)

Cerdá Iñesta J, Wirth F, Zahra G, Xuereb RG, Barbara C, Serracino-Inglott A. Pharmacogenetic testing for personalisation of statin therapy. EJHP. 2019;26(Suppl 1): A211.
(Poster Presentation, 24th Congress of the European Association of Hospital Pharmacy, 27-29 March 2019, Barcelona, Spain) – *This poster was selected for the Poster Walk.*

Cerdá Iñesta J, Wirth F, Serracino-Inglott A. Pharmacovigilance analysis of statin-associated muscle symptoms.
(Poster Presentation, 79th FIP World Congress of Pharmacy and Pharmaceutical Sciences, 22-26 September 2019, Abu Dhabi, United Arab Emirates)

References

Abd TT, Jacobson TA. Statin induced myopathy: A review and update. Expert Opin Drug Saf. 2011;10(3):373-87.

Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem. 1999;274(24):17159-63.

Agarwal V, Phung OJ, Tongbran V, Bhardwaj A, Coleman CI. Statin use and the prevention of venous thromboembolism: A meta-analysis. Int J Clin Pract. 2010;64(10):1375-83.

Anand K, Sketris I, Zhang Y, Levy A, Gamble JM. The impact of US FDA and Health Canada warnings related to the safety of high-dose simvastatin. Drugs Real World Outcomes. 2017;4(4):215-223.

Arnadottir M, Eriksson LO, Thysel H, Karkas JD. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron. 1993;65(3):410-3.

Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: A meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670-81.

Banach M, Rizzo M, Toth PP, Farnier M, Davidson MH, Al-Rasadi K, et al. Statin intolerance - an attempt at a unified definition. Position paper from an International Lipid Expert Panel. Arch Med Sci. 2015;14(6):935–55.

Barylski M, Nikfar S, Mikhailidis DP, Toth PP, Salari P, Ray KK, et al. Statins decrease all-cause mortality only in CKD patients not requiring dialysis therapy: A meta-analysis of 11 randomized controlled trials involving 21,295 participants. Pharmacol Res. 2013;72:35-44.

Bifulco M, Malfitano AM, Marasco G. Potential therapeutic role of statins in neurological disorders. Exp Rev Neurother. 2008;8(5):827-37.

Bitzur R, Cohen H, Kamari Y, Harats D. Intolerance to statins: Mechanisms and management. Diabetes Care. 2013;36(suppl 2):S325–30.

Birmingham BK, Bujac SR, Elsby R, Azumaya CT, Zalikowski J, Chen Y, et al. Rosuvastatin pharmacokinetics and pharmacogentics in Caucasian and Asian subjests residing in the United States. Eur J Clin Pharmacol. 2015;71(3):329-40.

Bruckert E, Hayem G, Dejager S, Yau C, Begaud B. Mild to moderate muscular symptoms with high-dosage statin therapy in hyperlipidemic patients – The PRIMO study. Cardiovasc Drugs Ther. 2005;19(6):403-14.

Brunham LR, Lansberg PJ, Zhang L, Miao F, Carter C, Hovingh GK, et al. Differential effect of the rs4149056 variant in *SLCO1B1* on myopathy associated with simvastatin and atorvastatin. Pharmacogenomics J. 2012;12(3):233-7.

Canestaro WJ, Austin MA, Thummel KE. Geneticc factors affecting statin concentrationns and subsequent myopathy: a HuGENet systematic review. Genet Med. 2014;16(11):810-9.

Carr D, O'Meara H, Jorgensen A, Campbell J, Hobbs M, McCann G, et al. *SLCO1B1* genetic variant associated with statin-induced myopathy: A proof-of-concept study using the Clinical Practice Research Datalink. Clin Pharmacol Ther. 2013;94(6):695-701.

Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. Eur Heart J. 2016;37(39):2999-3058

Chatzizisis YS, Koskinas KC, Misirli G, Vaklavas C, Hatzitolios A, Giannoglou GD. Risk factors and drug interactions predisposing to statin-induced myopathy: implications for risk assessment, prevention and treatment. Drug Saf. 2010;33(3):45-9.

Collins GS, Altman DG. Predicting the adverse risk of statin treatment: An independent and external validation of Qstatin risk scores in the UK. Heart. 2012;98(14):1091-7.

Collins R, Armitage J, Parish S, Sleight P, Peto R. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: A randomised placebo-controlled trial. Lancet. 2002;360(9326):7-22.

Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. Lancet. 2016;388(10059):2535-61.

Couvert P, Giral P, Dejager S, Gu J, Huby T, Chapman MJ, et al. Association between a frequent allele of the gene encoding OATP1B1 and enhanced LDL-lowering response to fluvastatin therapy. Pharmacogenomics. 2008;9(9):1217-27.

Danik JS, Chasman DI, Macfadyen JG, Nyberg F, Barrat BJ, Ridker PM. Lack of association between *SLCO1B1* polymorphisms and clinical myalgia following rosuvastatin therapy. Am Heart J. 2013;165(6):1008-14.

De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: A systematic review. Br J Clin Pharmacol. 2014;78:684-98.

Deedwania P, Barter P, Carmena R, Fruchart JC, Grundy SM, Haffner S, et al. Reduction of low-density lipoprotein cholesterol in patients with coronary heart disease and metabolic syndrome: Analysis of the Treating to New targets study. Lancet 2006;368(9539):919–28.

Deng JW, Song IS, Shin HJ, Yeo CW, Cho DY, Shon JH, et al. The effect of *SLCO1B1*15* on the disposition of pravastatin and pitavastatin is substrate dependent: The contribution of transporting activity changes by *SLCO1B1*15*. Pharmacogenet Genomics. 2008;18(5):424-33

Donnelly LA, Doney AS, Tavendale R, Lang CC, Pearson ER, Colhoun HM, et al. Common nonsynonymous substitution in *SLCO1B1* predispose to statin intolerance in routinely treated individuals with type 2 diabetes: A go-DARTS study. Clin Pharmacol Ther. 2011;89(2):210-6.

Duncan AJ, Hargreaves IP, Damian MS, Land JM, Heales SJ. Decreased ubiquinone availability and impaired mitochondrial cytochrome oxidase activity associated with statin treatment. Toxicol Mech Methods. 2009;19(1):44-50.

Farrugia G, Weinshilboum RM. Challenges in implementing genomic medicine: the Mayo Clinic Center for individualized medicine. Clin Pharmacol Ther. 2013;94:204-6

Fedson DS. Pandemic influenza: A potential role for statins in treatment and prophylaxis. Clin Infect Dis. 2006;43(2):199-205.

Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459-72.

Ferrari M, Guasti L, Maresca A, Mirabile M, Contini S, Grandi AM, et al. Association between statin-induced creatine kinase elevation and genetic polymorphisms in *SLCO1B1*, *ABCB1* and *ABCG2*. Eur J Clin Pharmacol. 2014;70:539-47.

Fiegenbaum M, da Silveira FR, Van der Sand CR, Van der Sand LC, Ferreira ME, Pires RC, et al, The role of common variants of ABCB1, CYP3A4, and CYP3A5 genes in lipid-lowering efficacy and safety of simvastatin treatment. Clin Pharmacol Ther. 2005;78(5):551-8.

Formea CM, Nicholson WT, McCullough KB, Berg KD, Berg ML, Cunningham JL, et al. Develpment and evaluation of a pharmacogenomics educational program for pharmacist. Am J Pham Educ. 2013;77(1):10.

Formea CM, Nicholson WT, Vitek CR, McCullough KB, Cunningham JL, Zeuli JD, et al. Implementation of a pharmacogenomics education program for pharmacist. Am J Health Syst Pharm. 2018;75(23):1939-1946.

Fu Q, Li YP, Gao Y, Yang SH, Lu PQ, Jia M, et al. Lack of association between *SLCO1B1* polymorphism and the lipid-lowering effects of atorvastatin and simvastatin in Chinese individuals. Eur J Clin Pharmacol. 2013;69(6):1269-74.

Fulcher J, Mihaylova B, O'Connel R, Emberson J, Blackwell L, Reith C, et al. Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials. Lancet. 2019;393(10170):407-415.

Ghatak A, Faheem O, Thompson PD. The genetics of statin-induced myopathy. Atherosclerosis. 2010;210(2):337-43.

Grapci AD, Dimovski AJ, Kapedanovska A, Vavlukis M, Eftimov A, Geshkovska NM, et al. Frequencies of single-nucleotide polymorphisms and haplotypes of the *SLCO1B1* gene in selected populations of the western balkans. Balkan J Med Genet. 2015;18(1):5-21.

Grundy SM. Approach to lipoprotein management in 2001 National Cholesterol Guidelines. Am J Cardiol. 2002;90(8A):11i–21i.

Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation. 2004;24(8):227–39.

Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA guideline on the management of blood cholesterol: A report of the American College of Cardiology/American Heart Association task force on cinical practice guidelines. Circulation. 2019;139(25):e1082-e1143.

Goldstein JL, Brown MS. A century of cholesterol and coronaries: From plaques to genes to statins. Cell. 2015;161(1):161-172.

Grimshaw JM, Eccles MP, Walker AE, Thomas RE. Changing physicians' behavior: What works and thoughts on getting more things to work. J Contin Educ Health Prof. 2002; 22(4):237–43.

Haga SB, Burke W, Ginsburg GS, Mills R, Agans R. Primary care physicians' knowledge of and experience with pharmacogenetic testing. Clin Genet. 2012; 82(4):388–94.

Haga SB, Lapointe NM. The potential impact of pharmacogenetic testing on medication adherence. Pharmacogenomics J. 2013;13:481-483.

Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. Pflugers Arch. 2004;447(5):653-65.

Haidar CE, Hoffman JM, Johnson SG, Relling MV, Crews KR. ASHP statement on the pharmacist's role in clinical pharmacogenomics. Am J Health-Syst Pharm. 2015;72(7):579-81.

Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identificatioin of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem. 1999;274(52):37161-68.

Hubacek JA, Dlouha D, Adamkova V, Zlatohlavek L, Vicklicky O, Hruba P, et al. *SLCO1B1* polymorphism is not associated with risk of statin-induced myalgia/myopathy in a Czech population. Med Sci Monit. 2015;21:1454-9.

Iwai M, Suzuki H, Ieiri I, Otsubo K, Sugiyama Y. Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). Pharmacogenet Genomics. 2004;14(11):749-57.

Iwere RB, Hewitt J. Myopathy in older people receiving statin therapy: A systematic review and meta-analysis. Br J Clin Pharmacol. 2015;80(3):363-371.

Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. Br J Pharmacol. 2009;158(3):693-705.

Kameyama Y, Yamashita K, Kobayashi K, Hosokawa M, Chiba K. Functional characterization of *SLCO1B1* (OATP-C) variants, *SLCO1B1*5*, *SLCO1B1*15* and *SLCO1B1*15+C1007G*, by using transient expression systems of HeLa and HEK293 cells. Pharmacogenet Genomics. 2005; 15(7):513-22.

Kapur NK, Musunuru K. Clinical efficacy and safety of statins in managing cardiovascular risk. Vasc Health Risk Manag. 2008;4(2):341-53.

Karlson BW, Palmer MK, Nicholls SJ, Lundman P, Barter PJ. Doses of rosuvastatin, atorvastatin and simvastatin that induce equal reductions in LDL-C and non-HDL-C: Results from the VOYAGER meta-analysis. Eur J Prev Cardiol. 2016;23(7):744-7.

Kavalipati N, Shah J, Ramakrishan A, Vasnawala H. Pleiotropic effects of statins. Ind J Endocrinol Metabol. 2015;19(5):554–62.

Kaysen GA. Lipid-lowering therapy in CKD: Should we use it and in which patients. Blood Purif. 2017;43(1-3):196-199.

Keen H, Krishnarajah J, Bates TR, Watts GF. Statin myopathy: The fly in the ointment for the prevention of cardiovascular disease in the 21st century? Expert Opin Drug Saf. 2014;13(9):1227-39.

Keltz E, Khan FY, Mann G. Rhabdomyolysis. The role of diagnostic and prognostic factors. Muscles Litaments Tendons J. 2014;3(4):303-12.

Keskitalo JE, Kurkinen KJ, Neuvoneni PJ, Niemi M. ABCB1 haplotypes differentially affect the pharmacokinetics of the acid and lactone forms of simvastatin and atorvastatin. Clin Pharmacol Ther. 2008;84(4):197-203.

Kitzmiller JP, Luzum JA, Dauki A, Krauss RM, Medina MW. Candidate-gene study of functional polymorphisms in *SLCO1B1* and CYP3A4/5 and the cholesterol-lowering response to simvastatin. Clin Transl Sci. 2017;10(3):172-77.

Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. 2000a;278(1):G156-64.

Konig J, Cui Y, Nies AT, Keppler D. Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. J Biol Chem. 2000b;275(30):23161-68.

Law M, Rudnicka AR. Statin safety: A systematic review. Am J Cardiol. 2006;97(8A):52C-60C.

Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol. 2005;45:89–118.

Li JH, Joy SV, Haga SB, Orlando LA, Kraus WE, Ginsburg GS, et al. Genetically guided statin therapy on statin perceptions, adherence, and cholesterol lowering: A pilot implementation study in primary care patients. J Pers Med. 2014;4:147-162.

Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, et al. *SLCO1B1* variants and statin-induced myopathy – a genomewide study. N Eng J Med. 2008;359(8):789-99.

Liu JE, Liu XY, Chen S, Zhang Y, Cai LY, Yang M, et al. SLCO1B1 521T>C polymorphism associated with rosuvastatin-induced myotoxicity in Chinese coronary artery disease patients: A nested case-control study. Eur J Clin Pharmacol. 2017;73:1409-16.

Maji D, Shaikh S, Solanki D, Gaurav K. Safety of statins. Indian J Endocrinol Metab. 2013;17(4):636-46.

Mancini GB, Tashakkor AY, Baker S, Bergeron J, Fitchett D, Frohlich J, et al. Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Working Group Consensus update. Can J Cardiol. 2013;29:1553-68.

Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Genest J, et al. Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Consensus Working Group Update . Can J Cardiol. 2016;32:S35–S65.

Marrs JC, Kostoff MD. Discontinuation of statins: What are the risks? Curr Atheroscler Rep. 2016;18(7):41.

Mastaglia FL. Iatrogenic myopathies. Curr Opin Neurol. 2010;23:45-49.

McCullough KB, Formea CM, Berg KD, Burzynski JA, Cunningham JL, Ou NN, et al. Assessment of the pharmacogenomics educational needs of pharmacists. Am J Pharm Educ. 2011;75(3):51.

McKenney JM, Davidson MH, Jacobson TA, Guyton JR. Final conclusions and recommendations of the National Lipid Association Statin Safety Assessment Task Force. Am J Cardiol. 2006;97(8A):89C-94C.

Mohaupt MG, Karas RH, Babiychuk EB, Sanchez-Freire V, Monastyrskaya K, Lyer L, et al. Association between statin-associated myopathy and skeletal muscle damage. CMAJ. 2009;181:E11-8.

Mombelli G, Pavanello C. Statin muscle toxicity and genetic risk factors. Int J Genomic Med. 2013;1:111.

Morimoto K, Ueda S, Seki N, Igawa Y, Kameyama Y, Shimizu A, et al. OATP-C (OATP01B1) *15 is associated with statin-induced myopathy in hypercholesterolemic patients. Clin Pharmacol Ther. 2005;77(2):P21-P21.

Morival C, Westerlynck R, Bouzille G, Cuggia M, Le Corre P. Prevalence and nature of statin drug-drug interactions in a university hospital by electronic health record mining. Eur J Clin Pharmacol. 2018;74(4):525-34.

Moßhammer D, Schaeffeler E, Schwab M, Morike K. Mechanisms and assessment of statin-related muscular adverse effects. Br J Clin Pharmacol. 2014;78(3):454-66.

Mulder AB, Van Lijf HJ, Bon MA, et al. Association of polymorphism in the cytochrome CYP2D6 and the efficacy and tolerability of simvastatin. Clin Pharmacol Ther. 2001;70:546-51.

Mwinyi J, Kopke K, Schaefer M, Roots I, Gerloff T. Comparison of *SLCO1B1* sequence variability among German, Turkish and African populations. Eur J Clin Pharmacol. 2008;64:257-66.

Naci H, Brugts J, Ades T. Comparative tolerability and harms of individual statins: A study-level network meta-analysis of 246,955 participants form 135 randomized, controlled trials. Circ Cardiovasc Qual Outcomes. 2013;6:390-99.

Needham M, Mastaglia F. Statin myotoxicity: A review of genetic susceptibility factors. Neuromuscul Disord. 2014;24:4-15

Niemi M. Role of OATP transporters in the disposition of drugs. Pharmacogenomics. 2007;8:787-802.

Niemi M, Kivisto KT, Diczfalusy U, Bodin K, Bertilsson L, Fromm MF, et al. Effect of *SLCO1B1* polymorphisms on induction of CYP3A4 by rifampicin. Pharmacogenet Genomics. 2006a;16:565-8.

Niemi M, Pasanen MK, Neuvonen PJ. *SLCO1B1* polymorphism and sex affect the pharmacokinetics of pravastatin but not fluvastatin. Clin Pharmacol Ther. 2006b;80(4):356-66.

Niemi M, Pasanen MK, Neuvonen PJ. Organic anion transporting polypeptide 1B1: A genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacol Rev. 2011;63:157-81.

Oh J, Ban MR, Miskie BA, Pollex RL, Hegele RA. Genetic determinants of statin intolerance. Lipids Health Dis. 2007;6:7.

Olsson S, Harrison-Woolrych M. The role and strategy of ISoP in global pharmacovigilance. 2018;40:740-3.

Oshiro C, Mangravite L, Klein T, Altman R. PharmGKB very important pharmacogene: *SLCO1B1*. Pharmacogenet Genomics. 2010;20:211-16.

Pasanen MK, Backman JT, Neuvonen PJ, Niemi M. Frequencies of single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide 1B1 *SLCO1B1* gene in a Finnish population. Eur J Clin Pharmacol. 2006a;62:409-15.

Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. *SLCO1B1* polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogent Genomics. 2006b;16(12):873-9.

Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M. Different effects of *SLCO1B1* polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin. Clin Pharmacol Ther. 2007;82(6):726-33.

Pasanen MK, Neuvonen PJ, Niemi M. Global analysis of genetic variation in *SLCO1B1*. Pharmacogenomics. 2008;9(1):19-33.

Pasternak RC, Smith SC, Barey-Merz CN, Grundy SM, Cleeman JI, Lenfant C. ACC/AHA/NHLBI clinical advisory on the use and safety of statins. J Am Coll Cardiol. 2002;40(3):567-72.

Patel J, Abd T, Blumenthal RS, Nasir K, Superko HR. Genetics and personalized medicine-A role in statin therapy: Curr Atheroscler Rep. 2014;16:384.

Patel HN, Ursan ID, Zueger PM, Cavallari LH, Pickard AS. Stakeholder views on pharmacogenomic testing. Pharmacotherapy. 2014; 34(2):151–65.

Postigo R, Brosch S, Slattery J, van Haren A, Dogne JM, Kurz X, et al. EudraVigilance medicines safety database: Publicly accessible data for research and public health protection. Drug Saf. 2018;41(7):665-675.

Puccetti L, Ciani F, Auteri A. Genetic involvement in statins induced myopathy. Preliminary data from an observational case-control study. Atheroschlerosis. 2010;211(1):28-9.

Rabar S, Harker M, O'Flynn N, Wierzbicki AS. Lipid modification and cardiovascular risk assessment for the primary and secondary prevention of cardiovascular disease: Summary of updated NICE guidance. BMJ. 2014;349:G4356.

Ramachandran R, Wierzbicki AS. Statins, mucle disease and mitochondria. J Clin Med. 2017;6(8):75.

Ramsey LB, Johnson SG, Caudle KE, Haidar CE, Voora D, Wilke RA, et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for *SLCO1B1* and simvastatin-induced myopathy: 2014 update. Clin Pharmacol Ther. 2014;96(4):423-28.

Rodriges AC, Curi R, Vecchia FD, Hirata MH, Hirata RDC. The expression of efflux and uptake transporters are regulated by statins in Caco-2 and HepG2 cells. Acta Pharmacol Sin. 2009;30(7):956-64.

Rohrbacher M, Kirchhof A, Skarke C, Geisslinger G, Lotsch J. Rapid indentification of three functionally relevant polymorphisms in the OATP1B1 transporter gene using Pyrosequencing. Pharmacogenomics. 2006;7(2):167-76.

Romaine SP, Bailey KM, Hall AS, Balmforth AJ. The influence of *SLCO1B1* (OATP1B1) gene polymorphisms on resonse to statin therapy. Pharmacogenomics J. 2010;10(1):1-11.

Rosenson RS, Baker SK, Jacobson TA, Kopecky SL, Parker BA. An assessment by the statin muscle safety task force: 2014 upddate. J Clin Lipidol. 2014;8(3 Suppl):S58-71.

Santos PC, Gagliardi AC, Miname MH, Chacra AP, Santos RD, Krieger JE, et al. *SLCO1B1* haplotypes are not associated with atorvastatin-induced mialgia in Brazilian patients with familial hypercholesterolemia. 2012;68:273-9.

Santos PC, Soares RA, Nascimiento RM, Machado-Coelho GL, Mill JG, Krieger JE, et al. *SLCO1B1* rs4149056 polymorphism associated with statin-induced myopathy is differently distributed according to ethnicity in the Brazilian general population: Amerindians as a high risk ethnic group. BMC Med Genet. 2011;12:136.

Sathasivam S. Statin induced myotoxicity. Eur J Intern Med. 2012;23(4):317-24.

Seithel A, Glaeser H, Fromm MF, Konig J. The functional consequences of genetic variations in transporter genes sencoding human organix anion-transporting polypeptide family members. Expert Opin Drug Metab Toxicol. 2008;4:51-64.

Selkirk CG, Weissman SM, Anderson A, Hulick PJ. Physicians' preparedness for integration of genomic and pharmacogenetic testing into practice within a major healthcare system. Genet Test Mol Bioma. 2013; 17(3):219–25.

Selva-O'Callaghan A, Alvarado-Cardenas M, Pinal-Fernández I, Trallero-Araguás E, Milisenda JC, Martínez MÁ, et al. Statin-induced myalgia and myositis: an update on pathogenesis and clinical recommendations. Expert Rev Clin Immunol. 2018;14(3):215-24.

Sewright KA, Clarkson PM, Thompson PD. Statin myopathy: Incidence, risk factors, and pathophysiology. Curr Atheroscler Rep. 2007;9(5):389-96.

Singh U, Devaraj S, Jialal I, Siegel D. Comparison effect of atorvastatin (10 versus 80 mg) on biomarkers of inflammation and oxidative stress in subjects with metabolic syndrome. Am J Cardiol. 2008;102(3):321-5.

Sortica AV, Fiegenbaum M, Lima LO, Van der Sand CR, Van der Sand LC, Ferreira ME, et al. *SLCO1B1* gene variability influences lipid-lowering efficacy on simvastatin therapy in Southern Brazilians. Clin Chem Lab Med. 2012;50(3):441-8.

St Sauver JL, Bielinski SJ, Olson JE, Bell EJ, Mc Gree ME, Jacobson DJ, et al. Integrating pharmacogenomics into clinical practice: promise vs reality. Am J Med. 2016;129(10):1093-99.

Stanek EJ, Sanders CL, Taber KA, Khalid M, Patel A, Verbrugge RR, et al. Adoption of pharmacogenomic testing by US physicians: Results of a nationwide survey. Clin Pharmacol Ther. 2012; 91(3):450–8.

Stewart A. *SLCO1B1* polymorphisms and statin-induced myopathy. PLoS Curr. 2013;4:5.

Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/Amerian Heart Association task force on practice guidelines. J Am Coll Cardiol. 2014;63(25 Pt B):2889-934.

Stringer HA, Sohi GK, Maguire JA, Cote HC. Decreased skeletal muscle mitochondrial DNA in patients with statin-induced myopathy. J Neurol Sci. 2013;325:142-7.

Stroes ES, Thompson PD, Corsini A, Vladutiu GD, Raal FJ, Ray KK, et al. Statin-associated muscle symptoms: impact on statin therapy-European Atherosclerosis Society Consensus panel statement on assessment, aetiology and management. Eur Heart J. 2015;36(17):1012-22.

Sychev D, Shuev G, Chertovskih J, Maksimova N, Grachev A, Syrkova O. The frequency of *SLCO1B1*5* polymorphism genotypes among Russian and Sakha (Yakutia) patients with Hypercholesterolemia. Pharmgenomics Pers Med. 2016;9:59-63.

Unertl KM, Field JR, Price L, Peterson JF. Clinician perspectives on using pharmacogenomics in clinical practice. Pers Med. 2015; 12(4):339–47.

Taber KA, Dickinson BD. Pharmacogenomic knowledge gaps and educational resource needs among physicians in selected specialties. Pharmgenomics Pers Med. 2014; 7:145–62.

Tachibana-limori R, Tabara Y, Kusuhara H, Kohara K, Kawamoto R, Nakura J, et al. Effect of genetic polymorphism of OATP-C (*SLCO1B1*) on lipid-lowering response to HMG-CoA reductase inhibitors. Drug Metab Pharmacokinel. 2004;19(5):375-80.

Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C: Identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. J Biol Chem. 2001;276(38):35669-75.

Thompson PD, Clarkson P, Karas RH. Statin-associated myopathy. JAMA. 2003;289:1681-90.

Tonelli M, Wanner C, Kidney Disease: Improving Global Outcomes Lipid Guideline Development Work Group Members. Lipid management in chronic kidney disease:

Synopsis of the kidney disease: Improving global outcomes 2013 clinical practice guidelines. Ann Intern Med. 2014;160(3):182

Torres PA, Helmstetter JA, Kaye AM, Kaye AD. Rhabdomyolysis: pathogenesis, diagnosis, and treatment. Ochsner J. 2015;15(1):58-69.

Toth PP, Patti AM, Giglio RV, Nikolic D, Castellino G, Rizzo M, et al. Management of statin intolerance in 2018: Still more questions than answers. Am J Cardiovasc Drugs. 2018;18(3):157-173.

Voora D, Shah SH, Spasojevic I, Ali S, Reed CR, Salisbury BA, et al. The *SLCO1B1*5* genetic variant is associated with statin-induced side effecs. J Am Coll Cardiol. 2009;54(17):1606-16.

Ward NC, Watts GF, Eckel RH. Statin Toxicity. Circ Res. 2019;124(2):328-50.

Weng TC, Yang YH, Lin SJ, Tai SH. A systematic review and meta-analysis on the therapeutic equivalence of statins. J Clin Pharm Ther. 2010;35(2):139-51.

Wilke R, Ramsey L, Johnson S, Maxwell WD, McLeod HL, Voora D, et al. The clinical pharmacogenomics implementation consortium: CPIC guideline for *SLCO1B1* and simvastatin-induced myopathy. Clin Pharmacol Ther. 2012;92:112-7

Xiang Q, Chen S, Ma L, Hu K, Zhang Z, Mu G, et al. Association between *SLCO1B1* T521C polymorphism and risk of statin-induced myopathy: a meta-analysis. 2018;18(6):721-729.

Zhang H, Plutzky J, Skentzos S, Morrison F, Mar P, Shubina M, et al. Discontinuation of statins in routine care settings: A cohort study. Ann Intern Med. 2013;158(7):526-34.

Zhou Z, Albarqouni L, Breslin M, Curtes AJ, Nelson M. Statin-associated muscle symptoms (SAMS) in primary prevention for cardiovascular disease in older adults: A protocol for a systematic review and meta-analysis of randomised controlled trials. BMJ open. 2017;7(9):e017587.

Appendices

Appendix 1

Data collection form

Data Collection Form

Pharmacogenetics in Statins Use

Judith Cerdá

in partial fulfilment of the Doctorate in Pharmacy(Pharm.D)

Section 1: Patient Demographic Information

Patient Study Number	ID Card Number	Patient initials	Date of recruitment
Telephone/Mobile	**Cardiologist**		
Procedure undertaken at Cath Suite at time of recruitment			

Age (at last birthday) in years	
Gender	○ Male ○ Female
Ethnicity	○ Caucasian ○ North African ○ Black/African American ○ Asian ○ Middle Eastern
Nationality	○ Maltese ○ Other _____

Section 2: Cardiac risk factors and social history

<table>
<tr><td>Family history of hypercholesterolaemia</td><td colspan="3">O Parent
O Sibling
O Don’t know
O No</td></tr>
<tr><td>Last lipid profile

Date of last lipid profile:</td><td colspan="3">TC:_____(2.0-5.0 mmol/L)
TGs: _____(0.1-2.26 mmol/L)
HDL:_____(0.9-1.45 mmol/L)
LDL:_____(< 2.0 mmol/L)
Number of months to date of recruitment: _____</td></tr>
<tr><td>Previous MI</td><td colspan="3">O Yes (Date_____)
O No</td></tr>
<tr><td>Previous PCI _____</td><td colspan="3">O Yes (Date_____)
O No</td></tr>
<tr><td>Previous CABG</td><td colspan="3">O Yes (Date_____)
O No</td></tr>
<tr><td colspan="4">O Hypertension</td></tr>
<tr><td colspan="4">O Diabetes mellitus</td></tr>
<tr><td>Weight (kg)</td><td>Height (m)</td><td colspan="2">BMI (kg/m²)</td></tr>
<tr><td colspan="2">Waist circumference (cm)</td><td colspan="2" rowspan="3">O Not recorded
O Underweight (<18.5)
O Normal weight (18.5-24.99)
O Obesity Class II (35-39.99)
O Pre-obesity (25-29.99)
O Obesity Class I (30-34.99)</td></tr>
<tr><td>Female</td><td>Male</td></tr>
<tr><td>O ≤ 80 cm
O > 80 cm</td><td>O ≤ 94 cm
O > 94 cm</td></tr>
<tr><td>Smoking</td><td colspan="3">O Active (No. of cigarettes/day_____)
O Past (Date/year stopped_____)
O Never</td></tr>
<tr><td>Alcohol consumption</td><td>O Regularly (daily)
O Socially (weekly)
O Socially (occasionally)</td><td colspan="2">No. of units (daily/weekly)
O 1-5
O 6-10
O >10</td></tr>
</table>

Section 3: Comorbidities

O GORD	O Congestive heart failure	O Arrhythmia
O Peripheral artery	O Stroke/TIA	O Asthma/COPD
O Venous thromboembolism	O Thyroid disorders	O None
O Others		

Section 4: Current Medications

	Class	Generic name	Dose	Dosage regimen	Start date
1	**STATIN**	**simvastatin**			
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					

Section 5: Investigations

LF **Ts** Date of last liver profile:	○ Albumin:_____(32-52 g/L) ○ Bilirubin:_____ (0-17.1 μmol/L) ○ Transaminases AP: _____(40-129 U/L) GGT:_____ (8-61 U/L) ALT:_____(10-50
Renal Function Date of last renal profile:	○ Serum Cr:_____(59-104 μmol/L) ○ Urea:_____(1.7-8.3 mmol/L) ○ eGFR:_____(> 60ml/min/1.73m^2)
Muscle marker Date: _____	○ CK:_____(22-198 U/L)

Section 6: SLC01B1 Genotyping and Clinical Recommendations

Normal function, Homozygous wild-type (TT)[1] Patient has 2 normal-function alleles	
Intermediate function, Heterozygous (TC)[2] Patient has 1 normal-function + 1 decreased-function allele	
Low function, Homozygous variant/mutant (CC)[2] Patient has 2 decreased-function alleles	

Myopathy Risk and Recommendations to Cardiologist

○

[1]Normal myopathy risk; prescribe desired starting dose of simvastatin and adjust dose based on disease-specific guidelines

○

[2]Mild or high myopathy risk; prescribe lower dose of simvastatin or consider an alternative statin (rosuvastatin) and consider routine CK surveillance

Ramsey L, Johnson S, Caudle K, Haidar C, Voora D, Wilke R *et al* The Clinical Pharmacogenetics Implementation Consortium Guideline for *SLCO1B1* and Simvastatin-Induced Myopathy. Clinical Pharmacology and Therapeutics. 2014;96(4):423-428.

Appendix 2

Ethics Approval

L-UNIVERSITÀ TA' MALTA
Msida MSD 2080 – Malta
DIPARTIMENT TAL-FARMA IJA

UNIVERSITY OF MALTA
Msida MSD 2080 – Malta
DEPARTMENT OF PHARMACY

Dear Professor Grech,

Kindly note that the study protocol approved by the University of Malta Research Ethics Committee (Protocol Number 17/2017) led by supervisor Professor Lilian M. Azzopardi, Head of the Department of Pharmacy, will be undertaken by Ms Judith Cerdá,

Judith Cerdá

Lilian M. Azzopardi

L-Università ta' Malta

Francesca Wirth <francesca.wirth@um.edu.mt>

Research Project Approval

Helen Grech <helen.grech@um.edu.mt> 8 October 2017 at 19:56
To: Francesca Wirth <francesca.wirth@um.edu.mt>
Cc: Victoria Perici <victoria.perici@um.edu.mt>

Dear Dr Wirth,

Pleas note that the request to change the candidate so that Judith Cerda takes over the proposed project (Faculty of Medicine & Surgery/17/2017), is approved by UREC.

Regards
Helen Grech

Professor Helen Grech
Chairperson, University Research Ethics Committee
Head, Department of Communication Therapy
Deputy Dean, Faculty of Health Sciences
University of Malta, MSD 2090
Tel: +356 2340 1858

[Quoted text hidden]

Appendix 3

Patient Information Sheets

INFORMAZZJONI GĦALL-PAZJENT/A

Jiena Judith Cerdá, spiżjara u studenta fid-Dipartiment tal-Farmaċija fl-Università ta' Malta. Bħalissa qed nagħmel proġett ta' riċerka għad-Dottorat fil-Farmaċija, intitolat *'Pharmacogenetics in Statins Use'* taħt is-superviżjoni prinċipali tal-Professur Anthony Serracino-Inglott u Dr Francesca Wirth mid-Dipartiment tal-Farmaċija fl-Università ta' Malta, b'kollaborazzjoni mad-Dipartimenti tal-Kardjoloġija u tal-Patoloġija fl-Isptar Mater Dei.

Inti ġejt identifikat/a biex tipparteċipa f'din ir-riċerka li tinvolvi dan li ġej:

L-għan tar-riċerka u l-benefiċċju għalik
Il-mediċina li inti qed tieħu biex tnaqqas l-ammont ta' kolesterol fid-demm, , '*simvastatin*, tigi mneħħija mill-ġisem permezz tal-enżimi tal-fwied. Jekk dawn l-enżimi ma jiffunzjonawx sew, il-mediċina '*simvastatin'* tibqa fil-ġisem f'livelli għoljin, li jafu jkunu ta' ħsara għal-muskoli, fost organi ohrajn. Din ir-riċerka se tiddetermina kif qegħdin jiffunzjonaw l-enżimi tiegħek. B'hekk il-konsulent tiegħek ikun f'pożizzjoni aħjar biex jiddetermina t-terapija li jkollok bżonn.

L-involviment tiegħek
- Ikun jeħtieġ li jittieħed kampjun tad-demm tiegħek darba mit-tabib/a jew infermier/a.
- Ikun jeħtieġ ukoll li l-konsulent/tabib tiegħek u jien insegwu l-każ tiegħek.

Informazzjoni importanti oħra
- Il-parteċipazzjoni tiegħek f'din ir-riċerka hija kompletament volontarja. L-informazzjoni miġbura tibqa' strettament kunfidenzjali u użata biss għar-riċerka skond *l-Att* dwar il-*Protezzjoni* u l-Privatezza tad-*Data* (Kap. 440).
- It-trattament tiegħek, bħala pazjent fl-isptar Mater Dei, bl-ebda mod ma jiġi affettwat jekk int tirrifjuta milli tipparteċipa.
- Inti tista' tieqaf milli tipparteċipa, fi kwalunkwe ħin, mingħajr ebda preġudizzju.
- Riżultati ta' din ir- riċerka mhux ha jaffettwaw it-trattament/servizz regolari li tirċievi.

Int ġentilment mitlub/a tiffirma l-formola ta' kunsens mehmuża jekk taċċetta li tipparteċipa f'din ir-riċerka.

Grazzi bil-quddiem għall-kooperazzjoni tiegħek.
Judith Cerdá
0173947A

PATIENT INFORMATION SHEET

I, Judith Cerdá, a Doctorate in Pharmacy student at the Department of Pharmacy, University of Malta, am currently undertaking a research project entitled '*Pharmacogenetics in statins use*' under the supervision of Professor Anthony Serracino-Inglott and Dr Francesca Wirth from the Department of Pharmacy, University of Malta, in collaboration with the Department of Cardiology and the Department of Pathology at Mater Dei Hospital.

You have been identified to participate in this research which involves the following:

Aim of research and how will you benefit?
Simvastatin, a drug you are taking to lower blood cholesterol levels, needs to be converted by liver enzymes to be eliminated by the body. If you have reduced functioning of these enzymes, simvastatin may remain in the body at higher levels, causing side-effects such as muscle pain. This research will determine the functioning of these enzymes so that your consultant cardiologist will be in a better position to individualise therapy according to your genetic make-up.

Your involvement
- Having a blood sample taken once by a physician or nurse at the Cardiology Department at Mater Dei Hospital
- Be followed-up by your consultant cardiologist and myself

Other important information
- Participation in this research is entirely voluntary. The information gathered will be kept strictly confidential and used solely for the purpose of the research according to the Data Protection Act (Chapter 440).
- Refusal to participate will in no way affect the treatment you receive as a patient at the Cardiology Department at Mater Dei Hospital.
- You may discontinue participation in the research at any time without any prejudice.
- Results of this research will not influence the routine treatment/service you receive.

Kindly sign the attached consent form if you agree to participate in this research.

Thank you in advance for your cooperation.

Judith Cerdá
0173947A

Appendix 4

Patient Consent Forms

PROPOSTA GĦALL-FORMULA TAL-KUNSENS

Jien/a ċittadin/a Malti/ja u għalaqt tmintax (18)-il sena.

Talbuni biex nieħu sehem fi studju riċerka bl-isem ta':

'Pharmacogenetics in Statins Use'

L-għan u d-dettalji tal-istudju spejgathomli Judith Cerdá li wkoll iċċaratli mistoqsijiet li għamilt.

Nagħti l-kunsens tiegħi lill-persuna responsabbli għal-din ir-riċerka biex tagħmel l-osservazjonijiet li hemm bżonn jew inkella tieħu l-kampjuni u nifhem li dan jista' jkun ta' skomdu għalija.

Jiena nifhem li r-riżultati ta' dan l-istudju jistgħu jintużaw għal skopijiet xjentifiċi u jista' jiġi ppubblikat rapport bil-miktub: jekk isir hekk b'ebda mod ma nista' nkun identifikat/a, individwalment jew bħala parti minn grupp, mingħajr il-kunsens tiehħi bil-miktub.

Jiena ma għandi l-ebda dmir li niehu sehem f'dan l-istudju u dan qed nagħmlu minn rajja.

Jiena nista', meta rrid, ma nkomplix niehu sehem fl-istudju, u mingħajr ma' nagħti raġuni. Jekk nagħmel hekk xorta nibqa' nieħu l-kura li ssoltu tingħatali.

Jiena nifhem li jekk ikun hemm xi kumplikazzjoniji jew effetti mhux mistennija waqt l-istudju, dawn jiġu mniżżla bil-miktub u jekk ikun hemm bżonn xi kura, tiġi mgħotija fis-Servizz Nazjonali tas-Saħħa.

Jiena qed nitħallas/**mhux qed nitħallas** biex nieħu sehem f'dan l-istudju.

Jekk ikolli xi diffikulta', nista' nistaqsi għal: judith.cerda.16@um.edu.mt/99747856

Firma tal-parteċipant _____

Isem tal-parteċipant _____

Numru ta' l-identita _____

Firma tal-persuna responsabbli għal din ir-riċerka _____

Persuna responsabbli għal din ir-riċerka Judith Cerdá – 0173947A

Date

CONSENT FORM

I am a Maltese citizen and am over eighteen (18) years of age.
I have been asked to participate in a research study entitled:

'Pharmacogenetics in Statins Use'

The purpose and details of the study have been explained to me by:
Judith Cerdá (Principal Investigator) and any questions which I raised have been adequately clarified.

I give my consent to the Principal Investigator either to make the appropriate observations, tests or both or take the necessary samples. I am aware of the inconveniences which this may cause.

I understand that the results of this study may be used for medical or scientific purposes and that the results achieved from the study in which I am participating may be reported or published: however, I shall not be personally identified in any way, either individually or collectively, without my express written permission.

I am under no obligation to participate in this study and am doing so voluntarily.

I may withdraw from the study at any time, without giving any reason. This will not affect in any way the care and attention and treatment normally given to me.

I understand that any complications and/or adverse effects which may arise during or as a consequence of the study will be recorded and any treatment which this may entail will be given within the Government Health Services.

I am/ **I am not** receiving any remuneration for participating in this study.

In case of queries during the study I may contact: judith.cerda.16@um.edu.mt/99747856

Signature of participant _____

Name of participant _____

ID of participant _____

Signature of Chief Investigator _____

Chief Investigator Judith Cerdá – 0173947A

Date

Appendix 5

Informative Letter for Cardiologist

***SLCO1B1* Genotyping Test Result**

Date:

Patient ID Card Number: Patient Name:

Last LDL-C level and date of test:

Attention: Dr

Your patient was genotyped for presence of the *SLCO1B1* rs4149056 C allele. Presence of this allele is associated with reduced hepatic uptake of simvastatin, resulting in increased concentration of simvastatin in the blood and greater muscle exposure, increasing the risk for simvastatin-induced myopathy.

RESULT (*SLCO1B1*)**: Carrier of one C allele (TC)**

According to this test result, the patient has predicted **increased concentration** of simvastatin due to intermediate *SLCO1B1* function and the predicted myopathy risk is **MILD**.

RECOMMENDATION *(CPIC guidelines)*[a]

Consider decreasing the dose of simvastatin to 20mg/day or consider an alternative statin (rosuvastatin) and consider routine CK monitoring.

Sincerely,

Judith Cerda Inesta
Doctorate in Pharmacy student

[a]Ramsey LB *et al*. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *SLCO1B1* and Simvastatin-Induced Myopathy: 2014 update. *Clin Pharmacol Ther* 2014;96(4):432-428.

***SLCO1B1* Genotyping Test Result**

Date:

Patient ID Card Number: Patient Name:

Last LDL-C level and date of test:

Attention: Dr

Your patient was genotyped for the *SLCO1B1* rs4149056 C allele. Presence of this allele is associated with reduced hepatic uptake of simvastatin, resulting in increased concentration of simvastatin in the blood and greater muscle exposure, increasing the risk for simvastatin-induced myopathy.

RESULT (*SLCO1B1* genotype)**: Carrier of two C alleles (CC)**

According to this test result, the patient has predicted **significantly increased concentration** of simvastatin due to low *SLCO1B1* function and the predicted myopathy risk is **HIGH**.

RECOMMENDATION *(CPIC guidelines)*[a]

Consider decreasing the dose of simvastatin to 20mg/day or consider an alternative statin (rosuvastatin) and consider routine CK monitoring.

Sincerely,

Judith Cerda Inesta
Doctorate in Pharmacy student

[a]Ramsey LB *et al*. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *SLCO1B1* and Simvastatin-Induced Myopathy: 2014 update. *Clin Pharmacol Ther* 2014;96(4):432-428.

Discussion with cardiologist and action taken

- **Recommendation considered:**

  O Yes

  O No

- If Yes:

  O Decrease dose O specify dose change _____

  O Change statin O specify statin change _____

  O CK monitoring

  O Follow up for muscle side effects

- If No, reason/s why:
  _____
  _____
  _____

- Other comments:

Appendix 6

Dissemination of study findings

Poster presentation at the International Pharmaceutical Federation World Congress of Pharmacy and Pharmaceutical Sciences, Glasgow, Scotland, September 2018

L-Università ta' Malta | Faculty of Medicine & Surgery | Department of Pharmacy

SLCO1B1 GENETIC POLYMORPHISMS IN CARDIAC PATIENTS ON SIMVASTATIN

Judith Cerdá Iñesta[1], Francesca Wirth[1], Graziella Zahra[2], Robert G. Xuereb[3], Christopher Barbara[2], Anthony Serracino-Inglott[1]

[1]Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[2]Molecular Diagnostics Unit, Department of Pathology, Mater Dei Hospital, Msida, Malta
[3]Cardiac Catheterisation Suite, Department of Cardiology, Mater Dei Hospital, Msida, Malta
email: judith.cerda.16@mt.edu.mt

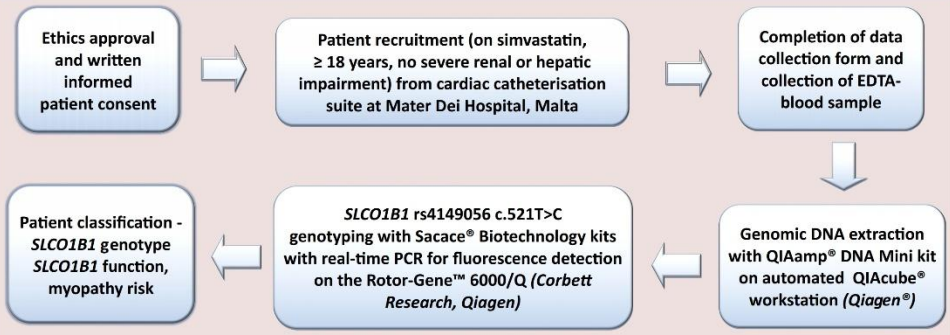
INTRODUCTION

The *SLCO1B1* rs4149056 c.521T>C gene variant is associated with higher serum simvastatin concentrations and increased risk of simvastatin-induced myopathy.[1]

AIM

To classify a cohort of cardiac patients on simvastatin according to *SLCO1B1* genotype, *SLCO1B1* function and myopathy risk

METHOD



RESULTS

- 110 patients (mean age 65.44 ±10.73 years, 81.8% (n=90) male, all Caucasian) were genotyped.
- 21.8% (n=24) of the patients were genotyped as carriers of one C allele (TC, heterozygous) or two C alleles (CC, homozygous variant), corresponding to intermediate and low *SLCO1B1* function, and mild and high myopathy risk, respectively (Table 1).
- 15 of the 24 patients genotyped as TC or CC were on a higher dose of simvastatin (40mg daily) than suggested by the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for SLCO1B1 and simvastatin-induced myopathy (20mg daily).[2]

Table 1. SLCO1B1 genotype, *SLCO1B1* function and myopathy risk (N=110)

SLCO1B1 genotype	Number of patients (%)	*SLCO1B1* function	Myopathy risk
TT	86 (78.2)	Normal	Normal
TC	22 (20.0)	Intermediate	Mild
CC	2 (1.8)	Low	High

CONCLUSION

In patients genotyped as TC and CC (24 patients in this study), the CPIC guideline suggests decreasing the dose of simvastatin from 40mg to 20mg/day or to consider prescribing an alternative statin (rosuvastatin or pravastatin).[1]

Pharmacogenetic testing for the *SLCO1B1* rs4149056 c.521T>C gene can be used to individualise statin therapy.

Funding: University of Malta Research Grant PHRRP12-17

REFERENCES

1. Link E, Parish S, Armitage J, Bowman L, Health S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy—a genomewide study. N Engl J Med. 2008;359(8):789-799.
2. Ramsey L, Johnson S, Caudle K, Haidar C, Voora D, Wilke R, et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1 and Simvastatin-Induced Myopathy. Clin Pharm Ther. 2014;96(4):423-428.

Oral presentation at the Maltese Cardiac Society Conference, Malta, October 2018

Pharmacogenetic Testing as a Tool in Precision Medicine for Statin Therapy

Judith Cerdá Iñesta[1], Francesca Wirth[1],
Graziella Zahra[2], Robert G. Xuereb[3],
Christopher Barbara[2], Anthony Serracino-Inglott[1]

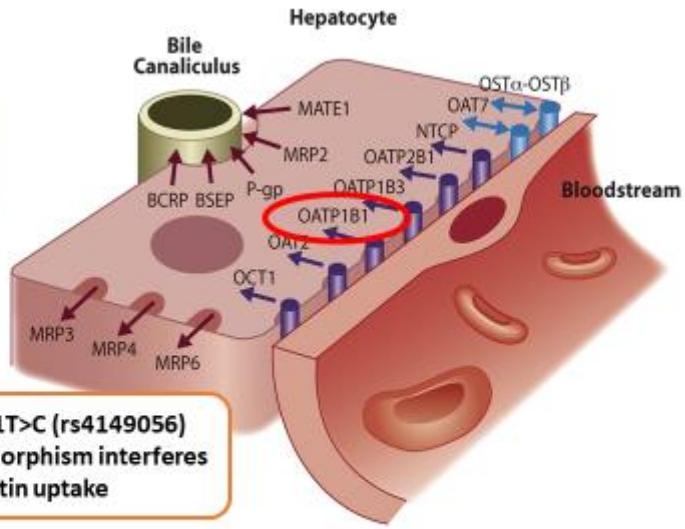
[1]Deparment of Pharmacy, University of Malta
[2]Department of Pathology, Mater Dei Hospital
[3]Deparment of Cardiology, Mater Dei Hospital

10m 00s

Background

OATP1B1

***SLCO1B1* gene**



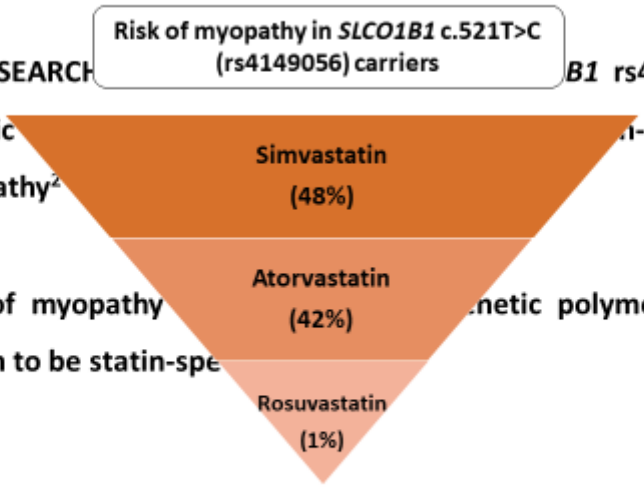
***SLCO1B1* c.521T>C (rs4149056) genetic polymorphism interferes with simvastatin uptake**

Figure 1: Transporters for the hepatic uptake of drugs[1]

[1]Niemi M, Pasanen MK, Neuvonen PJ. Organic anion transporting polypeptide 1B1: A genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacol Rev. 2011;63(1):157-81.

Background

- **2008 SEARCH** … ***B1*** **rs4149056 genetic** … **n-induced myopathy**[2]
- **Risk of myopathy** … **netic polymorphism shown to be statin-spe**



[2]Link E et al. SLCO1B1 variants and statin-induced myopathy - A genomewide study. N Engl J Med. 2008;360:789-99.

[3] Brunham LR et al. Differential effect of the rs4149056 variant in SLCO1B1 on myopathy associated with simvastatin and atorvastatin. Pharmacogenomics J. 2012;12(3):233-7.

Aims

- To classify a cohort of **cardiac patients** on **simvastatin** according to ***SLCO1B1* genotype** and ***SLCO1B1* function**
- To correlate *SLCO1B1* genotype and *SLCO1B1* function to **myopathy risk**

Methodology

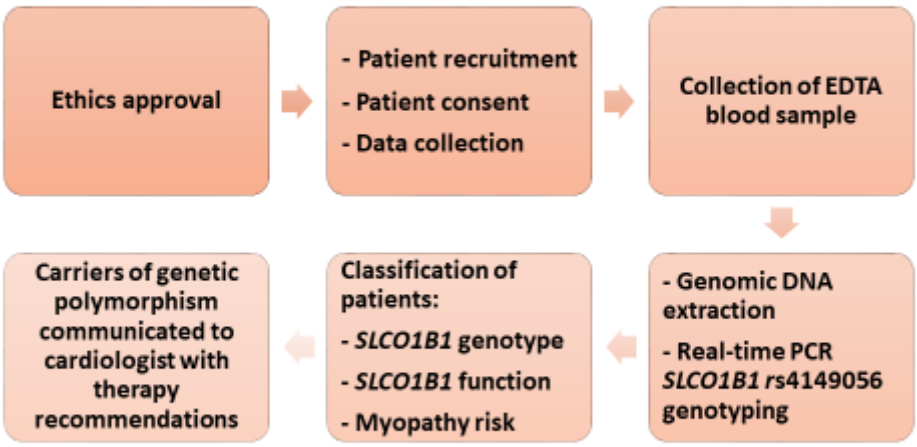


Figure 2: Methodology flowchart

Results

Table 1: Patient characteristics (N=110)

Age in years (mean ±SD)	**65.44 ±10.73**
Caucasian ethnicity (n, %)	**110, 100%**
Male gender (n, %)	**90, 81.8%**
Hypertension (n, %)	**85, 77.3%**
Diabetes Mellitus (n, %)	**41, 37.3%**
LDL-C in mmol/L (mean ±SD)	**2.41 ±1.03**
eGFR in ml/min/1.73m^2 (mean ±SD)	**84.41 ±22.45**

Results

Table 2: Patients classified according to *SLCO1B1* genotype and *SLCO1B1* function (N=110)

SLCO1B1 genotype	*SLCO1B1* function	Number of patients (%)	Reported genotype frequencies (%)[4]
TT Homozygous wild-type	Normal	86 (78.2)	55-88
TC Heterozygous	Intermediate	22 (20.0)	11-36
CC Homozygous variant	Low	2 (1.8)	0-6

[4]Ramsey L et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1 and Simvastatin-Induced Myopathy. Clin Pharm Ther. 2014;96(4):423-8.

Results and Discussion

Table 3: Dosing recommendations of simvastatin according to *SLCO1B1* genotype and myopathy risk (N=110)

SLCO1B1 genotype	Myopathy risk	Dosing recommendations of simvastatin[4]
TT (n=86)	Normal	Prescribe desired starting dose and adjust dose according to disease-specific guidelines
TC (n=22)	Mild	Prescribe lower dose of simvastatin (20mg daily) or consider rosuvastatin; Consider routine CK monitoring
CC (n=2)	High	

15 / 24 patients were on > 20mg simvastatin daily

Results and Discussion

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15 / 24 patients were on > 20mg simvastatin daily

Oral presentation at the Malta Medical School Conference, Malta, November 2018

X Malta Medical School Conference
2018

Pharmacogenetic Testing in Precision Medicine for Statin Use

Judith Cerdá Iñesta, Francesca Wirth, Graziella Zahra, Robert G. Xuereb, Christopher Barbara, Anthony Serracino-Inglott

Funding: University of Malta Research Grant (PHRRP12-17)

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Introduction

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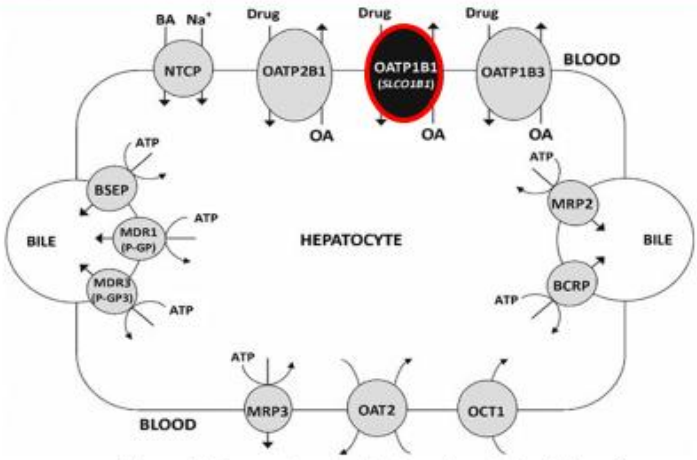

***SLCO1B1* c.521T>C (rs4149056) genetic polymorphism interferes with simvastatin uptake**

Figure 1: Transporters for the hepatic uptake of drugs[1]

[1] Romaine S, et al. The influence of SLCO1B1 (OATPB1) gene polymorphisms on response to statin therapy. Pharmacogenomics J. 2010;10(1):1-11.

Introduction

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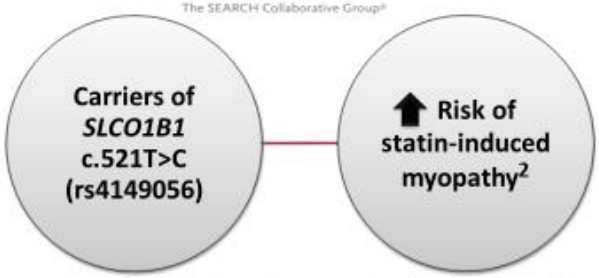
SLCO1B1 Variants and Statin-Induced Myopathy — A Genomewide Study

The SEARCH Collaborative Group[a]



[2]Link E et al. SLCO1B1 variants and statin-induced myopathy - A genomewide study. N Engl J Med. 2008;359(8):789-99.

Introduction

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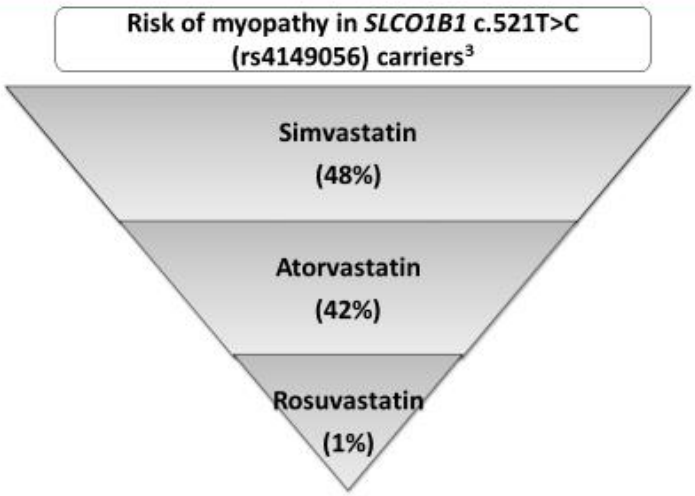

[3]Brunham L et al. Differential effect of the rs4149056 variant in SLCO1B1 on myopathy associated with simvastatin and atorvastatin. Pharmacogenomics J. 2012;12(3):233-7.

Aims

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- To classify a cohort of **cardiac patients** on **simvastatin** according to ***SLCO1B1* genotype** and ***SLCO1B1* function**

- To correlate *SLCO1B1* genotype and *SLCO1B1* function to **myopathy risk**

Methodology

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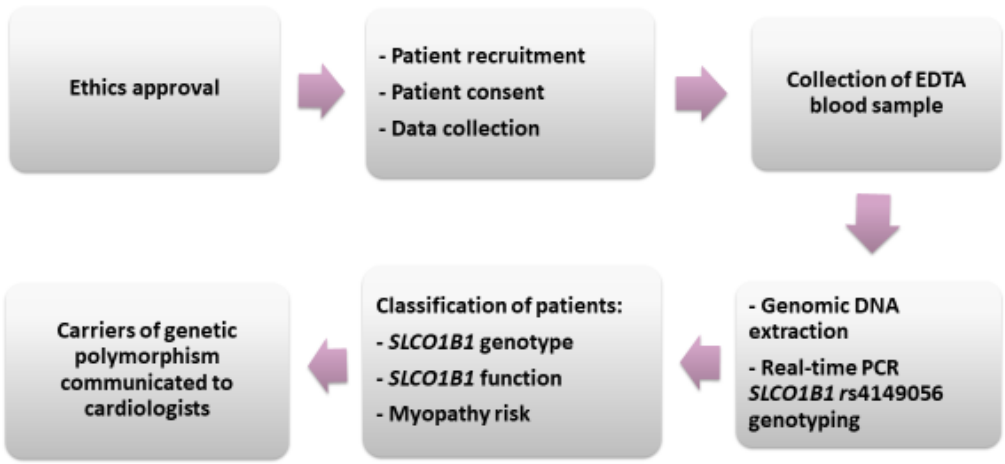

Results

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Table 1: Patient characteristics (N=110)

Age (mean ±SD)	65±10 years
Caucasian ethnicity n (%)	110 (100%)
Male gender n (%)	90 (81.8%)
Hypertension n (%)	85 (77.3%)
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Results

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Table 2: Patients classified according to *SLCO1B1* genotype and *SLCO1B1* function (N=110)

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Results and Discussion

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15 / 24 patients were on > 20mg simvastatin daily

Conclusion

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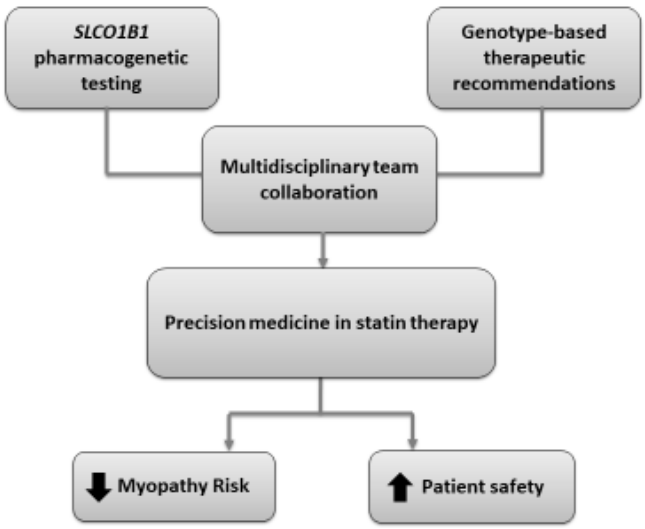
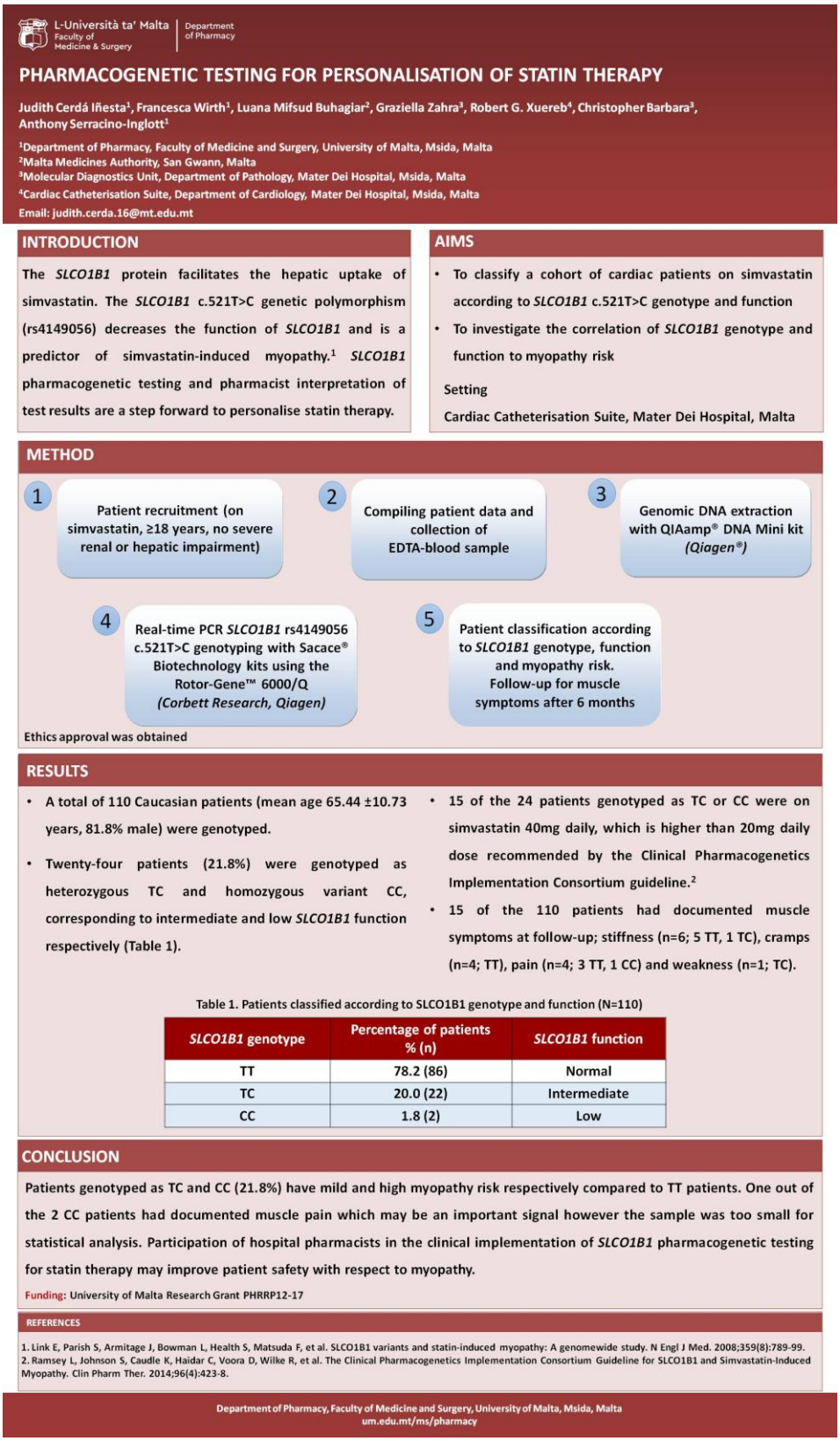


Poster presentation selected for poster walk at the 24th Congress of the European Association of Hospital Pharmacy, Barcelona, Spain, 27-29 March 2019.



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PHARMACOGENETIC TESTING FOR PERSONALISATION OF STATIN THERAPY

Judith Cerdá Iñesta[1], Francesca Wirth[1], Luana Mifsud Buhagiar[2], Graziella Zahra[3], Robert G. Xuereb[4], Christopher Barbara[3], Anthony Serracino-Inglott[1]

[1]Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[2]Malta Medicines Authority, San Gwann, Malta
[3]Molecular Diagnostics Unit, Department of Pathology, Mater Dei Hospital, Msida, Malta
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INTRODUCTION

The *SLCO1B1* protein facilitates the hepatic uptake of simvastatin. The *SLCO1B1* c.521T>C genetic polymorphism (rs4149056) decreases the function of *SLCO1B1* and is a predictor of simvastatin-induced myopathy.[1] *SLCO1B1* pharmacogenetic testing and pharmacist interpretation of test results are a step forward to personalise statin therapy.

AIMS

- To classify a cohort of cardiac patients on simvastatin according to *SLCO1B1* c.521T>C genotype and function
- To investigate the correlation of *SLCO1B1* genotype and function to myopathy risk

Setting

Cardiac Catheterisation Suite, Mater Dei Hospital, Malta

METHOD

1. Patient recruitment (on simvastatin, ≥18 years, no severe renal or hepatic impairment)
2. Compiling patient data and collection of EDTA-blood sample
3. Genomic DNA extraction with QIAamp® DNA Mini kit *(Qiagen®)*
4. Real-time PCR *SLCO1B1* rs4149056 c.521T>C genotyping with Sacace® Biotechnology kits using the Rotor-Gene™ 6000/Q *(Corbett Research, Qiagen)*
5. Patient classification according to *SLCO1B1* genotype, function and myopathy risk. Follow-up for muscle symptoms after 6 months

Ethics approval was obtained

RESULTS

- A total of 110 Caucasian patients (mean age 65.44 ±10.73 years, 81.8% male) were genotyped.
- Twenty-four patients (21.8%) were genotyped as heterozygous TC and homozygous variant CC, corresponding to intermediate and low *SLCO1B1* function respectively (Table 1).
- 15 of the 24 patients genotyped as TC or CC were on simvastatin 40mg daily, which is higher than 20mg daily dose recommended by the Clinical Pharmacogenetics Implementation Consortium guideline.[2]
- 15 of the 110 patients had documented muscle symptoms at follow-up; stiffness (n=6; 5 TT, 1 TC), cramps (n=4; TT), pain (n=4; 3 TT, 1 CC) and weakness (n=1; TC).

Table 1. Patients classified according to SLCO1B1 genotype and function (N=110)

SLCO1B1 genotype	Percentage of patients % (n)	*SLCO1B1* function
TT	78.2 (86)	Normal
TC	20.0 (22)	Intermediate
CC	1.8 (2)	Low

CONCLUSION

Patients genotyped as TC and CC (21.8%) have mild and high myopathy risk respectively compared to TT patients. One out of the 2 CC patients had documented muscle pain which may be an important signal however the sample was too small for statistical analysis. Participation of hospital pharmacists in the clinical implementation of *SLCO1B1* pharmacogenetic testing for statin therapy may improve patient safety with respect to myopathy.

Funding: University of Malta Research Grant PHRRP12-17

REFERENCES

1. Link E, Parish S, Armitage J, Bowman L, Health S, Matsuda F, et al. SLCO1B1 variants and statin-induced myopathy: A genomewide study. N Engl J Med. 2008;359(8):789-99.
2. Ramsey L, Johnson S, Caudle K, Haidar C, Voora D, Wilke R, et al. The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1 and Simvastatin-Induced Myopathy. Clin Pharm Ther. 2014;96(4):423-8.

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Poster presentation at the International Pharmaceutical Federation World Congress of Pharmacy and Pharmaceutical Sciences, Abu Dhabi, Scotland, September 2019

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PHARMACOVIGILANCE ANALYSIS OF STATIN-ASSOCIATED MUSCLE SYMPTOMS

Judith Cerdá Iñesta[1, 2], Francesca Wirth [1], Anthony Serracino-Inglott* [1, 2]

[1]Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta

[2]Malta Medicines Authority, Malta Life Sciences Park, San Ġwann, Malta

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INTRODUCTION

Muscle symptoms are clinically-relevant side-effects of statin therapy. Incidence of statin-associated muscle symptoms (SAMS), including myalgia, myopathy and rhabdomyolysis, varies with the different statins.[1]

AIM

To analyse adverse drug reaction (ADR) reports of SAMS with different statins (simvastatin, atorvastatin, rosuvastatin)

METHOD

ADR reports retrieved from EudraVigilance Data Analysis System (EVDAS)

↓

2014-2018 ADR reports of myalgia, myopathy and rhabdomyolysis with simvastatin, atorvastatin and rosuvastatin from 28 European Union countries

↓

Analysis of ADR reports according to SAMS, gender and statin dose

RESULTS

- The 4,164 ADR reports analysed were distributed as: Myalgia (78%, n=3,260 - atorvastatin 47%, simvastatin 32%, rosuvastatin 21%), Rhabdomyolysis (15%, n=643 - atorvastatin 48%, simvastatin 37%, rosuvastatin 14.5%) and Myopathy (6%, n=261 - atorvastatin 47.5%, simvastatin 34%, rosuvastatin 19%).
- Reports for all SAMS were significantly higher for atorvastatin compared to simvastatin and rosuvastatin (p=0.007).
- Incidence of rhabdomyolysis was significantly higher in males for atorvastatin (p<0.001) and simvastatin (p=0.003) and comparable between genders for rosuvastatin (p=0.103).
- The statin doses associated with SAMS were: Myalgia (simvastatin 20mg-49%, rosuvastatin 10mg-38%, atorvastatin 20mg-36%), Myopathy (simvastatin 40mg-39%, rosuvastatin 5mg, 10mg-33%, atorvastatin 10mg-30%), rhabdomyolysis (simvastatin 40mg-53%, atorvastatin 80mg-40%, rosuvastatin 20mg-38%).

Table 1: Analysis of ADR reports of myalgia, myopathy and rhabdomyolysis according to statin and dose (N=4,164)

ADR / Statin and Dose	Simvastatin (n=1380)				Atorvastatin (n=1963)				Rosuvastatin (n=812)			
	10mg	20mg	40mg	80mg	10mg	20mg	40mg	80mg	5mg	10mg	20mg	40mg
Myalgia (n=3,260)	130	516	381	24	461	556	378	140	230	255	150	36
Myopathy (n=261)	14	29	34	11	37	31	35	21	16	16	10	7
Rhabdomyolysis (n=643)	26	71	127	17	44	57	85	122	13	19	36	26

European Medicines Agency (EMA). EudraVigilance Data Analysis System (EVDAS); January 2019.

CONCLUSION

Myalgia was the most common statin-associated muscle symptom reported for the three statins. A higher number of ADR reports of myalgia with atorvastatin compared to simvastatin and rosuvastatin were observed.

REFERENCES

1. Morival C, Westerlynck R, Bouzille G, Cuggia M, Le Corre P. Prevalence and nature of statin drug-drug interactions in a university hospital by electronic health record mining. Eur J Clin Pharmacol. 2018;74(4):525-34.