Genetic Influences on the Pharmacokinetics and Pharmacodynamics of Statins

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ABBREVIATIONS AND DEFINITIONS

6 β -OHC/C	6 β -hydroxycortisol/cortisol
λ_z	Terminal elimination rate constant
ABCB1	ATP-binding cassette, sub-family B, member 1
ABCC2	ATP-binding cassette, sub-family C, member 2
ABCG2	ATP-binding cassette, sub-family G, member 2
ANOVA	Analysis of variance
AOR	Adjusted odds ratio
APOE	Apolipoprotein E
ARB	Angiotensin receptor blocker
AUC	Area under the plasma concentration-time curve
$AUC_{0-\infty}$ or AUC_{0-inf}	Area under the plasma concentration-time curve from time 0 to infinity
AUC_{0-t}	Area under the plasma concentration-time curve from time 0 to time t
BCRP	Breast cancer resistant protein
BMI	Body mass index
CETP	Cholesteryl ester transfer protein
CI	Confidence intervals
CK	Serum creatine kinase
CL/F	Apparent oral clearance
C_{max}	Peak plasma concentrations
CORONA	Controlled Rosuvastatin Multinational Trial in Heart Failure

CRP	C-reactive protein
C_t	Last quantifiable plasma concentration at time t
CV	Coefficient of variation
CVD	Cardiovascular disease
CYP	Cytochrome P450 enzyme
CYP2C9	Cytochrome P450 family 2, subfamily C, polypeptide 9
CYP2D6	Cytochrome P450 family 2, subfamily D, polypeptide 6
CYP3A4	Cytochrome P450 family 3, subfamily A, polypeptide 4
CYP3A5	Cytochrome P450 family 3, subfamily A, polypeptide 5
DBP	Diastolic blood pressure
EDTA	Ethylenediaminetetraacetic acid
EM	Extensive metabolizer
FDA	United States Food and Drug Administration
FH	Familial hypercholesterolaemia
GFJ	Grapefruit juice
GWAS	Genomewide association study
HDL-C	High-Density-Lipoprotein Cholesterol
HMG-Co A	3-hydroxymethyl-3-glutaryl coenzyme A
HMGCR	3-hydroxymethyl-3-glutaryl coenzyme A reductase
HNF1α or HNF1A	HNF1 homeobox A
HPS	Heart Protection Study
hsCRP	High-sensitivity C-reactive protein
IL6R	Interleukin 6 receptor
IM	Intermediate metabolizer
JUPITER	Justification for the Use of Statins in Prevention-

	an Intervention Trial Evaluating Rosuvastatin
K_{el}	Elimination rate coefficient
LDL-C	Low-Density-Lipoprotein Cholesterol
LDLR	Low-density-lipoprotein receptor
LEPR	Leptin receptor
Ln or ln	Natural logarithm based *e*
MDR1	P-glycoprotein
METEOR	Measuring Effects on intima media Thickness: an Evaluation Of Rosuvastatin
MR	Metabolic ratio
NCBI	National Center for Biotechnology Information, USA
NCEP	National Cholesterol Education Program
NS	Non-significant
P-Gp	P-glycoprotein
PCR	Polymerase chain reaction
PCSK9	Proprotein convertase subtilisin-like kexin type 9
PM	Poor metabolizer
PROVE-IT TIMI	Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocadial Infarction
OATP	Organic anion transporter polypeptide
RA	Rheumatoid arthritis
SBP	Systolic blood pressure
SD	Standard deviation
SLCO1B1	Solute carrier organic anion transporter family, member 1B1
SNP	Single nucleotide polymorphism

SPSS	Statistical Package for the Social Sciences
$t_{1/2}$	Elimination half-life
t_{max}	Time to reach maximum plasma concentration
TC	Total Cholesterol
TG	Triglycerides
UGT	UDP glucuronosyltransferase enzyme
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
ULN	Upper limit of normal
UM	Ultrarapid metabolizer
WHR	Waist-hip ratio

LIST OF PRESENTATIONS

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1. B Tomlinson, M Hu, VWL Mak, TTW Chu: **How to achieve regression of atherosclerosis.** *Journal of the Hong Kong College of Cardiology (2008) 16(Suppl 1): A52 – A61.*

2. M Hu, VWL Mak, TTY Chu, MMY Waye, B Tomlinson: **Pharmacogenetics of HMG-CoA reductase inhibitors: optimizing the presentation of coronary heart disease.** *Current Pharmacogenomics and Personalized Medicine (2009) 7(1): 1 – 26.* [Feature Article]

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11. VWL Mak, OQP Yin, MSS Chow, M Hu, B Tomlinson: **Does the cytochrome P450 2D6 polymorphism influence simvastatin plasma concentrations in Chinese subjects?** *Presented at 9th Hong Kong Diabetes and Cardiovascular Risk Factors – East Meets West Symposium, Sep 30 – Oct 1, 2007*

12. VWL Mak, OQP Yin, MSS Chow, M Hu, B Tomlinson: **Effects of the cytochrome P450 2D6*10 polymorphism on the pharmacokinetics of simvastatin and simvastatin acid in Chinese subjects.** *The Third Joint-Conference on Pharmacogenetics Central South University – Inje University – The Chinese University of Hong Kong, Changsa, China, Nov 18 – 22, 2007.*

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14. VWL Mak, M Hu, OQP Yin, MSS Chow, B Tomlinson: **Interaction of pitavastatin with grapefruit juice in subjects with different efflux transporter BCRP ABCG2 421C>A genotypes.** *Presented at International Symposium on Cardiovascular & Neurovascular Medicine (ISCNM) in conjunction with International Heart Failure Symposium, Hong Kong, Feb 22 – 24, 2008. International Journal of Cardiology (2008) Vol. 125, Suppl. 1: P327.*

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32. M Hu, MHK Lee, VWL Mak, B Tomlinson: **Determinants of hsCRP levels in Chinese patients treated with rosuvastatin.** *Presented at World Congress of Cardiology, Beijing, China, Jun 16- 19, 2010.*

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34. YJ Xiao, M Hu, VWL Mak, B Tomlinson: **Association of CYP3A activity with common *CYP3A* polymorphisms and lipid response to simvastatin in Chinese patients.** *Presented at 12th Hong Kong Diabetes & Cardiovascular Risk Factors East meets West Symposium, Oct 1 – 2, 2010.*

Abstract

The statins, or 3-hydroxymethyl-3-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, act on the rate limiting step in endogenous cholesterol synthesis. Their primary action results in reduction of plasma low-density lipoprotein cholesterol (LDL-C) levels and this is thought to be the major mechanism by which they reduce cardiovascular events. There are considerable differences between subjects in both the plasma levels of the statins and in their effects on LDL-C and other lipid parameters and some of this variation appears to be related to genetic differences in the pathways of drug metabolism and distribution and in the pathways involved in lipid metabolism.

This thesis describes a study of 270 patients recruited from the outpatient clinics at the Prince of Wales Hospital who were treated with simvastatin 40 mg daily for at least 4 weeks. Their mean (±SD) LDL-C baseline level was 5.38±1.68 mmol/L and the reduction in LDL-C after simvastatin treatment was 2.81±0.99 mmol/L or -47.1±12.5%.

The SNPs examined included those in the genes for the enzymes and transporters involved in the metabolic pathway or the distribution of simvastatin. Cytochrome P450 (CYP) enzymes are involved in hepatic and intestinal metabolism of several statins and simvastatin is known to undergo extensive metabolism via the CYP3A4/3A5 pathway. The common candidate SNPs in the CYP3A4/3A5 enzymes found in Chinese populations include *CYP3A4*1G, CYP3AP1*3* and

*CYP3A5*3*, which are associated with altered enzyme expression and activity. However, no statistically significant relationship was found between these SNPs and a potential phenotypic marker of enzyme activity, the urinary ratio of 6β-hydroxy-cortisol/cortisol (6β-OHC/C) concentrations. The analysis of lipid lowering responses in relation to individual SNPs or combinations from gene-gene interactions also revealed no statistically significant findings. In the subgroup of patients with familial hypercholesterolaemia, the *CYP3A4*1G*, *CYP3AP1*3* and *CYP3A5*3* polymorphisms appeared to have a small effect on the changes in LDL-C and total cholesterol with the subjects with the *CYP3A5*3* and *CYP3AP1*3* variants showing less reduction and those with the *CYP3A4*1G* variant showing more reduction than subjects with the wild-type genotype with a tendency for a gene-dose effect. It is difficult to interpret these findings and the significance may be related to multiple testing.

The variation in response may be related to variations in systemic or hepatic exposure to the drug, which in turn will be related to the pharmacokinetics. This is also likely to play a role in the adverse effects of myopathy and therapeutic tolerance. In a pharmacokinetic study in healthy male Chinese subjects, the common polymorphism of *CYP2D6*10* was analyzed in relation to the pharmacokinetics of lovastatin and simvastatin. There was a tendency for reduced clearance of simvastatin lactone by 30% ($P>0.05$) in subjects with the *CYP2D6*10/*10* genotype. With lovastatin, there were similar findings with 38.5-84.9% decrease in clearance which appeared to be related to enzyme activity according to genotype, with **5* carriers showing a greater decline in clearance than **10* carriers ($P<0.05$).

Polymorphisms in the drug transporters are likely to be more important with hydrophilic statins such as pitavastatin, which undergoes transporter mediated distribution. The *SLCO1B1 c.388A>G* polymorphism in the gene encoding the uptake transporter organic anion transporting polypeptide (OATP1B1) is common in Chinese and the variant was associated with increases of 63-68% in maximum plasma concentration and 44-47% in systemic exposure of both the lactone and acid compared to wild-type subjects ($P<0.05$). Co-administration of pitavastatin with grapefruit juice (GFJ) resulted in a small increase of the area under the plasma concentration time curve (AUC) by 15-16% for both the acid and lactone ($P<0.05$). However, there was no significant effect on the drug-food interaction in relation to relevant SNPs in the enzymes and transporters examined.

Clinical evidence suggested patients with lower plasma C-reactive protein (CRP) levels after statin therapy could have better clinical outcome. The last part of the study was to measure on-treatment high sensitivity CRP (hsCRP) levels among 229 Chinese patients with hyperlipidaemia undergoing treatment with simvastatin 40 mg daily. The patients were genotyped for 15 SNPs or haplotypes in 11 candidate genes that would have significant allele frequency among Chinese patients and may be linked to statin efficacy or hsCRP levels. The analysis suggested BMI is the largest single contributing factor of 15.0% of the variation in hsCRP levels, followed by plasma triglycerides levels contributing 4.7% and male gender 1.6% (all $P<0.05$). However comparisons of hsCRP levels among genotype groups did not reveal any significant findings, with or without adjustment with covariate genotypic or phenotypic factors. To further categorize

individuals as high or medium risk, we set a threshold hsCRP level of 1 mg/L as the benchmark for evaluation. The *CRPc.3872G>A* SNP was related to lower risk compared to the homozygous wild-type genotype (adjusted odds ratio AOR = 0.289; P = 0.014) after adjusting for phenotypic factors of age, gender, smoking status, BMI, waist circumference, hip circumference, plasma lipid profiles, co-existing disease and co-medications. Another marginal finding included the *HNF1A c.79A>C* SNP (AOR = 0.575; P = 0.118).

These results provide some insights into the pharmacokinetics and pharmacodynamics of statins and the pharamacogenetic relationships to candidate SNPs. Future research in this field should help to facilitate safer and more effective treatment with these commonly used medications, resulting in personalized therapy and optimal clinical benefits for patients with cardiovascular disease.

中文摘要

他汀類藥物，即3 -羥基-3甲基輔酶a還原酶抑製劑，通過作用於內源性膽固醇合成的限速步驟降低血中低密度脂蛋白膽固醇的水平進而減少心血管事件的發生。他汀類藥物的血藥濃度及其降膽固醇的療效存在相當大的個體差異，而部份的個體差異可能與藥物代謝和分佈以及脂質代謝途徑中的基因差異有關。

在本論文中涉及的一項研究中， 270位自威爾斯親王醫院門診招募的華人患者接受了爲期至少 4 周以上的每日辛伐他汀（simvastatin） 40毫克的藥物治療。 他們的平均低密度脂蛋白膽固醇水平（± 標準偏差）由服藥前的5.38 ± 1.68 mmol/L降至2.81 ± 0.99 mmol/L，降幅達-47.1 ± 12.5%。該項研究對与辛伐他汀藥物代謝與分佈相關的酶和藥物轉運蛋白的基因多態性進行了檢測。細胞色素P450（CYP）酶參與一些他汀類藥物在肝臟和腸道的代謝，而目前已知辛伐他汀主要通過CYP3A4/3A5的途徑代謝。在華裔人群中，常見的 *CYP3A4/3A5* 的基因多態性包括 *CYP3A4*1G, CYP3AP1*3* 以及 *CYP3A5*3*。這些單一核苷酸多態性伴有酶表達和活性的改變。但是本研究顯示這些基因多態性與一個潛在的酶活性表型標記，尿中6β-羥基皮質醇與皮質醇的比率（6β- hydroxy-cortisol / cortisol）無統計學上顯著相關性。這些基因多態性對辛伐他汀的降膽固醇的效用亦未發現有顯著作用。而在患有家族遺傳性高膽固醇血症的患者中 *CYP3A4*1G, CYP3AP1*3* 以及 *CYP3A5*3* 基因多態性似乎對辛伐他汀降低低密度脂蛋白膽固醇以及降低總膽固醇的作用有一定的影響。與帶有原生型基因型的家族遺傳性高膽固醇血症患者相比，帶有

*CYP3A5*3* 和 *CYP3AP1*3* 變異等位基因的家族遺傳性高膽固醇血症患者的降脂效果較差而那些帶有*CYP3A4*1G* 變異等位基因的家族遺傳性高膽固醇血症患者的降脂效果則較好，并趨于呈現基因-劑量效應。這一結果現時難以解釋，可能僅爲研究中多次測試下出現的偶然結果。

他汀類藥物降脂作用的個體差異可能與體內血藥濃度或肝臟內藥物濃度差異有關，而這些均與藥物代謝動力學相關。體內藥物濃度的差異還可能影響藥物肌肉毒性副作用以及藥物的耐受性。在一項對華裔男性健康志願者藥代動力學的研究中 我們評個了華裔人群中常見的*CYP2D6*10* 基因多態性與洛伐他汀及辛伐他汀藥代動力學的關係。帶有*CYP2D6*10/*10* 基因型的志願者體內辛伐他汀的清除率降低了30%（P>0.05）。而對洛伐他汀而言，帶有*CYP2D6* 變異等位基因志願者體內的藥物清除率降低了 38.5-84.9%，并與基因型/酶的活性呈相關性，*CYP2D6*5* 攜帶者體內的藥物清除率比**10* 攜帶者呈現更大的降幅 (P<0.05)。

藥物轉運蛋白的基因多態性對親水性他汀類藥物，例如：匹伐他汀（pitavastatin）可能尤爲重要，因爲這些他汀類藥物的分佈多由藥物轉運蛋白介導。溶質載體有機陰離子轉運多肽1B1（OATP1B1）*SLCO1B1 c.388A>G* 基因多態性在華裔人群中非常普遍。我們發現與原生型基因型志願者相比，帶有*SLCO1B1 c.388A>G* 變異等位基因志願者體內匹伐他汀的血藥濃度增加了44-47%，而血藥峰濃度升高了 63-68% (P<0.05). 同時服用匹伐他汀與西柚汁可令匹伐他汀藥時曲線下面積微升 15-16% (P<0.05)。但相關的酶和藥物轉運蛋白的基因多態性對這一藥物-食品相互作用無顯著作用。

臨床證據表明接受他汀類藥物治療后 C-反應蛋白水平較低的患者出現心血

管事件的風險較低。研究的最後一部份是對 229 位每日服用辛伐他汀 40 毫克的華裔高膽固醇患者服藥期間高敏感度 C-反應蛋白水平的測試。我們對這些病人的 11 個可能与他汀類藥物藥效以及 C-反應蛋白相關的基因的 15 個常見的基因多態性或單體型進行了檢測。研究結果顯示身體質量指數（BMI）與 C-反應蛋白水平高度相關，這一因素決定其 15%的變異。三酸甘油酯水平以及性別亦與 C-反應蛋白水平相關，分別可解釋 4.7%和 1.6%的變異 ($P<0.05$)。然而所檢測基因多態性并沒有發現影響 C-反應蛋白水平。我們採用 C-反應蛋白爲 1 mg/L 這一指標將患者分爲高/中和低心血管風險進行進一步研究， 結果顯示與原生型基因型相比，*CRP c.3872G>A* 基因多態性的變異等位基因伴有較低的風險 (被調整的勝算比 = 0.289; P = 0.014)，調整的因素包括年齡，性別，吸煙狀況，身體質量指數，腰圍，臀圍，血脂水平，其他疾病以及所服藥物。 另一個基因多態性 *HNF1A c.79A>C* 也趨向于伴有較低風險(被調整的勝算比 = 0.575; P = 0.118).

這些結果對他汀類藥物的藥代動力學和藥效學以及對相關的基因多態性的遺傳藥理學的提供了一些見解。這一領域未來的研究將有助於促進更安全和更有效的使用這些常用藥物，以達成心血管疾病患者的個體化治療以及取得最佳的臨床受益。

1. Introduction

1.1. Statins

The 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins are the most effective class of drugs for lowering low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) (NCEP, 2002). They exert their primary pharmacological effect inside the hepatocytes. Their clinical beneficial effects in hyperlipidaemia have been well proven for over 20 years with reductions of plasma TC and LDL-C leading to subsequent risk reduction of myocardial infarction or coronary heart disease (Baigent et al., 2005; Genser and Marz, 2006; Josan et al., 2008; LaRosa et al., 1999; Mills et al., 2008; Petretta et al., 2010; Ross et al., 1999; Vrecer et al., 2003). Henyan et al. suggested in their meta-analysis in 2007 that statin therapy significantly reduced the risk of developing cardiovascular events by 17% and ischaemic stroke by 21%; but not of haemorrhagic stroke (Henyan et al., 2007).

A prospective clinical trial with atorvastatin designed to evaluate the prevention of stroke by aggressive reduction of cholesterol levels, suggested a 16% reduction of incidence in stroke (fatal or non-fatal) after treatment (Armani and Toth, 2007). The JUPITER trial reported favourable findings with rosuvastatin 20 mg daily treatment in a group of subjects with lower LDL-C but raised high sensitivity C-reactive protein (hsCRP) levels. After 1.9 years of follow-up, the hazard ratio for the rosuvastatin treatment group to develop primary cardiovascular disease was 0.56 comparing to the placebo group (Ridker et al., 2008a). In the CORONA trial of rosuvastatin in heart failure, after follow-up for 36-months among the subgroup of subjects with raised hsCRP levels, there was an 11% reduction of total mortality in the treatment group of rosuvastatin 10 mg daily compared to the placebo group (McMurray et al., 2009). Another recently published study suggested a high dose atorvastatin may lead to favourable effects on angiogenic factors (Jaumdally et al., 2011). These data, and many others, are supportive of the beneficial effects of statins in cardiovascular disease management.

Statins were noted to slow the progression of atherosclerosis, which was suggested to correlate with intensive statin therapy and the degree of reduction of LDL-C. In the REVERSAL study, after 18-months follow-up the coronary atheroma volume was increased by 2.6% in the pravastatin treated group, but decreased by 0.2% in atorvastatin treated subjects (Nissen et al., 2005). This effect on non-calcified plaque size with atorvastatin has been reconfirmed in another study using computer tomographic scanning (Uehara et al., 2008). Another method to measure atheroma with carotid intima-media thickness (CIMT) using B-mode ultrasound has suggested a beneficial effect with rosuvastatin 40 mg daily (Crouse et al., 2007). Howard et al. suggested the role of statins in reducing LDL-C accompanied by lowering systolic blood pressure resulted in regression of CIMT and with a possible relationship to reduced cardiovascular events in diabetes (Howard et al., 2008).

The concern of adverse events with statins has been raised on many occasions (Silva et al., 2006). Silva et al. rated adverse event rates with individual statins, and suggested serious adverse events are not significant but could differ among the statins. de Denus et al. arrived at similar conclusion on the effect on liver function (de Denus et al., 2004). The association of statin and myopathy has been thoroughly studied over the years (Baker and Tarnopolsky, 2001; Sathasivam and Lecky, 2008). It has been suggested that the accumulation of statins inside skeletal muscle cells and the subsequent interference in the cholesterol production pathway or respiratory chain of the mitochondria may lead to muscle toxicity (Sirvent et al., 2008). Clinically speaking, the incidence of myotoxicity, ranging from mild myopathy to fatal rhadomyolysis should not be significant overall (Brown, 2008). A meta-analysis involving 7 clinical trials and over 29,000 patients, found the frequency of myalgia was no more than 4.8%, and that for more serious adverse effects of myopathy or rhabdomylosis was no more than 0.5% (Josan et al., 2008). A study using a genomewide association study (GWAS) approach of retrospective data to identify genetic markers of simvastatin-related myopathy concluded that a single nucleotide polymorphism (SNP) in the gene *SLCO1B1* encoding the hepatocyte uptake transporter organic anion-transporting

polypeptide 1B1 (OATP1B1 or SLCO1B1) had positive correlation with increased risk of myopathy (Link et al., 2008). Hence, there could be genetic factors in the pre-disposition of patients with some being more susceptible to statin-related adverse effects.

1.2. Genetic influence on clinical responses

Genetic variation could be the key to both statin efficacy and susceptibility to statin-induced adverse drug reactions. There have been reports on inter-ethnic diversity of the responses (LaRosa, 2000; Puccetti et al., 2007), and the genetic factors that would affect LDL-C levels have also been studied in GWAS (Sandhu et al., 2008). Hence, differences in the frequency of the underlying polymorphisms among ethnic groups may affect the successful hyperlipidaemic therapeutic management (Chasman et al., 2004; Mangravite et al., 2008; Tirona, 2005). Furthermore, environmental factors may also contribute to differences in LDL-C lowering with statin therapy (Hutz and Fiegenbaum, 2008; Mangravite et al., 2006).

Simon et al. reported a slight difference in LDL-C reduction between African Americans (-38.9%) vs. Whites (-42.1%) over 6-weeks treatment with simvastatin 40mg daily ($P<0.01$) (Simon et al., 2006). Similar results have been noted by Krauss et al. (-38.8% vs. -57.8%) with the same treatment regimen over the same period of time. In this study, the authors highlighted the HMG-CoA reductase (*HMGCR)* gene polymorphisms to be associated with reduced LDL-C lowering response to simvastatin, and suggested these effects were more evident among blacks (Krauss et al., 2008). There were other reports on *HMGCR* gene polymorphisms associated with lipid lowering responses (Kajinami et al., 2004b; Mangravite et al., 2008). However, a study in an elderly population suggested a significant relationship between the *LDLR* gene encoding the LDL-receptor and pravastatin responses but not with the *HMGCR* polymorphism (Polisecki et al., 2008). Another American study also identified the *LDLR* 3-UTR haplotype to be associated with smaller lipid-lowering response to simvastatin treatment. Gene-gene interaction with *HMGCR* haplotypes was suggested. Because of the differential prevalence of these haplotypes between ethnic groups, the authors suggested these might contribute to the differences in lipid lowering response between black and white races (Mangravite et al., 2010).

Allelic genotypes of the gene encoding apolipoprotein E were was also suggested to correlate with statin efficacy. After 12-months of treatment with pravastatin 40 mg daily, subjects with the *APOE*2* allele had significantly greater reductions than homozygous *APOE*3* subject or *APOE*4* carriers (36% vs. 27% vs. 26%; P = 0.04) (Ordovas et al., 1995). In another study with fluvastatin treatment over 12-weeks, subjects with homozygous *APOE*3* genotype had greater TC and LDL-C reductions than *APOE*4* subjects (20.4% vs. 15.4% and 28.8% vs. 22.7% respectively; P<0.05) (Ballantyne et al., 2000).

There were reports of other candidate genes which were proposed to affect the pharmacodynamic responses to statins. Fiegenbaum et al. suggested polymorphisms within sterol regulatory element-binding factors-1a and -2 (*SREBF-1a and SREBF-2*) and SREBF cleavage-activating protein (*SCAP*) genes could influence lipid-lowering response to simvastatin (Fiegenbaum et al., 2005b). In Japan, Takahashi-Yasuno et al. examined the pharmacogenetic influence of the leptin receptor and suggested a negative correlation between the Arg223Gln polymorphism at the encoding gene and significant elevation of serum TC and LDL-C levels among male patients after simvastatin treatment (Takahashi-Yasuno et al., 2003). All these genetic variations may be likely to contribute to inter-ethnic differences in statin response because of their diversity of allelic frequencies between ethnic groups.

1.3. Statin metabolic pathway and genetic factors

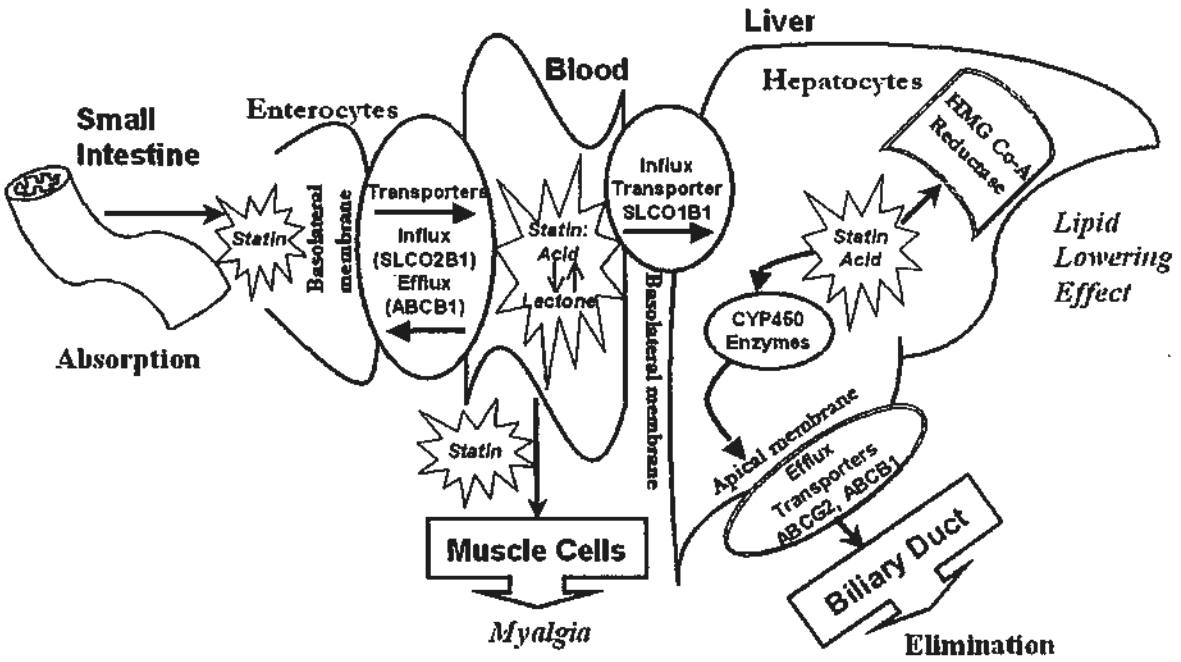
Another perspective to the pharmacogenetic influence on the beneficial and adverse clinical outcome with statins is from their pharmacokinetics.

Most statins undergo a complex metabolic pathway mediated by a number of cell membrane transporters and cytochrome P450 (CYP) enzymes. These enzyme and transporter proteins are the integral pathways of statins pharmacokinetics. Their encoding genes are likely be polymorphic, and hence subject to enhanced or reduced efficacy depending on the degree of gene expression and activity of the gene product. Even though the contributions of individual genetic determinants to overall response have been small or even not been conclusive, the combined effects of multiple genotypes may be more substantial (Wang et al., 2005a). Figure 1.1 summarized a schematic representation of a typical statin metabolic and distribution pathways, from the point of absorption at the enterocytes of the small intestine, which is modulated by influx and efflux transporters, then being taken up at the basolateral membrane of hepatocytes for the primary pharmacological effects at the HMG-CoA reductase enzyme, and finally eliminated by excretion via the biliary tract. Depending on the plasma levels, certain amount of statins may enter skeletal muscle cells by passive diffusion leading to myalgia side effects. The role of transporters is noted in the absorption, uptake, distribution and elimination of statins.

Ho et al. have highlighted the difference in frequency between European and African Americans on uptake transporter OATP1B1 (*SLCO1B1*) variants which consequently influence the disposition of hydrophilic pravastatin. In a pharmacokinetic study after a single 40 mg dose of pravastatin, *SLCO1B1*1a/*15* participants had 45% higher mean values of area under the plasma concentration-time curve (AUC) than *SLCO1B1*1a/*1a* subjects (P=0.013) and 80% higher AUC than *SLCO1B1*1b/*1b* (P = 0.001) carriers. The homozygous *SLCO1B1*15/*15* carriers had even higher AUC by 92% and 149% comparing to

*SLCO1B1*1a/*1a* (P = 0.017) and *SLCO1B1*1b/*1b* (P = 0.011) subjects, respectively (Ho et al., 2007).

Figure 1.1: Schematic representation of statin metabolic and distribution pathways



Lee et al. have reviewed the differences in disposition of rosuvastatin among ethnic Asian groups in the same environment in relation to a single nucleotide polymorphism (SNP) in the *SLCO1B1* gene. The results suggested there were inter-ethnic differences for the AUC values among Chinese, Malay and Asian-Indian subjects, the ratios being 2.31:1.91:1.63 accordingly, indicating that Chinese individuals could have twice the systemic exposure to rosuvastatin than ethnic Indian subjects. However, the *SLCO1B1* haplotypes analyzed could not explain the diversity in the pharmacokinetic parameters amongst the ethnic groups (Lee et al., 2005).

P-Glycoprotein (P-Gp, ABCB1 or MDR1) polymorphisms have also been studied and suggested to influence the lipid lowering efficacy of atorvastatin. A recent

study reported after a high dose of atorvastatin 80 mg daily for 6-weeks, patients with the *ABCB1c.3435CC* genotype showed less effective reductions in LDL-C compared to the *c.3435TT* or *c.3435TC* carriers (53% vs. 59%, respectively, P = 0.034) (Hoenig et al., 2011). In the same study, it was also suggested that the *T* alleles were found more frequently among patients with myalgia than non-myalgia patients (frequency 0.80 vs. 0.62; P=0.043). Such findings might be significant in clinical practice to evaluate the susceptibility to adverse events. However, these results on the pharmacodynamic effects were not found in a previous study in Brazil which involved atorvastatin 10 mg daily treatment for 4-weeks (Rodrigues et al., 2005). Another Brazilian study also noted the association of *ABCB1* gene polymorphisms with the efficacy of simvastatin, suggesting homozygous mutant carriers of *ABCB1c.1236TT* had greater reductions in TC and LDL-C compared to *c.1236CC* individuals (-29.0% vs. -24.2% and -39.6% vs. -33.8%, respectively; P = 0.042) (Fiegenbaum et al., 2005a). In another pharmacokinetic study, the AUC value for simvastatin acid was 60% higher and for atorvastatin was 55% higher with homozygous *ABCB1 TTT* carriers than with *CGC* subjects (P<0.05) (Keskitalo et al., 2008).

Statins also undergo metabolism through cytochrome P450 (CYP) enzyme mediated pathways. Wang et al. reported a minor allele of *CYP3A4*4* was associated with greater lipid lowering effect after treatment with simvastatin 20 mg daily for 4-weeks. The plasma TC level was lowered by a mean of 35.8% in the group with *CYP3A4*1/*4* compared to 22.0% in the *CYP3A4*1/*1* group (P = 0.0015) (Wang et al., 2005a). Another study in Chinese subjects suggested a *CYP3A4*1G* variant was associated with enhanced lipid lowering response to atorvastatin but not simvastatin. After treatment with atorvastatin 20 mg daily for 4-weeks, the mean percentage reduction in TC was 16.8% in the *CYP3A4*1/*1* carriers and 20.9% for *CYP3A4*1G/*1G* subjects (P<0.01) (Gao et al., 2008). The common metabolic pathway through CYP3A enzymes influenced by the *CYP3A5* and *CYP3A4* genes might contribute in altering effects from the polymorphisms of either gene.

The *CYP3A5* gene itself is highly polymorphic. A previous study among Caucasians had suggested that lovastatin, simvastatin and atorvastatin were less effective in lipid lowering in subjects who are *CYP3A5* gene expressors. The mean percentage reduction in serum total cholesterol from baseline was significantly smaller in *CYP3A5* expressors than in non-expressors (17% versus 31%, P = 0.026) (Kivisto et al., 2004). In the case of simvastatin, the difference was probably due to enhanced metabolism. A single dose pharmacokinetic study with 20 mg of simvastatin has reported the mean AUC as 4.94 ng·h/mL in the *CYP3A5*1/*1* carriers, significantly lower than the *CYP3A5*3/*3* carriers (16.35 ng·h/mL; P = 0.013) (Kim et al., 2007).

The genetic factors along altering enzyme activity in the metabolic pathway will affect the pharmacokinetics of statins and eventually the disposition of the metabolites, leading to modulation of clinical efficacy as well as adverse effects.

1.4. Anti-inflammatory effects of statins

The anti-inflammatory effects of statins have been considered to be related to their effects in modulation of kinase phosphorylation and protein prenylation (Montecucco and Mach, 2009). An early study with lovastatin reported 14.8% reduction in plasma C-reactive protein (CRP) levels, a biomarker of inflammation, and was independent of the changes in lipid parameters (Ridker et al., 2001). Another study showed that after 3-months treatment with simvastatin 10 mg daily there was a 74% reduction in high-sensitivity CRP (hsCRP) values to 0.3 mg/L (Kostakou et al., 2010). In the METEOR study, subjects with median baseline hsCRP of 1.4mg/L had rosuvastatin 40 mg daily treatment for 2-years. The resultant mean hsCRP levels was lowered by 36% and the change was not related to changes in LDL-C (de Denus et al., 2004). Another trial in a Chinese population reported 24% and 40% reductions in hsCRP values after 12-weeks treatment with atorvastatin and rosuvastatin, respectively (Qu et al., 2009). Significant reductions in hsCRP values in patients with carotid stenosis were also noted with different dosages (10-80 mg daily) of atorvastatin (Kadoglou et al., 2010), and the changes occurred in a dose dependent manner in high-risk subjects previously not on statin treatments (Gensini et al., 2010). High dose simvastatin 80 mg daily treatment for 4-weeks resulted in similar hsCRP reductions (23% vs. 30%) to those of combination simvastatin 10 mg and ezetimibe 10 mg in high risk subjects (medians 3.64 and 4.21 mg/L) (Araujo et al., 2010). Nevertheless, there were reports of lack of improvements on hsCRP values with some doses of statins such as simvastatin 40 mg monotherapy compared to the different combinations of rosuvastatin (5-20 mg) / fenofibric acid (135 mg) (Roth et al., 2010), or by comparing fluvastatin 80 mg therapy with placebo (Ostadal et al., 2010). Regarding co-medication regimens, changes in hsCRP values were comparable after the combination of simvastatin 20 mg with ezetimibe 10 mg or in triple combination with extended-release niacin 2 gm over a 64-weeks trial period (Fazio et al., 2010). Another study with patients preparing for carotid endarterectomy compared regimens of atorvastatin or chloestyramine after

12-weeks, failed to show statistically significant reductions of hsCRP values (Puato et al., 2010). These results suggested the probable direct influence from statins on hsCRP values were not affected by co-medication for other factors that might affect the lipid profiles.

In clinical settings, various randomized trials suggested short-term high-dose statins administration before coronary procedures improved clinical outcome in patients with acute coronary syndromes and/or high CRP levels (Mega et al., 2010). In fact, randomized clinical trials with statins aiming at either primary or secondary prevention have reported significant reductions in myocardial infarction, stroke and vascular events (Scandinavian-4S-Group, 1994; Shepherd et al., 1995), even though a clinical trial with rosuvastatin among dialysis patients failed to demonstrate the differences (Fellstrom et al., 2009). Drawing reference to the CORONA study involving treatment with rosuvastatin 10 mg daily in patients over 60-years old with chronic systolic heart failure, long term follow-up suggested a better cardiovascular outcome in the group with higher baseline hsCRP values (≥2.0 mg/L, median 5.5 mg/L; relative hazard ratio 0.87 vs. placebo group), while the plasma LDL-C levels were comparable in the groups with different hsCRP levels (McMurray et al., 2009). In summary, potent statins that were able to produce greater percentage reduction in plasma LDL-C and hsCRP levels were likely to achieve more reduction in cardiovascular risk (Ridker et al., 2008a; 2009). Targeting statin treatment based on plasma LDL-C levels alone may not provide an optimal risk reduction for all individuals (Stewart, 2009). These were clear suggestion that application of statin treatment would be beneficial to reduce cardiovascular risk for those even with low plasma LDL-C levels but with high hsCRP values (Ridker, 2010).

hsCRP may be an important biomarker for vascular inflammation and especially atherosclerosis, and therefore a useful predictor of cardiovascular risk. There are postulations that statins may have direct anti-inflammatory effects. Genetic influence on hsCRP in the circulation and the interaction with statins would be worthy of investigation as it may affect the overall clinical outcome.

1.5. Aims of this research

To reaffirm the genetic influence on the lipid lowering response to statins, we carried out a preliminary study by genotyping common polymorphisms in candidate genes and evaluated the differential LDL-C lowering response according to these genotypes. A total of 273 patients were recruited from the outpatient clinics and in particular the Lipid Clinic who had their plasma LDL-C levels assessed before and after at least 4-weeks treatment with simvastatin 40 mg daily. Simvastatin was chosen because it is the preferred statin for use in the Hong Kong Hospital Authority system, largely for economic reasons. The dose of 40mg simvastatin was chosen because many of these patients were also studied on a 10 mg dose of rosuvastatin, and simvastatin 40mg is required to produce a similar degree of lowering of LDL-C. The patients involved generally required this dose to reach the target LDL-C level. The mean duration of treatment was 12-weeks and the median duration was 6-weeks. Their mean±SD LDL-C baseline levels was 5.38±1.68 mmol/L, LDL-C changes after simvastatin treatment was 2.81±0.99 mmol/L, and the corresponding percentage changes was -47.1%±12.5%. There were 165 SNPs from 74 genes selected for our analysis (Hu et al., 2009a). The SNPs examined are listed in Table 1.1. The respective genes for these polymorphisms are involved in encoding proteins that are likely to be engaged in statin pharmacokinetics or pharmacodynamics. However, 17 of those SNPs failed to achieve reasonable analysis results due to having a minor allele frequency less than 1%, or departure of the genotype frequencies from Hardy-Weinberg equilibrium, or failure in genotyping. In Table 1.2 the 25 SNPs are listed that were found to associate with differences among genotype groups for the LDL-C baseline levels or on-treatment lowering responses, and were of statistical significance ($P<0.05$) or of marginal significance. In recognition of the small population size, and the multiple testing principles, these preliminary results were viewed as qualitative indicators to suggest possible genetic influence on clinical response to simvastatin in our group of Chinese patients. Additional objectives from this research work were to evaluate the pharmacological impact on statins

from candidate genetic factors that would be frequently encountered among Chinese population in Hong Kong. The following items were considered:

I. Evaluation of the effects of the common polymorphisms in Chinese in the genes encoding cytochrome P450 (CYP) 3A group metabolic enzymes on lipid lowering responses with simvastatin.
II. Evaluation of the influence of common Chinese polymorphism in *CYP2D6* on simvastatin pharmacokinetics.
III. Evaluation of the polymorphisms in the genes encoding influx and efflux transporters on the pharmacokinetics of statins. Study of gene-gene and gene-food interaction, considering pitavastatin as the substrate statin.
IV. Evaluation ~~of the~~ polymorphic changes with genes related to simvastatin pharmcodynamic effects on the influence towards its possible anti-inflammatory benefits, using plasma hsCRP as the biomarker.

Based on these findings, we anticipated being able to clarify how the common genetic variants in the local Chinese population would influence the lipid responses and possibly the adverse events with statin therapy.

Table 1.1: List of 165 single nucleotide polymorphisms of the 74 candidate genes/loci examined

Locus	Candidate Genes / Nearby Genes	Symbol	Polymorphism (Amino acid changes)	dbSNP
Genes encoding metabolic enzymes or cellular transporters, which potentially be involved in statin pharmacokinetics				
9q22	ATP-binding cassette, subfamily A, member 1	*ABCA1*	*T>C*	rs2472384
9q22	ATP-binding cassette, subfamily A, member 1	*ABCA1*	*c.1051G>A* (Arg219Lys)	rs2230806
9q22	ATP-binding cassette, subfamily A, member 1	*ABCA1*	*c.5155G>A* (Arg1587Lys)	rs2230808 [b]
9q22	ATP-binding cassette, subfamily A, member 1	*ABCA1*	*c.69C>T*	rs1800977
9q22	ATP-binding cassette, subfamily A, member 1	*ABCA1*	*c.378G>C*	rs1800978
7q21.1	ATP-binding cassette, sub-family B, member 1	*ABCB1*	*c.2677G>T*	rs2032582
7q21.1	ATP-binding cassette, sub-family B, member 1	*ABCB1*	*c.3435C>T*	rs1045642
7q21.1	ATP-binding cassette, sub-family B, member 1	*ABCB1*	*c.1236C>T*	rs1128503
10q24	ATP-binding cassette, sub-family C, member 2	*ABCC2*	*c.1249G>A* (Val417Ile)	rs2273697
10q24	ATP-binding cassette, sub-family C, member 2	*ABCC2*	*c.3563T>A* (Val1188Glu)	rs8187694 [a]
4q22	ATP-binding cassette, subfamily G, member 2	*ABCG2*	*c.421C>A* (Gln141Lys)	rs2231142
4q22	ATP-binding cassette, subfamily G, member 2	*ABCG2*	*c.34G>A*	rs2231137
2p21	ATP-binding cassette, subfamily G, member 5	*ABCG5*	Gln604Glu	rs6720173
2p21	ATP-binding cassette, subfamily G, member 8	*ABCG8*	Tyr54Cys	rs4148211
2p21	ATP-binding cassette, subfamily G, member 8	*ABCG8*	Thr400Lys	rs4148217

10q24	Cytochrome P450 family 2, subfamily C, polypeptide 19	*CYP2C19*	**3, c.636G>A*	rs4986893
10q24	Cytochrome P450 family 2, subfamily C, polypeptide 19	*CYP2C19*	**2, c.681G>A*	rs4244285
10q24	Cytochrome P450 family 2, subfamily C, polypeptide 9	*CYP2C9*	**3, c.1075A>C*	rs1057910
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	**10, c.100C>T*	rs1065852
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	*c.1934G>A*	rs5030866
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	*c.1846G>A/T*	rs3892097
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	*c.2850C>T*	rs16947
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	*c.4180G>C*	rs1135840
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	**5*, deletion	
7q21.1	Cytochrome P450 family 3, subfamily A, polypeptide 4	*CYP3A4*	**1G, c.20230G>A*	rs2242480
7q21.1	Cytochrome P450 family 3, subfamily A, polypeptide 5	*CYP3A5*	**3, c.6986A>G*	rs776746
7q21.1	Cytochrome P450 family 3, subfamily A, polypeptide 5	*CYP3A5*	**5, c.12952T>C*	rs80302149
7q21.1	Cytochrome P450, family 3, subfamily A, polypeptide 5, pseudogene 1	*CYP3AP1*	**3, c.-44G>A*	
8q11	Cytochrome P450, family 7, subfamily A, polypeptide 1	*CYP7A1*	*G>T*	rs3808607
1q23	Flavin containing monooxygenase 3	*FMO3*	Glu158Lys	rs2266782
1q23	Flavin containing monooxygenase 3	*FMO3*	Val257Met	rs1736557
1q23	Flavin containing monooxygenase 3	*FMO3*	Glu308Gly	rs2266780
8p22	N-acetyltransferase 2	*NAT2*	**6, c.590G>A* (Arg197Gln)	rs1799930
8p22	N-acetyltransferase 2	*NAT2*	**7, c.857G>A* (Gly286Glu)	rs1799931
14q24.1	Solute carrier family 10, member 1	*SLC10A1 (NTCP)*	**2, c.800C>T* (Ser267Phe)	rs2296651
4p16	Solute carrier family 2, member 9	*SLC2A9 (GLUT9)*	*c.45012T>C*	rs1014290
4p16	Solute carrier family 2, member 9	*SLC2A9 (GLUT9)*	*T>C*	rs12510549

6q26	Solute carrier family 22 (organic cation transporter), member 1	*SLC22A1 (OCT1)*	*c.480C>G* (Phe160Leu)	rs683369
6q26	Solute carrier family 22 (organic cation transporter), member 1	*SLC22A1 (OCT1)*	*c.1022C>T* (Pro341Leu)	rs2282143
6q26	Solute carrier family 22 (organic cation transporter), member 1	*SLC22A1 (OCT1)*	*c.1222A>G* (Met480Val)	rs628031
6q26	Solute carrier family 22 (organic cation transporter), member 2	*SLC22A2 (OCT2)*	*c.808G>T* (Ala270Ser)	rs316019
11q13.1	Solute carrier family 22 (organic anion/urate transporter), member 12	*SLC22CA12 (URAT1)*	*c.12515T>G*	rs893006
11q13.1	Solute carrier family 22 (organic anion/urate transporter), member 12	*SLC22CA12 (URAT1)*	*c.426T>C*	rs11231825
17q25.1	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	*SLC9A3R1 (NHERF1)*	*c.328C>G* (Leu110Val)	rs35910969 [a]
17q25.1	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	*SLC9A3R1 (NHERF1)*	*c.458G>A* (Arg153Gln)	rs41282065 [a]
17q25.1	Solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	*SLC9A3R1 (NHERF1)*	*c.672C>T*	rs35833270 [a]
9q34	Solute carrier family 34 (sodium phosphate), member 3 (Renal sodium phosphate cotransporters type 2)	*SLC34A3*	*c.1931T>C*	rs28407527
9q34	Solute carrier family 34 (sodium phosphate), member 3 (Renal sodium phosphate cotransporters type 2)	*SLC34A3*	*c.2704T>A*	rs35535797
9q34	Solute carrier family 34 (sodium phosphate), member 3 (Renal sodium phosphate cotransporters type 2)	*SLC34A3*	*c.4452T>A*	rs28542318 [a]
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*T>G*	rs2291073
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*C>A*	rs4149036
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*G>C*	rs4149080
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.388A>G* (Asn130Asp)	rs2306283
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.521T>C* (Val174Ala)	rs4149056

12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.571T>C*	rs4149057 [b]
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.597C>T*	rs2291075
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.-11187G>A*	rs4149015
12p12	Solute carrier organic anion transporter family, member 1B3	*SLCO1B3*	*c.344T>G*	rs4149117
12p12	Solute carrier organic anion transporter family, member 1B3	*SLCO1B3*	*c.699G>A*	rs7311358
11q13	Solute carrier organic anion transporter family, member 2B1	*SLCO2B1*	*c.1457C>T* (Ser486Phe)	rs2306168
2q37	UDP glucuronosyltransferase 1 family, polypeptide A1	*UGT1A1*	**60, c.-3279T>G*	rs4124874
2q37	UDP glucuronosyltransferase 1 family, polypeptide A1	*UGT1A1*	**6, c.211G>A* (Gly71Arg)	rs4148323
2q37	UDP glucuronosyltransferase 1 family, polypeptide A1	*UGT1A1*	*(TA)6>7, *28*	
2q37	UDP glucuronosyltransferase 1 family, polypeptide A6	*UGT1A6*	*c.541A>G* (Thr181Ala)	rs2070959
2q37	UDP glucuronosyltransferase 1 family, polypeptide A6	*UGT1A6*	*c.552A>C* (Arg184Ser)	rs1105879
4q13	UDP glucuronosyltransferase 2 family, polypeptide B7	*UGT2B7*	**2, c.802C>T* (His268Tyr)	rs7439366 [b]
4q13	UDP glucuronosyltransferase 2 family, polypeptide B7	*UGT2B7*	*c.-327A>G*	rs7662029
4q13	UDP glucuronosyltransferase 2 family, polypeptide B7	*UGT2B7*	*c.211G>T* (Ala71Ser)	rs12233719
4q13	UDP glucuronosyltransferase 2 family, polypeptide B7	*UGT2B7*	*c.−161T>C*	rs7668258

Genes encoding cellular receptors etc., which are potentially involved in statin pharmacodynamics				
17q23	Angiotensin I-converting enzyme	*ACE*	*G>A* (Ala157Ala)	rs4331
17q23	Angiotensin I-converting enzyme	*ACE*	*C>G*	rs4341
16p12.3	Acyl-CoA synthetase medium-chain family member 1	*ACSM1 (MACS1)*	*A>G*	rs163253 [c]
16p12.3	Acyl-CoA synthetase medium-chain family member 1	*ACSM1 (MACS1)*	*A>G*	rs151328
16p12.3	Acyl-CoA synthetase medium-chain family member 2B	*ACSM2B (MACS2)*	*C>T* (Leu513Ser)	rs1133607 [b]
16p13.11	Acyl-CoA synthetase medium-chain family member 3	*ACSM3 (SAH)*	*c.1077G>C* (Lys359Asn)	rs5716 [c]
16p13.11	Acyl-CoA synthetase medium-chain family member 3	*ACSM3 (SAH)*	*G>T*	rs886433
16p13.11	Acyl-CoA synthetase medium-chain family member 3	*ACSM3 (SAH)*	*c.-962 ins/del*	
3q27	Adiponectin	*ADIPOQ*	*c.-11377C>G*	rs266729
3q27	Adiponectin	*ADIPOQ*	*c.45T>G*	rs2241766
3q27	Adiponectin	*ADIPOQ*	*c.276G>T*	rs1501299
1p31.1	Angiopoietin-like 3	*ANGPTL3*	*C>G*	rs1748195
1q21	Apolipoprotein A-II	*APOA2*	*c.-265T>C*	rs5082
11q23	Apolipoprotein A-V	*APOA5*	*c.-1131T>C*	rs662799
2p24	Apolipoprotein B	*APOB*	Xba I, *c.7673C>T*	rs693
2p24	Apolipoprotein B	*APOB*	Ins/Del	
19q13.2	Apolipoprotein E	*APOE*	*c.472C>T* (Cys158Arg)	rs7412
19q13.2	Apolipoprotein E	*APOE*	*c.334T>C* (Cys112Arg)	rs429358
19q13	Apolipoprotein E/C-I/C-IV/C-II	*APOE/C1 /C4/C2*	*A>G*	rs4420638
17q11.2	Chemokine ligand 2	*CCL2 (MCP-1)*	*c.-3813C>T*	rs1860188
1p13	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)/Proline/serine-rich coiled-coil 1/Sortilin 1	*CELSR2 /PSRC1 /SORT1*	*C>T*	rs646776
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	*c.-971G>A*	rs4783961
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	*c.-629C>A*	rs1800775
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	*c.16G>A* (Ile405Val)	rs5882

16q21	Cholesteryl ester transfer protein, plasma	*CETP*	*c.2327G>A*	rs12149545
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	*c.2490C>A*	rs3764261
16q21	Cholesteryl ester transfer protein, plasma	*CETP*	TaqIB	rs708272
1q21	C-reactive protein, pentraxin-related	*CRP*	*c.3872G>A*	rs1205
1q21	C-reactive protein, pentraxin-related	*CRP*	*c.4284G>A*	rs2794521
1q21	C-reactive protein, pentraxin-related	*CRP*	*c.5237A>G*	rs2808630
11q13.5	Diacylglycerol O-acyltransferase homolog 2	*DGAT2*	*T>C*	rs3060
11q13.5	Diacylglycerol O-acyltransferase homolog 2	*DGAT2*	*C>T*	rs10899116
5q35.1	Dopamine receptor D1	*DRD1*	*c.-800T>C*	rs265981
5q35.1	Dopamine receptor D1	*DRD1*	*c.-94G>A*	rs5326
5q35.1	Dopamine receptor D1	*DRD1*	*c.-48G>A*	rs4532
4q42	Fibroblast growth-factor 23	*FGF23*	*c.716C>T* (Thr239Met)	rs7955866
1q41	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactos-aminyltransferase 2 (GalNAc-T2)	*GALNT2*	*T>G*	rs2144300
1q42	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactos-aminyltransferase 2 (GalNAc-T2)	*GALNT2*	*G>A*	rs4846914
1q42	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactos-aminyltransferase 2 (GalNAc-T2)	*GALNT2*	*G>A*	rs4846914
2p23	Glucokinase (hexokinase 4) regulator	*GCKR*	*C>T*	rs1260326
2p23	Glucokinase (hexokinase 4) regulator	*GCKR*	*G>A*	rs780094
2p23	Glucokinase (hexokinase 4) regulator	*GCKR*	*C>T*	rs1260326
2p23	Glucokinase (hexokinase 4) regulator	*GCKR*	*G>A*	rs780094
14q21	G Protein-coupled Receptor Kinase 4	*GRK4*	*c.425C>T* (Ala142Val)	rs1024323
16q12.2	Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	*HERPUD1*	*G>A*	rs9989419
5q13.3	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	*HMGCR*	*C>T*	rs3846662
5q13.3	3-hydroxy-4-methylglutaryl-Coenzyme A reductase	*HMGCR*	*A>T*	rs12654264
12q24.2	HNF1 homeobox A	*HNF1A*	Ile27Leu	rs1169288
12q24.2	HNF1 homeobox A	*HNF1A*	Ala98Val	rs1800574 [a]
6p21.2	Kinesin family member 6	*KIF6*	*c.2155T>C* (Trp719Arg)	rs20455
16q22.1	Lecithin-cholesterol acyltransferase	*LCAT*	*A>G*	rs255052

19p13.3	Low density lipoprotein receptor	*LDLR*	*c.2052T>C* (Val653Val)	rs5925
19p13.3	Low density lipoprotein receptor	*LDLR*	*c.1866C>T* (Asn591Asn)	rs688
19p13.3	Low density lipoprotein receptor	*LDLR*	*T>C*	rs1529729
19p13.3	Low density lipoprotein receptor	*LDLR*	*c.44857C>T*	rs1433099
19p13.3	Low density lipoprotein receptor	*LDLR*	*c.44964A>G*	rs2738466
1p31	Leptin receptor	*LEPR*	Gln223Arg	rs1137101
1p31	Leptin receptor	*LEPR*	Lys109Arg	rs1137100
15q21	Lipase, hepatic	*LIPC*	*C>T*	rs1532085
15q21	Lipase, hepatic	*LIPC*	*c.-514C>T*	rs1800588
18q21.1	Lipase, endothelial/ Acetyl-Coenzyme A acyltransferase 2	*LIPG /ACAA2*	*T>C*	rs4939883
18q21.1	Lipase, endothelial/ Acetyl-Coenzyme A acyltransferase 2	*LIPG /ACAA2*	*A>C*	rs506696
6q26	Lipoprotein, Lp(a)	*LPA*	*T>C*	rs3798220
6q26	Lipoprotein, Lp(a)	*LPA*	*G>C*	rs7765781
8p22	Lipoprotein lipase	*LPL*	*A>G*	rs331
8p22	Lipoprotein lipase	*LPL*	Ser447X	rs328
10q11.2	Mannose-binding lectin 2	*MBL2*	*c.8195C>G*	rs930507 [b]
10q11.2	Mannose-binding lectin 2	*MBL2*	*c.7204T>C*	rs1838065
7q11.23	MLX interacting protein-like	*MLXIPL*	*c.771G>C*	rs3812316
19p13	Neurocan/cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4	*NCAN /CILP2 /PBX4*	*G>T*	rs16996148
7p13	NPC1 (Niemann-Pick disease, type C1, gene)-like 1	*NPC1L1*	*c.1679C>G* (Leu272Leu)	rs2072183 [b]
7p13	NPC1 (Niemann-Pick disease, type C1, gene)-like 1	*NPC1L1*	*c.18975G>A*	rs4720470
7p13	NPC1 (Niemann-Pick disease, type C1, gene)-like 1	*NPC1L1*	*A>C*	rs2301935
1p32.3	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	*158C>T* (Ala53Val)	rs11583680
1p32.3	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	*c.658−7C> T*	rs2483205
1p32.3	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	*c.799+3A>G*	rs2495477
1p32.3	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	*c.1420G>A* (Val474Ile)	rs562556 [a]
1p32.3	Proprotein convertase subtilisin/kexin type 9	*PCSK9*	*c.2009G>A* (Gly670Glu)	rs505151
7q21.3	Paraoxonase 1	*PON1*	Gln192Arg	rs662

22q12	Peroxisome proliferator-activated receptor alpha	*PPARA*	Leu162Val	rs1800206 [a]
22q12	Peroxisome proliferator-activated receptor alpha	*PPARA*	*A>G*	rs4253776 [a]
22q12	Peroxisome proliferator-activated receptor alpha	*PPARA*	*C>G*	rs4253778 [a]
3p25	Peroxisome proliferator-activated receptor gamma	*PPARG*	*c.167C>G* (Pro12Ala)	rs1801282
4p15.1	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	*PPARGC1A*	*c.1546G>A*	rs8192678
3p22	Parathyroid hormone 1 receptor	*PTH1R*	*A>T*	rs4683301
7q11	Transducin (beta)-like 2 / MLX interacting protein-like	*TBL2 /MLXIPL*	*C>T*	rs17145738
8q24.13	Tribbles homolog 1 (Drosophila)	*TRIB1*	*A>G*	rs4518686
8q24.13	Tribbles homolog 1 (Drosophila)	*TRIB1*	*T>C*	rs10808546
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	BsmI *A>G*	rs1544410
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	ApaI *A>C*	rs7975232
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	TaqI *c.1056T>C*	rs731236
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	*c.-24817A>G*	rs2238136
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	*C>T*	rs2239185
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	*C>T*	rs7305032
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	*c.1270G>A*	rs11568820
12q13.11	Vitamin D (1,25-dihyroxyviatmin D3) Receptor	*VDR*	*c.3989A>G*	rs4516035
11q13	Uncoupling protein 3 (mitochondrial, proton carrier)	*UCP3*	*c.-55C>T*	rs1800849

[a] minor allele frequency <1%;
[b] Depart from Hardy-Weinberg equilibrium;
[c] Failed for genotyping.
Genotype data were not available for all polymorphisms in all subjects.
Ref: National Center for Biotechnology Information: www.ncbi.nlm.nih.gov

Table 1.2: Association of LDL-C response (mean±SD) and selected genetic factors after treatment with simvastatin 40 mg daily for at least 4-weeks

Gene	SNP	Geno-type	n	Baseline LDL-C (mmol/L)	After treatment LDL-C (mmol/L)	Percentage reduction in LDL-C
ABCB1	*c.69C>T*	*CC*	39	6.06±1.47	3.13±0.93	-48.1±9.2
	rs1800977	*CT*	129	5.35±1.81	2.81±1.03	-46.4±14.0
		TT	97	5.05±1.45	2.63±0.89	-47.5±11.7
P-value				0.006*	0.025*	0.664
	**6*					
NAT2	*c.590G>A*	*GG*	143	5.51±1.68	2.85±0.96	-47.3±13.1
	rs1799930	*GA*	105	5.41±1.75	2.83±1.07	-47.1±11.6
		AA	17	4.18±0.84	2.29±0.51	-44.1±13.4
P-value				0.009*	0.081	0.607
	**2*					
SLC10A1	*c.800C>T*	*CC*	222	5.31±1.65	2.74±0.98	-47.6±13.0
(NTCP)	rs2296651	*CT*	44	5.55±1.74	2.98±0.86	-44.9±9.5
		TT	3	6.77±0.90	4.27±1.45	-37.8±14.9
P-value				0.236	0.010*	0.186
SLC2A9	*T>C*	*TT*	112	5.23±1.75	2.82±1.07	-45.5±13.6
(GLUT9)	rs1014290	*TC*	118	5.50±1.68	2.85±0.97	-47.3±11.6
		CC	36	5.51±1.54	2.66±0.79	-51.2±10.7
P-value				0.445	0.587	0.058*
SLC22A2	*c.808 G>T*	*GG*	5	4.96±0.98	3.60±1.68	-26.7±35.8
(OCT2)	rs316019	*GT*	68	5.30±1.83	2.77±1.06	-47.2±11.5
		TT	194	5.39±1.63	2.79±0.93	-47.5±11.5
P-value				0.801	0.179	0.001*

SLC34A3	*c.1931T>C*	*TT*	70	5.26±1.65	2.77±0.91	-46.5±11.7
	rs28407527	*TC*	122	5.24±1.55	2.68±0.87	-48.1±10.7
		CC	77	5.73±1.88	3.07±1.16	-45.7±15.3
P-value				0.102	0.020*	0.391
SLCO1B1	*T>G*	*TT*	86	5.17±1.79	2.89±1.18	-43.4±15.1
	rs2291073	*TG*	144	5.49±1.55	2.76±0.90	-49.4±9.4
		GG	37	5.46±1.92	2.76±0.89	-47.6±12.4
P-value				0.354	0.603	0.001*
SLCO1B1	*C>A*	*CC*	46	5.14±1.66	2.91±1.25	-42.7±18.9
	rs4149036	*CA*	149	5.48±1.68	2.81±0.94	-48.0±10.7
		AA	72	5.38±1.74	2.74±0.93	-48.4±10.0
P-value				0.503	0.665	0.027*
SLCO1B1	*G>C*	*GG*	69	5.13±1.42	2.86±1.03	-43.6±16.2
	rs4149080	*GC*	141	5.56±1.79	2.83±0.99	-48.4±10.7
		CC	60	5.25±1.69	2.70±0.94	-47.9±10.9
P-value				0.175	0.615	0.026*
SLCO1B1	*c.597C>T*	*CC*	47	5.01±1.50	2.83±1.18	-43.0±18.8
	rs2291075	*CT*	142	5.51±1.74	2.81±0.95	-48.2±10.9
		TT	77	5.33±1.68	2.77±0.95	-47.6±10.0
P-value				0.211	0.928	0.041*
UGT1A6	*c.552A>C*	*AA*	169	5.35±1.59	2.80±0.94	-47.4±10.3
	rs1105879	*AC*	90	5.40±1.84	2.78±1.01	-47.2±13.4
		CC	9	5.56±1.85	3.23±1.47	-36.9±29.0
P-value				0.927	0.389	0.049*
Adiponectin	*c.-11377C>G*	*CC*	148	5.38±1.51	2.77±0.89	-48.0±10.5
	rs266729	*CG*	102	5.28±1.89	2.89±1.13	-44.5±14.5
		GG	19	5.81±1.81	2.64±0.98	-53.6±12.9
P-value				0.462	0.490	0.005*

APO B	*c.7673C>T*	*CC*	222	5.29±1.62	2.82±1.02	-46.3±12.8
Xba I	rs693	*CT*	43	5.86±2.00	2.77±0.87	-51.0±10.4
P-value				0.042*	0.768	0.024*
CRP	*c.5237A>G*	*AA*	169	5.58±1.80	2.88±1.09	-47.9±12.6
	rs2808630	*AG*	81	5.03±1.38	2.64±0.77	-46.8±10.4
		GG	12	5.36±1.78	2.93±0.99	-43.5±16.4
P-value				0.055	0.176	0.433
LDLR	*T>C*	*TT*	127	5.65±1.80	2.94±1.01	-47.2±10.7
	rs1529729	*TC*	117	5.11±1.46	2.68±0.90	-46.5±14.9
		CC	26	5.30±1.90	2.74±1.19	-49.0±8.1
P-value				0.042*	0.106	0.655
LIPC	*c.-514C>T*	*CC*	117	5.54±1.73	2.87±0.98	-47.5±10.7
	rs1800588	*CT*	110	5.38±1.71	2.87±1.04	-45.8±13.7
		TT	43	4.96±1.43	2.46±0.82	-49.2±13.5
P-value				0.160	0.041*	0.287
LIPG/ ACAA2	*T>C*	*TT*	7	7.20±2.10	3.31±0.82	-53.1±7.3
	rs4939883	*TC*	71	5.39±1.45	2.82±1.03	-47.8±12.7
		CC	190	5.32±1.72	2.79±0.98	-46.6±12.6
P-value				0.015*	0.388	0.348
NCAN/CILP 2/PBX4	*G>T*	*GG*	216	5.29±1.63	2.75±0.99	-47.5±12.7
	rs16996148	*GT*	53	5.74±1.86	3.06±0.98	-45.3±11.8
P-value				0.079	0.039*	0.265
NPC1L1	*c.18975G>A*	*GG*	124	5.26±1.56	2.71±0.90	-47.7±12.1
	rs4720470	*GA*	112	5.29±1.60	2.78±0.94	-46.9±11.0
		AA	34	6.13±2.18	3.27±1.30	-45.4±17.7
P-value				0.021*	0.011*	0.635

NPC1L1	*A>C*	*AA*	47	5.92±1.99	3.10±1.17	-47.2±12.3
	rs2301935	*AC*	138	5.11±1.51	2.69±0.91	-46.9±11.4
		CC	82	5.49±1.72	2.81±0.98	-47.3±14.6
P-value				0.013*	0.054	0.966
PPAR-G2	*c.167C>G*	*CC*	250	5.43±1.70	2.82±0.99	-47.3±12.3
	rs1801282	*CG*	19	4.62±1.23	2.57±0.88	-43.7±14.4
P-value				0.043*	0.295	0.220
VDR	ApaI	*AA*	20	4.66±1.05	2.20±0.57	-52.5±8.2
	rs7975232	*AC*	109	5.27±1.53	2.79±0.93	-46.0±12.2
		CC	136	5.56±1.80	2.90±1.03	-47.1±13.2
P-value				0.055	0.010*	0.102
VDR	*C>T*	*CC*	19	4.74±1.01	2.23±0.56	-52.6±8.4
	rs2239185	*CT*	111	5.16±1.42	2.73±0.88	-46.1±12.2
		TT	139	5.63±1.90	2.94±1.08	-47.2±13.0
P-value				0.022*	0.008*	0.109
VDR	*C>T*	*CC*	22	4.91±1.16	2.35±0.64	-51.8±8.3
	rs7305032	*CT*	100	5.15±1.42	2.74±0.88	-45.7±12.0
		TT	142	5.63±1.91	2.93±1.09	-47.3±13.3
P-value				0.037*	0.028*	0.109
APOE	*e2: TT*	*e2e2*	1	2.90	1.60	-44.8
c.112T>C	*e3: TC*	*e2e3*	17	5.74±2.19	3.25±1.24	-40.2±23.8
rs429358	*e4: CC*	*e3e3*	192	5.46±1.75	2.79±1.03	-48.3±11.2
c.158C>T		*e3e4*	53	5.11±1.24	2.77±0.72	-45.0±11.6
rs7412		*e4e4*	4	4.55±0.79	2.73±0.56	-39.6±10.3
		e2e4	2	4.85±1.06	2.15±0.35	-55.4±2.5
P-value:						
ANOVA				0.320	0.312	0.053
t-test	*e2e3 vs e3e4*			0.273	0.141	0.264
	e2e3 vs e3e3			0.537	0.087	0.181
	e3e3 vs e3e4			0.104	0.828	0.059

Genotype data were not available for all polymorphisms in all subjects.
Statistical comparison by Analysis of Variance (ANOVA) or Student's t-test.

*P-value<0.05 is statistically significant.

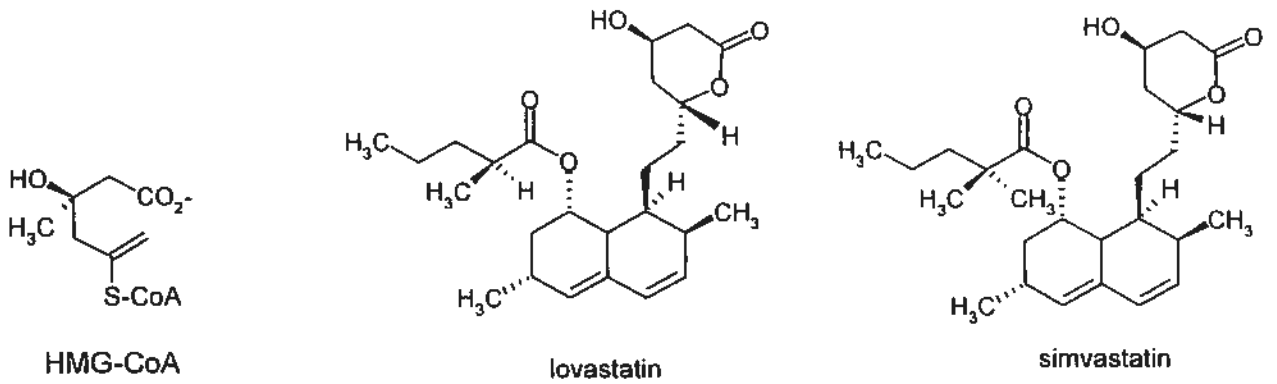
2. Study of the genetic influence on pharmacodynamic lipid lowering responses from candidate single nucleotide polymorphisms encoding CYP3A enzymes in Chinese patients on simvastatin therapy

2.1. Introduction

HMG-CoA reducatase inhibitors or statins are considered as one of the most effective classes of drugs for reducing low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) (NCEP, 2002). Genetic and environmental factors have been attributed to account for differences in LDL-C lowering responses to statin therapy (Hutz and Fiegenbaum, 2008; Mangravite et al., 2006). Interindividual differences in LDL-C reduction have been reported among different ethnic groups (LaRosa, 2000; Puccetti et al., 2007), as well as being confirmed by genomewide association studies (GWAS) (Sandhu et al., 2008).

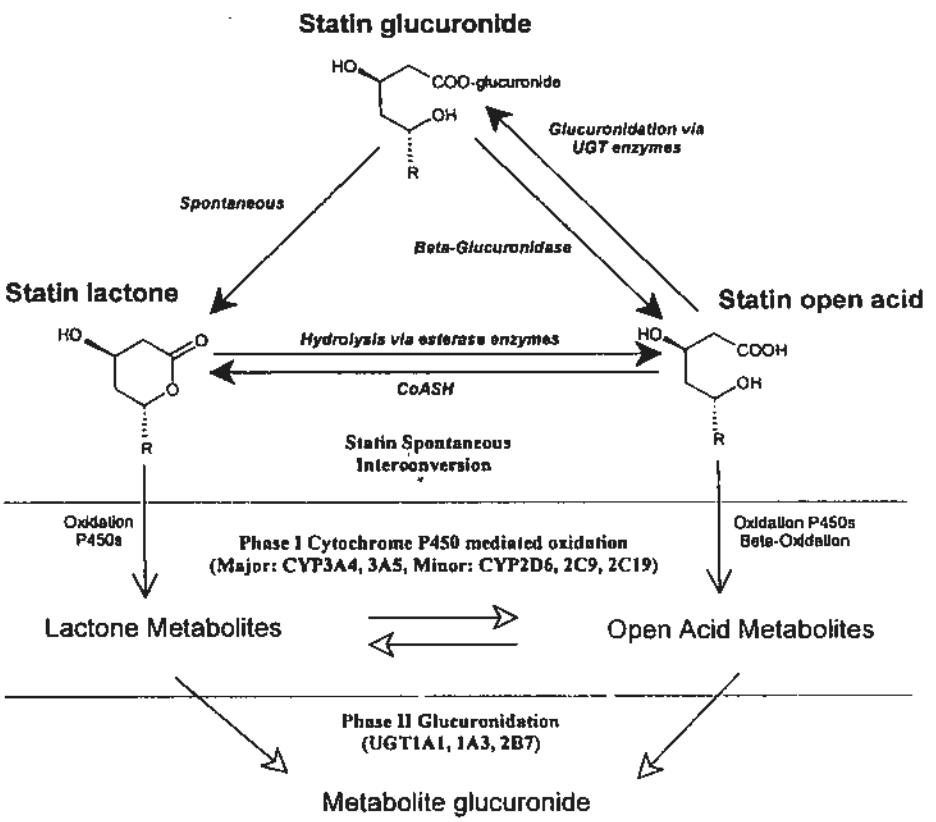
Lovastatin was the first compound introduced in the market. Simvastatin is a widely used statin in Hong Kong and many other countries. Lovastatin is a derivative of fungal products whereas simvastatin is semi-synthetic and structurally very similar and both are structurally related to the substrate of HMG-CoA in one part of the molecule (Figure. 2.1).

Figure 2.1: Chemical structure of HMG-CoA substrate, lovastatin and simvastatin



These two statins are administered as inactive prodrugs in the lactone forms and are transformed by hydrolysis into the biologically active acid form in the body whereas other newer statins are administered in the active open acid forms (Neuvonen et al., 2006; Shitara and Sugiyama, 2006). The systemic bioavailability of simvastatin and lovastatin is very low, usually ≤5%, which is mainly related to their extensive first-pass metabolism mediated by cytochrome P450 3A (CYP3A) enzymes in the intestine and liver (Williams and Feely, 2002). The hepatic CYP system is important to the metabolism of many statins including simvastatin (Mauro, 1993). Polymorphisms in the genes for the enzymes which determine statin disposition may directly influence the level of active metabolites at the intracellular site of action in the hepatocytes for their pharmacodynamic effect and may have a considerable impact towards interindividual clinical responses (Tirona, 2005). A possible model for simvastatin metabolism was shown in Figure. 2.2 (Prueksaritanont et al., 2002).

Figure 2.2: Metabolic pathway of statins (adopted from (Prueksaritanont et al., 2002)



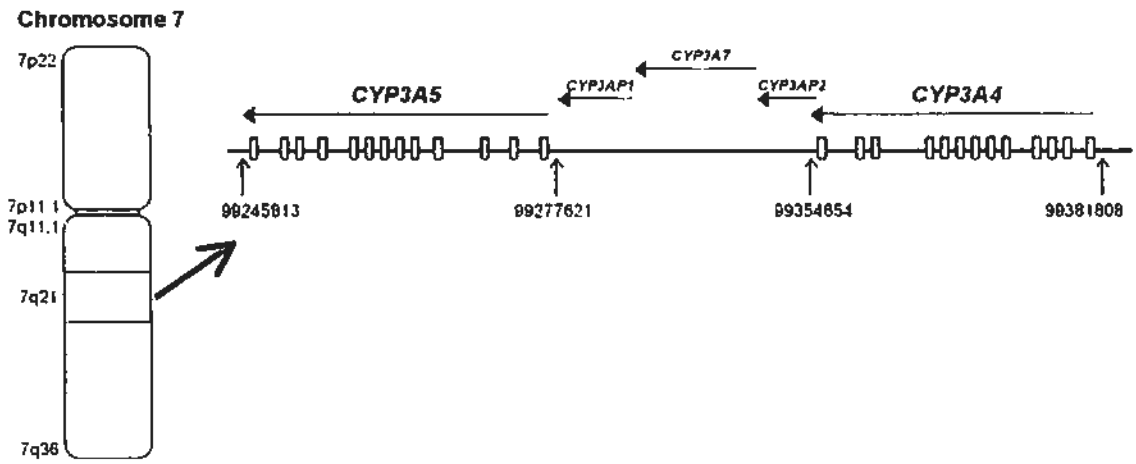
Hepatic metabolism of simvastatin acid is primarily by CYP3A4/5. CYP2D6 and other major CYP isoforms are not thought to be involved to a significant degree in the hepatic metabolism of simvastatin acid (Prueksaritanont et al., 2003; Transon et al., 1996). In fact, the complex interplay of CYP enzymes and cellular transporters further complicates the issues in the disposition of drugs inside the body (Bai, 2010). The variations in race and polymorphisms in the genes encoding CYP3A enzymes would result in pharmacokinetic and pharmacodynamic differences with statins and may eventually lead to variations in their safety, efficacy and clinical responses profiles (Maitland-van der Zee et al., 2000; Neuvonen et al., 2006). This study investigated the relationship of candidate SNPs and the lipid lowering responses after simvastatin treatment.

2.2. Cytochrome P450 3A isoenzymes and genetic influence

2.2.1. CYP3A4 enzyme

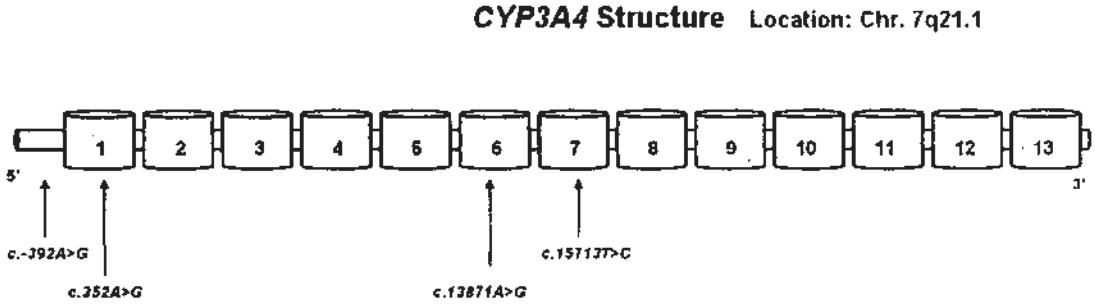
The cytochrome P450 superfamily is a diverse group of enzymes, belonging to the protein group of hemoproteins, and they are responsible for catalysis of the oxidation process within the metabolic pathway. Microsomal cytochrome P450 3A (CYP3A) enzymes, and especially CYP3A4 and CYP3A5, are considered as the most important subfamily of the enzymes, responsible for the metabolism of over 50% of pharmaceutical agents and xenobiotics (de Wildt et al., 1999; Dresser et al., 2000; Li et al., 1995; Shimada et al., 1994). There are marked interindividual variations in the CYP-mediated drug metabolism, which could in part be attributed to genetic influences. A previous study on CYP3A4 enzymes suggested 90% of the interindividual variations were from genetic origins (Ozdemir et al., 2000). Both CYP3A4 and CYP3A5 isoenzymes play important roles in the metabolism of simvastatin and lovastatin, while atorvastatin or fluvastatin were noted to be metabolized only by CYP3A4 but not 3A5 isoenzyme (Fischer et al., 1999; Park et al., 2008). CYP3A4 also showed higher affinity towards simvastatin lactone than CYP3A5 (Prueksaritanont et al., 1997). The *CYP3A4* and *CYP3A5* genes are located in chromosome 7q.21.1 (Figure 2.3). Their sequences show 90% similarity and share common substrate specificity. They are both highly polymorphic with significant ethnic differences in the frequency of the polymorphisms (Figure. 2.4 and 2.5) (Hu et al., 2009a).

Figure 2.3: Gene map of chromosome 7 and *CYP3A4* and *CYP3A5* with base no.



Ref: www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

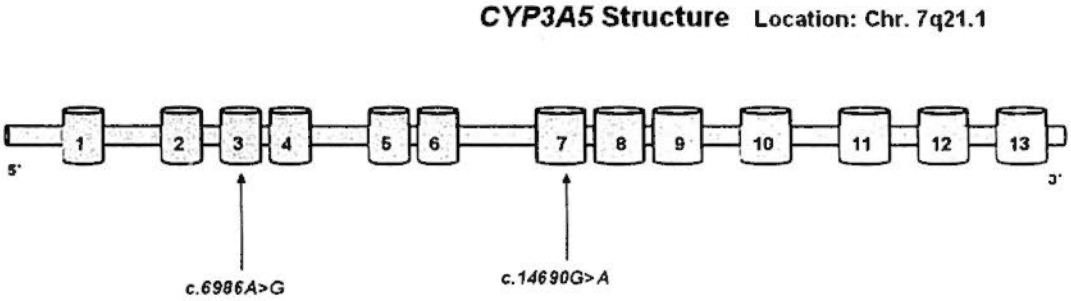
Figure 2.4: Schematic presentation of *CYP3A4* structure with SNPs location



Allele	Enzyme Activity	Frequency distribution		
		Whites	Blacks	Asians
**1B (c.-392A>G)*	Slightly decreased	3.9-4.2%	53-69%	0-4.7%
**2 (c.15713T>C)*	Substrate specific	2.7%	0%	0%
**4 (c.352A>G, c.13871A>G)*	Decreased	-	-	3.3%

Ref: 1. www.cypalleles.ki.se by Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee
2. www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

Figure 2.5: Schematic presentation of *CYP3A5* structure with SNPs location



Allele	Enzyme Activity	Frequency distribution		
		Whites	Blacks	Asians
**1* wild-type	Normal	0-15%	36-45%	23-40%
**3 (c.6986A>G)*	Severely Decreased	85-98%	27-84%	60-77%
**6 (c.14690G>A)*	Decreased	0	8.7%	1.6%

Ref: 1. www.cypalleles.ki.se by Human Cytochrome P450 (CYP) Allele Nomenclature Committee
2. www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

There had been reports of marked inter-individual variability in *CYP3A4* gene expression. However, in inter-ethnic studies, the polymorphisms in *CYP3A4* do not appear to be able to fully explain such variability (Xie et al., 2001). Out of the 28 *CYP3A4* single nucleotide polymorphisms (SNPs) identified, there were no associations between polymorphic changes and low hepatic CYP3A4 protein expression or in vivo enzyme activities (Lamba et al., 2002).

The importance of the CYP3A4 enzyme in the disposition of statins can be exemplified by the influence of specific enzyme inhibitors on the pharmacokinetics and clinical responses of statins. CYP3A4 represents 30-40% of total cytochrome P450 enzymes in liver and small intestine. Comparatively speaking, CYP3A5 is 83% homologous to the CYP3A4 enzyme but contributes a smaller proportion of the total enzyme activity (de Wildt et al., 1999). Simvastatin, lovastatin and atorvastatin were found to have significantly raised plasma concentrations with potent CYP3A4 inhibitors, whereas fluvastatin was less prone to pharmacokinetic interaction because of its pathway being mainly through the CYP2C9 isozyme (Neuvonen, 2010). In fact, individuals with both low hepatic or

gastrointestinal gene expression of CYP3A4 enzyme was suggested to have increased risk of myotoxicity due to potentially higher plasma statin concentrations (Ucar et al., 2000). However, an even stronger link with genetic variation in the gene encoding uptake transporter SLCO1B1 has been established in a GWAS (Link et al., 2008). In addition to these polymorphisms at selected loci, a literature review also suggested pre-existing conditions causing subclinical inherited muscle weakness may also contribute to susceptibility to myopathy (Ghatak et al., 2010).

Strong inhibitors of CYP3A enzymes like itraconazole could lead to increases of up to 20-fold in the plasma concentrations and area under curve (AUC) profiles of simvastatin, lovastatin and their active acid forms, thus enhancing systemic exposure of these statins and the risk of myotoxicity (Neuvonen et al., 2008), whereas concomitant treatment with itraconazole increased the AUC for both pravastatin and total circulating HMG Co-A reductase activity by only 1.7-fold, probably by inhibiting the P-glycoprotein transporter (Neuvonen et al., 1998). Concomitant use of the weak CYP3A4 inhibitor verapamil increased the AUC of simvastatin lactone and the acid form by approximately four-fold, and the maximum plasma concentrations (Cmax) by five- and three-fold respectively. Atorvastatin was noted to have a four-fold increase in these parameters when coadministered with mibefradil, and a 2.4-fold increase in Cmax and 47% increase in AUC when coadministered with itraconazole. Clarithromycin was also found to increase the AUC by 885% with simvastatin, 1,092% with simvastatin acid, 343% with atorvastatin and 110% with pravastatin (all $P<0.001$) (Jacobson, 2004). As pravastatin is not metabolised to a significant extent by the CYP enzymes, it is likely that the effects of clarithromycin on this statin and part of the effects on the other statins are related to inhibition of membrane transporters with clarithromycin.

Ingestion over a 3-day period of grapefruit juice, a CYP3A inhibitor probably owing to its furanocoumarin content, was found to be associated with 12 to15-fold increase in Cmax and 15-fold increase in AUC of lovastatin and its acid, probably

(mean values **1/*1* vs **1/*4*: -22.0% vs. -35.8%, P = 0.0015) and triglycerides (-25.1% vs. -38.1%, P = 0.034) but not for LDL-C (-36.8% vs. -29.0%, P = 0.0721). The *CYP3A4*4* allele was found to be associated with a lower urinary concentration ratio of 6β-hydroxycortisol/cortisol (56.6 vs 9.9; P=0.0039), which is a useful non-invasive assay for CYP3A enzyme activity. The authors suggested reduced CYP3A4 enzyme activity may be related to greater lipid lowering effects from simvastatin (Wang et al., 2005a). Another study with 423 Chinese hyperlipidaemic patients receiving atorvastatin therapy for 4-weeks, found the allele frequency for *CYP3A4*1G* to be 27.6%. Patients with the polymorphism had higher mean percentage reduction of plasma TC (**1G/*1G*:**1/*1G*:**1/*1*; 20.9%:17.8%:16.8%, respectively; P = 0.001) and were suggested to have a gene-dose-dependent effect. However, in the same study, no association with lipid lowering effects of simvastatin was found with the *CYP3A4*1G* polymorphism (Gao et al., 2008).

2.2.2. CYP3A5 enzyme

The carriers of the *CYP3A5*1* allele are the wild-type with full expression of the gene. *CYP3A5*3* is the variant SNP *c.6986G>A* within intron 3 resulting in alternative splicing and absence of gene expression (Hustert et al., 2001). This allele will lead to reduced enzyme expression and was noted to have a high frequency of about 76% among the Chinese population, 77% in Japanese and 85% in Caucasians (Balram et al., 2003). Consequently, this polymorphism was suggested to contribute substantially to inter-individual variability in pharmacokinetics and pharmacodynamics of cholesterol lowering therapy (Schmitz and Langmann, 2006). In a single-dose pharmacokinetic study with simvastatin 20 mg, the mean AUC for simvastatin in homozygous *CYP3A5*1/*1* carriers was significantly lower than *CYP3A5*3/*3* carriers (4.94 vs. 16.35 ng·/mL; P<0.05). The mean oral clearance was also significantly higher with homozygous *CYP3A5*1/*1* carriers (4.80 vs. 1.35 L/h; P<0.05) (Kim et al., 2007).

Statins that undergo CYP3A5 metabolism, including lovastatin, simvastatin and atorvastatin, were suggested to be less effective in *CYP3A5* expressors than in

due to the inhibitory effect on the intestinal CYP3A4 enzyme (Kantola et al., 1998). These interaction studies demonstrated the importance of the CYP3A4 isoenzyme in the metabolic pathway of respective statins, and hence the effects of corresponding inhibitors towards increased systemic exposure.

In a recent study in patients undergoing treatment with stable doses of atorvastatin, simvastatin, or lovastatin, an intronic polymorphism in *CYP3A4* affecting the expression of the enzyme was found to result in 40-80% reduction of statin doses among carriers of the mutated allele. These results suggested the role of this *CYP3A4* polymorphism and the subsequent enzyme expression in the clinical response (Wang et al., 2010). In a population-based cohort study of 1380 Caucasians on simvastatin or atorvastatin followed up for average of 5.3 years, the polymorphism *CYP3A4*1B* (*c.-392A>G*) was noted to be associated with two times lower risk of dose decrease or switching to another cholesterol lowering drug, which was interpreted by the authors to be due to less likelihood of adverse drug reaction from elevated plasma statin levels (Becker et al., 2010). In another study among 340 hyperlipidaemic patients, individuals homozygous for *CYP3A4*1B* alleles had significantly higher post-treatment LDL-C levels after atorvastatin 10 mg daily, but no difference in their respective absolute or percentage changes (Kajinami et al., 2004a). This polymorphism has been suggested to lead to 30% lower activity between the homozygous mutant compared to the wild-type individuals as measured by midazolam clearance (Wandel et al., 2000). However, a Brazilian study in 116 patients on simvastatin 20 mg daily treatment followed up to 6-months did not find a significant association between the *CYP3A4*1B* polymorphism and the efficacy or tolerance (Fiegenbaum et al., 2005a). Regarding another polymorphism *CYP3A4*4* (Ile118Val), the frequency of the mutant allele was only 3.32% in a trial with 211 Chinese hyperlipidaemic patients . In this trial, the lipid lowering effects from simvastatin were compared between a selected group of **1/*4* individuals with an equal number of wild-type **1/*1* patients. It was suggested that lipid lowering effects from treatment with simvastatin 20 mg daily for 4-weeks were greater in the group with the variant allele than the wild-type individuals for plasma TC

non-expressors, who were reported having 23% and 24% higher plasma TC and LDL-C levels after one-year statin therapy. The mean percentage reduction in serum TC from baseline was smaller in *CYP3A5* expressors of the wild-type *CYP3A5*1* allele, than in non-expressors with the *CYP3A5*3* allele (17% versus 31%, P = 0.026). However, those individuals under treatment with statins that may not undergo CYP3A5 metabolism, e.g. fluvastatin and pravastatin, did not have noted differences in hypolipidaemic efficacy (Kivisto et al., 2004). A study in 1902 subjects having 6-weeks therapy with atorvastatin 10 mg daily, found that those with *CYP3A5*1* or **3* alleles did not show significant differences in their clinical response (Thompson et al., 2005). A similar observation was noted among 116 Brazilian subjects after 6-months simvastatin 20 mg daily therapy. There was no significant association between tolerance or efficacy and *CYP3A5*1* or **3* alleles (Fiegenbaum et al., 2005a). In another clinical study of 601 patients presenting with acute myocardial infarction, after treatment with either rosuvastatin or simvastatin, carriers of at least 1 variant *CYP3A5*1* and/or *ABCG2 c.421C>A* allele were more likely to achieve the LDL-C target (odds ratio: 2.289; P = 0.017), suggesting enhanced lipid lowering responses to the statin therapy in these genotypes (Bailey et al., 2010). However, in a study comparing 15 healthy subjects and 14 patients experiencing atorvastatin-induced myopathy, the differences in systemic exposure to atorvastatin metabolites were not related to *CYP3A5* genotypes (Hermann et al., 2006). In another study, 68 patients developing atorvastatin-induced muscle damage had a similar proportion of *CYP3A5*3* allele carriers compared to the group of 69 control subjects. However, the same study also noted an association of elevated serum creatine kinase levels with the non-functional *CYP3A5*3* allele (Wilke et al., 2005). The relationship of *CYP3A5* genotypes, pharmacokinetic parameters, and clinical responses would appear to need further evaluation.

2.2.3. *CYP3AP1* pseudogene and CYP3A5 enzyme

CYP3AP1 is a pseudogene found among the *CYP3A* gene cluster. Pseudogenes are usually not functional, but can be important in gene conversion and recombination of a nearby gene, hence they are valuable points of reference in the genome

(Nelson et al., 2004). The allele *CYP3AP1*3* at *c.–44G>A* was found to have a complete concordance with the *CYP3A5*3* defective polymorphism, whereas the wild-type *CYP3AP1*1* is closely linked to *CYP3A5*1* (Kuehl et al., 2001) with strong *CYP3A5* expression (Finta and Zaphiropoulos, 2000) It also was suggested to correlate with *CYP3A4*1B*, even though the significance in alteration in enzyme activities is not clear (Eiselt et al., 2001; Garcia-Martin et al., 2002). A Chinese demographic study among 110 subjects suggested the mutant allele frequency was 72% (Chou et al., 2001).

In a 4-week study among 202 Chinese subjects on simvastatin 20 mg daily therapy, *CYP3AP1*3/*3* subjects were found to have greater LDL-C percentage reduction than the homozygous wild-type carriers (-28.6% vs. -25.5%), and this was of statistical significance in the female subgroup (P = 0.010). There were no significant findings in the LDL-C change in the same study with atorvastatin treatment. The mutant frequency in this study was 81%, in-line with expectations for a Chinese population (Li et al., 2011).

2.2.4. Phenotyping of CYP3A activity

CYP3A enzyme activities could be evaluated by phenotypic expressions of a substrate-metabolite ratio. CYP3A enzymes are specifically responsible for the unconjugated 6 β -hydroxylation of cortisol, and both cortisol and 6 β -hydroxycortisol are excreted in the urine (Yin et al., 2004). The concentration ratio of 6 β -hydroxycortisol (6 β -OHC) to cortisol (C) in urine has been extensively used as a non-invasive index (Chen et al., 2004). Reports among various applications including evaluation of the effect of the menopause or menstrual cycle-related changes in CYP3A4 activities (Burstein et al., 1998), effects of liver cirrhosis on hepatic CYP3A activities (Shibuya et al., 2003), in vivo confirmation of inhibitory effects of thyroid hormones on CYP3A enzyme expression (Takahashi et al., 2010), studies on induction of CYP3A4 activity by St John's Wort (Roby et al., 2000) or oxcarbazepine (Hogler et al., 2010), inhibition of CYP3A activity by clarithromycin (Furuta et al., 2003) or grapefruit juice (Seidegard et al., 1998), establishment of bioequivalence between two

itraconazole products (Estevez-Carrizo et al., 2005) and for studies of various inhibitory or inducing activities with CYP3A enzymes (Eeckhoudt et al., 2001; Galteau and Shamsa, 2003; Keung et al., 1999; Saruwatari et al., 2003; Ushiama et al., 2002). The procedure had been considered simple as it involved only urine collection (El Desoky et al., 2005; Rais et al., 2006). However, the circadian variation must be noted in finalizing the protocol to avoid false results (Ohno et al., 2000). There were also suggestions that the ratio could be significantly higher with female than male gender, and the intraindividual variations could be as high as 20-fold (Lutz et al., 2010). A previous study with a 20 mg daily dose has suggested simvastatin to be an inducer resulting in a mild increase of (+24%) the 6β-OHC/C ratio after 17-days of treatment (P = 0.0125), while there was no similar observation with pravastatin (Horsmans et al., 1993). A recent publication evaluating the interaction effect of genetic polymorphisms after single intake of grapefruit juice among Chinese populations suggested that individuals with *CYP3A5*3/*3* and *ABCB1 TTT/TTT* haplotypes had 2.4 times the reduction of the urinary 6β-OHC/C ratio compared to those with *CYP3A5*1/*3* and *ABCB1 CGC/CGC* haplotypes (P<0.01) (Li et al., 2010a). In the study on lipid lowering response of *CYP3A4*4* subjects with reduced enzyme activities, it was noted there was a significantly lower urinary 6β-OHC/C ratio in subjects with heterozygous **1/*4* minor alleles compared to homozygous wild-type **1/*1* subjects (9.9 vs. 56.6; P= 0.0039) (Wang et al., 2005a).

The objective of our study is to elucidate the influence of CYP3A gene polymorphisms among Chinese population, their influence on the urinary 6β-OHC/C ratio, and subsequently to review their relationship on their respective LDL-C lowering responses after simvastatin 40 mg daily treatment.

2.3. Patients and Methods

2.3.1. Study population and design

The recruited subjects were aged over 18 years, all of Han Chinese origin with no known ancestors of other ethnic origin. Their baseline LDL-C > 2.6 mmol/L, considered at increased cardiovascular disease (CVD) risk because of the presence of established atherosclerotic disease, having diabetes or known to have familial hypercholesterolaemia (FH). Study subjects were recruited from those attending the Outpatient Clinics (mainly the Lipid Clinic) at the Prince of Wales Hospital who were suitable for treatment with simvastatin 40mg and who consented to take part in the study. Consecutive suitable patients were invited to participate. Baseline demographic characteristics were recorded before treatment was initiated with simvastatin 40 mg daily. Patients taking gemfibrozil or diltiazem or other strong inhibitors of CYP3A enzymes were not included in the study, in order to avoid any significant pharmacokinetic or pharmacodynamic interaction with simvastatin

All patients were advised to continue with their usual diet and lifestyle activities during the study. It was intended that they should not change their lifestype during the study treatment period as this would influence the lipid response. Education on the reasons for taking lipid lowering treatment and importance of maintaining treatment was given to improve drug compliance. Participants were interviewed and counseled by one of the investigators or a trained research nurses at the start and at a scheduled follow-up visit after at least 4 weeks treatment with simvastatin. Adherence to therapy was assessed by asking patients about their medication-taking behaviour in a non-judgmental manner and tablet counting and subjects with poor adherence (reported taking <80% or >120% of the prescribed number of tablets) would be excluded.

Fasting blood samples were collected for the measurement of lipid profiles and laboratory safety data including serum alanine aminotransferase (ALT), CK, and

creatinine. LDL-C concentrations were calculated according to the Friedewald formula or directly measured if triglycerides level was greater than 4.5 mmol/L. All biochemistry tests were performed by standard methods in the Chemical Pathology laboratory at the Prince of Wales Hospital, which has international laboratory accreditation. Safety was assessed in all participants by recording abnormal laboratory data and adverse events.

This pharmacogenetic study was approved by the local Clinical Research Ethics Committee. The study was performed in accordance with the Declaration of Helsinki ICH GCP guidelines.

2.3.2. SNP selection and genotyping

Candidate genes and single nucleotide polymorphisms (SNP) on *CYP3A5*3 (c.6986A>G)*, *CYP3A4*1G (c.20230G>A)* and *CYP3AP1*3 (c.-44G>A)* were genotyped. In brief, DNA was extracted from subject's plasma samples and genotyping was performed in the Genome Research Centre, University of Hong Kong, using the mass-spectroscopy based, high-throughput MassARRAY iPLEXTM platform (Sequenom, San Diego, CA). All SNPs were checked for compliance with Hardy-Weinberg equilibrium ($P>0.05$).

2.3.3. Phenotyping method

The procedure for phenotyping of CYP3A activity was based on the non-invasive method of urine sample collection and analysis by an ultra performance liquid chromatography (UPLC) method of urinary concentration of 6β-hydroxycortisol and cortisol developed by Xiao Yajie as part of her Ph.D. studies, which is more practical than invasive methods for the patients studied (Xiao et al., 2011). This involved a solid phase extraction procedure applied to urine samples modified from one previously described (Homma et al., 2000). To each 2 mL urine sample the internal standard (IS) dexamethasone (10 ng/mL, 20 μL) was added and the sample was alkalinized by adding 200 μL of 1 M NaOH. The mixture was vortex-mixed and loaded onto the Oasis HLB cartridge, which had been pretreated with 1 mL of methanol followed by 1 mL of distilled water. The cartridges were

subsequently washed with 3 mL of water followed by 3 mL of hexane. Cortisol, cortisone, 6β-hydroxy cortisol and dexamethasone were subsequently eluted with 5 mL of ethyl acetate and the effluents were dried by a speedvac and reconstituted in 100 μL of 10% acetonitrile.

The analytes were separated using the Acquity UPLC System from Waters Corporation (Milford, MA, USA) which consisted of Binary Solvent Manager, Sample Manager, and Tunable UV Detector. The separation was performed at 40 °C using the Acquity UPLC BEH C18 column from Waters (1.7μm, 50mm × 2.1mm i.d.), protected by a 0.2μm stainless steel filter inside an in-line filter holder and a Waters Van GuardTM Pre-column (1.7μm, 5mm × 2.1mm i.d.). The mobile phase consisted of (A) water (containing 0.01% formic acid) and (B) methanol. A gradient elution program was applied as follows: 0–0.5min 0% B: 0.5–2.5 min linear increased from 0–40% B; 2.5–5.0 min 40% B; 5.0–6.5 min linear increased from 40–60% B; 6.51 min decreased from 60–0% B; the composition was held at 0% B for a further 0.5 min for re-equilibration, giving a total run time of 7 min. The retention time of the last eluted analyte was 6.1 min. UV detection was performed at 245nm. The flow rate was 0.5 mL/min and the injection volume was 20μL.

The stock standard solutions of 1000 ng/mL for each standard were prepared in pure methanol and these were diluted to the working concentrations of the mixture of standard solutions daily with pure methanol. Calibration standards were prepared by spiking active carbon-treated human urine with the working standard solutions. Calibration curves of 8 concentrations of cortisol from 5 to 200 ng/mL, cortisone and and 6β-hydroxy cortisol from 10 to 1000 ng/mL were extracted and assayed. In all cases the internal standard was added (10 ng/mL, 20 μL) prior to the extraction procedure. Peak areas of cortisol, cortisone, 6β-hydroxy-cortisol and internal standard were measured. The calibration graphs were obtained by an unweighted least-squares linear fitting of the peak area ratios of glucocorticoids to the internal standard versus the concentration of cortisol, cortisone and 6β-hydroxy-cortisol on each analysis of the standard mixtures. The limit of

detection (LOD) was calculated by interpolating the value obtained from multiplying 3 times the signal-to-noise ratio in the calibration curve.

Quality control (QC) samples used for recovery assay were prepared at a low (7.5 ng/mL for cortisol, 15 ng/mL for both cortisone and 6β-hydroxy-cortisol), a medium (75 ng/mL for cortisol, 300 ng/mL for both cortisone and 6β-hydroxy-cortisol), and a high (150 ng/mL for cortisol, 850 ng/mL for both cortisone and 6β-hydroxy-cortisol) concentrations by spiking the normal human urine with the appropriate working standard solution. Five samples of each concentration were prepared and these were then carried through the sample preparation procedure described above. Recovery (%) was calculated by comparing the peak area ratios of glucocorticoids to the internal standard in spiked urine samples with those of the controls. For intra- and inter-day accuracy and precision assays, five QC samples were prepared at the three above concentrations by spiking the active carbon-treated human urine with the appropriate working standard solution. Intra-day precision and accuracy were evaluated on the same day, whereas inter-day precision and accuracy was evaluated on three separated days. Precision was calculated in terms of relative standard deviation % (RSD%).

The retention times of 6β-hydroxycortisol, cortisone, cortisol and dexamethasone were 2.3, 4.2, 4.6 and 6.1 min, respectively. No interfering peak was found with the three analytes or the internal standard. A good correlation was found between the observed peak area ratios (A) and the theoretical concentration (C). Unweighted least-squares regression analysis gave typical regression lines: C = 0.0105A − 0.073 (r^2 = 0.9997) for cortisol, C = 0.0072A − 0.035 (r^2 = 0.9999) for cortisone and C = 0.0057A − 0.0165 (r^2 = 0.9997) for 6β-hydroxycortisol. The LOD was 3 ng/mL, for cortisol, 5 ng/mL, and 5 ng/mL for cortisone and 6β-hydroxycortisol, respectively. The general recoveries and accuracies of the three analytes determined at low, medium and high concentrations were all above 90%. The method showed a good overall intra-day (7.25%) and inter-day (8.75%) variation, with an accuracy >90%.

2.3.4. Statistical analysis

Study variables were presented as mean±standard deviation, or geometric means and 95% confidence intervals (CI) for skewed variables and n (%) for categorical variables.

The percentage reduction in plasma total cholesterol (TC), low-density lipoprotein (LDL-C), and triglycerides and the increase in high-density lipoprotein (HDL-C) were assessable endpoints. Since the primary outcome was LDL-C reduction upon treatment with simvastatin, the comparison was mainly performed on this parameter.

Individual demographic and phenotypic characteristics were compared with LDL-C percentage change by univariate analysis of variance. Significant findings were then mapped on impact analysis, by linear regression for scale variables, or Student's t-test on binominal variables.

Population characteristics, baseline and after treatment lipid profiles of groups of patients divided according to each individual candidate SNP were evaluated by analysis of variance (ANOVA) for continuous variables, or Chi-Square tests for categorical variables. Linkage disequilibrium between candidate SNPs was tested by Chi-Square tests and evaluated by Spearman correlation coefficients. Genetic influence on lipid responses in the whole population was analysed by ANOVA for each SNP, or by Student's t-test of either dominant or recessive genotype models based on population size among genotype groups. Sub-group analyses on lipid changes among genotypes were performed by non-parametric Mann-Whitney test or Kruskal-Wallis test because of the small population size. Non-parametric bivariate correlation analyses were performed between plasma lipid response with individual SNPs to obtain the Spearman correlation factors to identify the effect size.

The sample size was estimated based on previous similar studies. A clinically

significant effect of a genetic variation might be predicted to alter the LDL-C reduction by about 7%, which would be equivalent to doubling or halving the dose of the statin.

The analysis of urinary 6β-hydroxycortisol/cortisol (6β-OHC/C) concentration ratio was first investigated on the population distribution. Because of the skewed distribution, the 6β-OHC/C ratio was natural logarithmically transformed to fit for a normal distribution pattern. Statistical analysis was based on Ln(6β-OHC/C) ratio.

All statistical analyses were conducted using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). $P<0.05$ was reported as statistically significant.

2.4. Results

2.4.1. Baseline characteristics of participants

A total of 273 eligible patients with good adherence to simvastatin treatment were included in the study. Their baseline demographic and phenotypic characteristics were listed in Table 2.1. The mean (±SD) age was 55.6±11.0 years and 41.8% were male (n = 114). Familial hypercholesetrolaemia (FH) was diagnosed in 145 subjects (53.1%), hypertension in 130 subjects (47.6%), history of cardiovascular disease in 38 subjects (13.9%) and diabetes mellitus in 56 subjects (20.5%). Comparison between genders suggested male subjects were on average taller (+8.4%), had greater mean body weight (+22.2%), waist circumference (+8.8%), waist/hip ratio (+5.8%), but less in body fat % (-27.6%), and these were all of statistical significance (P<0.001). The male subjects also tended to include more drinkers (15.8% vs. 1.3%; P<0.001) and smokers (24.6% vs. 1.9%; P<0.001). Their baseline lipid profiles were not of statistically significant difference, except that the mean HDL-C level was higher with the female populations (1.61 mmol/L vs. 1.37 mmol/L; P<0.001).

Except for the frequency of patients with a history of cardiovascular disease (P<0.05), there were no significant differences of baseline demographic characteristics or in the lipid profiles before and after treatment among genotype groups for each of three candidate SNPs *CYP3A4*1G* (Table 2.2), *CYP3A5*3* (Table 2.3) and *CYP3AP1*3* (Table 2.4). Their mutant frequencies were 26.6%, 73.3% and 72.8% respectively, and were tested for compliance with Hardy-Weinberg equilibrium.

Table 2.1: Demographic, phenotypic characteristics and baseline lipid profiles of the study population (n = 273)

	All Subjects (n=273)	Male Subjects (n=114)	Female Subjects (n=159)	
Demographic Characteristics	**Mean±SD**			**P-value**
Age (years)	55.6±11.0	54.8±10.9	56.2±11.1	0.300
Body weight (kg)	64.5±13.0	72.2±12.0	59.1±10.8	<0.001*
Body height (m)	1.60±0.08	1.67±0.06	1.54±0.06	<0.001*
Body mass index (kg/m^2)	25.1±4.1	25.7±3.7	24.7±4.3	0.072
Waist circumference (cm)	85.8±11.4	90.1±10.5	82.8±11.0	<0.001*
Hip circumference (cm)	97.4±7.7	98.5±6.6	96.7±8.3	0.069
Waist/hip ratio	0.88±0.07	0.91±0.06	0.86±0.08	<0.001*
Body fat (%)	29.8±8.1	24.7±5.2	34.1±7 5	<0.001*
Phenotypic Characteristics	**n (%)**			**P-value**
Current drinker	20 (7.3)	18 (15.8)	2 (1.3)	<0.001*
Current smoker	31 (11.4)	28 (24.6)	3 (1.9)	<0.001*
FH	145 (53.1)	55 (48.2)	90 (56.6)	0.172
Hypertension	130 (47.6)	57 (50.0)	73 (45.9)	0.505
History of CVD	38 (13.9)	21 (18.4)	17 (10.7)	0.069
Diabetes mellitus	56 (20.5)	28 (24.6)	28 (17.6)	0.161
History of RA	18 (6.6)	4 (3.5)	14 (8.8)	0.082
Baseline lipid profiles (mmol/L)	**Mean±SD**			**P-value**
Total Cholesterol	7.71±1.70	7.47±1.45	7.89±1.84	0.043
LDL-C	5.38±1.68	5.24±1.45	5.49±1.82	0.205
HDL-C	1.51±0.39	1.37±0.32	1.61±0.40	<0.001
Triglycerides	1.95±1.47	2.03±1.35	1.90±1.54	0.454

Statistical tests by Student's t-test or Chi-square tests between male and female subjects.
*P-value<0.05 is statistically significant.
FH, familial hypercholesterolaemia; CVD, cardiovascular disease; RA, rheumatoid arthritis;
HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2.2: Characteristics and baseline lipid profiles of patients stratified by *CYP3A4*1G*

	All Genotypes (n=271)	**1/*1* (n=145)	**1/*1G* (n=108)	**1G/*1G* (n=18)	P-value
Characteristics					
Age (years)	55.6±11.0	55.4±11.5	55.6±10.9	58.2±6.2	0.598
Male, n (%)	113 (41.7)	58 (40.0)	50 (46.3)	5 (27.8)	0.280
Body weight (kg)	64.5±13.0	64.5±12.7	64.6±13.8	62.8±11.2	0.852
Body height (m)	1.60±0.08	1.60±0.08	1.60±0.09	1.57±0.08	0.293
Body mass index (kg/m^2)	25.1±4.1	25.1±3.9	25.1±4.3	25.6±4.4	0.875
Waist circumference (cm)	85.8±11.4	85.7±10.6	85.3±12.3	86.2±10.9	0.830
Hip circumference (cm)	97.5±7.7	97.4±7.7	97.2±8.5	97.7±7.1	0.881
Waist/hip ratio	0.88±0.07	0.88±0.07	0.88±0.07	0.88±0.08	0.887
Body fat (%)	29.8±8.1	29.5±7.9	29.8±7.9	31.8±10.3	0.625
Current drinker, n (%)	20 (7.4)	10 (6.9)	9 (8.3)	1 (5.6)	0.869
Current smoker, n (%)	31 (11.4)	15 (10.3)	13 (12.0)	3 (16.7)	0.706
FH, n (%)	145 (53.5)	78 (53.8)	60 (55.6)	7 (38.9)	0.420
Hypertension, n (%)	129 (47.6)	75 (51.7)	48 (44.4)	6 (33.3)	0.236
History of CVD, n (%)	38 (14.0)	17 (11.7)	15 (13.9)	6 (33.3)	0.045*
Diabetes mellitus, n (%)	56 (20.7)	32 (22.1)	22 (20.4)	2 (11.1)	0.554
History of RA, n (%)	18 (6.6)	7 (4.8)	9 (8.3)	2 (11.1)	0.397
Urinary ratio of 6β-hydroxycortisol/ cortisol, (geometric mean, 95% CI)	3.92 (3.76, 4.10) (n=233)	3.86 (3.65, 4.09) (n=129)	4.05 (3.76, 4.35) (n=89)	3.74 (3.15, 4.42) (n=15)	0.507¶
Baseline lipid levels (mmol/l)					
Total Cholesterol	7.72±1.71	7.81±1.77	7.67±1.59	7.30±1.86	0.449
LDL-C	5.39±1.68	5.50±1.76	5.31±1.58	5.02±1.67	0.432
HDL-C	1.51±0.38	1.51±0.38	1.51±0.36	1.58±0.54	0.728
Triglycerides	1.95±1.47	2.01±1.68	1.91±1.19	1.79±1.12	0.771
Lipid levels on treatment (mmol/l)					
Total Cholesterol	4.91±1.00	5.00±1.00	4.85±1.04	4.58±0.74	0.162
LDL-C	2.81±0.99	2.87±1.07	2.78±0.91	2.51±0.74	0.330
HDL-C	1.47±0.37	1.49±0.38	1.44±0.35	1.44±0.38	0.551
Triglycerides	1.45±0.79	1.49±0.79	1.40±0.81	1.38±0.65	0.614

Data are given as mean ± SD or n (%) except for urinary ratio of 6β-hydroxycortisol/cortisol as geometric means and 95% CI. Statistical comparison among by ANOVA for continuous variable, or Chi-Square test for categorical variables, except ¶ ANOVA comparison on natural logarithmic values. *P-value<0.05 is statistically significant.

Table 2.3: Characteristics and baseline lipid profiles of patients stratified by *CYP3A5*3*

	All Genotypes (n=273)	**1/*1* (n=18)	**1/*3* (n=110)	**3/*3* (n=145)	P-value
Characteristics					
Age (years)	55.6±11.0	56.8±10.2	56.2±10.8	55.1±11.3	0.661
Male, n (%)	114 (41.8)	8 (44.4)	48 (43.6)	58 (40.0)	0.820
Body weight (kg)	64.5±13.0	61.0±10.1	64.7±14.6	64.7±11.9	0.530
Body height (m)	1.60±0.08	1.60±0.08	1.59±0.09	1.60±0.08	0.904
Body mass index (kg/m^2)	25.1±4.1	23.9±3.3	25.3±4.3	25.1±4.0	0.400
Waist circumference (cm)	85.8±11.4	82.6±9.8	85.6±11.8	86.3±11.2	0.487
Hip circumference (cm)	97.4±7.7	95.3±5.7	97.3±8.1	97.8±7.6	0.436
Waist/hip ratio	0.88±0.07	0.87±0.07	0.88±0.07	0.88±0.08	0.804
Body fat (%)	29.8±8.1	28.6±7.1	30.0±7.9	29.8±8.3	0.861
Current drinker, n (%)	20 (7.3)	3 (16.7)	5 (4.5)	12 (8.3)	0.153
Current smoker, n (%)	31 (11.4)	3 (16.7)	14 (12.7)	14 (9.7)	0.569
FH, n (%)	145 (53.1)	9 (50.0)	56 (50.9)	80 (55.2)	0.767
Hypertension, n (%)	130 (47.6)	6 (33.3)	50 (45.5)	74 (51.0)	0.308
History of CVD, n (%)	38 (13.9)	5 (27.8)	21 (19.1)	12 (8.3)	0.010*
Diabetes mellitus, n (%)	56 (20.5)	3 (16.7)	16 (14.5)	37 (25.5)	0.091
History of RA, n (%)	18 (6.6)	2 (11.1)	10 (9.1)	6 (4.1)	0.209
Urinary ratio of 6β-hydroxycortisol/ cortisol, (geometric mean, 95% CI)	3.93 (3.77, 4.10) (n=234)	3.64 (3.07, 4.31) (n=13)	4.04 (3.78, 4.31) (n=96)	3.89 (3.66, 4.12) (n=125)	0.478¶
Baseline lipid levels (mmol/l)					
Total Cholesterol	7.71±1.70	7.37±1.60	7.66±1.71	7.80±1.71	0.551
LDL-C	5.38±1.68	5.12±1.47	5.34±1.67	5.45±1.71	0.679
HDL-C	1.51±0.39	1.54±0.50	1.54±0.39	1.49±0.37	0.483
Triglycerides	1.95±1.47	1.81±1.25	1.90±1.39	2.01±1.55	0.756
Lipid levels on treatment (mmol/l)					
Total Cholesterol	4.91±1.00	4.51±0.77	4.85±1.05	5.01±0.98	0.102
LDL-C	2.81±0.99	2.51±0.73	2.75±0.92	2.89±1.06	0.223
HDL-C	1.47±0.37	1.44±0.32	1.48±0.40	1.47±0.35	0.847
Triglycerides	1.44±0.79	1.30±0.55	1.36±0.83	1.53±0.78	0.164

Data are given as mean ± SD or n (%), except for urinary ratio of 6β-hydroxycortisol/cortisol as geometric means and 95% CI. Statistical comparison among by ANOVA for continuous variable, or Chi-Square test for categorical variables, except ¶ ANOVA comparison on natural logarithmic values. *P-value<0.05 is statistically significant.

Table 2.4: Characteristics and baseline lipid profiles of patients stratified by *CYP3AP1*3*

	All Genotypes (n=267)	**1/*1* (n=20)	**1/*3* (n=105)	**3/*3* (n=142)	P-value
Characteristics					
Age (years)	55.5±11.0	56.8±9.8	56.0±10.8	55.0±11.3	0.681
Male, n (%)	113 (42.3)	9 (45.0)	45 (42.9)	59 (41.5)	0.948
Body weight (kg)	64.6±13.0	61.2±10.2	64.7±14.8	65.0±11.9	0.485
Body height (m)	1.60±0.08	1.59±0.08	1.59±0.09	1.60±0.08	0.744
Body mass index (kg/m^2)	25.1±4.1	24.1±3.2	25.3±4.3	25.1±4.0	0.474
Waist circumference (cm)	85.9±11.4	83.5±9.7	85.6±12.0	86.5±11.2	0.511
Hip circumference (cm)	97.5±7.7	95.5±5.9	97.2±8.1	98.0±7.5	0.368
Waist/hip ratio	0.88±0.07	0.87±0.07	0.88±0.07	0.88±0.08	0.895
Body fat (%)	29.8±8.1	28.9±6.8	29.8±7.8	29.8±8.5	0.921
Current drinker, n (%)	20 (7.5)	3 (15.0)	5 (4.8)	12 (8.5)	0.229
Current smoker, n (%)	31 (11.6)	3 (15.0)	14 (13.3)	14 (9.9)	0.621
FH, n (%)	143 (53.6)	9 (45.0)	54 (51.4)	80 (56.3)	0.543
Hypertension, n (%)	125 (46.8)	8 (40.0)	48 (43.8)	71 (50.0)	0.514
History of CVD, n (%)	37 (13.9)	7 (35.0)	17 (16.2)	13 (9.2)	<0.010*
Diabetes mellitus, n (%)	54 (20.2)	4 (20.0)	15 (14.3)	35 (24.6)	0.134
History of RA, n (%)	18 (6.7)	2 (10.0)	10 (9.5)	6 (4.2)	0.216
Urinary ratio of 6β-hydroxycortisol/ cortisol, (geometric mean, 95% CI)	3.66 (3.15, 4.26) (n=230)	3.66 (3.13, 4.29) (n=15)	4.04 (3.77, 4.34) (n=91)	3.66 (3.13, 4.29) (n=124)	0.420¶
Baseline lipid levels (mmol/l)					
Total Cholesterol	7.71±1.69	7.30±1.56	7.67±1.70	7.80±1.69	0.442
LDL-C	5.38±1.66	5.06±1.43	5.34±1.66	5.46±1.70	0.579
HDL-C	1.51±0.38	1.49±0.50	1.55±0.38	1.49±0.36	0.458
Triglycerides	1.96±1.48	1.88±1.22	1.90±1.41	2.01±1.56	0.837
Lipid levels on treatment (mmol/l)					
Total Cholesterol	4.91±1.00	4.52±0.74	4.85±1.03	5.01±0.98	0.091
LDL-C	2.81±0.98	2.52±0.70	2.75±0.90	2.89±1.07	0.211
HDL-C	1.47±0.37	1.39±0.33	1.49±0.40	1.47±0.35	0.550
Triglycerides	1.44±0.80	1.37±0.60	1.35±0.83	1.52±0.79	0.212

Data are given as mean ± SD or n (%), except for urinary ratio of 6β-hydroxycortisol/cortisol as geometric means and 95% CI. Statistical comparison among by ANOVA for continuous variable, or Chi-Square test for categorical variables, except ¶ ANOVA comparison on natural logarithmic values. *P-value<0.05 is statistically significant.

2.4.2. Lipid responses and safety

In 270 patients with plasma LDL-C data available, simvastatin treatment significantly reduced LDL-C by -47.1±12.5% (95% CI: -48.6%, -45.6%). Among the demographic and phenotypic characteristics analyzed, only age was found to be significantly associated with LDL-C percentage reduction (P = 0.003) (Table 2.5). However, the association between advancing age and LDL-C percentage reduction was relatively weak (regression coefficient: –0.202, R^2 = 0.03) (Graph 2.1). Simvastatin was well tolerated in all study participants. No patients had clinically relevant elevations in CK, ALT or creatinine, and no muscle problem was observed.

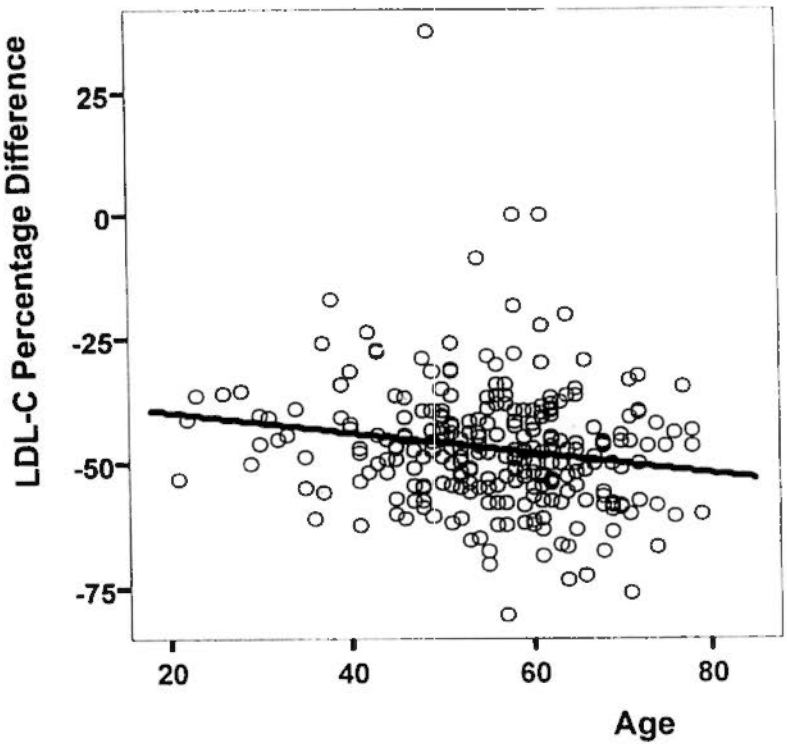
Table 2.5: Demographic and phenotypic characteristics and their association with percentage change in LDL-C upon treatment with simvastatin 40 mg daily for at least four weeks (n = 270)

	n	Mean±SD	95% CI	
LDL-C Change (%)	**270**	**-47.1±12.5**	**-48.6%, -45.6%**	
Demographic Characteristics	**n = 270**	**Range**	**P-value**	
Age (years)	55.7±11.0	21-79	0.003*	
Body weight (kg)	64.4±13.0	39.2-113.0	0.776	
Body height (m)	1.60±0.08	1.40-1.81	0.498	
Body mass index (kg/m^2)	25.1±4.1	16.6-42.3	0.747	
Waist circumference (cm)	85.8±11.4	60.5-123.8	0.590	
Hip circumference (cm)	97.4±7.7	83.0-129.0	0.868	
Waist/hip ratio	0.88±0.08	0.69-1.11	0.324	
Body fat (%)	29.8±8.1	11.5-57.7	0.629	
Phenotypic Characteristics	**n (%)**	**Presence (LDL-C %-change)**	**Absence (LDL-C %-change)**	**P-value**
Male Gender	112 (41.5)	-47.0±13.5	-47.1±11.8	0.938
Current drinker	20 (7.4)	-46.8±12.3	-47.1±12.5	0.908
Current smoker	30 (11.1)	-47.6±14.0	-47.0±12.3	0.820
FH	144 (53.3)	-46.8±11.4	-47.4±13.6	0.714
Hypertension,	127 (47.0)	-47.9±12.4	-46.4±12.6	0.322
History of CVD	38 (14.1)	-45.1±12.6	-47.4±12.5	0.304
Diabetes mellitus	54 (20.0)	-46.3±17.6	-47.3±10.9	0.591
History of RA	18 (6.7)	-46.2±15.9	-47.1±12.2	0.759

Data are given as mean±SD or n(%). Statistical tests by Linear Regression Analysis for continuous variables of Demographic Characteristics, and Student's t-tests for categorical variable of Phenotypic Characteristics. *P-value<0.05 is statistically significant.

Graph 2.1: Linear regression plot of LDL-C percentage reduction vs. age (years) after simvastatin 40mg daily treatment for at least 4-weeks (P<0.01)

Linear regression equation:
LDL-C Percentage Difference = (-35.80) + (-0.20 * Age); $R^2 = 0.03$



2.4.3. Genetic influence on lipid responses to simvastatin

2.4.3.1. Individual polymorphisms

Analysis on candidate SNPs of *CYP3A4*1G, CYP3A5*3* and *CYP3AP1*3* did not reveal significant differences of lipid responses to simvastatin treatment among each of the 3 genotype groups or by dominant or recessive genotype models, except for HDL-C changes in *CYP3A4*1G* analysis comparing subjects with homozygous **1/*1* vs. carriers of **1G* (+0.3% vs. -3.7%, P = 0.043) (Table 2.6). Bivariate correlation analysis did not deliver significant findings between individual SNPs with lipid responses, except for weak effects between TC percentage reduction and *CYP3A5*3* polymorphism (Spearman coefficient: +0.120, P = 0.048) and HDL-C percentage changes for *CYP3A4*1G* polymorphism (Spearman coefficient: -0.127, P = 0.036) Table 2.7.

2.4.3.2. Gene-gene interactions of individual SNPs

Further analysis was performed on gene-gene interactions between the SNPs under discussion. Linkage disequilibrium between the candidate SNPs were tested by Chi-Square tests. The correlation of *CYP3AP1*3* and *CYP3A5*3* was noted to have a high Spearman coefficient of +0.978 (P<0.001), suggesting the two SNPs are in tight linkage disequilibrium (Table 2.8). The other two combinations also showed significant relationship (Spearman correlation coefficients = -0.773 and -0.762, respectively), but not as strong as the above pairings. Gene-gene interaction analysis was performed by combining alleles of *CYP3A4*1G* and *CYP3A5*3*. Lipid responses were mapped in each group for comparison (Table 2.9).

Table 2.6: Genotype association of candidate SNPs with plasma lipid responses to at least four weeks of treatment simvastatin 40 mg/day (calculated as % change from baseline)

Genotypes of candidate SNPs	TC	LDL-C	HDL-C	Triglycerides
Total population (n=270)	-35.7±8.5 (-36.7,-34.6)	-47.1±12.5 (-48.6,-45.6)	-1.4±15.1 (-3.2, +0.4)	-16.5±20.2 (-20.7, -12.4)
CYP3A4 *1G (c.20230 G>A)				
**1/*1* (n=145)	-35.0±8.3 (-36.4,-33.7)	-47.1±13.4 (-49.3,-44.9)	+0.3±16.0 (-2.4, +2.9)	-13.5±39.1 (-19.9, -7.1)
**1/*1G* (n=108)	-36.3±8.5 (-37.9,-34.7)	-46.9±10.9 (-49.0,-44.8)	-3.3±13.1 (-5.8, -0.8)	-20.5±28.6 (-26.0, -15.1)
**1G/*1G* (n=18)	-35.1±12.0 (-41.0,-29.1)	-47.7±14.8 (-55.0,-40.4)	-5.9±15.8 (-13.8, +1.9)	-9.0±39.1 (-28.4, +10.5)
P-value on all groups	0.508[a]	0.971[a]	0.112[c]	0.226[c]
P-value (**1/*1*vs.[**1/*1Gor*1G/*1G*])	0.306[b]	0.966[b]	0.043[d]*	0.311[d]
CYP3A5 *3 (c.6986A>G)				
**1/*1* (n=18)	-37.6±8.7 (-41.9,-33.2)	-50.2±9.6 (-54.9,-45.4)	-3.7±15.5 (-11.4, +4.0)	-14.4±28.4 (-28.6, -0.3)
**1/*3* (n=110)	-35.9±9.2 (-37.7,-34.2)	-47.2±12.1 (-49.5,-44.9)	-3.5±12.4 (-5.8, -1.1)	-21.9±27.9 (-27.1, -16.6)
**3/*3* (n=145)	-35.0±8.1 (-36.3,-33.7)	-46.6±13.1 (-48.7,-44.4)	+0.3±16.5 (-2.5, +3.0)	-12.0±40.3 (-18.6, -5.4)
P-value on all groups	0.417[a]	0.507[a]	0.227[c]	0.101[c]
P-value ([**1/*1or*1/*3*] vs. **3/*3*)	0.277[b]	0.476[b]	0.085[d]	0.101[d]
CYP3AP1*3 (c.-44G>A)				
**1/*1* (n=20)	-36.9±8.6 (-40.9,-32.9)	-49.2±9.7 (-53.8,-44.7)	-3.4±14.7 (-10.3, +3.5)	-15.3±27.2 (-28.0, -2.5)
**1/*3* (n=105)	-35.9±9.4 (-37.8,-34.1)	-47.2±12.3 (-49.6,-44.8)	-3.5±12.4 (-5.9, -1.1)	-22.3±28.2 (-27.8, -16.9)
**3/*3* (n=142)	-35.1±8.1 (-36.4,-33.7)	-46.6±13.2 (-48.8,-44.4)	+0.1±16.8 (-2.7, +2.9)	-12.1±40.4 (-18.8, -5.4)
P-value on all groups	0.565[a]	0.678[a]	0.257[c]	0.086[c]
P-value ([**1/*1or*1/*3*] vs. **3/*3*)	0.333[b]	0.556[b]	0.103[d]	0.089[d]

Data are given as mean±SD (95% CI). Statistical comparison by [a]ANOVA Analysis of Variance, on dominant model for *CYP3A4*1G*, or recessive model for *CYP3A5*3* and *CYP3AP1*3* by [b]Student's t-tests, or non-parametric [c]Kruskal-Wallis test or [d]Mann-Whitney test. *P-values<0.05 is statistically significant.

Table 2.7: Bivariate correlation analysis on genotype-lipid response for all patients after simvastatin 40 mg/day treatment for at least four weeks

Spearman correlation coefficient	*CYP3A4 *1G (c.20230 G>A)*	P-value	*CYP3AP1 *3 (c.-44 G>A)*	P-value	*CYP3A5 *3 (c.6986 A>G)*	P-value
Plasma Lipid Response (% change)	(n=271)		(n=267)		(n=273)	
Total Cholesterol	-0.096	0.114	+0.108	0.079	+0.120	0.048*
LDL-C	-0.003	0.959	+0.051	0.411	+0.066	0.283
HDL-C	-0.127	0.036*	+0.094	0.125	+0.101	0.096
Triglycerides	0.042	0.495	+0.081	0.185	+0.078	0.196
Urinary ratio of 6β-hydrocortisol/ cortisol	+0.016 (n=233)	0.805	-0.020 (n=230)	0.758	-0.009 (n=234)	0.895

*P-value<0.05 is statistically significant.

127 patients (47% of population) were carriers of both *CYP3A4*1/*1* and *CYP3A5*3/*3* alleles, and 84 patients (31%) were carriers of *CYP3A4*1/*1G* and *CYP3A5*1/*3*. Their LDL-C reductions were similar at -46.7% and -46.9%, respectively. Other groups constituted a combination of 22% among the whole population. There was no significant finding between each sub-group on Bonferroni post-hoc comparisons.

2.4.3.3. Gene-gender interactions of individual SNPs

There was no significant finding on gene-gender interactions in lipid responses with the *CYP3A4*1G* polymorphism (Table 2.10). Plasma triglyceride changes were smaller among male subjects of homozygous *CYP3A5*3/*3* genotype (-4.7%, P = 0.050) (Table 2.11) or *CYP3AP1*3/*3* carriers (-4.9%, P = 0.032) (Table 2.12). The findings would need confirmation as noted with apparent greatest effect from heterozygous groups.

Table 2.8: Correlation analysis among the candidate SNPs by Chi-square tests

n	***CYP3A4*1G***	****1/*1***	****1/*1G***	****1G/*1G***
CYP3AP1*3				
**1/*1*		0	8	12
**1/*3*		17	81	6
**3/*3*		124	16	0

Spearman correlation coefficient = -0.773 (P<0.001)

n	***CYP3A4*1G***	****1/*1***	****1/*1G***	****1G/*1G***
CYP3A5*3				
**1/*1*		0	7	11
**1/*3*		18	83	7
**3/*3*		126	17	0

Spearman correlation coefficient = -0.762 (P<0.001)

n	***CYP3A5*3***	****1/*1***	****1/*3***	****3/*3***
CYP3AP1*3				
**1/*1*		18	2	0
**1/*3*		0	103	1
**3/*3*		0	2	139

Spearman correlation coefficient = +0.978 (P<0.001)

Table 2.9: Gene-gene interaction between *CYP3A4*1G* and *CYP3A5*3* with plasma lipid responses (calculated as % change from baseline) and urinary ratio of 6β-hydrocortisol/cortisol after at least four weeks of treatment simvastatin 40 mg/day

Genotypes of candidate genes	TC (% change)	LDL-C (% change)	HDL-C (% change)	Triglycerides (% change)	Urinary ratio of 6β-hydrocortisol/ cortisol
*CYP3A4*1/*1* & *CYP3A5*1/*3* (n=18)	-35.0±8.7 (-39.3, -30.7)	-50.4±12.1 (-56.6, -44.2)	-3.0±11.8 (-8.8, +2.9)	-21.5±25.6 (-34.3, -8.8)	4.11 (3,46, 4.90) (n=17)
*CYP3A4*1/*1* & *CYP3A5*3/*3* (n=127)	-35.0±8.3 (-36.5, -33.6)	-46.7±13.5 (-49.1, -44.3)	+0.7±16.5 (-2.2, +3.6)	-12.4±40.6 (-19.5, -5.3)	3.85 (3.62, 4.09) (n=111)
*CYP3A4*1/*1 G* & *CYP3A5*1/*1* (n=7)	-38.3±5.9 (-43.8, -32.8)	-48.7±9.4 (-57.4, -40.0)	-0.1±11.8 (-11.0, +10.9)	-16.0±32.5 (-46.1, +14.1)	3.68 (1.83, 7.42) (n=4)
*CYP3A4*1/*1 G* & *CYP3A5*1/*3* (n=84)	-36.4±8.9 (-38.4, -34.5)	-46.9±11.4 (-49.4, -44.4)	-3.4±12.7 (-6.2, -0.7)	-23.7±25.1 (-29.1, -18.2)	4.03 (3.73, 4.35) (n=72)
*CYP3A4*1/*1 G* & *CYP3A5*3/*3* (n=17)	-34.8±7.6 (-38.7, -30.9)	-46.3±9.7 (-51.3, -41.3)	-4.3±16.0 (-12.5, +3.9)	-6.8±39.1 (-27.0, +13.3)	4.25 (3.28, 5.49) (n=13)
*CYP3A4*1G/*1G* & *CYP3A5*1/*1* (n=11)	-37.1±10.4 (-44.1, -30.1)	-51.1±10.0 (-57.8, -44.4)	-6.0±17.6 (-17.8, +5.9)	-13.4±27.1 (-31.7, +4.8)	3.62 (3.07, 4.26) (n=9)
*CYP3A4*1G/*1G* & *CYP3A5*1/*3* (n=7)	-31.9±14.4 (-45.2, -18.6)	-42.3±19.9 (-60.8, -23.9)	-5.8±13.7 (-18.4, +6.8)	-1.9±54.8 (-52.6, +48.8)	3.92 (2.50, 6.15) (n=6)
P-value	0.801	0.821	0.404	0.297	0.855 ¶

Data are given as mean±SD (95% confidence intervals), except for urinary ratio of 6β-hydrocortisol/cortisol as geometric means (95% confidence intervals). Statistical comparison by ANOVA Analysis of Variance and Bonferroni post-hoc test, except ¶ statistical comparison on naturally logarithmic transformed data . No statistical significance found (P>0.05).

Table 2.10: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3A4 *1G* genotype groups in patients stratified by gender

		**1/*1*	**1/*1G*	**1G/*1G*	P-value[a]	P-value[b]
Male subjects		(n=58)	(n=50)	(n=5)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.68±0.86	-2.77±0.83	-2.38±1.14	0.880	0.800
	LDL-C	-2.52±1.01	-2.42±0.90	-2.42±0.91	0.576	0.295
	HDL-C	+0.01±0.19	-0.06±0.22	+0.02±0.15	0.258	0.187
	TG	-0.40±1.33	-0.60±0.96	-0.20±0.36	0.246	0.217
Percentage changes from baseline (mean±SD)						
	TC	-34.8±7.5	-37.1±7.8	-34.3±11.3	0.425	0.193
	LDL-C	-46.5±16.0	-47.1±10.8	-49.8±6.7	0.801	0.864
	HDL-C	+1.8±15.8	-3.4±14.3	+2.4±15.4	0.305	0.201
	TG	-8.5±48.3	-20.8±30.9	-11.1±21.3	0.335	0.304
Female subjects		(n=87)	(n=58)	(n=13)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.89±1.24	-2.86±1.10	-2.85±1.40	0.926	0.703
	LDL-C	-2.69±1.22	-2.61±1.08	-2.55±1.31	0.999	0.973
	HDL-C	-0.03±0.25	-0.06±0.19	-0.20±0.28	0.113	0.127
	TG	-0.59±1.47	-0.43±0.61	-0.48±1.10	0.717	0.749
Percentage changes from baseline (mean±SD)						
	TC	-35.2±8.8	-35.6±9.2	-35.4±12.7	0.573	0.338
	LDL-C	-47.5±11.5	-46.8±11.1	-46.9±17.1	0.746	0.996
	HDL-C	-0.7±16.2	-3.3±12.1	-9.1±15.3	0.147	0.120
	TG	-16.9±31.4	-20.3±26.7	-8.1±44.8	0.555	0.617

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on dominant genotype model (**1/*1* vs [**1/*G or 1G/1G*]) by Mann-Whitney test. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.
Genotype data were not available for all polymorphisms in all subjects.

Table 2.11: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3A5 *3* genotype groups in patients stratified by gender

		**1/*1*	**1/*3*	**3/*3*	P-value[a]	P-value[b]
Male subjects		(n=8)	(n=48)	(n=58)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.69±1.01	-2.81±0.86	-2.62±0.82	0.641	0.361
	LDL-C	-2.58±0.93	-2.53±0.93	-2.40±0.97	0.928	0.762
	HDL-C	+0.04±0.14	-0.05±0.20	-0.01±0.21	0.265	0.460
	TG	-0.45±1.06	-0.73±1.26	-0.28±1.02	0.042*	0.038*
Percentage changes from baseline (mean±SD)						
	TC	-35.4±9.0	-37.3±7.8	-34.6±7.5	0.184	0.066
	LDL-C	-47.5±7.1	-48.2±11.2	-46.0±15.8	0.709	0.424
	HDL-C	+4.4±13.6	-3.3±13.7	+1.1±16.3	0.251	0.405
	TG	-11.6±31.8	-25.8±24.1	-4.7±49.4	0.063	0.050
Female subjects		(n=10)	(n=62)	(n=87)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.99±1.12	-2.80±1.19	-2.90±1.21	0.857	0.747
	LDL-C	-2.64±1.05	-2.60±1.19	-2.67±1.19	0.951	0.766
	HDL-C	-0.23±0.29	-0.06±0.19	-0.03±0.26	0.118	0.154
	TG	-0.56±1.09	-0.40±0.62	-0.62±1.47	0.886	0.996
Percentage changes from baseline (mean±SD)						
	TC	-39.3±8.6	-34.8±10.1	-35.3±8.5	0.235	0.398
	LDL-C	-52.3±11.0	-46.5±12.8	-47.0±11.1	0.227	0.550
	HDL-C	-10.1±14.5	-3.6±11.5	-0.3±16.7	0.151	0.138
	TG	-16.7±26.9	-18.8±30.4	-16.8±32.2	0.727	0.639

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on recessive genotype model ([**1/*1 or *1/*3*] vs. **3/*3*) by Mann-Whitney test. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.
Genotype data were not available for all polymorphisms in all subjects.

Table 2.12: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3AP1*3* genotype groups in patients stratified by gender

		**1/*1*	**1/*3*	**3/*3*	P-value[a]	P-value[b]
Male subjects		(n=9)	(n=45)	(n=59)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.57±1.02	-2.84±0.87	-2.62±0.82	0.642	0.376
	LDL-C	-2.43±0.97	-2.55±0.94	-2.41±0.96	0.940	0.836
	HDL-C	+0.04±0.14	-0.05±0.21	-0.01±0.21	0.295	0.511
	TG	-0.47±0.99	-0.77±1.29	-0.27±1.02	0.032*	0.022*
Percentage changes from baseline (mean±SD)						
	TC	-34.6±8.8	-37.4±7.9	-34.7±7.5	0.202	0.085
	LDL-C	-46.1±7.8	-48.1±11.2	-46.1±15.7	0.725	0.601
	HDL-C	+3.6±12.9	-3.4±14.2	+1.0±16.2	0.272	0.416
	TG	-13.9±30.5	-26.8±24.2	-4.9±49.0	0.047*	0.032*
Female subjects		(n=11)	(n=60)	(n=83)		
Changes from baseline in mmol/l (mean±SD)						
	TC	-2.95±1.07	-2.80±1.21	-2.91±1.21	0.903	0.858
	LDL-C	-2.63±1.00	-2.59±1.21	-2.68±1.18	0.977	0.865
	HDL-C	-0.20±0.28	-0.06±0.18	-0.03±0.26	0.187	0.179
	TG	-0.54±1.04	-0.40±0.63	-0.63±1.50	0.915	0.925
Percentage changes from baseline (mean±SD)						
	TC	-38.8±8.3	-34.8±10.3	-35.4±8.6	0.350	0.442
	LDL-C	-51.8±10.6	-46.6±13.1	-47.0±11.2	0.315	0.543
	HDL-C	-9.1±14.1	-3.5±11.0	-0.6±17.2	0.242	0.177
	TG	-16.4±25.6	-19.0±30.6	-17.2±32.2	0.699	0.681

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on recessive genotype model ([**1/*1 or *1/*3*] vs. **3/*3*) by Mann-Whitney test. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.
Genotype data were not available for all polymorphisms in all subjects.

2.4.3.4. Gene-disease interactions of individual SNPs

Gene-disease associations with lipid responses were further analyzed in the three candidate SNPs. Patients were identified for their medical conditions of Familial Hypercholesterolaemia (FH), Hypertension, Cardiovascular Disease, Diabetes or Rheumatoid Arthritis. Significant findings were noted in FH patients.

Demographic characteristics and lipid profiles of the patient group diagnosed with FH were further analyzed (Table 2.13). FH patients were found to be of younger age (53.5 vs. 58.2 years, $P<0.001$), and body weight and other metabolic parameters were also lower with these patients compared to non-FH group. Baseline and on-treatment TC (8.70 vs. 6.59 and 5.44 vs. 4.29 mmol/L, respectively), LDL-C (6.40 vs. 4.22 and 3.37 vs. 2.16 mmol/L, respectively), HDL-C (1.57 vs. 1.45 and 1.55 vs. 1.39 mmol/L, respectively) were higher with the FH patients (all $P<0.001$), but triglycerides were lower (1.64 vs. 2.29 and 1.23 vs. 1.64 mmol/L, respectively, $P<0.001$). These figures were as expected for elevated plasma lipid levels with FH patients.

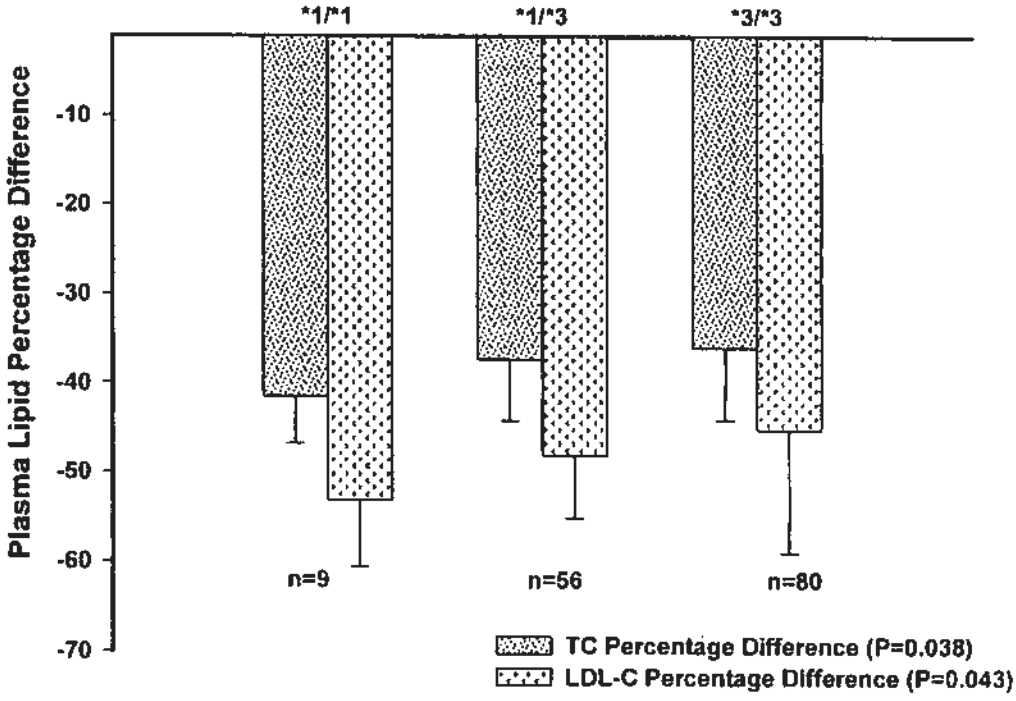
Table 2.13: Patient characteristics and baseline lipid profiles stratified by diagnosis of FH

	Whole population (n=271)	FH subjects (n=144)	Non-FH subjects (n=127)	P-value
Characteristics				
Age (years)	55.6±11.0	53.5±12.4	58.2±8.5	<0.001*
Male, n (%)	113 (41.7)	55 (38.2)	58 (45.7)	0.213
Body weight (kg)	64.5±13.0	61.7±10.9	67.5±14.4	<0.001*
Body height (m)	1.60±0.08	1.60±0.08	1.59±0.09	0.689
Body mass index (kg/m^2)	25.1±4.1	24.1±3.6	26.3±4.3	<0.001*
Waist circumference (cm)	85.8±11.4	82.6±9.9	89.4±11.9	<0.001*
Hip circumference (cm)	97.5±7.7	96.2±6.5	98.8±8.7	0.008*
Waist/hip ratio	0.88±0.07	0.86±0.07	0.90±0.07	<0.001*
Body fat (%)	29.8±8.1	28.8±7.6	31.0±8.6	0.067
Current drinker, n (%)	20 (7.4)	12 (8.3)	8 (6.3)	0.523
Current smoker, n (%)	31 (11.4)	12 (8.3)	19 (15.0)	0.087
Hypertension, n (%)	128 (47.2)	39 (27.1)	89 (70.1)	<0.001*
History of CVD, n (%)	38 (14.0)	9 (6.3)	29 (22.8)	<0.001*
Diabetes mellitus, n (%)	55 (20.3)	15 (10.4)	40 (31.5)	<0.001*
History of RA, n (%)	18 (6.6)	0 (0.0)	18 (14.2)	<0.001*
Urinary ratio of 6β-hydroxycortisol/ cortisol, (geometric mean, 95% CI)	3.93 (3.76, 4.10) (n=227)	3.87 (3.67, 4.09) (n=130)	4.00 (3.72, 4.29) (n=97)	0.480 ¶
Baseline lipid levels (mmol/l)				
Total Cholesterol	7.72±1.71	8.70±1.56	6.59±1.04	<0.001*
LDL-C	5.39±1.68	6.40±1.54	4.22±0.91	<0.001*
HDL-C	1.51±0.38	1.57±0.36	1.45±0.40	0.006*
Triglycerides	1.95±1.47	1.64±0.93	2.29±1.84	<0.001*
Lipid levels on treatment (mmol/l)				
Total Cholesterol	4.91±1.00	5.44±0.93	4.29±0.63	<0.001*
LDL-C	2.81±0.99	3.37±0.94	2.16±0.55	<0.001*
HDL-C	1.47±0.37	1.55±0.37	1.39±0.36	<0.001*
Triglycerides	1.45±0.79	1.23±0.57	1.64±0.85	<0.001*

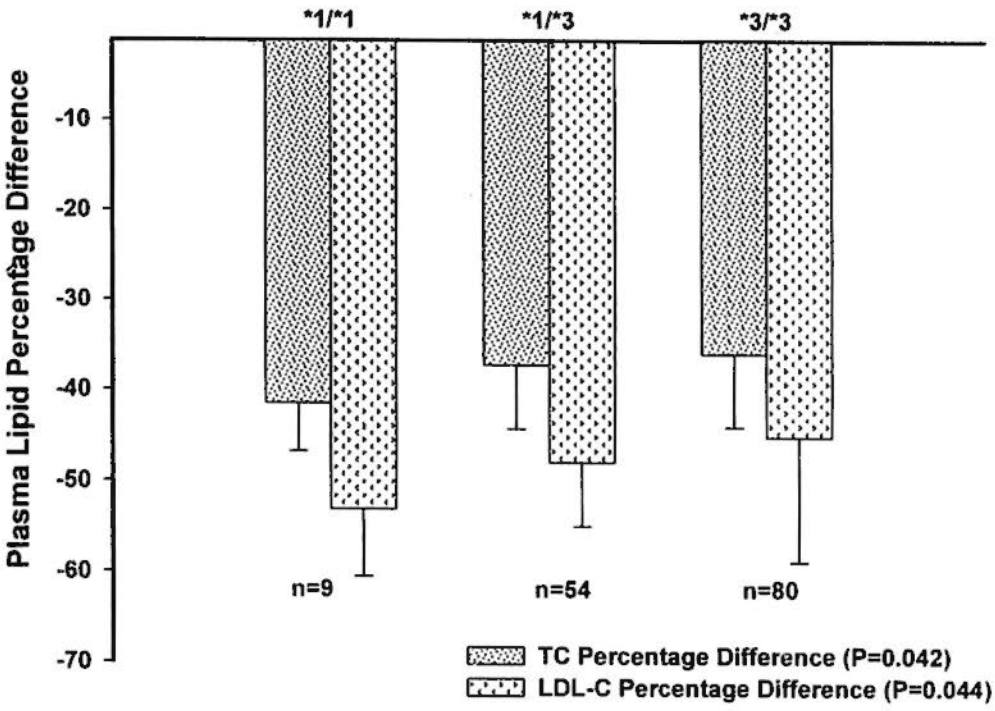
Data are given as mean ± SD or n (%), except for urinary ratio of 6β-hydroxycortisol/cortisol as geometric means and 95% CI. Statistical comparison among by Student's t-test for continuous variable, or Chi-Square test for categorical variables, except ¶ Student's t-test comparison on natural logarithmic values.
*P-value<0.05 is statistically significant.

Homozygous *CYP3A5*3/*3* and *CYP3AP1*3/*3* carriers had reduced TC and LDL-C lowering responses (P<0.05) (Graph 2.2 and 2.3), but this became non-significant when comparing the dominant genotype model against carriers with at least one copy of wild-type **1* allele (P>0.05) (Table 2.14 and 2.15). Plasma triglyceride lowering was more significant with the heterozygous *CYP3A5*1/*3* and *CYP3AP1*1/*3* groups (P<0.05).

Graph 2.2: TC and LDL-C percentage change vs. genotype groups of *CYP3A5 *3* in patients diagnosed as Familial Hypercholesterolaemia (P<0.05 on both comparisons)



Graph 2.3: TC and LDL-C percentage change vs. genotype groups of *CYP3AP1 *3* in patients diagnosed as Familial Hypercholesterolaemia (P<0.05 on both comparisons)



Graph 2.4: TC and LDL-C percentage change vs. genotype groups of *CYP3A4 *1G* in patients diagnosed as Familial Hypercholesterolaemia (P<0.05 on both comparisons)

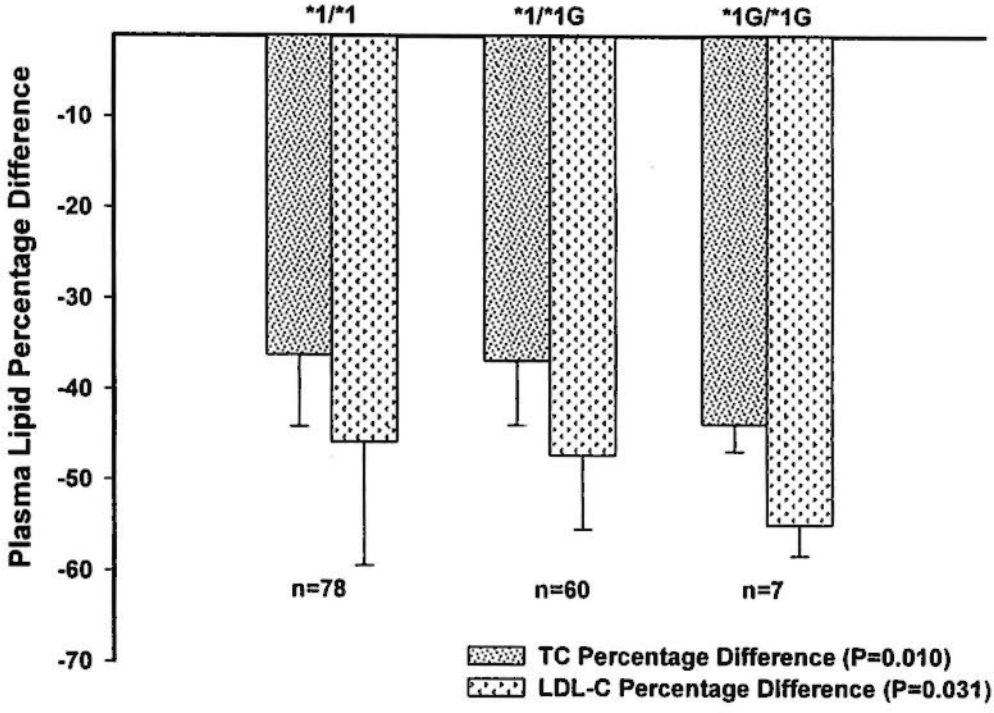


Table 2.14: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3A5*3* genotype groups in patients of different phenotypes

	% Change	**1/*1*	**1/*3*	**3/*3*	P-value[a]	P-value[b]
FH patients		(n=9)	(n=56)	(n=80)		
	TC	-41.5±5.3	-37.3±6.9	-36.0±8.1	0.038*	0.054
	LDL-C	-53.1±7.4	-48.1±7.0	-45.2±13.7	0.043*	0.064
	HDL-C	-6.9±14.9	-1.4±12.6	+0.3±16.6	0.497	0.397
	TG	-9.6±32.4	-23.9±21.7	-12.7±32.9	0.042*	0.055
Non-FH patients		(n=9)	(n=54)	(n=65)		
	TC	-33.7±10.0	-34.5±11.0	-33.8±8.1	0.811	0.534
	LDL-C	-47.3±10.9	-46.3±15.8	-48.2±12.1	0.902	0.655
	HDL-C	-0.5±16.3	-5.6±12.0	+0.2±16.5	0.183	0.112
	TG	-19.3±24.8	-19.7±33.2	-11.0±48.1	0.771	0.575
Hypertensive patients		(n=6)	(n=50)	(n=74)		
	TC	-41.0±8.5	-34.9±10.3	-35.5±8.6	0.168	0.707
	LDL-C	-51.4±13.4	-45.7±13.5	-49.0±11.5	0.230	0.273
	HDL-C	-2.7±15.1	-5.7±12.9	-0.8±17.0	0.309	0.140
	TG	-34.8±30.2	-24.6±28.1	-16.0±36.6	0.332	0.184
Atherosclerotic CVD patients		(n=5)	(n=21)	(n=12)		
	TC	-36.9±12.7	-34.2±10.5	-30.7±10.1	0.167	0.076
	LDL-C	-49.9±8.3	-43.6±13.2	-45.9±13.3	0.687	0.889
	HDL-C	-0.6±18.9	-8.5±12.7	+14.5±19.5	<0.010*	<0.010*
	TG	-30.1±36.6	-15.2±27.3	-11.0±68.8	0.589	0.466
Diabetes mellitus patients		(n=3)	(n=16)	(n=37)		
	TC	-37.0±11.4	-33.8±14.2	-35.1±9.8	0.835	0.703
	LDL-C	-42.5±14.4	-45.9±18.5	-46.7±17.8	0.664	0.882
	HDL-C	+3.6±19.6	-8.7±8.5	-1.3±17.8	0.369	0.328
	TG	-60.7±13.9	-27.5±28.7	-16.6±48.1	0.082	0.210
Rheumatoid arthritis patients		(n=2)	(n=10)	(n=6)		
	TC	-29.6±1.1	-30.9±13.3	-32.5±4.1	0.777	0.820
	LDL-C	-41.8±3.3	-42.8±19.3	-53.4±10.1	0.337	0.213
	HDL-C	0.0±0.0	-2.4±11.7	-2.8±16.0	0.958	0.892
	TG	-20.6±13.4	-27.7±29.4	+16.6±56.5	0.135	0.067

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on recessive genotype model ([**1/*1 or *1/*3*] vs **3/*3*) by Mann-Whitney test. FH, familial hypercholesterolaemia; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.

Table 2.15: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3AP1*3* genotype groups in patients of different phenotypes

	% Change	**1/*1*	**1/*3*	**3/*3*	P-value[a]	P-value[b]
FH patients		(n=9)	(n=54)	(n=80)		
	TC	-41.5±5.3	-37.3±7.0	-36.0±8.1	0.042*	0.060
	LDL-C	-53.1±7.4	-48.0±7.0	-45.2±13.7	0.044*	0.071
	HDL-C	-6.9±14.9	-1.2±12.2	+0.0±17.0	0.534	0.445
	TG	-9.6±32.4	-24.8±21.2	-13.3±32.6	0.034*	0.051
Non-FH patients		(n=11)	(n=51)	(n=62)		
	TC	-33.2±9.1	-34.6±11.3	-33.9±8.1	0.731	0.584
	LDL-C	-46.1±10.5	-46.4±16.1	-48.5±12.3	0.729	0.527
	HDL-C	-0.6±14.6	-5.8±12.3	+0.1±16.6	0.150	0.125
	TG	-19.9±22.6	-19.7±34.1	-10.5±48.8	0.721	0.531
Hypertensive patients		(n=8)	(n=46)	(n=71)		
	TC	-38.5±8.7	-34.8±10.6	-35.8±8.7	0.529	0.929
	LDL-C	-48.7±12.7	-45.8±14.0	-49.2±11.6	0.374	0.222
	HDL-C	-2.3±12.9	-5.6±12.7	-1.3±17.5	0.396	0.244
	TG	-31.8±26.6	-26.0±27.9	-15.9±36.9	0.305	0.130
Atherosclerotic CVD patients		(n=7)	(n=17)	(n=13)		
	TC	-35.2±10.9	-33.9±11.2	-31.8±10.5	0.459	0.224
	LDL-C	-47.2±8.7	-43.2±14.3	-46.6±12.9	0.923	0.937
	HDL-C	-0.7±15.5	-8.9±12.0	+10.9±22.7	0.027*	0.011*
	TG	-28.0±30.6	-16.9±27.5	-11.7±65.9	0.784	0.582
Diabetes mellitus patients		(n=4)	(n=15)	(n=35)		
	TC	-34.8±10.3	-34.2±14.7	-35.5±9.8	0.953	0.800
	LDL-C	-40.7±12.3	-46.7±19.0	-46.8±18.4	0.326	0.815
	HDL-C	+2.0±16.3	-9.0±8.7	-1.1±18.3	0.341	0.370
	TG	-53.5±18.3	-27.2±29.6	-17.0±49.1	0.151	0.243
Rheumatoid arthritis patients		(n=2)	(n=10)	(n=6)		
	TC	-29.6±1.1	-30.9±13.3	-32.5±4.1	0.777	0.820
	LDL-C	-41.8±3.3	-42.8±19.3	-53.4±10.1	0.337	0.213
	HDL-C	0.0±0.0	-2.4±11.7	-2.8±16.0	0.958	0.892
	TG	-20.6±13.4	-27.7±29.4	+16.6±56.5	0.135	0.067

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on recessive genotype model ([**1/*1 or *1/*3*] vs **3/*3*) by Mann-Whitney test. FH, familial hypercholesterolaemia; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.

Table 2.16: Association of plasma lipid responses to simvastatin 40 mg/day treatment and *CYP3A4*1G* genotype groups in patients of different phenotypes

	% Change	**1/*1*	**1/*1G*	**1G/*1G*	P-value[a]	P-value[b]
FH patients		(n=78)	(n=60)	(n=7)		
	TC	-36.2±7.9	-36.8±7.1	-43.8±3.0	0.010*	0.137
	LDL-C	-45.8±13.6	-47.2±8.2	-54.9±3.4	0.031*	0.283
	HDL-C	+1.0±16.9	-2.2±11.7	-9.7±17.6	0.145	0.100
	TG	-13.6±32.0	-20.4±24.6	-22.4±36.3	0.277	0.114
Non-FH patients		(n=67)	(n=48)	(n=11)		
	TC	-33.7±8.5	-35.6±10.1	-29.5±12.3	0.285	0.508
	LDL-C	-48.7±13.1	-46.6±13.7	-43.1±17.4	0.474	0.246
	HDL-C	-0.7±15.1	-4.7±14.6	-3.5±14.9	0.401	0.201
	TG	-13.4±46.2	-20.7±33.2	-0.4±40.0	0.253	0.969
Hypertensive patients		(n=75)	(n=48)	(n=6)		
	TC	-35.3±9.0	-35.9±10.1	-36.3±9.7	0.768	0.471
	LDL-C	-49.3±12.2	-45.8±12.9	-47.8±11.3	0.366	0.170
	HDL-C	-1.9±16.3	-5.0±14.3	+0.2±14.7	0.431	0.348
	TG	-15.8±37.2	-25.1±26.2	-31.3±33.7	0.382	0.185
Atherosclerotic CVD patients		(n=17)	(n=15)	(n=6)		
	TC	-32.5±9.9	-34.2±11.4	-34.0±11.9	0.635	0.352
	LDL-C	-46.7±11.6	-41.7±14.9	-49.4±7.4	0.478	0.794
	HDL-C	+7.2±21.2	-6.8±14.0	-4.5±16.0	0.124	0.042*
	TG	-12.7±59.4	-20.2±29.5	-14.0±30.1	0.572	0.486
Diabetes mellitus patients		(n=32)	(n=22)	(n=2)		
	TC	-35.1±10.6	-34.4±12.1	-35.4±15.7	0.999	0.987
	LDL-C	-47.0±19.6	-45.4±14.9	-43.5±20.2	0.767	0.512
	HDL-C	-3.2±16.9	-3.2±14.7	-1.1±25.2	0.996	0.934
	TG	-16.9±50.8	-26.3±29.2	-57.4±17.9	0.282	0.476
Rheumatoid arthritis patients		(n=7)	(n=9)	(n=2)		
	TC	-31.4±6.4	-34.7±7.8	-15.5±18.8	0.227	0.930
	LDL-C	-51.4±13.1	-48.0±10.5	-19.7±27.9	0.123	0.179
	HDL-C	+2.4±9.3	-3.8±13.2	-11.5±16.3	0.485	0.328
	TG	-8.3±38.6	-19.4±47.9	+7.2±52.6	0.469	0.479

Data are given as mean ± SD. Statistical comparison among genotype groups by non-parametric [a]Kruskal-Wallis test, and [b]on dominant genotype model (**1/*1* vs. [**1/*G or 1G/1G*]) by Mann-Whitney test. FH, familial hypercholesterolaemia; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. *P-value<0.05 is statistically significant.

Among the group of FH patients, TC and LDL-C lowering response were higher comparing homozygous *CYP3A4*1G* carriers with **1/*1* individuals (-37.6% vs. -36.2%; and -48.1% vs. -45.8% respectively, P<0.05), but not significant when comparing on a dominant genotype model (Table 2.16, Graph 2.4). Among the 36 patients with a history of atherosclerotic CVD, HDL-C responses were noted to have significant differences on genotypes with all three SNPs (P<0.05).

Bivariate correlations analysis among FH patients between lipid responses and genotype groups confirmed only weak correlations (Spearman coefficients <0.20; P<0.05) pairing TC percentage reduction with each of the three SNPs, as well as LDL-C percentage reduction with *CYP3A5*3* and *CYP3AP1*3* polymorphisms respectively (Spearman coefficients +0.180 and +0.178 respectively, P<0.05) (Table 2.17). No other significant finding was noted in the analysis.

Table 2.17: Bivariate correlation analysis on genotype-lipid response for FH patients after simvastatin 40 mg/day treatment for at least four weeks

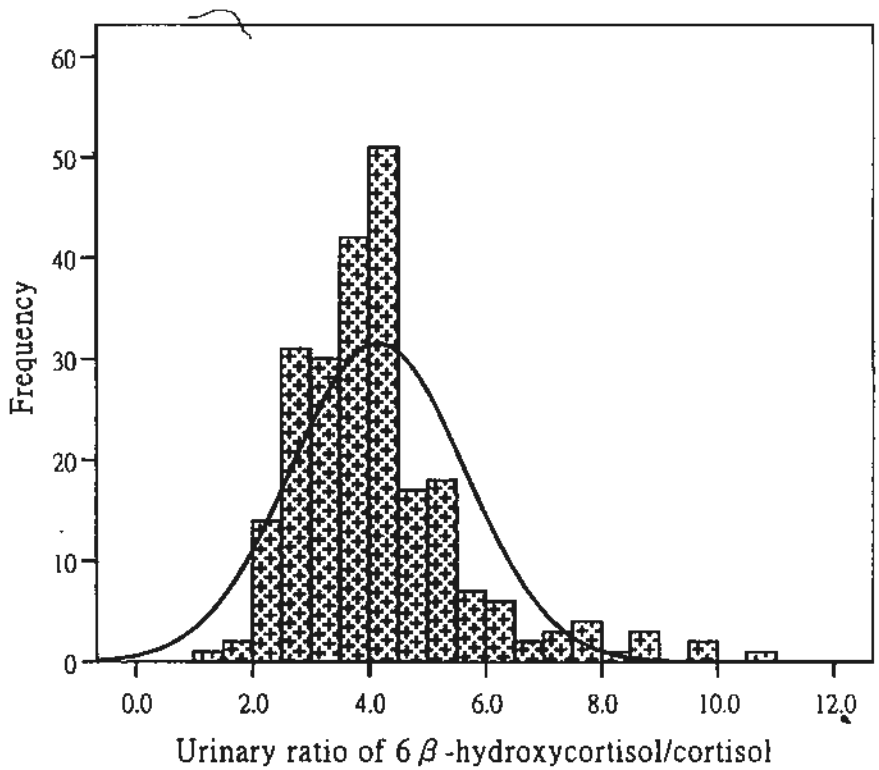
Spearman correlation coefficient	***CYP3A4 *1G (c.20230 G>A)***	**P-value**	***CYP3A5 *3 (c.6986 A>G)***	**P-value**	***CYP3AP1 *3 (c.-44 G>A)***	**P-value**
Plasma Lipid Response (% change)	(n=145)		(n=145)		(n=143)	
Total Cholesterol	-0.163	0.050*	+0.186	0.025*	+0.183	0.029*
LDL-C	-0.126	0.133	+0.180	0.031*	+0.178	0.034*
HDL-C	-0.151	0.069	+0.083	0.320	+0.077	0.362
Triglycerides	-0.126	0.131	+0.130	0.120	+0.132	0.117
Urinary ratio of 6β-hydrocortisol/ cortisol	+0.014 (n=132)	0.870	-0.020 (n=132)	0.818	-0.034 (n=130)	0.703

*P-value<0.05 is statistically significant.

2.4.4. CYP3A activity in terms of urinary 6 β-hydroxycortisol/cortisol (6 β-OHC/C) concentration ratio and lipid responses to simvastatin

Only 234 patients had data available for analysis for the urinary 6 β-hydroxycortisol/cortisol (6 β-OHC/C) concentration ratio. The results were found to be as expected on a skewed population distribution as shown in Graph 2.5. The natural logarithmic transformation to obtain a normal distribution curve (Graph 2.6) was applied for statistical analysis. The geometric mean of the studied population was 3.93 (95%CI: 3.77, 4.10; n = 234).

Graph 2.5: Urinary ratio of 6 β-hydroxycortisol/cortisol distribution curve



2.5. Discussion

The candidate gene approach to identify the pharmacodynamic influence on statin responses began with selection of SNPs along the metabolic pathway. Through identification of critical steps, this selection could narrow down to key proteins and their encoding genes. A recent publication has emphasized the greater complexity and the roles played by gene-gene, gene-diet and gene-environment interactions in epidemiology of lipid metabolism and related cardiovascular outcomes (Ordovas et al., 2011). Even though it would be possible for a GWAS to examine the majority of SNPs over the whole geneome, critical pathway analysis could offer a more pragmatic approach.

The influence on drug response from genetic polymorphisms could also result from pharmacokinetic influences on drug metabolism, pharmacodynamic gene-drug interactions at receptor or enzyme sites of action, or modifying factors in the disease pathway (Maitland-van der Zee et al., 2002). The study described in this chapter examined the genetic effects of the major drug metabolism pathway for simvastatin on the lipid lowering responses.

CYP3A enzymes are responsible for phase 1 oxidative metabolism of a large number of drugs. The two isoforms of CYP3A4 and 3A5 have been attributed with over 70% of the activity of CYP3A. A previous study examined the possibility of using single midazolam concentrations between 2 and 5 hours to predict midazolam AUC as a marker of CYP3A activity and inhibition of the enzyme with an extract of Ginkgo biloba, but the large intersubject variability altered the prediction of optimal midazolam sampling times and the authors concluded that midazolam AUC was preferable for assessing CYP3A activity in drug-drug interaction studies (Penzak et al., 2008). The site of action for simvastatin is the inhibition of HMG-CoA reductase within hepatocytes. The CYP3A enzymes are the major metabolic enzymes in these cells and are known to be the major pathway of metabolism for both simvastatin lactone and acid, and

hence their activities could be influential on simvastatin's pharmacological profile.

The relationship of polymorphisms among the genes encoding CYP3A enzymes and the phenotypic expression in urinary 6 β-OHC/C concentration ratio has not been clearly defined. There were studies which suggested reduced CYP3A4 enzyme activities from genetic mutations could result in a lower urinary 6 β -OHC/C ratio. To the contrary, a trial in 16 healthy volunteers reported urinary 6 β -OHC/C ratio correlated poorly with CYP3A4 activities comparing to quantification using midazolam as the probe drug (Hu et al., 2009b). A similar report describing CYP3A4 inhibition by fluvoxamine when comparing between this urinary ratio against midazolam metabolism suggested the high intra-individual variability from the 6 β -OHC/C ratio prevented that from becoming an optimal tool to quantify CYP3A activities (Chen et al., 2006). A recent Chinese report using clarithromycin as the inhibitor and comparing midazolam metabolism and the urinary 6 β-OHC/C ratio also failed to find correlations (Luo et al., 2009). Results from our study among 234 patients who underwent the urinary cortisol metabolic test did not find correlations between urinary 6 β-OHC/C ratio and polymorphisms at individual *CYP3A* SNPs. as well as the gene-gene interaction analysis between *CYP3AP1*3* and *CYP3A5*3*. The natural logarithmic transformed population curve was normally distributed, suggesting the tests were likely to be representative. Hence our results could indicate that the overall determinants of the urinary 6 β -OHC/C ratio, a phenotypic biomarker for CYP3A enzyme activities, would be more complex due to the interplay of a number of isoenzymes.

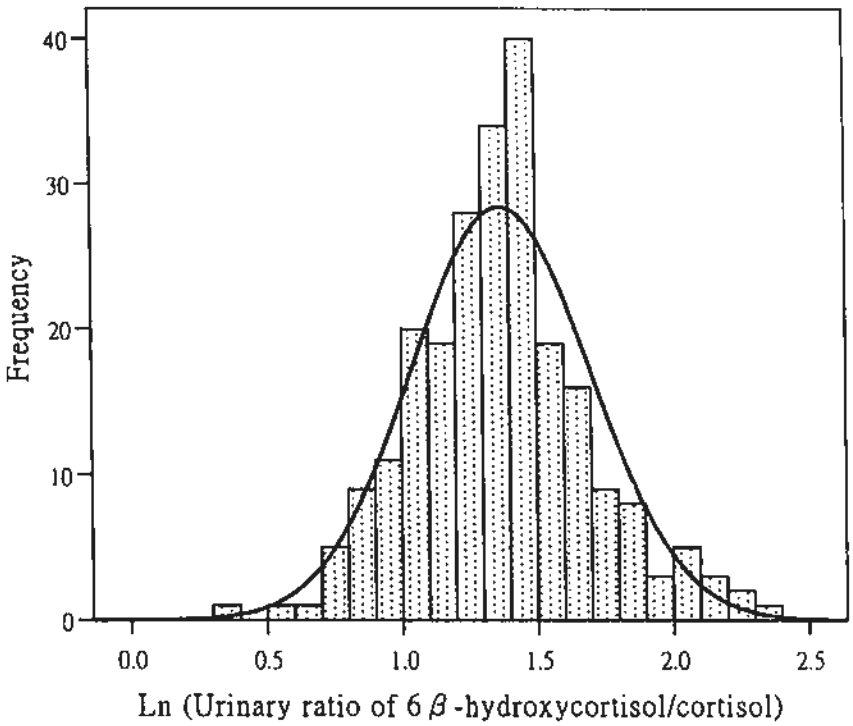
In our study of 270 patients, there were no significant differences of lipid responses comparing genotype groups among individual SNPs. The patient demographics, phenotypic characteristics and baseline lipid levels did not have significant differences and therefore could be assumed to be homogeneous groups. Our findings were likely to be typical of a Chinese population. The allele

frequency of *CYP3A4*1G* in this patient group was 26.6%, similar to a previous study in a Chinese population which suggested a gene-dose response relationship with atorvastatin and the *CYP3A4*1G* polymorphism (Gao et al., 2008). However, that correlation was not observed in our study group. A possible explanation is simvastatin may be affected to a different extent to atorvastatin. The affinity of atorvastatin to CYP3A4 enzymes had been suggested (Jacobsen et al., 2000) to explain the interindividual variability of lipid responses (Park et al., 2008). Simvastatin was suggested as a lesser inhibitor to CYP3A4 enzymes (Sakaeda et al., 2006) and hence could be less affected by the corresponding variations in enzyme activities comparing to atorvastatin.

The *CYP3A5*3* polymorphism had been noted to relate to pharmacokinetic variations in simvastatin, contributing to increased plasma levels and systemic exposure in terms of AUC values (Kim et al., 2007), and possibly leading to increased lipid lowering responses (Kivisto et al., 2004). The causal relationship between simvastatin systemic exposure and myotoxicity from the interaction with CYP3A inhibitors has been suggested (Neuvonen et al., 2006). The frequency of the *CYP3A5*3* allele in our study population was 73.3%, comparable to previously reported values for a Chinese population (Balram et al., 2003). Our analysis did not reveal any significant effect of this polymorphism in relation to the lipid lowering responses. Since the pharmacokinetic study mentioned above involved a single dose protocol, it is possible that the role of a single polymorphism on the overall pharmacodynamic response may be compensated with repeated dosing and on multiple pathways.

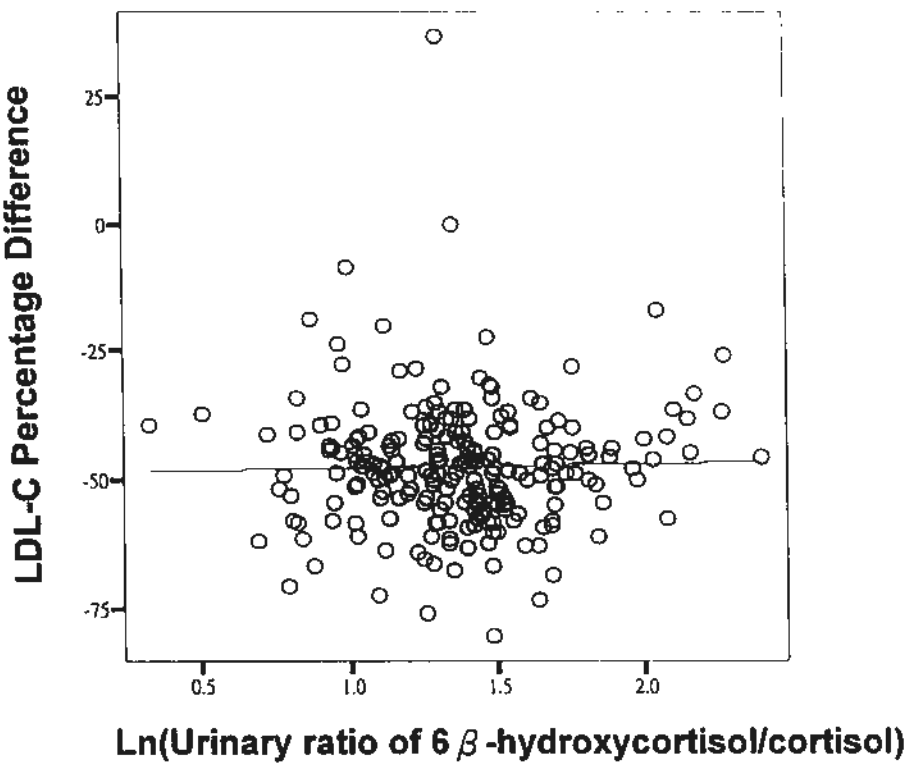
The combined effects of multiple genetic variants to explain the variability to lipid responses had been proposed. Chasman et al. proposed a combination of two HMG-Co polymorphism for better prediction of lipid lowering responses after pravastatin therapy (Chasman et al., 2004). A recent study on variants among 18 SNPs in 6 genes reported a three-loci interaction model, but this did not include SNPs studied with *CYP3A4* gene (Poduri et al., 2010). We have mapped our candidate SNPs and confirmed the suggestion that *CYP3AP1*3* and *CYP3A5*3*

Graph 2.6: Natural logarithmic transformed urinary 6 β - hydroxycortisol / cortisol concentration ratio distribution curve



There were no significant differences of urinary 6 β -OHC/C ratio among genotype groups of the individual candidate SNPs (Table 2.2-2.4), or in the gene-gene interaction analysis (Table 2.9). Geometric means of the individual genotype groups were comparable. Linear regression analysis of Ln(6 β -OHC/C) ratio vs. LDL-C percentage changes did not reveal statistical significance (P = 0.735) (Graph 2.7), suggesting the absence of relationship between this CYP3A phenotypic marker and LDL-C reduction responses from simvastatin treatment.

Graph 2.7: Linear regression plot of LDL-C percentage difference vs. Ln (Urinary ratio of 6 β-hydroxycortisol/cortisol) after simvastatin 40 mg daily treatment for at least 4-weeks (P=0.735)



are tightly linked. Our gene-gene interaction study worked on the combination from the SNP *CYP3A5*3* with the third candidate of *CYP3A4*1G*. No significant difference in lipid responses was found, suggesting the metabolic pathways of simvastatin inside the hepatocytes might be more complex.

A previous study in a Chinese population did not report gender differences in lipid lowering responses with statins (Tavintharan et al., 2007). In another large scale pharmacogenetic study, female subjects were found to have slightly greater LDL-C reduction with atorvastatin (Thompson et al., 2005). A previous study in Japanese on low dose simvastatin did not note difference in response to LDL-C reduction, even though the incidence of coronary events was lower with the female gender (Sasaki et al., 2006). We stratified our data for gene-gender analysis. No significant difference was found, except for plasma triglycerides reduction in male subjects with *CYP3A5*3* and *CYP3AP1*3* polymorphism, probably due to chance finding from baseline levels in the patient categories analyzed and from performing multiple comparisons.

One of the factors that was suggested to contribute to inter-individual variations in statin responses could be polymorphisms in genes that may influence protein expression involved in the underlying disease conditions (Hutz and Fiegenbaum, 2008; Puccetti et al., 2007). New genetic loci have been described in recent publications (Waterworth et al., 2010) and could be worthy of investigation for effects on statin responses.

Gene-disease interactions on population stratification could give a convenient indicator how individual SNPs might interact to deliver benefits to individuals. In a Chinese study with diabetic patients, Apolipoprotein E (APOE) e3/e4 subjects on treatment with with simvastatin or lovastatin were noted to have better lipid lowering responses than e2/e3 patients (-48% vs -28%; $P<0.05$) (Tavintharan et al., 2007), while no such interaction was noted with atorvastatin (Christidis et al., 2006). In a particular study on APOE e3 individuals in a group of patients with FH and apoe3e3 phenotype, female FH patients were found to respond better than

male FH patients to simvastatin therapy (De Knijff et al., 1990). A recent study with rosuvastatin has suggested a 2.6% smaller reduction of LDL-C in Chinese FH patients compared to those without FH (Hu et al., 2010b).

In our study with further stratification on gene-disease interactions analysis, FH subjects showed some statistically significant differences in their lipid lowering responses, while patients with other conditions with increased cardiovascular risk did not have such findings. There were significant differences in the demographic profiles between FH and non-FH patients, largely attributed to the inherited nature of the disease. FH patients were younger, and apparently had less exposure to other metabolic disorders. The frequencies of diabetes and hypertension were much lower in the FH than the non-FH group. Both baseline and on-treatment lipid parameters were higher in FH than non-FH subjects, except for triglyceride levels. TC and LDL-C reductions were greater with carriers of homozygous *CYP3A4*1G/*1G*, *CYP3A5*1/*1* and *CYP3AP1*1/*1*, suggesting the importance of these enzyme activities towards simvastatin in the lipid lowering responses in FH patients. The findings in our study that the subjects with *CYP3A4*1G/*1G* genotype had greater lipid lowering responses were similar to those reported in another study on atorvastatin (Gao et al., 2008), while that on *CYP3A5*1/*1* was in contrast to a previous report (Kivisto et al., 2004). The comparisons under their respective dominant or recessive models were not of statistical significance, suggesting further research would be warranted to provide more definitive results. A similar study with fluvastatin in FH patients suggested polymorphic SNPs at *CETP* and *ABCB1* genes were associated with differences in lipid lowering responses (Bercovich et al., 2006). FH is a common inherited disorder. The genetic basis of FH can usually be traced to a single genetic origin, leading to the mode of inheritance as autosomal dominant or recessive. Mutations at the *LDLR* gene, apolipoprotein B-100 (*APOB*) gene and *PCSK9* gene have been related to different FH genetic classifications (Izar et al., 2010). Detailed studies suggested the *LDLR* gene mutation diversity among FH patients would result in variations of lipid response to simvastatin treatment (Miltiadous et al., 2005; Vohl et al., 2002). A recent publication suggested an interaction between FH patients and a SNP with

ABCA1 leading to increased coronary heart disease risk, which could been eliminated by statins (Versmissen et al., 2011). Therefore, interpretation of our study results could be seen as indicative that the simvastatin lipid lowering response would be largely unaffected by *CYP3A* polymorphisms, unless the individuals were diagnosed with familial hypercholestolaemia due to certain genetic disorders.

This study has several limitations. The subjects were identified and recruited on an opportunistic basis and it is not a proper prospective randomized study. The period of treatment was not the same for all subjects because of practical reasons. It was not possible to control for changes in diet or compliance to medication during the period of treatment, but using a relatively short treatment period and excluding subjects who reported poor medication compliance were adopted to try to reduce these problems. Overall results would warrant further research for confirmation of findings, and to investigate possible interactions with other disease modifying genes.

2.6. Conclusion

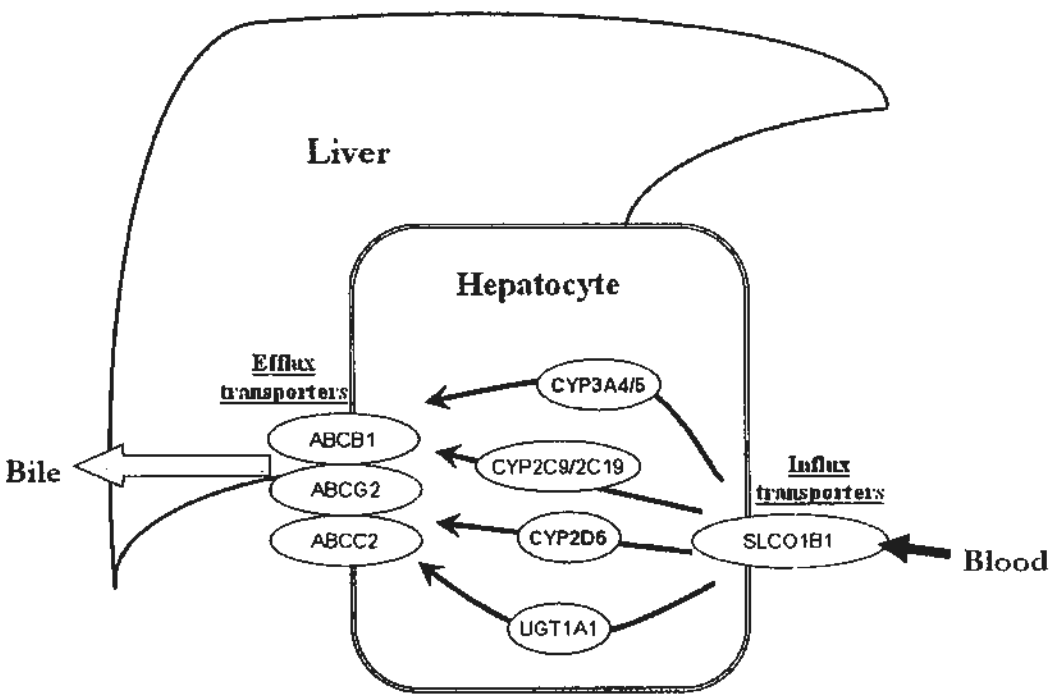
The cytochrome P450 enzyme system plays important roles in hepatic metabolism of xenobiotics. CYP3A isoenzymes are the major CYP enzymes because of their abundance in hepatocytes. We examined the role of candidate SNPs in *CYP3A4*1G, CYP3AP1*3* and *CYP3A5*3* which were previously noted to be associated with altered CYP3A4/3A5 enzyme expressions and activities and which may probably affect simvastatin metabolism. No statistically significant relationship was noted between these SNPs and phenotypic expressions in terms of the non-invasive urinary 6 β -OHC/C concentration ratio. Low-density lipoprotein cholesterol (LDL-C) reduction is the key clinical endpoint measurement with statins. Our analysis did not find any statistically significant relationship between LDL-C lowering and individual SNPs, gene-gene combinations, gene-gender interactions or urinary 6 β -OHC/C ratio. However, working on gene-disease interactions suggested patients with familial hypercholesterolaemia had significant variations relating the candidate SNPs for TC and LDL-C reductions. In conclusion, *CYP3A* SNPs may not be influential factors in the lipid lowering responses to simvastatin treatment. Previous studies had suggested *CYP3A4/5* polymorphisms might affect the pharmacokinetic profile of simvastatin, but this did not appear to be relevant to our findings. A noteworthy finding was the significant relationship among familial hypercholesterolaemic (FH) patients and greater lipid lowering response in carriers of homozygous *CYP3A4*1G/*1G, CYP3A5*1/*1* and *CYP3AP1*1/*1*, which may suggest a gene-disease interaction. Whether the interaction is on a pharmacodynamic basis on simvastatin response, or a gene-gene interaction involving *CYP3A* polymorphisms and FH genetic disorder would warrant further research, which could lead to future personalized therapy.

3. Impact of *CYP2D6* polymorphisms on the pharmacokinetics of lovastatin and simvastatin in Chinese Subjects

3.1. Introduction

It is generally accepted that simvastatin is metabolized predominantly by Cytochrome P-450 (CYP) 3A enzymes (Gruer et al., 1999). The pathways involved in the metabolism and disposition of simvastatin are shown in Figure 3.1, including the uptake from the blood stream into hepatocytes via uptake transporters SLCO1B1, then within the hepatocyte simvastatin undergoes oxidative metabolism or glucuronidation, before elimination via efflux transporters into the bile. The CYP3A4/5 enzymes constitute the most important pathway for the oxidative metabolism (Hu et al., 2009a).

Figure 3.1: Possible metabolic pathway of simvastatin



Ref: 1) www.pharmgkb.org by PharmGKB Project, USA
2) Hu M. et al. Current Pharmacogenomics and Personalized Medicine 2009; 7: 1-26.

The interactions from concomitant uses of simvastatin and CYP3A inhibitors leading to myopathy and myalgia have been frequently reported (Kanathur et al., 2001). The importance of CYP3A enzyme-mediated drug-drug interactions have been emphasized (Worz and Bottorff, 2001), and this should be considered in daily practice as a precaution towards myotoxicity (Molden et al., 2008). Case reports of myopathy in Chinese subjects from co-medication with simvastatin and diltiazem have suggested that the likely cause is through CYP3A inhibition (Hu et al., 2011a). Although myopathy is typically associated with high plasma concentrations of the active drug or active metabolites, the exact mechanism of statin-related myopathy remains uncertain (Thompson et al., 2003). In various studies on CYP3A inhibitors and statins, large increments in systemic exposure have been observed, such as 4-5-fold with cerivastatin when co-administered with gemfibrozil which has been linked to a high incidence of myopathy and rhabdomyolysis (Backman et al., 2002). Similar interactions with gemfibrozil result in 2.2-fold increase of systemic exposure with rosuvastatin (Schneck et al., 2004) and 2-3-fold with simvastatin (Backman et al., 2000; Neuvonen et al., 2006).

There have been previous reports suggesting that CYP2D6 activity may also affect the pharmacokinetics and pharmacodynamics of simvastatin (Mulder et al., 2001; Nordin et al., 1997; Vermes and Vermes, 2004; Zuccaro et al., 2007). It is well known that the encoding gene of *CYP2D6* is highly polymorphic. The aim of this study was to evaluate the effects of the common polymorphisms in *CYP2D6* found in Chinese subjects on simvastatin and lovastatin pharmacokinetics in healthy volunteers in Hong Kong.

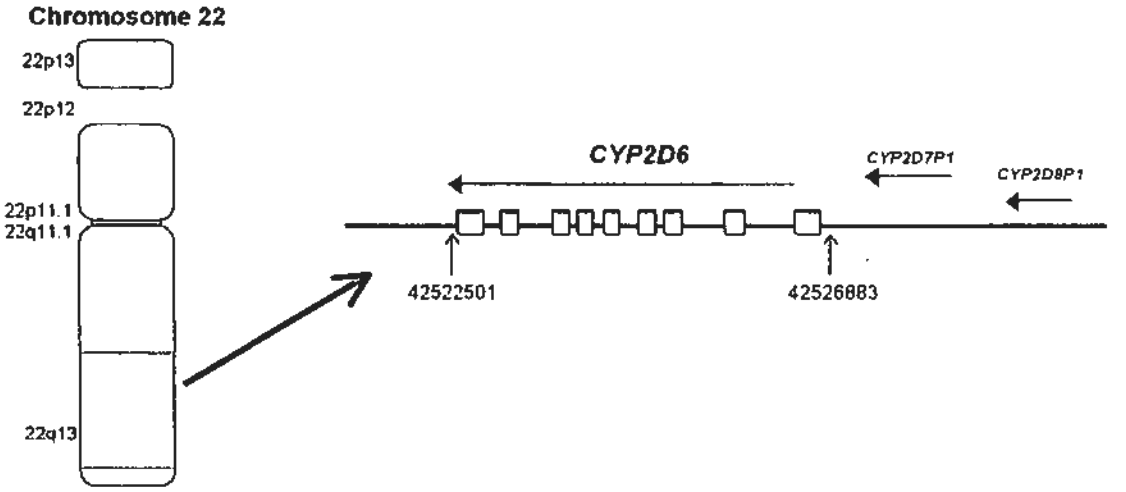
3.2. CYP2D6 isoenzyme

Cytochrome P450 (CYP) 2D6 has been widely studied for its involvement in pharmacogenetic effects on several drugs. CYP2D6 is mainly found in liver, but it is also be found in the lungs and heart (Frank et al., 2007). Within the hepatic CYP families, CYP2D6 accounts for only a small percentage (2-4%) of the total enzymes, but it appears to be responsible for metabolism of 20-25% of drugs and xenobiotics (Cascorbi, 2003; Frank et al., 2007). The list of drugs metabolized through this pathway includes antidepressants, antipsychotics, antiemetics, antihistamines, opioids, nicergoline and tamoxifen (Zhou, 2009b).

3.2.1. *CYP2D6* genetic polymorphism

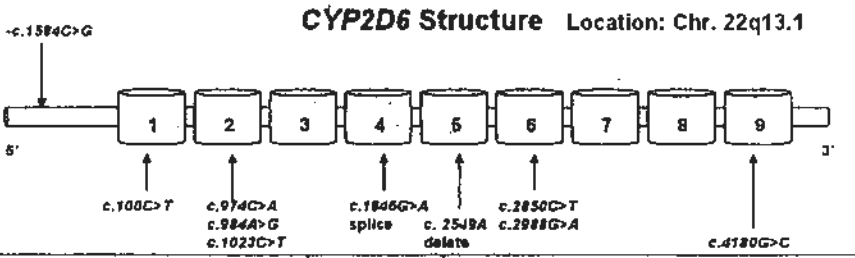
The *CYP2D6* gene is located in chromosome 22q13.1 (Figure 3.2). It is highly polymorphic with more than 90 allelic variants identified (Daly et al., 1996; Ingelman-Sundberg and Evans, 2001; Meyer and Zanger, 1997). It has also been noted there is wide inter-individual and inter-ethnic variability due to these polymorphisms (Figure 3.3). About 7%-10% of Caucasians lack CYP2D6 activity due to gene deletions or loss-of-activity mutations, whereas up to 29% of Ethiopians display gene duplications, leading to elevated so-called ultrarapid metabolization rates (Cascorbi, 2003). In a study with 119 Hong Kong Chinese subjects, as many as 65% of them were noted to be carriers of *CYP2D6*10*, an allele associated with reduced enzyme activity (Garcia-Barcelo et al., 2000). This allele codes for a protein with CYP2D6 enzyme activity regarded as an "intermediate metaboliser" compared comparing to wild-type enzyme activity with the **1* or **2* alleles. Such a high frequency is likely to have significant impact on regular clinical practice for the drugs which are metabolized through this enzyme.

Figure 3.2: Gene Map of chromosome 22 and *CYP2D6* with base no.



Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

Figure 3.3: Schematic presentation of *CYP2D6* structure with SNPs location

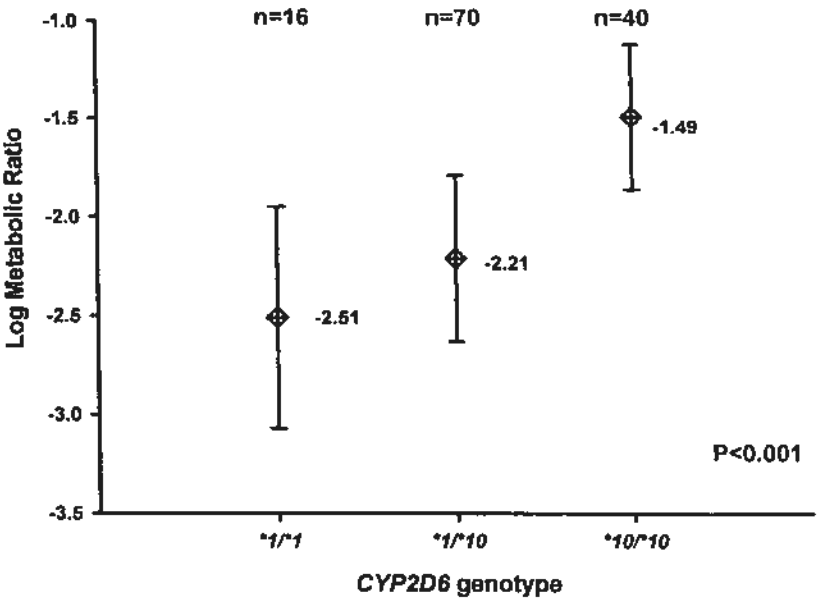


Allele	Enzyme Activity	Frequency distribution		
		Whites	Blacks	Asians
*1 wild-type	Normal	33.4-83.8%	27.8-90.4%	22.7-49.0%
*2 (c.-1584C>G, c.2850C>T, c.4180G>C)	Normal	32.4-35.3%	9.9-40.0%	8.0-13.4%
*3 (c.2549A del)	Inactive	0.0-2.5%%	0.0-1.0%	0.0%
*4 (c.100C>T, c.974C>A, c.984A>G, c.1846G>A splice, c.4180G>C)	Inactive	11.3-28.6%	0.9-9.3%	0.2-0.8%
*5 gene deletion	No Activity	0.6-7.3%	3.3-9.0%	1.2-6.2%
*10 (c.100C>T, c.4180G>C)	Decreased	1.4-6.1%	1.0-8.6%	38.1-70.0%
*17 (c.1023C>T, c.2850C>T, c.4180G>C)	Decreased	0.0-1.1%	9.0-34.0%	0.0%
*41 (c.-1584C>G, c.2850C>T, c.2988G>A, c.4180G>C)	Decreased	10-20%	-	

Ref: 1. www.cypalleles.ki.se by Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee
2. www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

The genotypes for *CYP2D6* are classified according to their mean effect on the phenotype and can be differentiated into four subgroups: poor (PM), intermediate (IM), extensive (EM), and ultrarapid metabolizers (UM). Phenotyping can be performed in vivo by evaluation of the Metabolic Ratio (MR) from urinary recovery of oxidative metabolites and the original probe drugs, e.g. debrisoquine or dextromethorphan. The CYP2D6 enzymatic activity classified as PM was noted in less than 1% of Asians (Kim et al., 2004), but up to 10% of Europeans (Fux et al., 2005). These individuals were devoid of activity for drug metabolism via CYP2D6-dependent pathways. The UM phenotype is at the other end of spectrum, and is due to co-existence of multiple *CYP2D6* gene copies. The IM and EM phenotypes with a certain degree of residual activity are phenotypes occurring because their alleles result in reduced enzyme activities (Zanger et al., 2004). There is a clear overlap of the Metabolic Ratios in IM and EM subjects, whereas PM and UM were quoted to differ from EM individuals by 5- to 15-fold values of Metabolic Ratio (Zhou, 2009a). The *CYP2D6 *1* and **2* alleles are the wild-types with "normal" enzyme activities, the**5* alleles are the PM type because of gene deletion, while the **10* carriers are expected to be IM type. In a study using the MR as evaluated by urinary recovery of dextromethorphan vs. the metabolite dextrophan, to be the phenotypic measurement of CYP2D6 enzymatic activity in Chinese subjects, it was found that the mean metabolic ratio of homozygous **10/*10* and heterozygous **1/*10* individuals were 0.042 and 0.009 respectively, which would be 6.1 and 1.3 times that of the homozygous **1/*1* carriers (mean MR=0.007), respectively (Cai et al., 2007) (Figure 3.4). Hence, the IM status of *CYP2D6*10* carriers and the high allele frequency among Asians prompted further investigation into the role of this polymorphism in the pharmacokinetic and pharmacodynamic of various drugs. There have been previous reports on this allele with haloperidol (Park et al., 2006), tramodol (Li et al., 2010c; Wang et al., 2006) and metoprolol (Jin et al., 2008), suggesting the presence of the *CYP2D6 *10* allele will affect the metabolic clearance of the respective drugs.

Figure 3.4: Metabolic Ratio (MR) and the influence from *CYP2D6* polymorphism



Metabolic Ratio determined using dextramethorphan as probe drug.
Adapted from: Cai WM et al. Clin Pharmacol Ther 2007; 81(1): 95-98.

3.2.2. *CYP2D6* polymorphisms and statins

As mentioned above, simvastatin is predominantly metabolized by Cytochrome P-450 (CYP) 3A enzymes, but there have been previous reports suggesting that CYP2D6 activity may also influence the pharmacokinetics and pharmacodynamics of simvastatin. A pilot study has linked CYP2D6 enzyme activity with the change in plasma cholesterol levels resulting from treatment with simvastatin. In this report, the metabolic ratio was calculated from debrisoquine hydroxylation, an accepted phenotyping method for CYP2D6 enzyme activity, and this suggested greater changes in cholesterol levels after treatment with simvastatin were related to reduced CYP2D6 enzyme activity presumably resulting from genetic influence (Nordin et al., 1997).

Previous reports which examined the cholesterol-lowering effects of simvastatin in relation to CYP2D6 activity in Caucasians have pointed out the consequence of *CYP2D6* genotype on efficacy and primarily tolerability of simvastatin in these patient groups. It was suggested that carriers of *CYP2D6*4* alleles resulting in PM

phenotypes could have a larger decrease of 0.23 vs. 0.10 mmol/L per mg of simvastatin in total cholesterol levels compared to the wild-types, but this was not associated with tolerability in these reports (Mulder et al., 2001; Zuccaro et al., 2007). Conversely, studies of the influence of CYP2D6 enzyme activity on the pharmacokinetics of simvastatin *in vitro* failed to show any association with hepatic metabolism of simvastatin acid (Prueksaritanont et al., 2003). These clinical studies were performed in Caucasians in whom the alleles show wide inter-individual variability due to the genetic polymorphisms. The *CYP2D6*4* allele was found in less than 1% of Asian populations. On the other hand, the mislabeled "intermediate metabolizers" with *CYP2D6*10* alleles will actually result in lower CYP2D6 enzyme activities. The *CYP2D6*10* allele has the same single nucleotide polymorphism of *CYP2D6 c.100C>T* as the *CYP2D6*4* allele. Another poor metabolizer variant is the *CYP2D6*5* allele resulting in gene deletion of *CYP2D6* which would be found in no more than 10% of the population and is probably less frequent among Asians than Caucasians (Bertilsson et al., 2002). The high prevalence of *CYP2D6*10* alleles in Chinese populations may significantly influence the systemic exposure to simvastatin and lovastatin in this population if these drugs are influenced by this pathway (Owen et al., 2009; Sistonen et al., 2009). It was on this basis that we attempted to evaluate the relationship of *CYP2D6* polymorphisms with the major pharmacokinetic parameters of simvastatin and lovastatin in Chinese subjects. The protocol of this study was to examine the possible variations among pharmacokinetic parameters in relation to the major genotype groups of *CYP2D6* for both the parent drug simvastatin lactone and its major active metabolite simvastatin acid.

3.2.3. Simvastatin and lovastatin

Lovastatin is a fungal product derivative and simvastatin is semi-synthetic and they are structurally closely related. These two statins are administered as inactive prodrugs in the lactone forms and are transformed into the biologically active acid form in the body whereas other newer statins are given in the active open acid form (Neuvonen et al., 2006; Shitara and Sugiyama, 2006). The systemic bioavailability of simvastatin and lovastatin is very low, usually ≤5%, which is mainly related to their extensive first-pass metabolism mediated by CYP3A enzymes in the intestine and liver (Williams and Feely, 2002).

Lovastatin is a natural product isolated from the fungus *Aspergillus terreus*, whereas simvastatin is a semi-synthetic analogue with an additional methyl group forming a 2,2-dimethyl butyrate side chain which results in increased potency of 2- to 3-fold in clinical usage (Christians et al., 1998). In 1998, the FDA placed a ban on the sale of dietary supplements derived from red yeast rice, which naturally contains cholesterol-lowering amounts of monacolin K, that is chemically identical to lovastatin, arguing that products containing prescription agents require drug approval (McCarthy, 1998). This ban was subsequently rescinded, in light of the law that natural products are not patentable.

Both statins are administered as inactive prodrugs in the lactone form and are transformed into the biologically active acid form in the plasma and liver and probably other tissues. They undergo extensive first-pass metabolism largely mediated by CYP3A in the intestine and liver resulting in low systemic bioavailability of ≤5% (Williams and Feely, 2002). Lovastatin is oxidized by rat and human liver microsomes at the 6'-position resulting in 6' beta-hydroxy-, 6'-exomethylene- and 6'-methoxy-lovastatin (Wang et al., 1991). Similarly, simvastatin lactone and acid were metabolized mainly at the 6' alpha-methyl group of the 6'chiral center of the naphthalene ring through the intermediate 6'-exomethylene product to 6' beta-hydroxy- and 6' beta-carboxy-simvastatin acid (Vickers and Duncan, 1991; Vickers et al., 1990a) which have about half the

potency of simvastatin acid to inhibit HMG-CoA reductase activity. In human liver microsomes, ≥ 80% of simvastatin acid metabolism was catalysed by CYP3A4/5 with a minor contribution from CYP2C8 but not by CYP2D6 (Prueksaritanont et al., 2003). This was confirmed in another study which also showed that the metabolic clearance of simvastatin lactone was 70-fold higher than that of the acid (Fujino et al., 2004) supporting earlier work that the lactone is metabolized much more effectively than the acid (Vickers et al., 1990b). Simvastatin undergoes some dehydrogenation at the 3"-positon of the side chain and both lovastatin and simvastatin also undergo beta-oxidation of the dihydroxy heptanoic or heptanoic acid side chain in rodents but not in humans (Prueksaritanont et al., 2001).

Lovastatin acid and simvastatin acid showed moderate affinity for dextromethorphan O-demethylation (CYP2D6), as well as CYP3A4 and CYP2C9 in one study (Transon et al., 1996) but this has not been found in other studies .

3.3. Methods

3.3.1. Study design

Two separate single-dose pharmacokinetic studies were performed with lovastatin and simvastatin, respectively. Study protocols were approved by the local Clinical Research Ethics Committee. Written informed consent was obtained from all subjects.

3.3.2. Study subjects

In the lovastatin pharmacokinetic study, 23 healthy male Chinese volunteers (aged 21 to 26 years, weight 55.6 to 70.6 kg) were recruited after prescreening for *CYP2D6* genotypes. These subjects were classified into five different *CYP2D6* genotype groups: group I, *CYP2D6 wt/wt* (**1* or **2*), n=4; group II, *CYP2D6 wt/*10*, n=7; group III, *CYP2D6*10/*10*, n=7; group IV, *CYP2D6*5/*10*, n=3; group V, *CYP2D6*5/*5*, n=2. In the simvastatin pharmacokinetic study, a total of 16 healthy male Chinese volunteers (aged 20 to 25 years, weight 56.2 to 79.9 kg) were recruited without pre-screening of *CYP2D6* genotype, and this group of subjects represented a random population in terms of genotype distribution which was identified retrospectively (group I, *CYP2D6 wt/wt*, n=4; group II, *CYP2D6 wt/*10*, n=6; and group III, *CYP2D6*10/*10*, n=6). No *CYP2D6*5* allele was detected in this group of subjects. The demographic characteristics of the study subjects are shown in Table 3.1.

All subjects were non-smokers and in good health as determined from their medical history, physical examination, ECG evaluation and routine laboratory tests (blood chemistry, haematology and urine analysis). All subjects were required not to take any prescription or nonprescription medication 2 weeks before and throughout the study. They were instructed to abstain from grapefruit, grapefruit juice, herbal dietary supplements, and caffeine-containing beverages including coffee and tea 3 days before the study and during the study period.

Table 3.1: Demographic characteristics of study subjects

Characteristics	*CYP2D6* genotypes				
	wt/wt	*wt/*10*	**10/*10*	**5/*10*	**5/*5*
Lovastatin study	(n=4)	(n=7)	(n=7)	(n=3)	(n=2)
Age (years)	22.2 ± 1.9	22.2 ± 1.0	23.1 ± 1.4	23.7 ± 2.1	22.0 ± 0.0
Body weight (kg)	60.2 ± 1.2	64.6 ± 5.7	60.9 ± 3.8	66.1 ± 1.6	65.4 ± 3.1
Simvastatin study	(n=4)	(n=6)	(n=6)		
Age (years)	22.8 ± 2.2	22.0 ± 1.3	23.3 ± 0.8		
Body weight (kg)	64.4 ± 6.3	71.0 ± 6.8	70.3 ± 3.4		

Data are given as mean ± SD. *wt:* wild-type genotype of **1 or *2*

3.3.3. *CYP2D6* genotyping

A 10-ml blood sample was obtained from each subject and DNA was isolated using an extraction kit (QIAamp Blood Mini Kit, Qiagen). The *CYP2D6*5* allele was detected using long PCR with two primer sets, 2D6-F/-R and 2D6*5-F/-R, as described previously (Stamer et al., 2002). The *CYP2D6*10* allele was identified as the *c.100C>T* mutation with use of the method described by Wang et al (Wang et al., 1993). The mutated alleles (**10, *5*) and the wild-type (**1, *2*) constituted the predominant alleles in the Chinese population based on the previous studies reported in the literature (Garcia-Barcelo et al., 2000; Wang et al., 1993).

3.3.4. Pharmacokinetic studies

After an overnight fast, subjects received a single 40 mg (Mevacor, Merck Sharp & Dohme) oral dose of lovastatin with 240 ml of water. Standardized meals were served 4 and 10 h after dosing. Venous blood samples were collected at pre-dose (0 h) and at 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24 h post dosing. All blood samples were centrifuged immediately after collection, and the separated plasma samples were stored at –80 °C until analysis. The pharmacokinetic study of simvastatin was conducted in the same manner as that for lovastatin but using a single 40 mg dose of simvastatin (Zocor, Merck Sharp & Dohme).

The subjects were observed by research nurses during the pharmacokinetic studies and they were asked to report any adverse effects.

3.3.5. Determination of lovastatin, simvastatin and their metabolites

Plasma concentrations of lovastatin and lovastatin acid were determined by an LC-MS-MS method as described previously using simvastatin and simvastatin acid as the internal standards for lovastatin and lovastatin acid, respectively (Calaf et al., 1997). The analytes were extracted from plasma using a solid-phase extraction method, and quantified by the mass spectrometer using multiple reaction monitoring mode. The lower limit of quantification was 0.2 ng/ml for both lovastatin and lovastatin acid. The coefficient of variation (CV) of intra- and inter-day assay was < 8.6% and < 10.4% for lovastatin, and < 12.9% and < 13.4% for lovastatin acid, respectively. The accuracy of the assay ranged from 84.4% to 113.9%.

Plasma concentrations of simvastatin lactone and simvastatin acid were determined by a liquid chromatography-tandem mass spectrometry (LC-MS) using a method modified from previous studies (Wu et al., 1997; Zhao et al., 2000). Lovastatin and lovastatin acid were used as the internal standards for simvastatin and simvastatin acid, respectively. Simvastatin acid and its internal standard (lovastatin acid) were detected in negative mode (-4500 V). The lower limit of quantification was 0.5 ng/ml for both simvastatin and simvastatin acid. The CV of intra- and inter-day assay was < 8.1% and < 8.3% for simvastatin, and < 7.9% and < 8.0% for simvastatin acid, respectively. The accuracy of the assay ranged from 92.8% to 107.1%.

3.3.6. Pharmacokinetic Analysis

Pharmacokinetic parameters of lovastatin, simvastatin and their metabolites were calculated using the non-compartmental method, with the aid of the WinNolin program (Version 2.1, Pharsight Corp.). Peak plasma concentrations (C_{max}) of lovastatin, simvastatin and their metabolites were obtained directly from the

observed concentration-time data. The terminal elimination rate constant (λ_Z) was estimated by linear regression of the terminal portion of the concentration-time curve, and the elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated using the trapezoidal rule, and extrapolated to infinity. The apparent oral clearance (CL/F) was calculated as Dose/ $AUC_{0-\infty}$.

3.3.7. Statistical Analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise indicated and were analyzed using the statistical program SPSS 14.0 for Windows (SPSS Inc., Chicago, USA). The C_{max} and $AUC_{0-\infty}$ values were logarithmically transformed before analysis. The pharmacokinetic variables of lovastatin, simvastatin and their metabolites were compared between the *CYP2D6* genotypes using one-way ANOVA followed by Bonferroni post hoc test. To test the trend of changes in lovastatin pharmacokinetic parameters (C_{max}, $AUC_{0-\infty}$ and CL/F) in relation to the *CYP2D6* mutant alleles, the Jonckheere Terpstra trend test was performed. In the trend test, the order of subjects with zero, one or two mutant allele was defined *a priori*, according to the usual "gene-dose" effects observed for CYP isoenzymes. $P<0.05$ was considered statistically significant.

3.4. Results

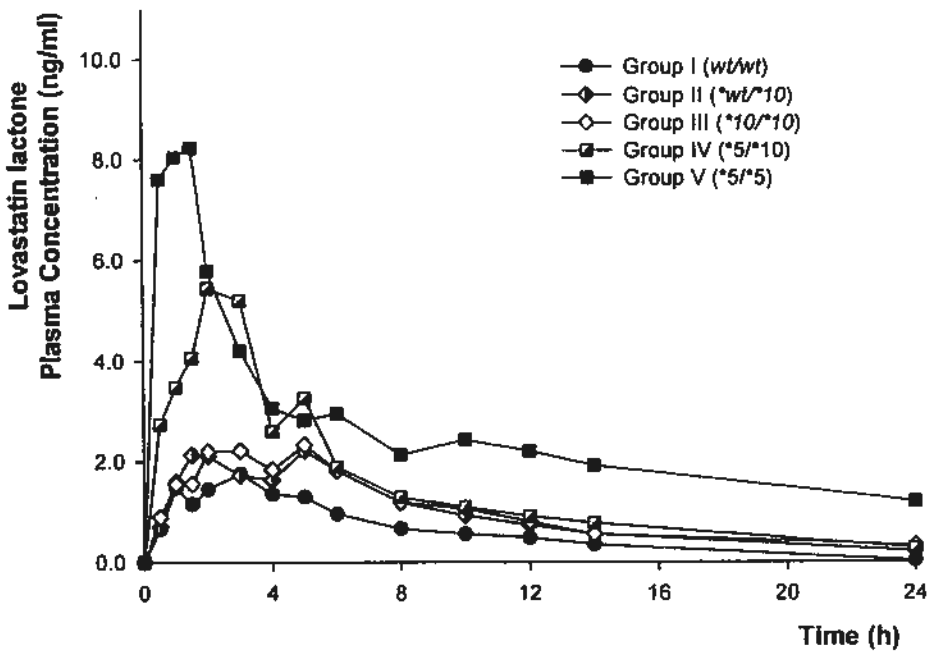
All subjects completed the study with compliance to the protocol. No clinically important adverse effects were observed.

3.4.1. Pharmacokinetics of lovastatin

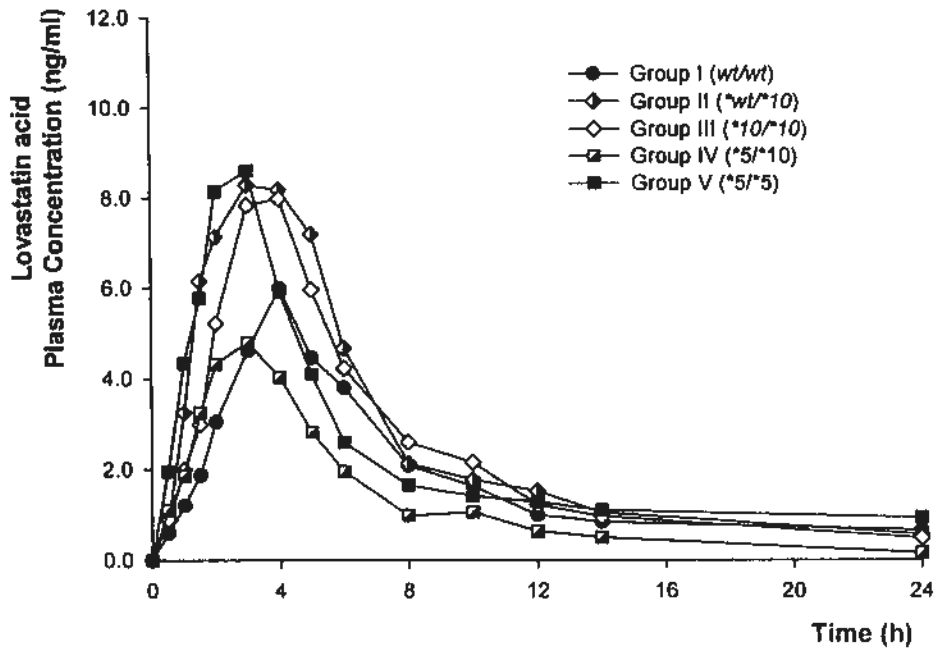
The mean plasma concentration-time profiles of lovastatin lactone and acid in the different *CYP2D6* genotypes are shown in Graph 3.1a and 3.1b, respectively.

Graph 3.1: Mean plasma concentration-time profiles of a) lovastatin lactone and b) lovastatin acid after a single oral dose of 40 mg lovastatin in subjects with different *CYP2D6* genotypes

a) Lovastatin lactone



b) Lovastatin acid



Major pharmacokinetic parameters of lovastatin lactone and acid are shown in Table 3.2. Significant differences were observed in the C_{max} and $AUC_{0-\infty}$ of lovastatin lactone among different genotype groups (P = 0.005 and P <0.001, respectively). Compared to the CYP2D6 *wt/wt* group, the $AUC_{0-\infty}$ values for lovastatin lactone increased (P <0.001) on average (95% CI) by ratios of 1.68 (0.28-3.08), 2.15 (0.75-3.55), 2.62 (0.91-4.33), 5.80 (3.86-7.74) in the *wt/*10*, **10/*10*, **10/*5* and **5/*5* genotype groups respectively and the values of lovastatin lactone plasma clearance (CL/F) were reduced on average (95% CI) by 38.5% (3.9%-73.2%), 52.2% (17.6%-86.8%), 62.1% (19.9%-104.3%), 84.4% (36.5%-132.3%) in these genotype groups, respectively (Table 3.2). The $t_{1/2}$ of lovastatin lactone was also significantly different among different genotype groups (P = 0.001). There was a significant trend in the changes in C_{max}, $AUC_{0-\infty}$ and CL/F of lovastatin lactone among the *CYP2D6* genotype groups, suggesting a gene-dose effect for the pharmacokinetics of lovastatin lactone (Graph 3.2a, 3.2b and 3.2c).

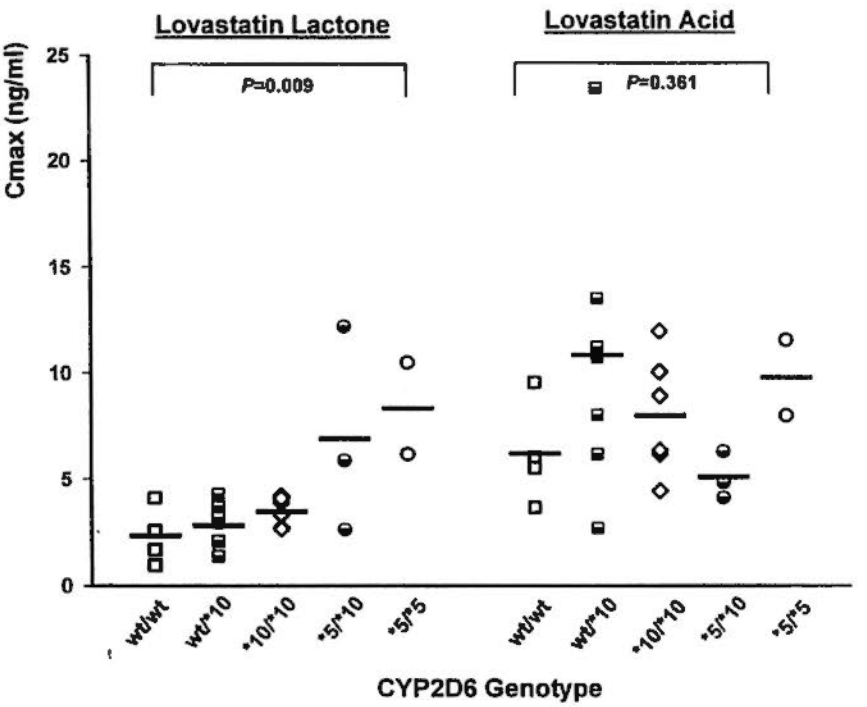
Table 3.2: Pharmacokinetic parameters of lovastatin lactone and acid according to *CYP2D6* genotype groups

Pharmacokinetic parameters	*CYP2D6* Genotype Groups					P
	wt/wt (n=4)	*wt/*10* (n=7)	**10/*10* (n=7)	**5/*10* (n=3)	**5/*5* (n=2)	
Lovastatin lactone						
C_{max} (ng/ml)	2.34 ± 1.36	2.81 ± 1.03	3.45 ± 0.63	6.89 ± 4.87	8.33 ± 3.05*	0.009
t_{max} (h)	3.63 ± 1.70	2.86 ± 1.75	3.21 ± 1.47	1.67 ± 1.26	1.25 ± 0.35	0.179
AUC_{0-t} (ng·h/ml)	12.38 ± 3.95	21.50±9.94††	26.98±6.66*	35.91±16.05*	61.88±12.76**	<0.001
$AUC_{0-\infty}$ (ng·h/ml)	15.03 ± 3.00	25.21±11.55††	32.32±8.63§*	39.34±15.27*	87.17±15.81**	<0.001
CL/F by Body Weight (L/h/kg)	45.61 ± 9.55	28.04±8.87†††	21.80±6.55§§*	17.30±7.58**	7.12±0.95***	<0.001
Lovastatin acid						
C_{max} (ng/ml)	6.19 ± 2.46	10.83 ± 6.61	7.98 ± 2.82	5.08 ± 1.11	9.78 ± 2.53	0.361
t_{max} (h)	4.50 ± 1.00	3.50 ± 1.50	3.00 ± 0.58	2.50 ± 0.87	2.50 ± 0.71	0.205
AUC_{0-t} (ng·h/ml)	41.35 ±19.30	60.00 ± 27.04	52.99 ± 24.08	29.63 ± 6.54	56.88 ± 12.57	0.262
$AUC_{0-\infty}$ (ng·h/ml)	44.86 ±20.33	67.36 ± 24.94	63.45 ± 27.70	34.90 ± 5.07	73.17 ± 26.09	0.118
CL/F by Body Weight (L/h/kg)	18.10 ±10.10	10.34 ± 3.89	11.50 ± 3.48	17.56 ± 2.19	8.86 ± 2.76	0.088

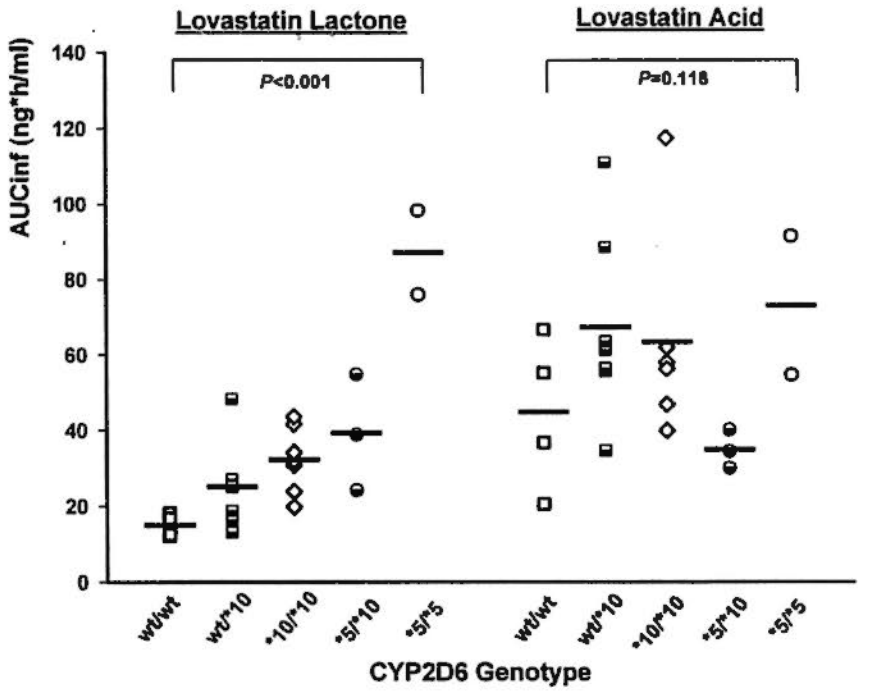
Data are presented as mean ± SD. * P<0.05 vs. *wt/wt*; ** P <0.01 vs. *wt/wt*; *** P <0.001 vs. *wt/wt*. † P <0.05 vs. **5/*5*; †† P <0.01 vs. **5/*5*; ††† P <0.001 vs. **5/*5*

Graph 3.2: Relationships between a) C_{max}, b) $AUC_{0-\infty}$ and c) CL/F of lovastatin lactone and acid and the *CYP2D6* genotypes

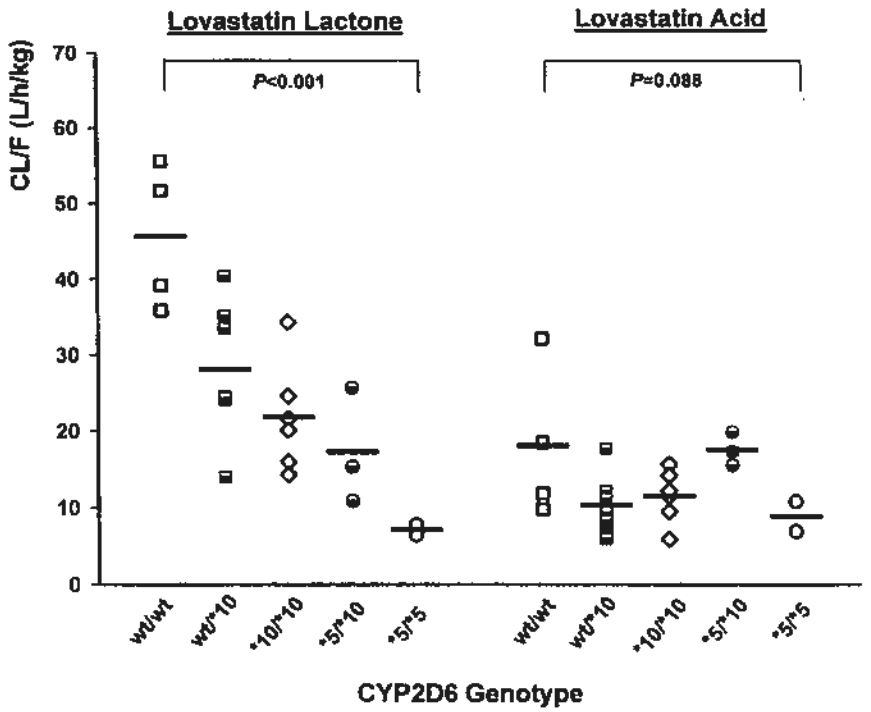
a) C_{max}



b) $AUC_{0-\infty}$



c) CL/F



However, the differences in plasma concentrations of lovastatin acid were not statistically significant among the different genotype groups (Table 3.2). The $AUC_{0-\infty}$ values for lovastatin acid tended to be higher in the groups with mutant alleles but there was a greater variation than that for lovastatin lactone and the differences were not statistically significant.

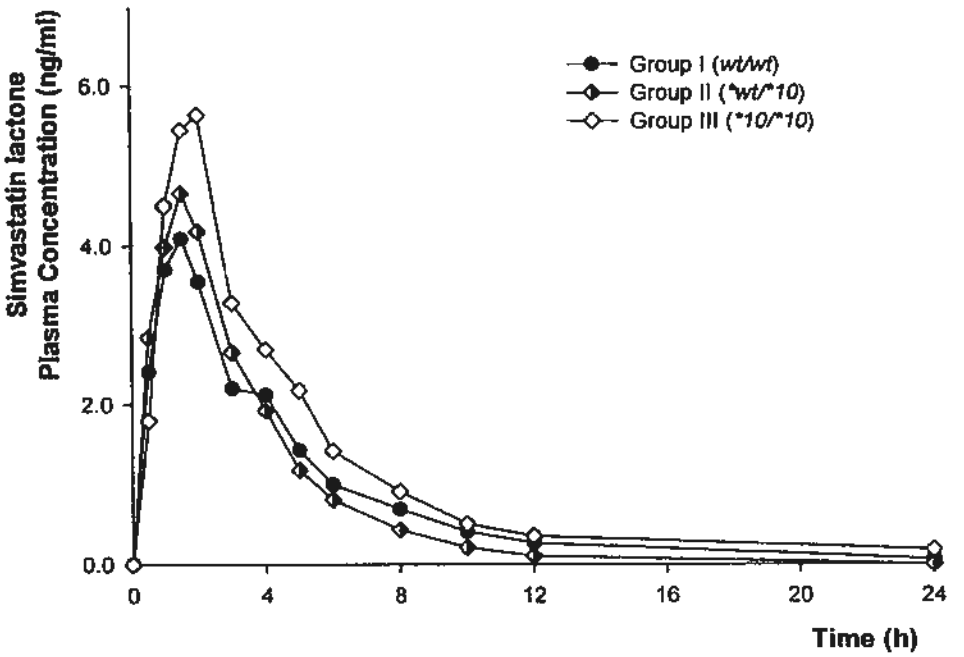
3.4.2. Pharmacokinetics of simvastatin

The mean plasma concentration-time profiles of simvastatin lactone and acid in the different *CYP2D6* genotypes are shown in Graph 3.3a and 3.3b, respectively.

There was no significant difference in the pharmacokinetic parameters for simvastatin and simvastatin acid among the three genotype groups (Table 3.3), although subjects with **10/*10* genotype tended to have an increased systemic exposure (C_{max} and $AUC_{0-\infty}$) to simvastatin lactone and a reduced CL/F compared to those with one or two copies of wild-type alleles but this was not significant (Graph 3.4a, 3.4b and 3.4c). No effect of *CYP2D6* genotypes was observed for the pharmacokinetics of simvastatin acid.

Graph 3.3: Mean plasma concentration-time profiles of a) simvastatin lactone and b) simvastatin acid after a single oral dose of 40 mg simvastatin in subjects with different *CYP2D6* genotypes

a) Simvastatin lactone



b) Simvastatin acid

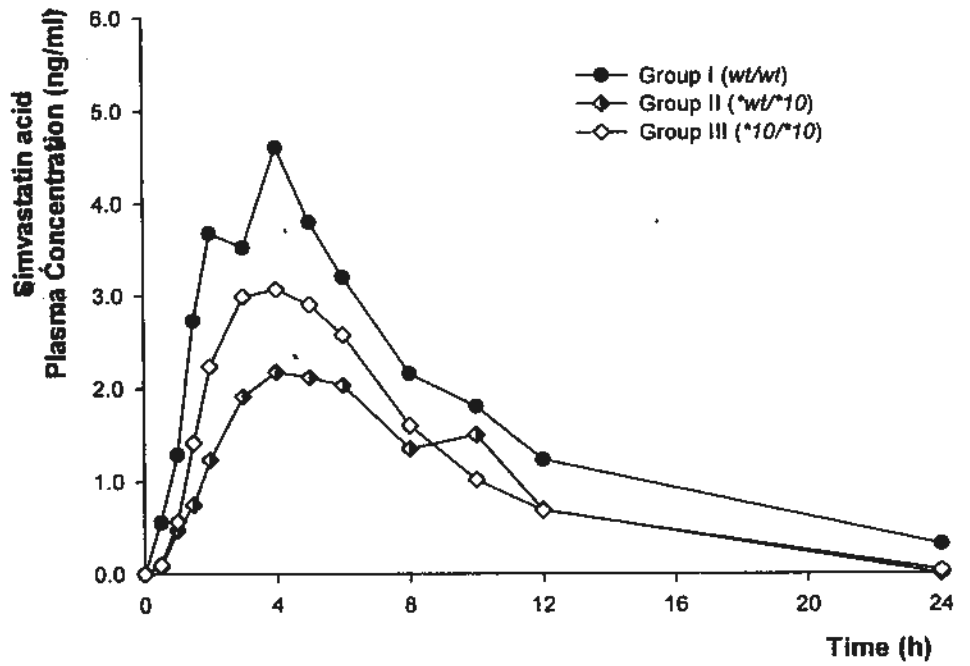


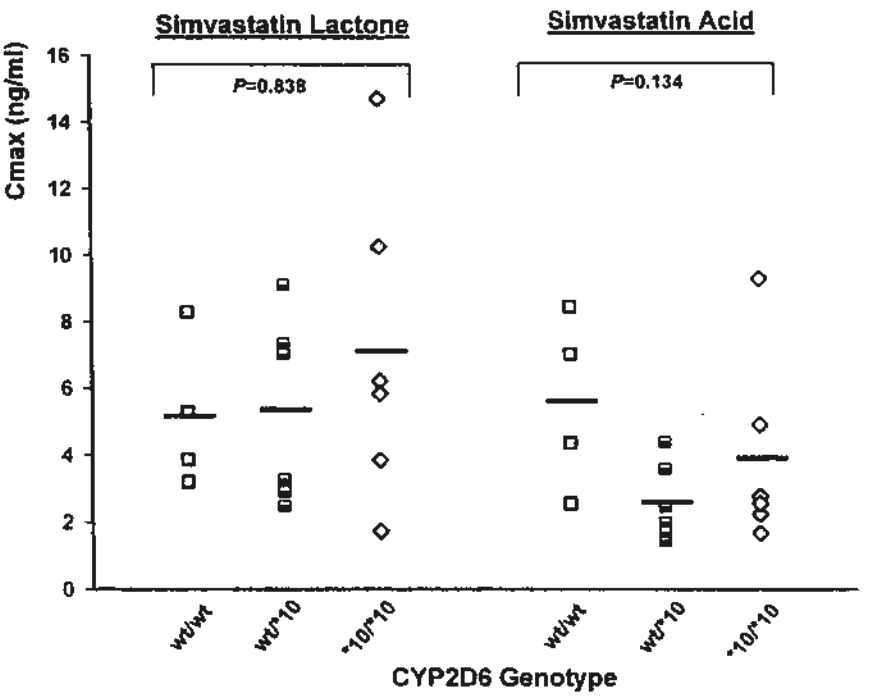
Table 3.3: Pharmacokinetic parameters of simvastatin lactone and acid according to *CYP2D6* genotype groups

Pharmacokinetic parameters	*CYP2D6* Genotype Groups			P-value
	wt/wt (n=4)	*wt/*10* (n=6)	**10/*10* (n=6)	
Simvastatin lactone				
C_{max} (ng/ml)	5.17 ± 2.26	5.36 ± 2.80	7.10 ± 4.68	0.838
t_{max} (h)	1.80 ± 1.53	1.67 ± 0.82	2.50 ± 1.23	0.395
AUC_{0-t} (ng·h/ml)	19.41 ± 5.87	17.81 ± 9.57	26.97 ± 9.95	0.230
$AUC_{0-\infty}$ (ng·h/ml)	20.85 ± 5.66	18.35 ± 9.67	31.42 ± 15.32	0.138
CL/F by Body Weight (L/h/kg)	30.93 ± 4.42	38.97 ± 20.89	21.31 ± 8.40	0.100
Simvastatin acid				
C_{max} (ng/ml)	5.61 ± 2.65	2.60 ± 1.16	3.91 ± 2.87	0.134
t_{max} (h)	3.75 ± 1.26	5.50 ± 2.35	4.67 ± 1.51	0.312
AUC_{0-t} (ng·h/ml)	38.69 ± 20.67	17.34 ± 7.47	23.56 ± 9.53	0.064
$AUC_{0-\infty}$ (ng·h/ml)	42.41 ± 20.61	22.11 ± 6.92	26.21 ± 7.87	0.068
CL/F by Body Weight (L/h/kg)	18.07 ± 9.23	27.16 ± 5.85	23.18 ± 5.88	0.117

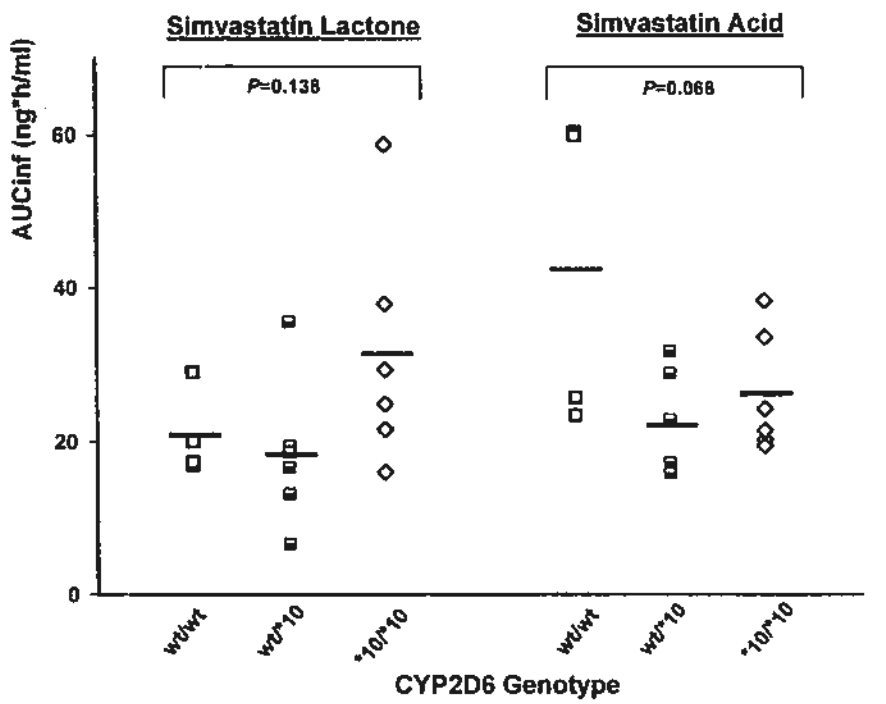
Data are presented as mean ± SD. Statistical comparison by Analysis of Variance (ANOVA). No statistical significance was found.

Graph 3.4: Relationships between a) C_{max}, b) $AUC_{0-\infty}$ and c) CL/F of simvastatin lactone and acid and the *CYP2D6* genotypes

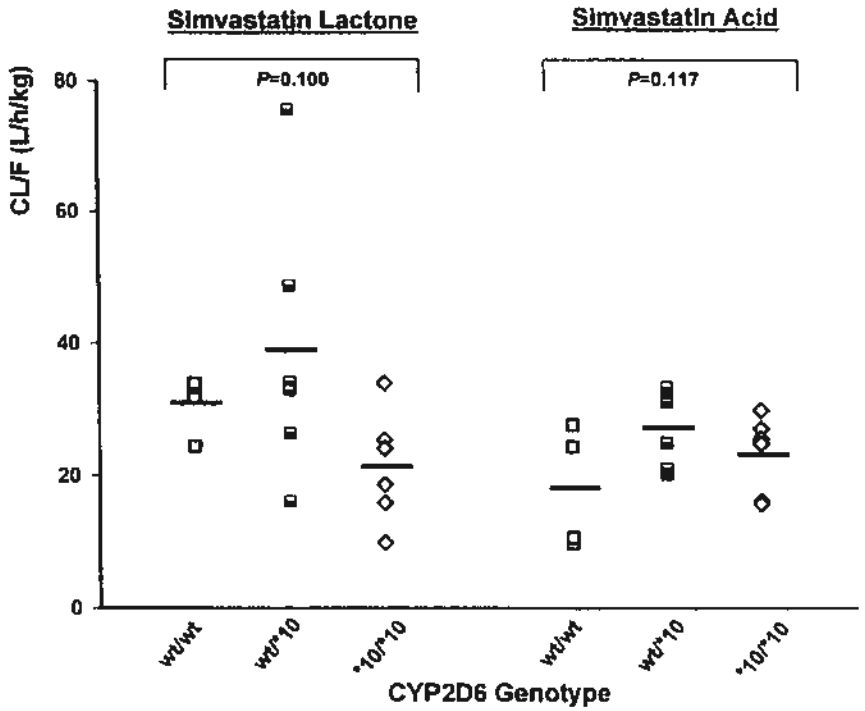
a) C_{max}



b) $AUC_{0-\infty}$



c) CL/F


Simvastatin Lactone
Simvastatin Acid
P=0.100
P=0.117
CL/F (L/h/kg)
80
60
40
20
0
wt/wt
wt/*10
*10/*10
wt/wt
wt/*10
*10/*10
CYP2D6 Genotype

3.5. Discussion

Myopathy and rhabdomyolysis are the most important side effects of the statins and are often related to increased systemic exposure from drug interactions or genetic predisposition. A genomewide association study (GWAS) to examine genetic determinants of myopathy with high doses of simvastatin identified a single strong association with a single-nucleotide polymorphism (SNP) in the *SLCO1B1* gene encoding the organic anion-transporting polypeptide OATP1B1 (Link et al., 2008). This SNP was in nearly complete linkage disequilibrium with the nonsynonymous *c.521T>C* polymorphism which is known to affect the pharmacokinetics of simvastatin acid but not the parent drug simvastatin lactone (Pasanen et al., 2006).

In the following chapter of this thesis, we have looked at the influence of pharmacogenetics of genes encoding drug transporters and the pharmacokinetics of another statin, pitavastatin. The current study focused on the gene *CYP2D6* which will affect the activity of the CYP2D6 enzyme and subsequently could affect the metabolic profile of simvastatin and lovastatin. These results suggest that polymorphisms in *CYP2D6* may influence the pharmacokinetics of lovastatin lactone and possibly simvastatin lactone but not their active acid metabolites in Chinese subjects. In comparison to values in the *CYP2D6* wild-type genotype, the $AUC_{0-\infty}$ and CL/F of lovastatin were significantly increased (67.8% - 480%) and decreased (38.5% - 84.4%), respectively, in genotype groups among carriers with *CYP2D6*10* or more significantly with **5* alleles. The apparent gene-dose effect demonstrated for lovastatin lactone pharmacokinetics suggests that the CYP2D6 enzyme may play a role in the disposition of the lovastatin parent drug.

Although there was no statistically significant difference in the pharmacokinetics of simvastatin lactone among the three *CYP2D6* genotype groups, which may be related to the small number of subjects and high inter-individual variation in each genotype group, subjects with **10/*10* genotype appeared to have a mean (95%CI) increase in systemic exposure (C_{max} and $AUC_{0-\infty}$) to simvastatin lactone compared

to those with wild-type alleles by 37.4% (84.4% - 159%) and 50.7% (47.6% - 149%), respectively. It is likely that the *CYP2D6*5* polymorphism may influence the pharmacokinetics of simvastatin to a greater extent as observed with lovastatin, since simvastatin is chemically very similar to lovastatin and undergoes similar metabolism (Neuvonen et al., 2006; Shitara and Sugiyama, 2006). In fact, some previous studies demonstrated that patients with defective mutated alleles of *CYP2D6* (**3* and **4*) or deletion of the gene (**5*) had greater lipid-lowering responses to simvastatin and increased risk of side effects (Mulder et al., 2001; Zuccaro et al., 2007), suggesting the involvement of CYP2D6 in the metabolism of simvastatin. However, both **3* and **4* alleles are very uncommon among Asian populations (0.0-0.8%; Figure 3.3). It was not possible to identify subjects with these genotypes and the effect of the *CYP2D6*10* polymorphism is the most important in our population.

Although lovastatin and simvastatin are generally considered to be metabolized predominantly via CYP3A4, an early *in vitro* study has shown that lovastatin and simvastatin both have a moderate affinity for CYP2D6 in addition to CYP3A4 and CYP2C9 (Transon et al., 1996) but this was not identified in subsequent *in vitro* studies (Fujino et al., 2004; Prueksaritanont et al., 1997; Prueksaritanont et al., 2003). Prueksaritanont et al reported that the metabolism of simvastatin acid in human liver microsomes was predominantly catalysed (≥80%) by CYP3A4/5, with a minor contribution from CYP2C8, whereas CYP2D6 and other major CYP isoforms were not involved in the hepatic metabolism of simvastatin acid (Prueksaritanont et al., 2003). Another *in vitro* study also showed that CYP2D6 did not play a role in the metabolism of simvastatin lactone or acid (Fujino et al., 2004). However, *in vitro* data does not always reflect *in vivo* conditions. For example, haloperidol, a commonly used antipsychotic drug, has been shown to be extensively metabolized by CYP3A4 but not CYP2D6 in *in vitro* studies. However, various *in vivo* pharmacogenetic studies have indicated that the *CYP2D6* polymorphisms affect the metabolism and disposition of haloperidol, suggesting the involvement of CYP2D6 in the *in vivo* biotransformation of haloperidol (Kudo and Ishizaki, 1999). There was no study to report whether

CYP2D6 polymorphisms would affect the pharmacokinetics of lovastatin and simvastatin, but several studies have suggested an increased cholesterol-lowering efficacy with simvastatin treatment in subjects with *CYP2D6* genotypes associated with decreased enzyme activities (Mulder et al., 2001; Nordin et al., 1997; Zuccaro et al., 2007). However, all these *in vivo* studies with *CYP2D6* polymorphisms only involved a small number of patients (n<60) and the results were not conclusive. Furthermore, Geisel et al did not find such an association in a study that included 41 patients with primary hypercholesterolaemia (Geisel et al., 2002). Further large scale studies are needed to verify the associations between *CYP2D6* polymorphisms and responses to simvastatin and lovastatin.

In the present study, the *CYP2D6* polymorphisms were only associated with the pharmacokinetics of lovastatin lactone but not the acid and a similar trend was observed with simvastatin lactone, suggesting that the CYP2D6 enzyme is involved in the metabolism of the parent lactone forms of lovastatin and simvastatin, but not their active acid metabolites. This is supported by previous reports showing that the acid forms of statins are much poorer substrates than their corresponding lactones, for the CYP450 enzymes (Fujino et al., 2004; Prueksaritanont et al., 2003) and the metabolic clearances of the simvastatin lactone was found to be 70-fold higher than that of simvastatin acid (Fujino et al., 2004). It has also been shown that lovastatin and simvastatin lactones, rather than their acid forms, are subject to oxidative metabolism to form various metabolites including 6-hydroxy, 6-exomethylene and 3-hydroxy derivatives (Greenspan et al., 1988; Mauro, 1993). At first sight, the greater effect of the *CYP2D6* genotype on the systemic exposure of lovastatin lactone rather than lovastatin acid might appear to be of less clinical significance. However, it may be of greater significance in respect of the side effect of muscle toxicity as penetration of statins into skeletal muscle seems to be largely dependent on their lipid solubility. The lactone forms of simvastatin and lovastatin are much more lipid soluble than their active hydroxyl acid forms and they can be converted into the acid forms in most cells of the body (Serajuddin et al., 1991). A significantly higher potency of the lactone forms of statins, than the respective acid forms, to induce myotoxicity was

observed in human skeletal muscle cells *in vitro* (Skottheim et al., 2008). Therefore, the increased systemic exposure to lactone forms of these 2 statins in the subjects with the *CYP2D6* poor metabolizers genotype may be associated with increased risk of statin-induced myopathy.

Our study with lovastatin has shown that subjects with **5* variant alleles had a markedly reduced CL/F and increased systemic exposure ($AUC_{0-\infty}$) for lovastatin lactone, by factors of up to 6.4- and 5.8-fold, respectively suggesting the *CYP2D6* poor metaboliser genotype can significantly influence the pharmacokinetics of lovastatin and probably simvastatin and these pharmacokinetic differences could lead to an increased risk of muscle toxicity when higher doses are used in subjects who are *CYP2D6* poor metabolisers. The intermediate metaboliser genotype (**10*) had limited impact on the pharmacokinetics of these 2 statins and the changes in the pharmacokinetic parameters in relation to the *CYP2D6*10* may not be of great clinical significance for individual patients. However, it has been reported that there was a trend for greater simvastatin exposure in Chinese and Japanese than Caucasians with a ratio (90% CI) of AUC_{0-t} of 1.23 (0.96 - 1.58) and 1.12 (0.87 - 1.44) in Chinese and Japanese compared to Caucasians, respectively (Birmingham et al., 2008), which may be related to the high prevalence of the **10* allele in Asian populations associated with reduced CYP2D6 activity.

To our knowledge, this may be the first report on the association between *CYP2D6* polymorphisms and the pharmacokinetics of lovastatin and potentially simvastatin. The findings of our studies were similar with both statins, suggesting a similar role of the CYP2D6 enzyme in their disposition pathway. However, in addition to lack of poor metabolisers in the simvastatin study, the small sample size and high inter-subject variability within each genotype group these are likely to lead to insufficient power to detect a statistically significant difference among genotype groups. Several other limitations of the study need to be considered. First of all, we did not evaluate or control for the influence of polymorphisms in *CYP3A4* or *SLCO1B1* which may be important for their respective pharmacokinetics. However, the prevalence of *CYP3A4* polymorphisms is very

low in Chinese populations and the function of these mutations is uncertain even though it may have an effect on simvastatin lipid lowering effects (Hu et al., 2009a; Wang et al., 2005a), whereas, the functional *c.521T>C* polymorphism in *SLCO1B1* was only found to affect the pharmacokinetics of simvastatin acid but not the parent simvastatin lactone (Pasanen et al., 2006), so these polymorphisms are unlikely to influence the current results. However, although CYP3A5 exhibited lower affinity for simvastatin than CYP3A4 (<1/3) (Prueksaritanont et al., 1997), the common *CYP3A5*3* polymorphism has been shown to affect the disposition of simvastatin (Kim et al., 2007), but this was not evaluated in the current analysis. Secondly, the determination of the total active HMG-CoA reductase inhibitors in addition to their plasma concentrations would be desirable. Furthermore, future studies to examine the lipid-lowering efficacy in relation to *CYP2D6* polymorphisms in a larger number of subjects would be needed to confirm the pharmacodynamic and clinical consequences of *CYP2D6* polymorphism-related differences in statin pharmacokinetics.

3.6. Conclusion

Although the use of lovastatin has declined with the availability of more potent statins, red yeast rice products are still widely used worldwide, even in the United States, where the consumption of red yeast rice products was reported as growing nearly 80% from 2005 to 2008 with annual sales of $20 million. Reductions in LDL-C of up to 30% with red yeast rice products have been reported in statin-intolerant patients (Becker et al., 2009; Halbert et al., 2010). The inclusion of lovastatin in red yeast rice and the lack of regulation with dietary products have raised concerns with FDA and among healthcare professionals on lovastatin related adverse effects (Klimek et al., 2009). In China, yeast rice products have been extensively used for therapy of patients with cardiovascular disorders for thousands of years (Li et al., 2010b). Therefore, it is important to identify genetic factors which may influence the pharmacokinetics of lovastatin and the safety of red yeast rice products, although red yeast rice products usually contain a low dose of lovastatin (<10mg) and we are not aware of any reports of myopathy with the use of these products (Gordon et al., 2010). There have been suggestions that these red yeast rice products may contain other active ingredients similar to lovastatin that act synergistically to lower LDL-C (Li et al., 2005). Therefore, our study in subjects of Chinese ethnics origins may be influenced by their daily diets which would largely be rice based.

In conclusion, this study demonstrated that the *CYP2D6*5* variant influenced the disposition of lovastatin lactone, but a minor effect of *CYP2D6 *10* on the disposition of lactones of lovastatin and simvastatin in Chinese populations cannot be ruled out. The results of this study may be more important for Caucasian populations in which the *CYP2D6* poor metaboliser genotype is more prevalent than in Asians. Further studies in Caucasian subjects are warranted to verify the impact of *CYP2D6* polymorphisms on the pharmacokinetics of statins.

4. Study of the influence on the pharmacokinetics of pitavastatin by single nucleotide polymorphisms (SNPs) in candidate genes for transporters and cytochrome P450 3A5, and possible food-gene interaction with Grapefruit Juice

4.1. Introduction

Statins have high inter-individual variability in terms of lipid lowering efficacy, as well as tolerance profile. Higher doses and drug interactions are associated with liver and skeletal muscle damage, which in the most severe cases can lead to rhabdomyolysis and acute renal failure (Thompson et al., 2003). The incidence of rhabdomyolysis with simvastatin was noted to be 0.05% in the Heart Protection Study (HPS) among 10,269 patients taking simvastatin 40 mg/day for 5 years (Heart Protection Study Collaborative Group, 2002). An American cohort study suggested the incidence of 0.44/10,000 persons-years of treatment with statin monotherapy, but this was raised to 5.98 in combination with fibrates (Graham et al., 2004). The occurrence of statin-induced myopathic adverse effects, ranging from mild myalgia to rhadomyolysis had been suggested as dose-related, plasma-concentration dependent and there are particular concerns with interacting concomitant medications, in high-risk individuals, or with high doses (Ballantyne et al., 2003; Hu et al., 2009a; Neuvonen et al., 2006; Rosenson, 2004), but the exact cellular mechanism of statin-related myopathy remains uncertain (Thompson et al., 2003)

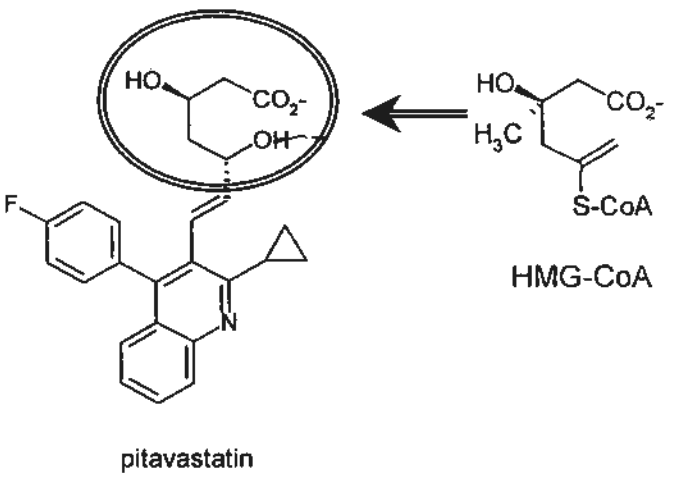
Individual statins differ in their pharmacokinetic characteristics (Neuvonen et al., 2008; Schachter, 2005; White, 2002; Williams and Feely, 2002). Simvastatin and lovastatin are administered as lactones, while others are given in the active acid form. CYP3A4 and CYP2C9 are involved in the oxidative metabolic clearance of certain statins. The role of drug transporters in drug disposition is being increasingly recognized. The influence from polymorphic changes of the related genes could affect transporter activity and hence the pharmacokinetic profiles of substrate drugs (Niemi, 2010; Petzinger and Geyer, 2006).

In a British genomewide association study (GWAS) with 12,000 patients, among patients using an 80 mg dose of simvastatin, myopathy was more common in individuals with a single nucleotide polymorphism (SNP) in the gene *SLCO1B1* encoding hepatic uptake transporter OATP1B1 (organic anion-transporting polypeptide 1B1). OATP1B1 is generally considered as liver-specific, even though OATP1B1 mRNA expression had been identified in human intestinal biopsies (Glaeser et al., 2007; Uno and Yasui-Furukori, 2006). The *SLCO1B1* SNP identified in the GWAS is in close linkage disequilibrium with a nonsynonymous SNP *c.521T>C*. The analysis concluded that those patients with a copy of the *C* allele would have an odds ratio of 4.5 for developing myopathy compared to those to the *T* allele, and that for homozygous *CC* carriers the odds ratio was 16.9 compared to *TT* individuals (The-SEARCH-Collaborative-Group, 2008). This chapter describes a study of the genetic influence from polymorphisms within genes encoding selected transporters including *SLCO1B1*, *ABCB1* and *ABCG2* on the pharmacokinetics of the new statin pitavastatin.

4.2. Pitavastatin – active hydroxy acid

Pitavastatin, a novel potent hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (Figure 4.1), is administered orally as the active acid and undergoes reversible conversion into the inactive lactone.

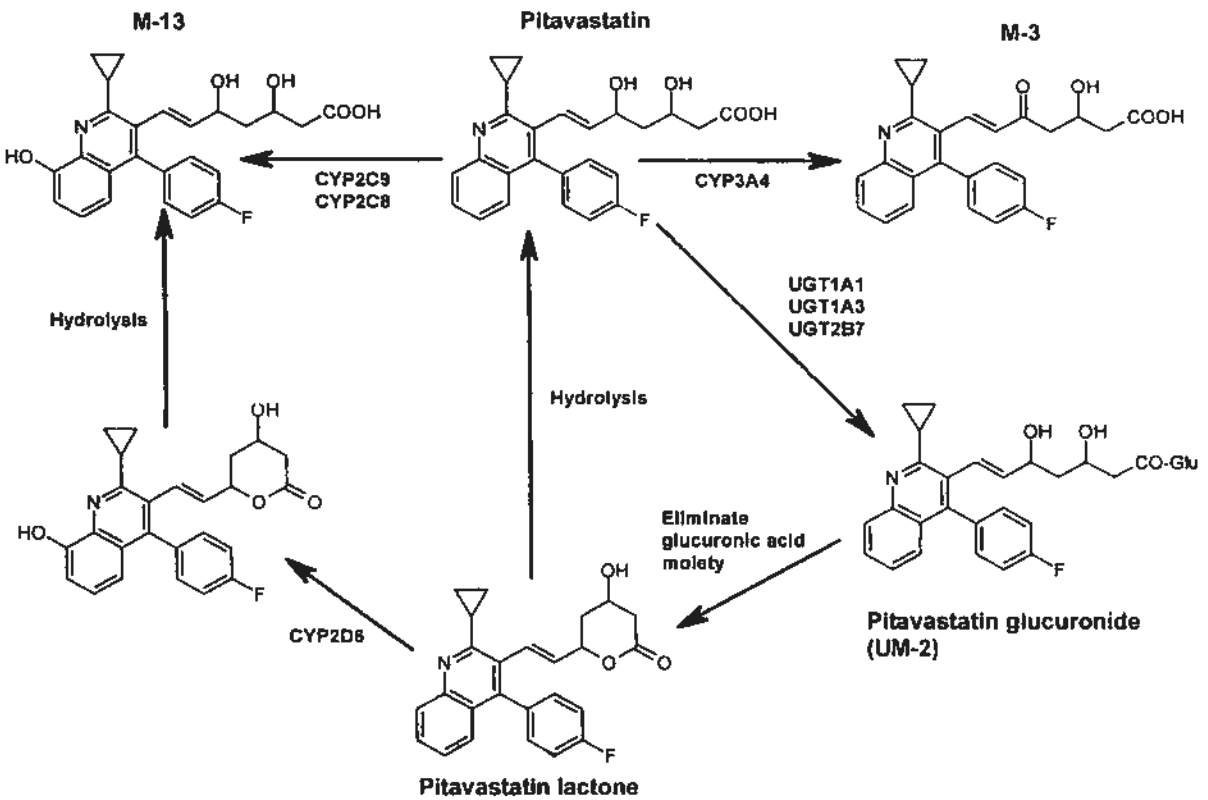
Figure 4.1: Pitavastatin structure and comparison to HMG-CoA substrate



pitavastatin

A recent study has suggested a 2 mg dose of pitavastatin would have comparable lipid lowering efficacy and reduction in proinflammatory markers with 2.5 mg of rosuvastatin in Japanese patients (Yanagi et al., 2011). The partition coefficient (n-octanol/phosphate buffer, pH 7.0) of pitavastatin is 31.7, can be regarded as lipophilic and comparable to atorvastatin (34.0) (Kajinami et al., 2003). The drug is nearly 99% protein bound, and undergoes glucuronidation and non-enzymic conversion into the inactive lactone metabolite (Fujino et al., 2003; Mukhtar et al., 2005; Yamada et al., 2003) (Figure 4.2).

Figure 4.2: Pitavastatin metabolic pathway



Adapted from Fujino H et al., *Xenobiotica* 2003; 33: 27-41.

Pitavastatin is minimally metabolized in the liver and is mainly excreted unchanged in the faeces via the bile (Catapano, 2010; Fujino et al., 2003). CYP3A4 is one of the Cytochrome P450 enzymes in the metabolic pathway but it only has a minor contribution in overall clearance. Its elimination is mainly unchanged through the liver and biliary pathway. P-glycoprotein (gene *ABCB1*) mediated transport does not play a major role in its disposition but organic anion transporting polypeptide (OATP) 1B1 (gene *SLCO1B1*) is the most important transporter for its hepatic uptake and the efflux transporter breast cancer resistance protein (BCRP, gene *ABCG2*) contributes to its biliary excretion (Fujino et al., 2005; Hirano et al., 2005; Hirano et al., 2004). *In vitro* studies have confirmed that pitavastatin acid but not the lactone is a substrate for these SLC and ABC transporters (Fujino et al., 2005). Polymorphisms in *SLCO1B1* leading to reduced

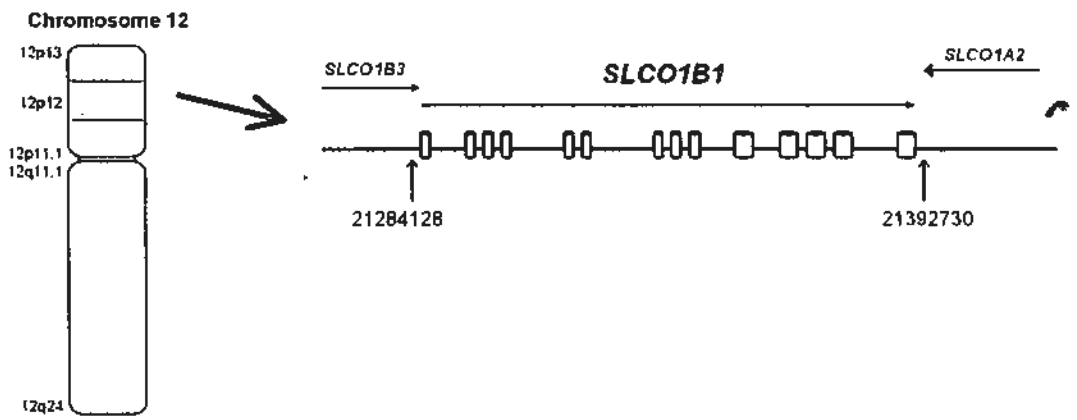
transporter efficacy have been noted in *in vitro* study to significantly affect cellular uptake of pitavastatin (Choi et al., 2011) and are known to influence its pharmacokinetics (Chung et al., 2005; Deng et al., 2008; Wen and Xiong, 2010b), but the common *ABCG2* variant *c.421C>A*, did not have a significant effect (Ieiri et al., 2007). In the present study we have considered the differential substrate specificity between acid and lactone when reviewing the pharmacokinetic profile in relation to genetic influences. The following sections describe the relevant transporters and their role in pitavastatin disposition.

4.3. Genetic influence

4.3.1. Uptake transporter – Organic Anion Transporting Polypeptide 1B1 (OATP1B1) and gene *SLCO1B1*

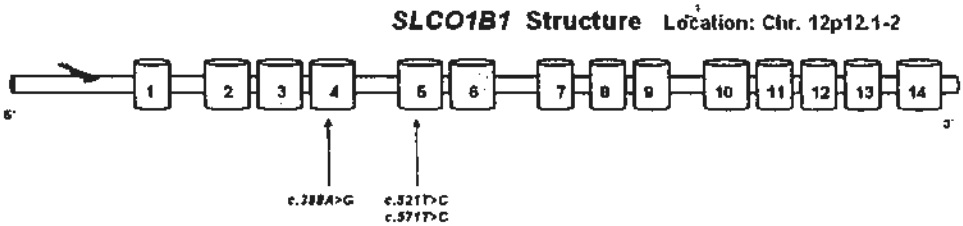
OATP1B1 (gene *SLCO1B1*) consists of 691 amino acids and has been shown to be involved in the hepatic uptake of statins (Hsiang et al., 1999). The protein is located in the sinusoidal membrane of the hepatocytes. It plays an important role in transmembrane transport of drugs. Drug-drug interactions from inhibitors like cyclosporin could cause significant changes in pharmacokinetic profiles leading to adverse effects (Kalliokoski and Niemi, 2009). The encoding gene *SLCO1B1* is located in chromosome 12p12.1-2 (Figure 4.3) (Hagenbuch and Meier, 2004) and is highly polymorphic (Figure 4.4). Respective polymorphisms could lead to changes in transporter efficacy, and subsequently large inter-individual variations of pharmacokinetic parameters and drug exposure.

Figure 4.3: Gene map of chromosome 12 and *SLCO1B1* with base no.



Ref: www.ncbi.nlm.nlh.goy by National Center for Biotechnology Information

Figure 4.4: Schematic presentation of *SLCO1B1*



Allele	Frequency distribution		
	Whites	Blacks	Asians
*1a			35.2% (Japanese)
*1b (c.388A>G)	30.0-37%	74.0 %	87 % (Malay), 79.6% (Chinese) 57% (Indian), 53.7% (Japanese)
*5 (c.521T>C)	14.0%	2.0%	11 % (Malay), 13% (Chinese) 6.5% (Indian), 0.7-16 (Japanese)
*15 (c.388A>G, c.521T>C)			7 % (Malay), 8.5% (Chinese) 3.4% (Indian), 10.3% (Japanese)

Ref: www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

The SNP *SLCO1B1 c.388A>G* (Asn130Asp) is common in all populations, and especially among Asians (60-90%) (Hu et al., 2009a; Jada et al., 2007). Another SNP *c.521T>C* (Val174Ala) is also relatively common in Asians and Europeans (10-20%), but less in Africans (1.9%) (Pasanen et al., 2008). This particular SNP *c.521T>C* was the one identified in the SEARCH study to be associated with myopathy with high doses of simvastatin. The SNP should lead to reduced uptake activity of the transporter. In a clinical study, a Japanese retrospective analysis suggested *c.521C* carriers had a greater lipid lowering response than *c.521T* carriers (-22.3% vs. -16.5%) (Tachibana-Iimori et al., 2004). A pharmacokinetic study with simvastatin suggested *c.521CC* carriers had 120% and 221% increases in area under the plasma concentration-time curve (AUC) compared to *c.521TC* and *c.521TT* carriers, while peak plasma concentration (C_{max}) increased by 162% and 200% , respectively (Pasanen et al., 2006). In other studies, *c.521CC* carriers were found to have 100% increase of AUC with atorvastatin and 65% with

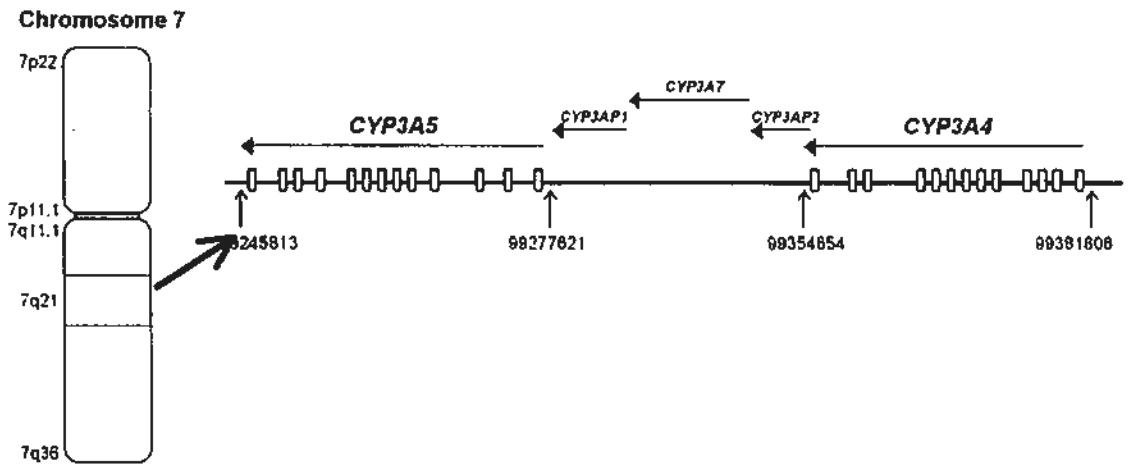
rosuvastatin (Pasanen et al., 2007) compared to the wild-type. The combination of the *c.388A>G* with the *c.521T>C* polymorphism results in four distinct haplotypes: **1a(c.388A-c.521T); *1b(c.388G-c.521T); *5(c.388A-c.521C)* and **15(c.388G-c.521C)* with differences in transporter efficacy which may differ for different substrates (Nishizato et al., 2003; Nozawa et al., 2002). Choi et al. reported that in their study with rosuvastatin subjects with one or two copies of the **15* haplotype had 14% and 72% increases, respectively, in AUCs (Choi et al., 2008). Heterozygous **15* carriers were reported to have 93% higher pravastatin AUC levels than non-carriers (Niemi et al., 2004) and homozygous **15* subjects had AUC levels, which were higher by 92%-149% (Ho et al., 2007). Lee et al. recently suggested that homozygous **15* carriers had 85% and 124% greater AUC values with atorvastatin than heterozygous **15* and homozygous wild-type carriers, respectively (Lee et al., 2010). With pitavastatin acid, the AUC was increased by 77%-200% among subjects with *SLCO1B1*15* alleles compared to homozygous **1b/*1b* subjects (Ieiri et al., 2007). Deng et al. reported that subjects with homozygous *SLCO1B1*15* alleles had lower oral clearance (CL/F) of pitavastatin compared to **1a/*1a* subjects and both C_{max} and AUC levels of pitavastatin acid were higher by 2.6-3.1-fold. (Deng et al., 2008) Chung et al. showed that subjects with one copy of the**15* allele had significantly higher values of dose-normalized AUCs by 25-75% and higher C_{max} by 61-123% compared to the wild-type **1a* or **1b* alleles for pitavastatin acid but not pitavastatin lactone (Chung et al., 2005). Recently, a study in Chinese subjects reported the findings that in subjects with at least one copy of *c.388G*, plasma pitavastatin acid showed higher C_{max} by 71%, AUCs by 81-85% and 35% lower oral clearance (Wen and Xiong, 2010a). These findings had been confirmed with *in vitro* studies that pitavastatin acid, but not the lactone, was a substrate for OATP1B1 (Fujino et al., 2005). However, a recent Chinese study failed to note differences of lipid responses among the genotype groups of the two *SLCO1B1*

SNPs identified (Yang et al., 2010). The authors attributed this to possible compensatory mechanisms from pitavastatin lactone as the concentrations of the lactone were independent from changes in transporter efficacy due to the polymorphism, or the alterations in pharmacokinetic profiles were not sufficient to affect pharmacodynamic responses.

4.3.2. Cytochrome P450 CYP3A5 isoenzyme

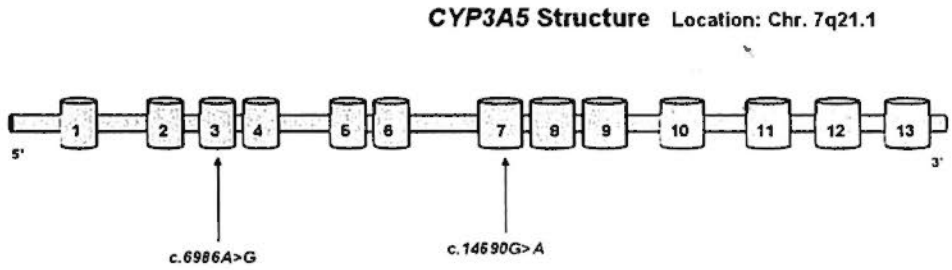
CYP3A5 isoform is an important cytochrome P450 enzyme in drug metabolism (Hustert et al., 2001; Williams and Feely, 2002). The encoding gene *CYP3A5* is highly polymorphic and shares the regulatory part of the encoding gene for CYP3A4 enzyme with similar substrate specificities (Lin et al., 2002) (Figure 4.5 and 4.6).

Figure 4.5: Gene map of chromosome 7 and *CYP3A4* and *CYP3A5* with base no.



Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

Figure 4.6: Schematic presentation of *CYP3A5*



Allele	Enzyme Activity	Frequency distribution		
		Whites	Blacks	Asians
*1 wild-type	Normal	0-15%	36-45%	23-40%
*3 (c.6986A>G)	Severely Decreased	85-98%	27-84%	60-77%
*6 (c.14690G>A)	Decreased	0	8.7%	1.6%

Ref: 1. www.cypalleles.ki.se by Human Cytochrome P450 (CYP) Allele Nomenclature Committee
2. www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

Inhibition of the CYP3A4 enzyme has been noted as the major determinant in drug interactions with statins leading to adverse events (Worz and Bottorff, 2001). CYP3A5 enzymes have been noted to play a significant role in metabolism of some statins (Prueksaritanont et al., 1997) but probably the role is minor for pitavastatin (Mukhtar et al., 2005). The frequency of variant alleles shows interethnic differences, with the wild-type *CYP3A5*1* allele being more common in Africans than Caucasians and Asians (Roy et al., 2005) It has been suggested that only 20% of the general population has hepatic CYP3A5, while the rest are non-expressor due to a genetic mutation (Daly et al., 2006). In individuals who express CYP3A5, the related percentage contribution to total hepatic CYP3A enzyme activity is still unclear, partly because CYP3A5 is mainly found in the gut and hence may not significantly contribute to hepatic metabolism (Westlind-Johnsson et al., 2003). The *CYP3A5*3* allele resulting from the *c.6986A>G* polymorphism at intron 3 is associated with a truncated protein and loss of function (Kuehl et al., 2001) and is widely found among Chinese (76%), Japanese (77%) and Caucasians (85%), but is not so common in

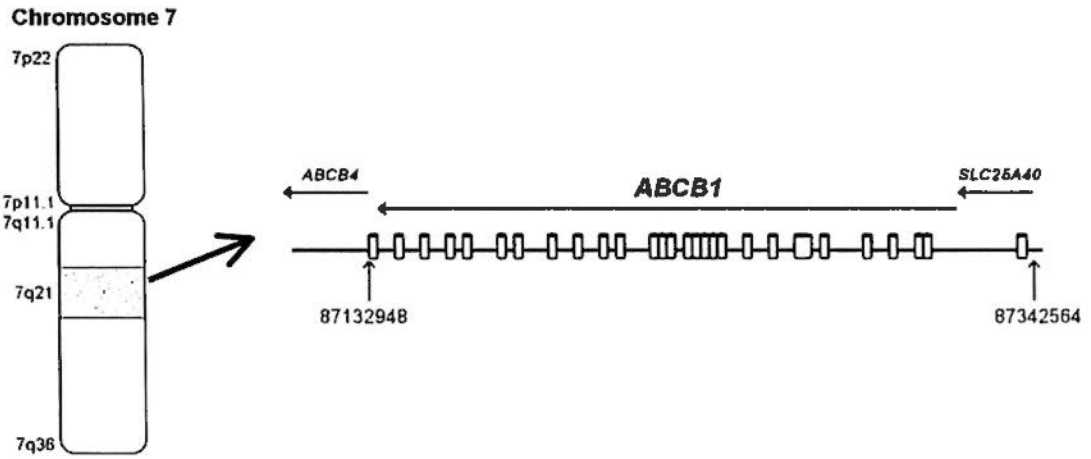
African-Americans (48%) (Balram et al., 2003). Kim et al. have shown that simvastatin pharmacokinetic parameters were affected by the *CYP3A5*3* polymorphism. Systemic exposure of simvastatin in terms of AUC values increased by 230%, and oral clearance decreased by 70% (Kim et al., 2007). Kivisto et al. studied the influence on lipid lowering efficacy with statins by the *CYP3A5* polymorphism and suggested expressors of the gene may show reduced clinical response compared to homozygous *CYP3A5 *3/*3* subjects. The mean reduction of plasma cholesterol was 17% in *CYP3A5* expressors, compared to 30% among non-expressors (Kivisto et al., 2004).

The CYP3A5 pathway is further complicated by gene-gene interactions. One observation was that subjects with the *ABCB1 3435T* allele, among those carrying *CYP3A5*1* had lower blood pressure than those with either allele. The possible gene-gene interaction of the two alleles could result in additive effects on blood pressure (Bochud et al., 2009). Yoo et al. also noted that *CYP2C19* polymorphisms affected metabolism of cilostazol only in individuals with the non-expressor *CYP3A5*3/*3* genotype (Yoo et al., 2009).

4.3.3. Efflux transporter – P-glycoprotein

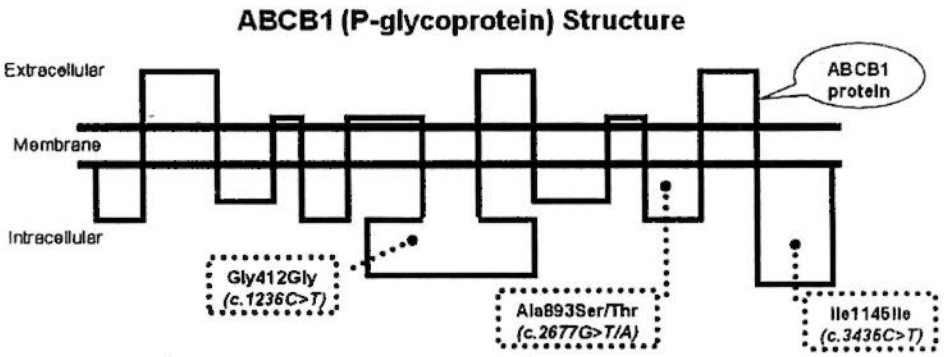
P-glycoprotein, also known as MDR1 or ABCB1, is an efflux transporter, which is expressed in multiple organs in humans, including the small intestine, liver, kidney and brain. The protein is encoded by gene *ABCB1* which is highly polymorphic (Figure 4.7). The expression of *ABCB1* was found to be 7-fold higher in small-intestine enterocyte homogenates than in the liver (von Richter et al., 2004), it contributes not only to limiting the oral bioavailability by reducing absorption of substrates from the gut but also to the excretion of substrates from the liver into the bile (Figure 4.8).

Figure 4.7: Gene map of chromosome 7 and ABCB1 with base no.



Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

Figure 4.8: Protein structure of P-glycoprotein (MDR1 or ABCB1), key amino acids and respective SNPs in the formation of influential haplotype



Encoding Haplotype	Frequency distribution		
	Whites	Blacks	Asians
CGC	32.5-46.0%	43.6-79.3%	22.7-28.5% (Malay), 17.6-23.5% (Indian) 16.8-30.1% (Chinese), 18.6-20.7% (Korean)
TTT	23.1-42.2%	6.5-8.7%	32.9-49.5% (Malay), 31.4-56.1% (Indian) 33.3-45.3% (Chinese), 32.2-41.4% (Korean)
Others	15.5-36.8%	16.2-47.7%	22.0-44.4% (Malay), 20.5-51.1% (Indian) 24.6-47.5% (Chinese), 37.9-49.2% (Korean)

Adapted from

1) Wang D and Sadee W, *The AAPS Journal* 2006; 8(3) Article 61

2) Fung KL and Gottesman, *Biochimica et Biophysica Acta* 2009; 1794: 860-871.

P-glycoprotein is increasingly being recognized as an important element in the disposition of drugs and subsequent bioavailability because of its broad range of substrates (Marzolini et al., 2004) and its role in the intestinal enterocytes as the efflux transporter (Fromm, 2003) contributing to drug resistance. The encoding gene *ABCB1* is located on chromsome 7p21.12. It has 28 exons and is highly polymorphic. Its polymorphisms result in differences in mRNA expression (Wang and Sadee, 2006) and substrate specificity through affecting protein function (Fung and Gottesman, 2009). Variation in P-glycoprotein activity will influence pharmacokinetics by altering intestinal absorption and/or biliary excretion (Kondo et al., 2004). The 3 common polymorphisms in the *ABCB1* gene are *c.1236C>T, c.2677G>T/A* and *c.3435C>T*. Together they account for most of the haplotypes among Eurpoeans, African Americans and Asians but are different in frequencies (Sai et al., 2003; Tang et al., 2002; Xie et al., 2001). In Chinese, their expected frequencies of minor alleles are 68%, 44% and 40%, respectively, and the *2677A* polymorphism allele frequency is 6% (Tang et al., 2002). These three SNPs are known to be in tight linkage disequilibrium in some populations. It has been suggested from data in *in vitro* studies that altered P-glycoprotein activity is significantly associated with individual SNPs or haplotypes (Salama et al., 2006). The SNP *c.3435C>T* is usually associated with decreased gene expression and mRNA stability (Wang et al., 2005b; Wang and Sadee, 2006) and could also change protein structure and function (Fung and Gottesman, 2009). The other prominent SNP *c.2677G>T* is linked to amino acid changes resulting in reduced transporter activities (Choudhuri and Klaassen, 2006; Fromm, 2002), but not necessary a higher drug plasma level as observed with a study with citalopram (Nikisch et al., 2008). *In vitro* studies have suggested that certain statins might not be major substrates for p-Glycoprotein (Chen et al., 2005; Huang et al., 2006), and in particular lovasatin, simvastatin and atorvastatin which would lead to lack of

interaction with inhibitors of this transporter like grapefruit juice (Chen et al., 2004). However, a Finnish study suggested *ABCB1 TTT/TTT* carriers had 60% and 55% greater AUC values than *CGC/CGC* carriers for simvastatin acid and atorvastatin respectively. The half-life for atorvastatin was 24% longer, but there was no change with the lactone metabolites (Keskitalo et al., 2008). Another Korean study on atorvastatin suggested the *c.2677TT-c.3435TT* diplotype was related to a longer elimination half-life by approximately 40% (Lee et al., 2010). The present study focused on the *TTT* vs. *CGC* haplotypes, but also analyzed the effect of individual SNPs to evaluate their possible influence.

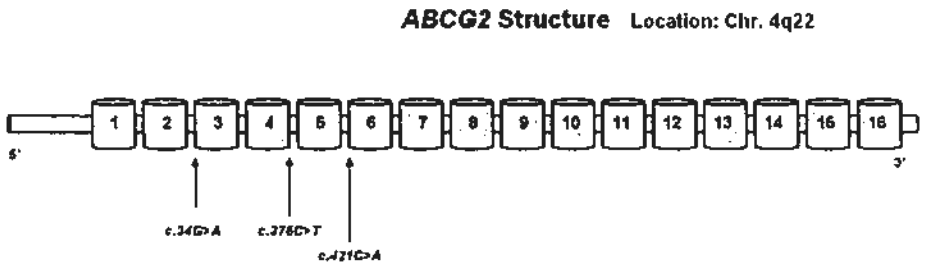
4.3.4. Efflux transporter – Breast cancer resistance protein (BCRP) and gene *ABCG2*

Breast cancer resistance protein (BCRP), encoded by gene *ABCG2* (Figure 4.9), is an efflux transporter located in the apical brush-border membrane of enterocytes of the small intestine, the bile canalicular membrane of hepatocytes for biliary excretion and also the endothelial cells that form the blood-brain barrier (Vlaming et al., 2009). *ABCG2* is a highly polymorphic gene (Figure 4.10).

Figure 4.9: Gene map of chromosome 4 and *ABCG2* with base no.

Ref: www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

Figure 4.10: Schematic presentation of *ABCG2*



Allele	Enzyme Activity	Frequency distribution		
		Whites	Blacks	Asians
c.34G>A (Val-12Met)	Uncertain	2-10.3%	4%	15-36%
c.421C>A (Gln141Lys)	Decreased	9.0-14%	0%	28-35%
c.376C>T (Gln126 Term)	Lack of function	0%	NA	0.4-1.9%

Ref: www.ncbi.nlm.nih.gov by National Center for Biotechnology Information

Most statins are substrates of BCRP (Kitamura et al., 2008). The SNP at *c.421C>A* has been noted to reduce transporter efficacy (Robey et al., 2009). This SNP or *c.421C>A* polymorphism is a commonly found allele in Chinese and Japanese (about 35%) (de Jong et al., 2004). A study on rosuvastatin noted the *c.421AA* carriers had 100% and 144% higher AUC values than *c.421CA* and *c.421CC* carriers, respectively (Keskitalo et al., 2009b). The results confirmed an earlier report from a Chinese group of similar 76-78% increase of AUC values of rosuvastatin in *c.421A* carriers compared with *c.421CC* subjects (Zhang et al., 2006). Keskitalo et al. reported their study on simvastatin showing 111% larger AUC values for lactone metabolites in subjects with *c.421AA* compared to *c.421CC* carriers. Similar findings with fluvastatin showed 97% and 72% higher AUC values in *c.421AA* carriers compared to *c.421CA* and *c.421CC* subjects, respectively. However, there were no significant effect on the pharmacokinetics of simvastatin acid or pravastatin (Keskitalo et al., 2009a). A previous pharmacokinetic study with pitavastatin did not demonstrate a statistically significant effect of this polymorphism in *ABCG2* (Ieiri et al., 2007). We have included an examination of this SNP in the present study because of its reported effects with other statins.

4.3.5 Food-genotype interaction from inhibitory activities – Grapefruit Juice

Food-statin interactions have been suggested to be of clinical significance. Grapefruit juice (GFJ) contains the furanocoumarin 6'7' dihydroxybergamottin and the flavonoids naringenin and naringin which have been found to be inhibitors of the cytochrome P450 (CYP) 3A4 enzymes and certain drug transporters (Chen et al., 2004; de Castro et al., 2008; Farkas and Greenblatt, 2008; Glaeser et al., 2007; Kirby and Unadkat, 2007). Downregulation of the enzymes and transporters can lead to changes in bioavailability. Various reports suggested GFJ as a possible source of interaction with statins which may have clinical consequences (Araujo

et al., 2010; Farkas and Greenblatt, 2008; Kiani and Imam, 2007; Lim et al., 2003; Neuvonen et al., 2008). Administration with GFJ increased the plasma levels of the acid and lactone of lovastatin, simvastatin and atorvastatin, probably by decreasing CYP3A4-mediated metabolism in the small intestine, but it had no significant effect on the pharmacokinetics of pravastatin (Kantola et al., 1998; Lilja et al., 1998; 1999). After repeated GFJ intake, Ando et al. found that the AUC with atorvastatin increased by 83% after GFJ intake (Ando et al., 2005). Another study with GFJ also demonstrated a 2.5-fold increase in AUC of atorvastatin, but no change in the major pharmacokinetic parameters with pravastatin (Lilja et al., 1999). The AUC with lovastatin was found to increase by 15-fold but the half-life unchanged (Kantola et al., 1998). Lilja et al. studied repeated administration of GFJ with simvastatin and demonstrated an increase of AUC values by 16-fold and 7-fold, and those of C_{max} by 9-fold and 7-fold, for the lactone and acid, respectively (Lilja et al., 1998). These data have indicated a vastly increased systemic exposure of statins with concurrent administration of GFJ. As for pitavastatin, repeated doses of GFJ for 4 days had a small effect on the pharmacokinetics with a pitavastatin 4 mg daily dose, increasing the mean area under the plasma concentration-time curve ($AUC_{0\text{-}24h}$) by 13% (95% CI –3%-29%) in 8 healthy Japanese male subjects and this was compared to the effect of an increase of 83% (95% CI 23%-144%) for atorvastatin acid (Ando et al., 2005). This study and other data suggest that pitavastatin undergoes very limited CYP3A4-mediated metabolism (Mukhtar et al., 2005). GFJ is also thought to influence certain intestinal drug transporters. It has been shown that GFJ resulted in reduced plasma exposure to fexofenadine after oral intake, probably because of inhibition of the OATP1A2 intestinal uptake transporter, although short-term administration of GFJ did not affect the expression of OATP1A2 in the duodenum of healthy volunteers or of MDR1 (P-glycoprotein, ABCB1) which co-localized with OATP1A2 to the brush border domain of enterocytes.(Glaeser

et al., 2007) Increased systemic exposure to the P-glycoprotein substrate talinolol was seen in rats when it was administered with GFJ suggesting inhibition of intestinal secretion of the drug through the ABCB1 pathway (Spahn-Langguth and Langguth, 2001), but GFJ significantly reduced talinolol bioavailability in humans suggesting that it may preferentially inhibit an intestinal uptake transporter rather than ABCB1.(Schwarz et al., 2005)

Recent *in vitro* studies showed that pitavastatin is a substrate for human OATP1A2, OATP2B1, and MDR1 and that naringin from GFJ inhibited transport of pitavastatin by the rat transporters, Oatp1a5 and Mdr1a, in a concentration-dependent manner so that lower concentrations inhibited Oatp1a5-mediated intestinal uptake in the *in situ* closed loop perfusion model, whereas higher concentrations inhibited Mdr1a-mediated intestinal efflux increasing overall intestinal absorption of pitavastatin, suggesting a paradoxical effect of GFJ on pitavastatin absorption (Shirasaka et al., 2010).

Our study looked at the changes after repeated doses of GFJ to evaluate if there would be differential effects between genotypes.

4.4. Methods

4.4.1. Study methods and design

The study design adopted a pharmacogenetic approach to examine possible gene-gene and gene-food interactions. The influence of the SNPs at *SLCO1B1 c.388A>G* and *c.521T>C* on pitavastatin pharmacokinetics was examined. Pitavastatin is a substrate for the transporter protein OATP1B1, the genotypic effects of these SNPs on the drug have been reported previously. Analysis of the effects of polymorphisms with *CYP3A5*3 (c.6986A>G), ABCB1 (c.1236C>T, c.2677G>T/A, c.3435C>T)* and *ABCG2 (c.421C>A)* were evaluated. Respective SNPs were genotyped. The effects of repeated dosing with grapefruit juice (GFJ) were evaluated on gene-food interaction by looking at the incremental changes among genotype groups. Pharmacokinetic parameters in terms of plasma pitavastatin acid and lactone levels were measured after administration with water or after repeated doses of GFJ.

4.4.2. Protocol

The study was performed in an open, randomized, two-phase crossover design with a wash-out interval of at least 3-weeks. The protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Twelve healthy male Chinese volunteers were randomized to receive either "double-strength" GFJ or water 200 mL 3 times a day for 2 days before taking pitavastatin as a 2 mg tablet. "Double-strength" GFJ was prepared by diluting 12 ounces [355 mL] Minute Maid Frozen concentrated grapefruit juice, Coca Cola Foods, Houston, Texas with 355 mL water. This resulted in a liquid which was double the strength recommended for the ordinary drink, and should provide a

high concentration of the active ingredients in the intestine. The same volumes of this GFJ or water were given 0.5 and 1.5 hours after taking pitavastatin and 3 times at approximately 8 hours intervals on the following day. Plasma concentrations of pitavastatin acid and pitavastatin lactone were measured over 48 hours after the dose. After a wash-out period of at least 3 weeks, the volunteers were given the alternative regimen with GFJ or water according to the same timetable. Blood samples were taken to determine the pharmacokinetics after a second dose of 2 mg pitavastatin. During each sampling period, 10 ml of blood was collected into light protected tubes containing sodium heparin. There were 10 time points: pre dose (0 h), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 32 and 48 h after oral dosing. After sample collection, the tubes were stored in a box containing ice cubes and subsequently centrifuged at 3000 rpm for 10-minutes at 4°C within 15-minutes after collection. Plasma was aliquoted, protected from light and placed in tubes, which were packed and stored at -20°C until analysis.

The analytical method used for the assay of pitavastatin acid and lactone was previously developed by Kowa Company and further validated by SRL Inc. Hachioji Laboratory of Japan.

Plasma samples were extracted with methyl *tert*-butyl ether and then the extract was subjected to methylation with diazomethane to prevent the mutual conversion between pitavastatin acid and lactone. The extract was injected into a column-switching HPLC system. The limits of quantification for both pitavasatin acid and lactone were 0.5 ng/mL.

Throughout the 3-weeks wash-out periods, subjects followed restrictions to avoid beverages and food intake including grapefruit juice and/or citrus fruits. During the study periods, citrus fruits, alcohol, xanthine-containing beverages and herbal

supplements were prohibited. On the day of drug administration, water and other beverage intake were not allowed from 1 hour before until 1 hour after the administrations of GFJ and test drug. Standard meals were served at 4 and 8 hours after drug intake.

The subjects were observed by research nurses during the pharmacokinetic studies and they were asked to report any adverse effects.

4.4.3. Genotyping

A 10 mL blood sample was drawn from each subject and DNA was extracted from peripheral blood leucocytes using the traditional phenol chloroform method or High Pure PCR Template Preparation Kits (Roche). Genotyping for *SLCO1B1 (c.388A>G, c.521T>C; *1a, *1b, *5, *15)*, *CYP3A5*3 (c.6986A>G)*, *ABCB1 (c.1236C>T, c.2677G/A>T, c.3435C>T)* and *ABCG2 (c.421C>A)* polymorphisms was performed in the Genome Research Centre, University of Hong Kong using the mass-spectroscopy based, high-throughput MassARRAY iPLEX^{TM} platform (Sequenom, San Diego, CA).

4.4.4. Pharmacokinetic analysis

The pharmacokinetic parameters of pitavastatin acid and lactone were calculated using non-compartmental methods with the aid of the computer program WinNolin (version 2.1, Pharsight Corporation). Peak plasma concentrations (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the observed concentration-time data. The terminal elimination rate constant (λ_z) was estimated by linear regression of the terminal portion of the concentration-time curve, and the elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. Systemic exposure to the pitavastatin acid and lactone were evaluated by the calculation of area under the plasma concentration-time curve (AUC) using the linear trapezoidal rule and $AUC_{0-\infty}$ was calculated as $AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$

where C_t is the last quantifiable concentration. The apparent oral clearance (CL/F) was calculated as Dose/ $AUC_{0-\infty}$

4.4.5. Statistical analysis

All genotypes were checked for confirmation not to deviate from Hardy-Weinberg equilibrium by Chi-square analysis. All continuous variables were expressed as mean±SD with range. Categorical variable t_{max} was expressed as median and range. Analysis of variance (ANOVA) and Student's t-test were performed for independent values comparison with genotypes as factors and plasma pharmacokinetic parameters as the dependent variables, except for t_{max} values which were compared by non-parametric Wilcoxon signed-rank test. Food-gene interactions were evaluated by changes between administration by water and grapefruit juice and were compared by non-parametric Mann-Whitney U tests. For comparison of gene-gene interactions between *SLCO1B1 c.388A>G* with *CYP3A5*3* or *ABCG2 c.421C>A* or *ABCB1 CGC/TTT* haplotypes, non-parametric Mann-Whitney U tests or Kruskal-Wallis tests were used for comparison between subgroups. A P-value of <0.05 was considered statistically significant for all tests, otherwise they were considered as non-significant (NS). Statistical calculations were performed using SPSS Version 14.0 software (SPSS Inc., Illinois, USA)

4.5. Results

4.5.1. Overall evaluation

All 12 subjects completed the study without adverse effects. Mean±SD age was 22.7±1.3 years (range 21-25 years), body weight 65.8±10.0 kg (range 53.4-74.3 kg), and height 172.5±6.9 cm (range 163.3-187.2 cm). Demographic and genotype data were listed in Table 4.1. Repeated administration with grapefruit juice (GFJ) resulted in modest variations in the pharmacokinetic parameters (Table 4.2). Mean C_{max} was lower by 14% for pitavastatin lactone ($P<0.05$), $AUC_{0\text{-}48h}$ values were higher by 16% and 15%, and CL/F values were lower by 10% and 15% for pitavastatin acid and lactone, respectively ($P<0.05$). The analysis of the major pharmacokinetic parameters according to genotypes when administered with water are shown in Table 4.3. All genotype distributions did not deviate from the Hardy-Weinberg equilibrium. The respective frequencies were compatible with expectations for a Chinese population.

Table 4.1: Demographics of subjects, pharmacokinetic parameters for pitavastatin acid and lactone when administered with water and genotypes

ID	Demographic Characteristics			Pitavastatin Acid (administered with water)		Pitavastatin Lactone (administered with water)		*SLCO1B1* genotypes		*CYP3A5* genotype	Efflux transporters genotypes	
	Age (yrs)	Body weight (kg)	Height (cm)	C_{max} (ng/ml)	AUC_{0-48h} (ng×h/ml)	C_{max} (ng/ml)	AUC_{0-48h} (ng×h/ml)	*c.388 A>G*	*c.521 T>C*	**3 (c.6986 A>G)*	*ABCB1* haplotype	*ABCG2 c.421 C>A*
1	22	54.4	163.3	30.4	75.05	23.3	151.15	*AG*	*TT*	**1/*3*	*TTT/TGC*	*CA*
2	24	74.3	178.6	32.8	74.42	16.4	161.30	*AG*	*TT*	**3/*3*	*CGC/CGC*	*CC*
3	23	59.3	168.6	47.1	110.22	30.3	238.82	*GG*	*TT*	**3/*3*	*TTT/TGC*	*CC*
4	21	63.1	169.8	31.6	88.35	17.1	167.52	*AG*	*TT*	**1/*3*	*TTT/TTT*	*AA*
5	25	59.1	170.3	47.7	107.70	39.6	234.60	*AG*	*TC*	**1/*3*	*TTT/TTT*	*CA*
6	23	53.4	167.3	24.0	73.42	22.9	179.40	*GG*	*TT*	**3/*3*	*TGC/TGC*	*CC*
7	23	87.8	171.0	50.7	97.60	25.5	146.50	*GG*	*TC*	**1/*1*	*CGC/TGC*	*CA*
8	22	72.0	174.0	63.5	85.15	30.7	177.02	*GG*	*TT*	**3/*3*	*CGC/TAC*	*CA*
9	23	59.4	167.4	53.0	127.52	31.2	207.12	*GG*	*TT*	**3/*3*	*CGC/TGT*	*CC*
10	21	67.4	187.2	40.1	104.28	28.1	223.12	*GG*	*TT*	**3/*3*	*CGC/CAC*	*CC*
11	21	71.3	182.0	21.1	53.52	13.0	116.02	*AA*	*TT*	**3/*3*	*CGC/TGC*	*AA*
12	24	64.7	170.0	19.5	49.22	18.3	118.68	*AG*	*TT*	**1/*3*	*TTT/TGC*	*CA*
	22.7 ± 1.3	**65.8 ± 10.0**	**172.5 ± 6.9**	**38.5 ± 14.0**	**87.20 ± 23.47**	**24.7 ± 7.7**	**176.77 ± 41.82**					

Data are presented as mean±SD

AUC_{0-48h}, area under plasma concentration-time curve from 0-48 hours;

C_{max}, peak plasma concentration.

Table 4.2: Pharmacokinetic profiles of pitavastatin acid and pitavastatin lactone according to mode of administration with a single dose of 2 mg pitavastatin

Pitavastatin Acid

Parameters	Water (W)	Grapefruit Juice (GFJ)	P-value
t_{max} (h)	0.75 (0.5-1)[a]	1 (1-2)[a]	P=0.007[b] #
C_{max} (ng/ml)	38.5 ± 14.0 (19.5-63.5)	33.2 ± 10.1 (16.5-46.4)	P=0.117
AUC_{0-24h} (ng·h/ml)	84.9 ± 22.5 (49.2-125.5)	95.6 ± 29. 5 (56.1-167.6)	P=0.022 #
AUC_{0-48h} (ng·h/ml)	87.2 ± 23.5 (49.2-127.5)	100.8 ± 33.4 (56.1-179.6)	P=0.023 #
AUC_{0-t} (ng·h/ml)	83.0 ± 24.0 (45.0-125.5)	96.2 ± 33.4 (50.7-174.0)	P=0.025 #
$AUC_{0-\infty}$ (ng·h/ml)	93.7 ± 24.4 (49.7-129.8)	105.5 ± 34.3 (56.0-182.7)	P=0.037 #
CL/F (L/h)	23.0 ± 7.4 (15.4-40.2)	20.8 ± 6.8 (11.0-35.7)	P=0.015 #
$t_{1/2}$ (h)	9.1 ± 3.4 (4.6-16.9)	9.2 ± 3.4 (4.1-15.2)	P=0.930

Pitavastatin lactone

Parameters	Water (W)	Grapefruit Juice (GFJ)	P-value
t_{max} (h)	1 (1-1.5)[a]	2 (1.5-3)[a]	P=0.002[b] #
C_{max} (ng/ml)	24.7 ± 7.7 (13.0-39.6)	21.2 ± 5.4 (12.7-28.4)	P=0.021 #
AUC_{0-24h} (ng·h/ml)	155.0 ± 37.6 (99.1-209.6)	172.5 ± 44.8 (117.7-270.8)	P=0.041 #
AUC_{0-48h} (ng·h/ml)	176.8 ± 41.8 (116.0-238.8)	202.5 ± 53.1 (141.7-321.2)	P=0.024 #
AUC_{0-t} (ng·h/ml)	174.1 ± 43.7 (109.9-238.8)	201.0 ± 54.0 (141.7-321.2)	P=0.025 #
$AUC_{0-\infty}$ (ng·h/ml)	186.2 ± 43.4 (118.7-250.0)	217.6 ± 54.1 (161.6-339.1)	P=0.016 #
CL/F (L/h)	11.3 ± 2.8 (8.0-16.9)	9.6 ± 2.0 (5.9-12.4)	P=0.004 §
$t_{1/2}$ (h)	12.4 ± 4.0 (7.1-19.6)	15.2 ± 4.6 (8.3-26.5)	P=0.071

Data are given as mean±SD and range, except for t_{max} as Median and range[a]. Apparent oral clearance (CL/F) is calculated by Dose/ $AUC_{0-\infty}$. Statistical comparison by Student's t-test except Wilcoxon signed-rank test for t_{max}[b]. # P<0.05 and §P<0.01 are significant.

Table 4.3: C_{max} and AUC_{0-48h} of pitavastatin acid and pitavastatin lactone comparison among genotypes (administration with water)

	Pitavastatin Acid			Pitavastatin Lactone		
	C_{max} (ng/ml)	AUC_{0-48h} (ng×h/ml)	$t_{1/2}$ (hr)	C_{max} (ng/ml)	AUC_{0-48h} (ng×h/ml)	$t_{1/2}$ (hr)
SLCO1B1 c.521T>C	p.Val174Ala					
c.521TT (n=10)	36.3±14.5	84.1±24.6	9.0±3.8	23.1±6.7	174.0±40.7	12.4±4.0
c.521TC (n=2)	49.2	102.7	9.6	32.6	190.6	11.9
SLCO1B1 c.388A>G	p.Asn130Asp					
c.388AA/AG (n=6)	30.5±10.1	74.7±21.8	8.4±2.3	21.3±9.6	158.2±43.2	14.6±4.0
c.388GG (n=6)	46.4±15.4	99.7±19.0	9.8±4.4	28.1±3.3	195.3±34.0	10.1±2.6
P-value	0.043 #	0.060	0.505	0.130	0.129	0.043 #
SLCO1B1 haplotype:	**1a: c.388A-c.521T* (**1a*: 130Asn174Ala) **1b: c.388G-c.521T* (**1b*: 130Asp174Val)					
c.388AA/AG -c.521TT (n=5)	27.1±6.3	68.1±16.3	8.1±2.4	17.6±3.7	142.9±24.1	14.4±4.4
c.388GG -c.521TT (n=5)	45.5±14.8	100.1±21.3	9.9±5.0	28.6±3.4	205.1±27.0	10.5±2.7
P-value	0.033 #	0.028 #	0.476	0.001 §	0.005 §	0.132
*CYP3A5*3:*	*c.6986A>G* (Premature codon stop)					
**1/*1* or **1/*3* (n=5)	36.0±13.0	83.6±22.7	8.4±2.2	24.8±9.0	163.7±43.4	13.8±4.2
**3/*3* (n=7)	40.2±15.5	89.8±25.5	9.6±4.2	24.7±7.4	186.1±41.3	11.3±3.7
P-value	0.628	0.673	0.557	0.983	0.385	0.302
ABCB1 haplotype:	*c.1236C>T, c.2677G/A>T, c.3435C>T* (Wobble and Ala893Ser)					
CGC carrier (n=6)	43.5±15.3	90.4±25.6	7.9±2.4	24.2 ± 7.7	171.8±39.4	10.7±4.3
TTT carrier (n=5)	35.3±12.0	86.1±25.2	9.0±2.9	25.7 ± 9.3	182.2±52.8	14.4±3.3
Others (n=1)	24.0	73.4	16.9	22.9	179.4	11.9
P-value *CGC* vs *TTT* carriers	0.353	0.786	0.506	0.766	0.719	0.152
ABCG2 c.421C>A	p.Gln141Lys					
c.421CC (n=5)	39.4±11.5	98.0±23.6	11.1±4.0	25.8±6.2	202.0±31.6	12.4±4.0
c.421CA (n=5)	42.4±17.4	82.9±22.5	7.7±2.8	27.5±8.1	165.6±43.8	12.9±4.8
c.421AA (n=2)	26.4±7.4	70.9±24.6	7.6±0.6	15.1±2.9	141.8±36.4	10.8±3.0
P-value	0.426	0.370	0.241	0.141	0.171	0.839

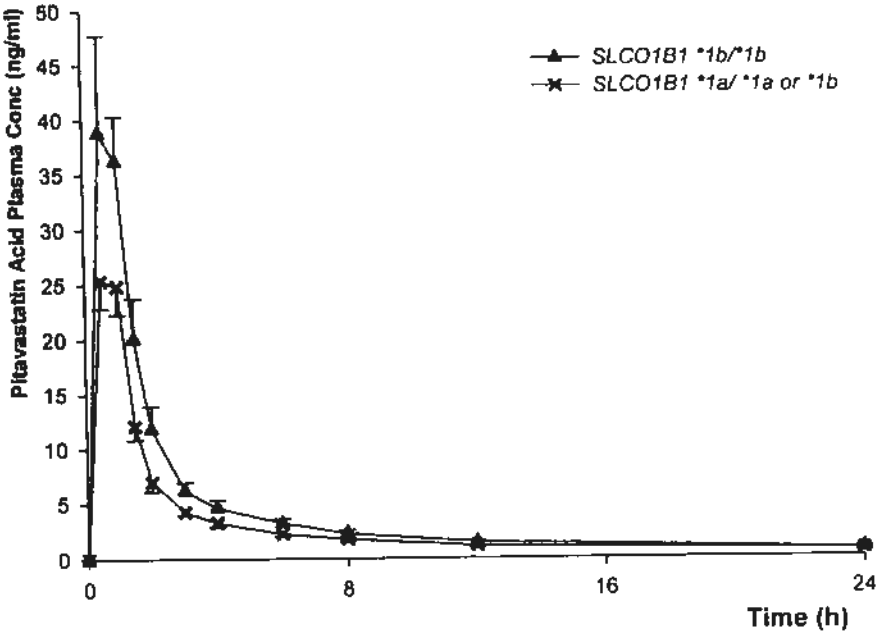
C_{max}: peak plasma concentration; AUC_{0-48h}: area under the plasma concentration-time curve from time 0 to 48 h. Data are given as mean±SD and range, comparison by Student's t-test or ANOVA (Analysis of Variance). # $P<0.05$ and §$P<0.01$ are statistically significant.

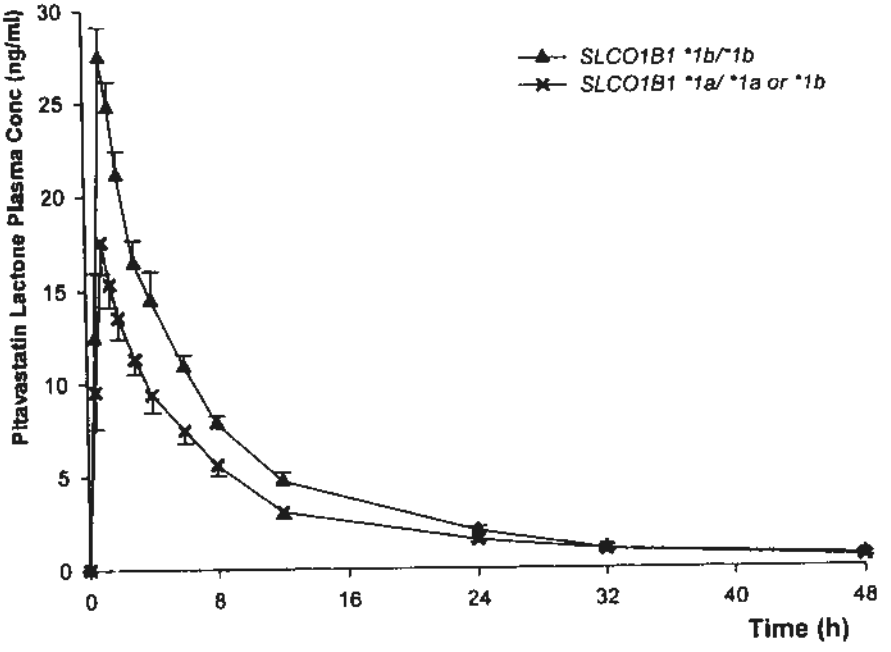
4.5.2. Genetic influence from *SLCO1B1 c.521T>C* and *c.388A>G*, haplotype **1a, *1b* and **15*

There were only two heterozygous *SLCO1B1 c.521TC* carriers. By excluding subjects with *c.521TC* polymorphisms, and hence **15* haplotype, the plasma concentration time profiles were compared between the haplotype groups *SLCO1B1*1a* and **1b* for both pitavastatin acid and lactone when administered with water only (Graph 4.1) and after repeated administration with GFJ (Graph 4.2).

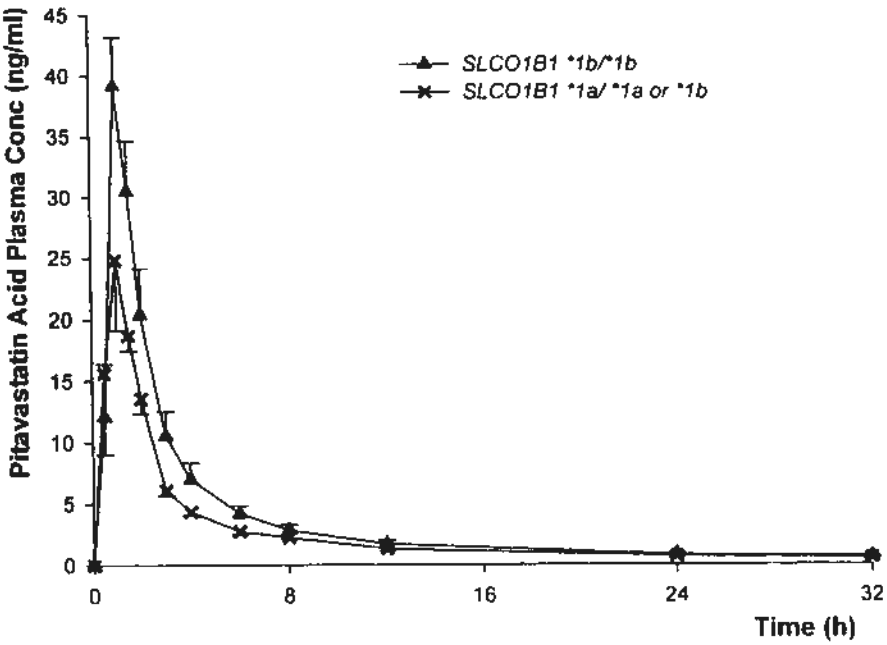
Homozygous **1b/*1b* carriers showed higher plasma concentrations than **1a* carriers for both acid and lactone under both conditions. Table 4.4 listed the comparison of major pharmacokinetic parameters between genotype groups for the SNP *c.521T>C* polymorphism. There were higher trends for C_{max} (Graph 4.3), $AUC_{0\text{-}48h}$ (Graph 4.4) and $AUC_{0\text{-}\infty}$ (Graph 4.5) values with *c.521TC* than *c.521TT* for both pitavastatin acid and lactone, but these were not tested statistically due to the small group sizes.

Graph 4.1: Plasma concentration–time plot (mean ± SEM) for pitavastatin acid and lactone as administered with water for *SLCO1B1 *1b/*1b (c.521TT-c.388GG;* n=5) vs. **1a/*1a or *1b (c.521TT-c.388AA/AG;* n=5)





Graph 4.2: Plasma concentration–time plot (mean ± SEM) for pitavastatin acid and lactone as administered with GFJ for *SLCO1B1 *1b/*1b (c.521TT-c.388GG;* n=5) vs. **1a/*1a or *1b (c.521TT-c.388AA/AG;* n=5)



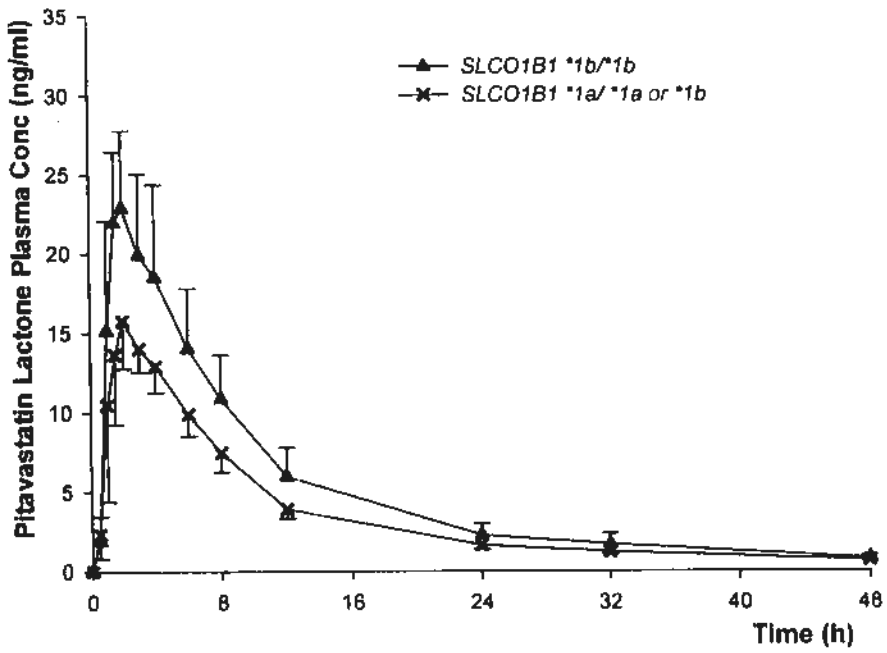
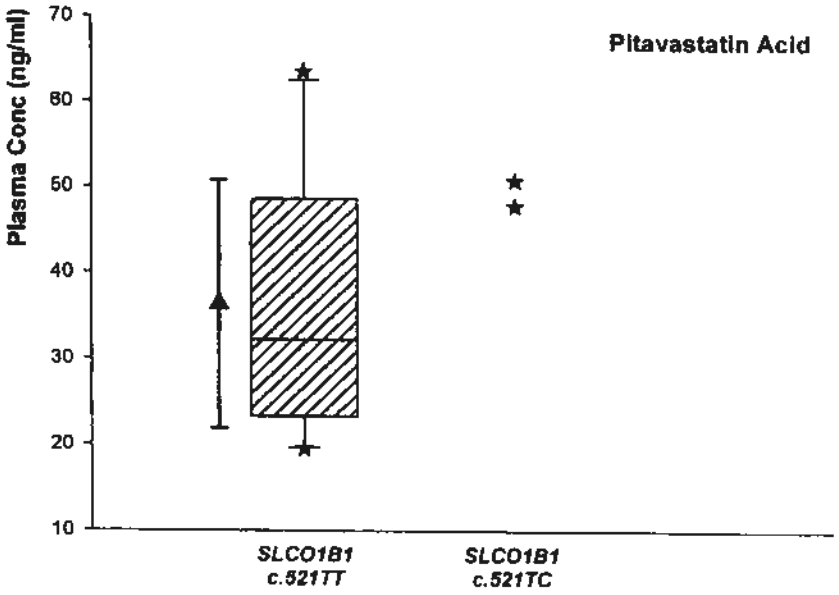


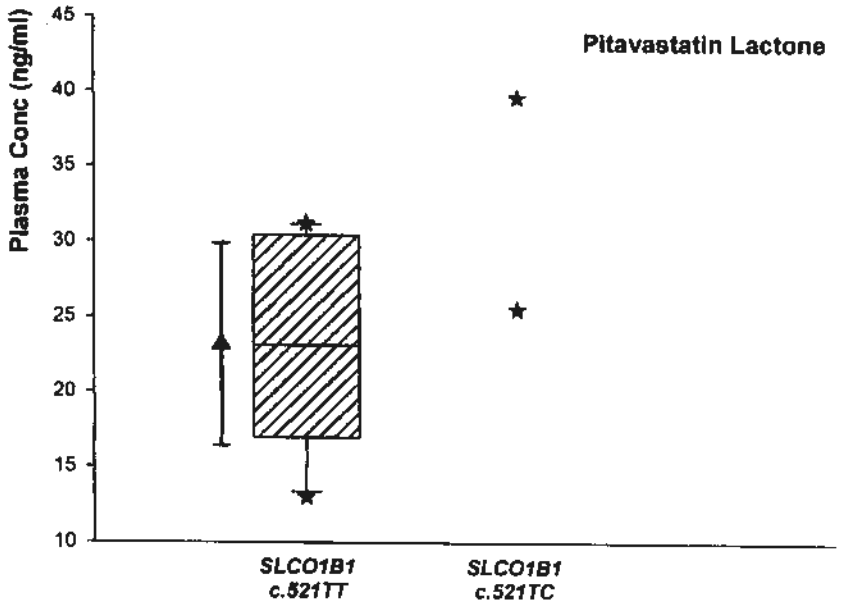
Table 4.4: Pharmacokinetic parameters according to *SLCO1B1 c.521T>C* genotypes, and the changes after administration with Grapefruit Juice (GFJ)

Genotype	*c.521TT (n=10)*	*c.521TC (n=2)*	*c.521TT (n=10)*	*c.521TC (n=2)*
Administration			*Changes after consumption with GFJ*	
Pitavastatin Acid				
C_{max} (ng/ml)	36.3 ± 14.5 (19.5 – 63.5)	49.2 (47.7, 50.7)	-3.2 ± 10.4 (-24.6 - +10.8)	-15.6 (-18.2, -13.0)
AUC_{0-24h} (ng×h/ml)	82.2 ± 23.9 (49.2 – 125.5)	98.7 (97.6, 99.7)	+11.5 ± 14.1 (-8.0 - +42.1)	+6.3 (-5.2, +17.8)
AUC_{0-48h} (ng×h/ml)	84.1 ± 24.6 (49.2 – 127.5)	102.7 (97.6, 107.7)	+14.6 ± 17.2 (-7.2 - +52.1)	+8.3 (-11.2, +27.8)
AUC_{0-t} (ng×h/ml)	80.5 ± 25.5 (45.0 – 125.5)	95.6 (87.4, 103.7)	+13.3 ± 16.5 (-8.0 - +48.5)	+12.4 (-9.2, +34.0)
$AUC_{0-\infty}$ (ng×h/ml)	90.4 ± 25.5 (49.7 – 129.8)	110.4 (110.0, 110.9)	+13.2 ± 17.0 (-5.5 - +52.9)	+4.5 (-11.5, +20.5)
Apparent oral clearance (L/h)	24.0 ± 7.8 (15.4 – 40.2)	18.1 (18.0, 18.2)	-2.6 ± 2.5 (-5.5 - +1.9)	-0.4 (-2.9, +2.1)
$t_{1/2}$ (h)	9.0 ± 3.8 (4.6 – 16.9)	9.6 (9.2, 10.0)	+0.1 ± 3.5 (-7.8 - +5.1)	+0.1 (-3.2, +3.4)
t_{max} (h)	*0.75 (0.5 – 1)*	*0.75 (0.5, 1)*	*1 (1 – 2)**	*1.25 (1, 1.5)**
Pitavastatin Lactone				
C_{max} (ng/ml)	23.1 ± 6.7 (13.0 – 31.2)	32.6 (25.5, 39.6)	-2.7 ± 3.9 (-9.3 - +3.1)	-7.3 (-11.9, -2.6)
AUC_{0-24h} (ng×h/ml)	152.0 ± 36.8 (99.1 – 209.6)	170.1 (132.9, 207.4)	+21.9 ± 25.2 (-17.0 - +80.4)	+4.7 (-22.5, 13.2)
AUC_{0-48h} (ng×h/ml)	174.0 ± 40.7 (116.0 – 238.8)	190.6 (146.5, 234.6)	+30.5 ± 34.3 (-17.4 - +114.0)	+2.1 (-18.5, 22.8)
AUC_{0-t} (ng×h/ml)	171.5 ± 42.3 (109.9 – 238.8)	187.4 (140.1, 234.6)	+32.2 ± 36.3 (-17.4 - +120.4)	+0.5 (-18.5, +19.6)
$AUC_{0-\infty}$ (ng×h/ml)	183.6 ± 41.4 (118.7 – 250.0)	198.8 (149.5, 248.2)	+36.3 ± 39.2 (-19.6 - +130.2)	+7.5 (-15.6, +30.6)
Apparent oral clearance (L/h)	11.4 ± 2.8 (8.0 – 16.9)	10.7 (8.1, 13.4)	-1.9 ± 1.6 (-4.5 - +0.8)	-0.9 (-2.3, +0.5)
$t_{1/2}$ (h)	12.4 ± 4.0 (7.1 – 19.6)	11.9 (8.1, 15.7)	+3.0 ± 5.4 (-5.6 - +13.7)	+2.1 (+0.6, +3.7)
t_{max} (h)	*1 (1 – 1.5)*	*1.25 (1, 1.5)*	*2 (1.5 – 3)**	*1.75 (1.5, 2)**

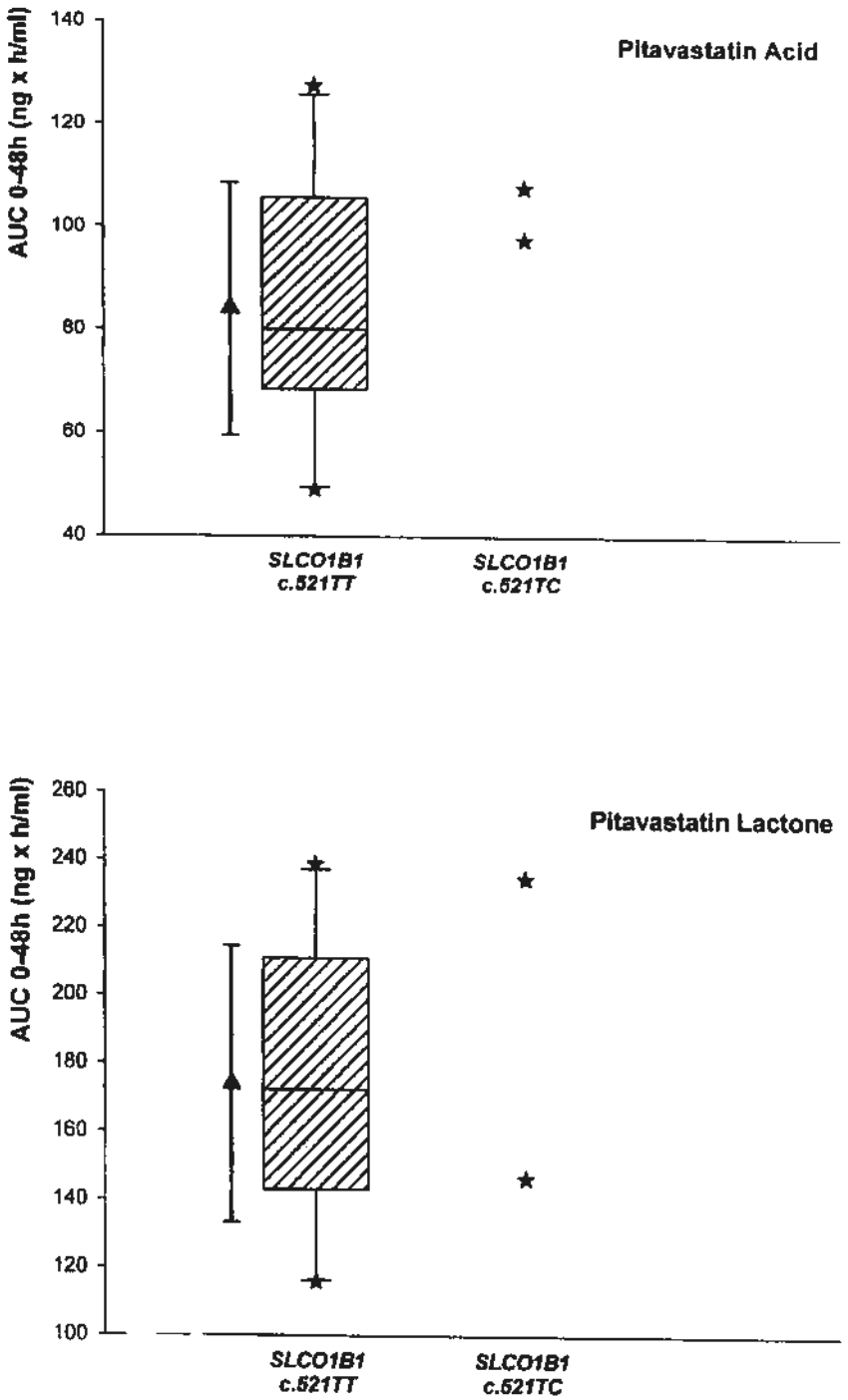
Data are given as mean±SD and range, except t_{max} data as median (range) * t_{max} data are as administered under GFJ. Apparent oral clearance is calculated by Dose / $AUC_{0-\infty}$

Graph 4.3: C_{max} as administered with water for pitavastatin acid and lactone according to *SLCO1B1 c.521T>C* genotypes. Mean ± SD with boxplots showing median and outlier for data reading with *c.521TT* group

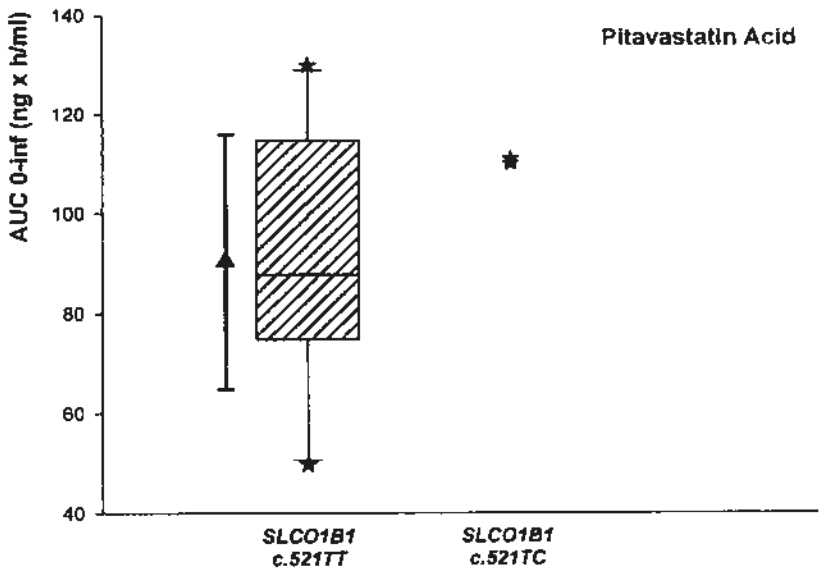


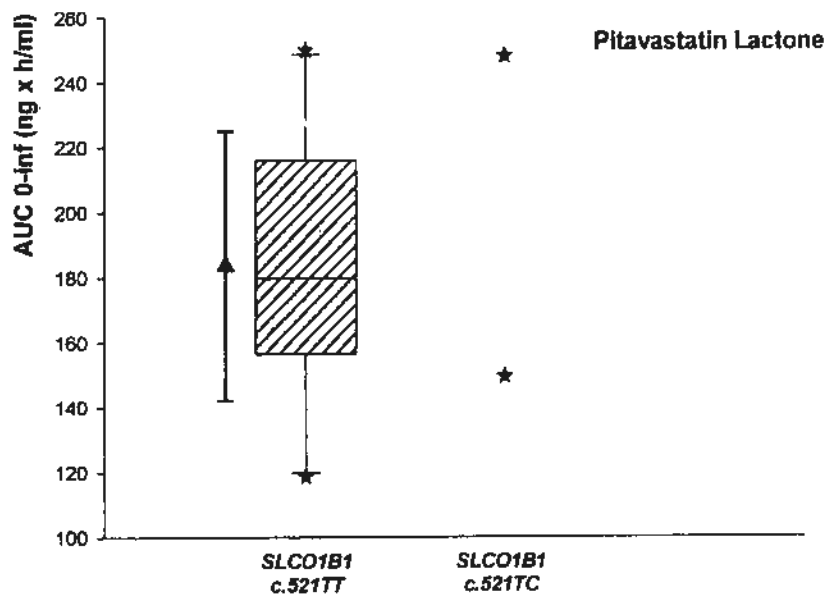


Graph 4.4: AUC $_{0\text{-}48h}$ as administered with water for pitavastatin acid and lactone according to *SLCO1B1 c.521T>C* genotypes. Mean ± SD with boxplots showing median and outlier for data reading with *c.521TT* group



Graph 4.5: AUC $_{0-\infty}$ as administered with water for pitavastatin acid and lactone according to *SLCO1B1 c.521T>C* genotypes. Mean ± SD with boxplots showing median and outlier for data reading with *c.521TT* group

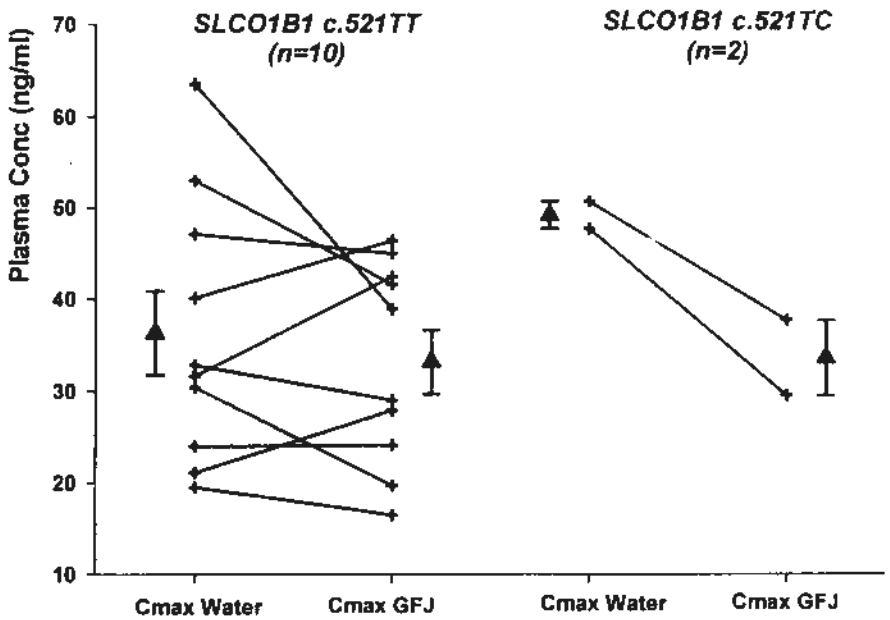




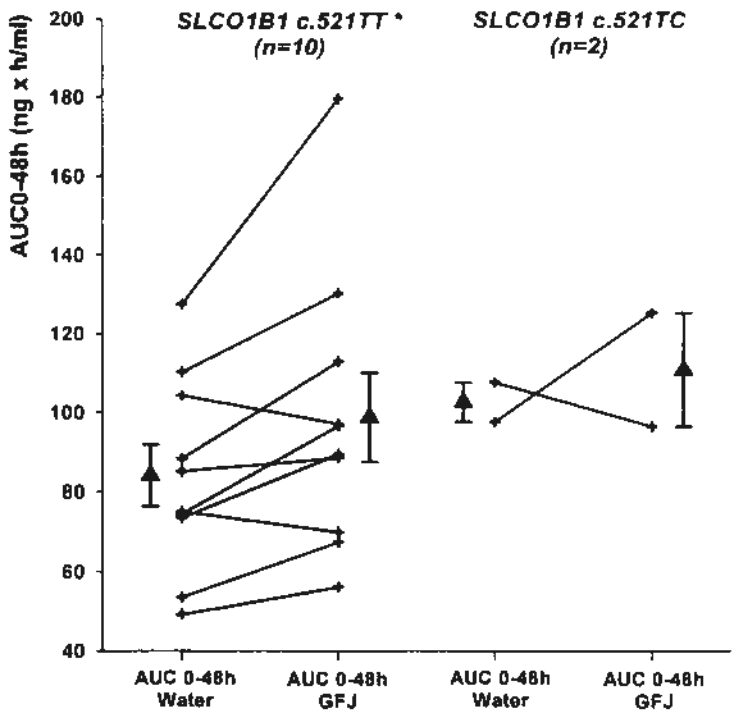
Testing the changes for *c.521TT* subjects between administration with water and GFJ for pitavastatin acid, there were no statistical significance of C_{max} (Graph 4.6), but there were higher AUCs after administration with GFJ ($P<0.05$, Graph 4.7 and 4.8).

Table 4.5 listed the comparison and according to SNP *SLCO1B1 c.388A>G*. Trends were noted for higher C_{max} and AUCs with *c.388GG* individuals than *c.388A* carriers.

Graph 4.6: Individual C_{max} of pitavastatin acid comparison between administration with water and GFJ according to *SLCO1B1 c.521T>C* genotypes with mean ± SD. No statistical significance by Student's t-test on paired values



Graph 4.7: Individual AUC $_{0\text{-}48h}$ of pitavastatin acid comparison between administration with water and GFJ according to *SLCO1B1 c.521T>C* genotypes with mean ± SD. Statistical comparison by Student's t-test on paired values. *P<0.05 is statistically significant



Graph 4.8: Individual AUC $_{0\text{-}\infty}$ of pitavastatin acid comparison between administration with water and GFJ according to *SLCO1B1 c.521T>C* genotypes with mean ± SD. Statistical comparison by Student's t-test on paired values. *P<0.05 is statistically significant

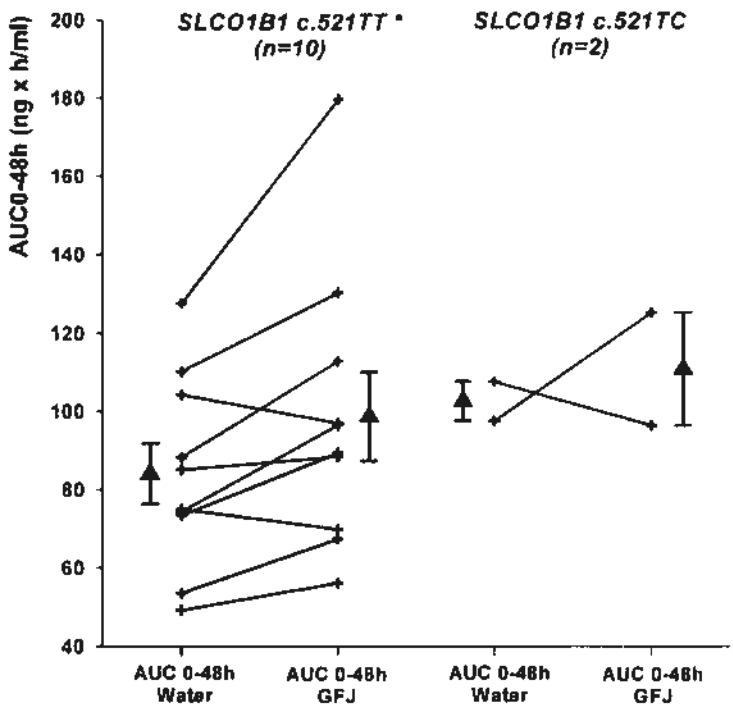


Table 4.5: Pharmacokinetic parameters according to *SLCO1B1 c.388A>G* genotypes, and the changes after administration with Grapefruit Juice (GFJ)

Genotype	*c.388AA (n=1)*	*c.388AG (n=5)*	*c.388GG (n=6)*	*c.388AA (n=1)*	*c.388AG (n=5)*	*c.388GG (n=6)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	21.1	32.4±10.1 (19.5–47.7)	46.4±13.4 (24.0–63.5)	+6.8	-5.0±10.7 (-18.2-+10.8)	-7.5±11.1 (-24.6-+6.3)
AUC_{0-24h} (ng×h/ml)	53.5	75.8±18.8 (49.2–99.7)	97.8±18.9 (70.2–125.5)	+13.8	+7.4±16.0 (-5.6-+16.6)	+14.1±16.8 (-8.0-+42.1)
AUC_{0-48h} (ng×h/ml)	53.5	78.9±21.4 (49.2–107.7)	99.7±19.0 (73.4–127.5)	+13.8	+7.4±16.0 (-11.2-+24.6)	+18.7±20.6 (-7.2-+52.1)
AUC_{0-t} (ng×h/ml)	46.9	75.8±21.5 (45.0–103.7)	95.1±20.2 (70.2–125.5)	+13.2	+6.9±14.7 (-9.2-+22.2)	+18.4±20.7 (-8.0-+48.5)
$AUC_{0-\infty}$ (ng×h/ml)	58.3	83.4±22.3 (49.7–110.9)	108.2±17.4 (85.7–129.8)	+11.1	+6.6±15.7 (-11.5-+26.6)	+16.2±20.1 (-4.6-+52.9)
Apparent oral clearance (L/h)	34.3	25.7±8.5 (18.0–40.2)	18.9±3.2 (15.4–23.3)	-5.5	-1.9±3.5 (-4.8-+2.1)	-1.9±1.7 (-4.5-+0.8)
$t_{1/2}$ (h)	7.2	8.6±2.4 (4.7–10.6)	9.8±4.5 (4.6–16.9)	-1.8	+0.0±3.1 (-3.2-+5.1)	+0.5±4.2 (-7.8-+3.4)
t_{max} (h)	*1*	*1 (0.5–1)*	*0.5 (0.5–1)*	*1**	*1.5 (1 –2)**	*1 (1–1)**
Pitavastatin Lactone						
C_{max} (ng/ml)	13.0	22.9±9.7 (16.4–39.6)	28.1±3.3 (22.9–31.2)	-0.3	-3.6±5.6 (-11.9-+3.1)	-3.9±4.0 (-9.3-+2.6)
AUC_{0-24h} (ng×h/ml)	104.2	143.1±39.6 (99.1–207.4)	173.5±27.9 (132.9–209.6)	+18.7	+7.4±16.0 (-22.5-+24.8)	+18.7±20.6 (-17.0-+80.4)
AUC_{0-48h} (ng×h/ml)	116.0	166.7±42.4 (118.7–234.6)	195.3±34.0 (146.5–238.8)	+32.7	+14.7±18.7 (-18.5-+27.2)	+33.8±45.5 (-17.4-+114.0)
AUC_{0-t} (ng×h/ml)	111.2	164.9±45.0 (109.9–234.6)	192.3±36.1 (140.1–238.8)	+37.5	+19.7±20.8 (-18.5-+31.8)	+39.4±51.7 (-17.4-+120.4)
$AUC_{0-\infty}$ (ng×h/ml)	118.7	180.0±42.9 (130.1–248.2)	202.5±37.3 (149.5–250.0)	+42.9	+19.7±20.8 (-15.6-+38.3)	+39.4±51.7 (-19.6-+130.2)
Apparent oral clearance (L/h)	16.9	11.6±2.6 (8.1–15.4)	10.2±2.0 (8.0–13.4)	-4.5	-1.5±1.4 (-3.5-+0.5)	-1.4±1.6 (-3.7-+0.8)
$t_{1/2}$ (h)	8.7	15.8±3.0 (12.8–19.6)	10.1±2.6 # (7.1–13.9)	+6.2	+2.8±7.3 (-5.6-+13.7)	+2.4±3.0 (-2.2-+5.4)
t_{max} (h)	*1*	*1 (1 –1.5)*	*1 (1 –1.5)*	*2**	*2 (1.5–3)**	*1.75(1.5–2)**

Data are given as mean±SD and range, statistical comparison (*GG vs AG*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test.

# P<0.05 is statistically significant. * t_{max} data are as administered under GFJ. Apparent oral clearance is calculated by Dose / $AUC_{0-\infty}$

Statistical comparison between the six subjects with homozygous *SLCO1B1 c.388GG*, with those of least one copy of *c.388A*, C_{max} of pitavastatin acid was 46.4±13.4 vs. 30.5±10.1 ng/ml, $AUC_{0-\infty}$ was 108.2±17.4 vs. 79.3±22.4 ng×h/ml ($P<0.05$), and C_{max} of lactone was 28.1±3.3 vs. 21.3±9.6 ng/ml, $AUC_{0-\infty}$ was 202.5 ±37.3 vs. 169.8±45.8 ng×h/ml (NS) (Table 4.6). By excluding the two subjects with *c.521TC*, and hence **15* haplotype, Table 4.7 listed comparisons between *c.388GG* (n=5) and *c.388AA/AG* subjects (n=5). Mean C_{max} value for pitavastatin acid was higher by 68%, AUC_{0-48h} by 47% and CL/F were lower by 34% in *c.388GG* compared to *c.388AA/AG* subjects ($P<0.05$). Similar variations were noted for pitavastatin lactone values, with mean C_{max} higher by 63%, AUC_{0-48h} by 44% ($P<0.01$), $AUC_{0-\infty}$ by 38% ($P<0.05$) (Graph 4.9-4.11) and CL/F lower by 29% ($P<0.05$). However, the changes in pharmacokinetic parameters due to administration with GFJ did not differ significantly between the two haplotype groups.

Table 4.6: Pharmacokinetic parameters comparison on a recessive model according to *SLCO1B1 c.388A>G* genotypes, and the changes after administration with Grapefruit Juice (GFJ)

Genotype	*c.388AA/AG* *(n=6)*	*c.388GG* *(n=6)*	*c.388AA/AG* *(n=6)*	*c.388GG* *(n=6)*
Administration			*Changes after consumption with GFJ*	
Pitavastatin Acid				
C_{max} (ng/ml)	30.5 ± 10.1 (19.5 – 47.7)	46.4 ± 13.4 # (24.0 – 63.5)	-3.0 ± 10.7 (-18.2 - +10.8)	-7.5 ± 11.1 (-24.6 - +6.3)
AUC_{0-24h} (ng×h/ml)	72.1 ± 19.1 (49.2 – 99.7)	97.8 ± 18.9 # (70.2 – 125.5)	+7.2 ± 10.4 (-5.7 - +16.6)	+14.1 ± 16.8 (-8.0 - +42.1)
AUC_{0-48h} (ng×h/ml)	74.7 ± 21.8 (49.2 – 107.7)	99.7 ± 19.0 (73.4 – 127.5)	+8.5 ± 14.5 (-11.2 - +24.6)	+18.7 ± 20.6 (-7.2 - +52.1)
AUC_{0-t} (ng×h/ml)	71.0 ± 22.6 (45.0 – 103.7)	95.1 ± 20.2 (70.2 – 125.5)	+7.9 ± 13.4 (-9.2 - +22.2)	+18.4 ± 20.7 (-8.0 - +48.5)
$AUC_{0-\infty}$ (ng×h/ml)	79.3 ± 22.4 (49.7 – 110.9)	108.2 ± 17.4 # (85.7 – 129.8)	+7.3 ± 14.1 (-11.5 - +26.6)	+16.2 ± 20.1 (-4.6 - +52.9)
Apparent oral clearance (L/h)	27.2 ± 8.4 (18.0 – 40.2)	18.9 ± 3.2 (15.4 – 23.3)	-2.5 ± 3.5 (-5.5 - +2.1)	-1.9 ± 1.7 (-4.5 - +0.8)
$t_{1/2}$ (h)	8.4 ± 2.3 (4.7 – 10.6)	9.8 ± 4.4 (4.6 – 16.9)	-0.3 ± 2.9 (-3.2 - +5.1)	+0.5 ± 4.2 (-7.8 - +3.4)
t_{max} (h)	*1 (0.5 – 1)*	*0.5 (0.5 – 1)*	*1.5 (1 – 2)**	*1 (1 – 1)**
Pitavastatin Lactone				
C_{max} (ng/ml)	21.3 ± 9.6 (13.0 – 39.6)	28.1 ± 3.3 (22.9 – 31.2)	-3.1 ± 5.2 (-11.9 - +3.1)	-3.9 ± 4.0 (-9.3 - +2.6)
AUC_{0-24h} (ng×h/ml)	136.5 ± 38.8 (99.1 – 207.4)	173.5 ± 27.9 (132.9 – 209.6)	+12.1 ± 17.2 (-22.5 - +24.8)	+22.8 ± 33.8 (-17.0 - +80.4)
AUC_{0-48h} (ng×h/ml)	158.2 ± 43.2 (116.0 – 234.6)	195.3 ± 34.0 (146.5 – 238.8)	+17.7 ± 18.3 (-18.5 - +32.7)	+33.8 ± 45.5 (-17.4 - +114.0)
AUC_{0-t} (ng×h/ml)	155.9 ± 45.8 (109.9 – 234.6)	192.3 ± 36.1 (140.1 – 238.8)	+20.0 ± 19.9 (-18.5 - +37.5)	+33.8 ± 48.3 (-17.4 - +120.4)
$AUC_{0-\infty}$ (ng×h/ml)	169.8 ± 45.8 (118.7 – 248.2)	202.5 ± 37.3 (149.5 – 250.0)	+23.6 ± 20.9 (-15.6 - +42.9)	+39.4 ± 51.7 (-19.6 - +130.2)
Apparent oral clearance (L/h)	12.5 ± 3.2 (8.1 – 16.9)	10.2 ± 2.0 (8.0 – 13.4)	-2.0 ± 1.8 (-4.5 - +0.5)	-1.4 ± 1.6 (-3.7 - +0.8)
$t_{1/2}$ (h)	14.6 ± 4.0 (8.7 – 19.6)	10.1 ± 2.6 # (7.1 – 13.9)	+3.3 ± 6.7 (-5.6 - +13.7)	+2.4 ± 3.0 (-2.2 - +5.4)
t_{max} (h)	*1 (1 – 1.5)*	*1 (1 – 1.5)*	*2 (1.5 – 3)**	*1.75 (1.5 – 2)**

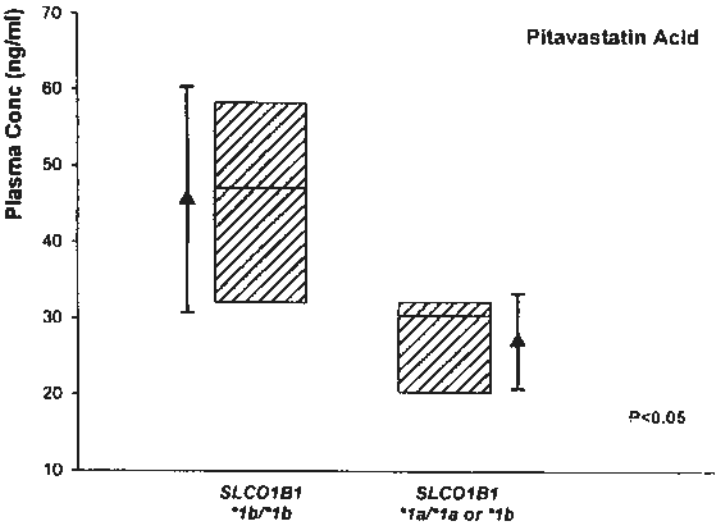
Data are given as mean±SD and range, statistical comparison (*GG vs AG*) by Student's t-test, except t_{max} as median (range) and compared by Mann-Whitney U Test. # P<0.05 is statistically significant. *t_{max} data are as administered under GFJ.

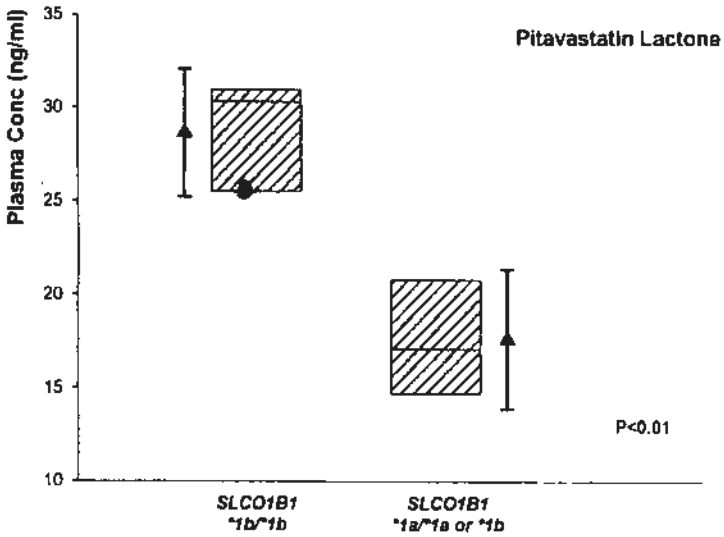
Table 4.7: Pharmacokinetic parameters comparison on a recessive model according to *SLCO1B1 c.388A>G* genotypes (excluding subjects *c.521TC/CC*), and changes after administration with GFJ

Genotype	*c.388AA/AG* (*n=5*)	*c.388GG* (*n=5*)	*c.388AA/AG* (*n=5*)	*c.388GG* (*n=5*)
Administration			*Changes after consumption with GFJ*	
Pitavastatin Acid				
C_{max} (ng/ml)	27.1 ± 6.3 (19.5 – 32.8)	45.5 ± 14.8 # (24.0 – 63.5)	+0.0 ± 8.7 (-10.7 - +10.8)	-6.3 ± 12.0 (-24.6 - +6.3)
AUC $_{0\text{-}24h}$ (ng×h/ml)	72.1 ± 19.1 (49.2 – 86.0)	97.8 ± 18.9 # (70.2 – 125.5)	+7.2 ± 10.4 (-5.7 - +16.6)	+14.1 ± 16.8 (-8.0 - +42.1)
AUC $_{0\text{-}48h}$ (ng×h/ml)	68.1 ± 16.3 (49.2 – 88.4)	100.1 ± 21.3 # (73.4 – 127.5)	+12.4 ± 12.1 (-5.3 - +24.6)	+16.9 ± 22.4 (-7.2 - +52.1)
AUC $_{0\text{-}t}$ (ng×h/ml)	71.0 ± 22.6 (45.0 – 86.0)	95.1 ± 20.2 # (70.2 – 125.5)	+7.9 ± 13.4 (-5.7 - +22.2)	+18.4 ± 20.7 (-8.0 - +48.5)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	72.9 ± 18.1 (49.7 – 92.9)	107.8 ± 19.4 # (85.7 – 129.8)	+11.1 ± 12.0 (-5.5 - +26.6)	+15.4 ± 22.3 (-4.6 - +52.9)
Apparent oral clearance (L/h)	29.0 ± 7.9 (21.5 – 40.2)	19.1 ± 3.5 # (15.4 – 23.3)	-3.4 ± 3.0 (-5.5 - +1.9)	-1.7 ± 1.9 (-4.5 - +0.8)
$t_{1/2}$ (h)	8.1 ± 2.4 (4.7 – 10.6)	9.9 ± 5.0 (4.6 – 16.9)	+0.3 ± 2.8 (-1.8 - +5.1)	-0.1 ± 4.4 (-7.8 - +2.9)
t_{max} (h)	*1 (0.5 – 1)*	*0.5 (0.5 – 1)*	*1.0 (1 – 2)**	*1 (1 – 1)**
Pitavastatin Lactone				
C_{max} (ng/ml)	17.6 ± 3.7 (13.0 – 23.3)	28.6 ± 3.4 § (22.9 – 31.2)	-1.3 ± 3.3 (-5.1 - +3.1)	-4.1 ± 4.4 (-9.3 - +2.6)
AUC $_{0\text{-}24h}$ (ng×h/ml)	136.5 ± 38.8 (99.1 – 141.9)	173.5 ± 27.9 § (155.8 – 209.6)	+12.1 ± 17.2 (-15.5 - +24.8)	+22.8 ± 33.8 (-17.0 - +80.4)
AUC $_{0\text{-}48h}$ (ng×h/ml)	142.9 ± 24.1 (116.0 – 167.5)	205.1 ± 27.0 § (177.0 – 238.8)	+25.0 ± 5.0 (+20.0 - +32.7)	+36.0 ± 50.5 (-17.4 - +114.0)
AUC $_{0\text{-}t}$ (ng×h/ml)	155.9 ± 45.8 (109.9 – 167.5)	192.3 ± 36.1 § (171.4 – 238.8)	+20.0 ± 19.9 (-20.1 - +37.5)	+33.8 ± 48.3 (-17.4 - +120.4)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	154.1 ± 27.9 (118.7 – 179.5)	213.2 ± 30.0 # (180.1 – 250.0)	+31.4 ± 9.2 (+19.9 - +42.9)	+41.1 ± 57.6 (-19.6 - +130.2)
Apparent oral clearance (L/h)	13.4 ± 2.6 (11.1 – 16.9)	9.5 ± 1.3 # (8.0 – 11.1)	-2.5 ± 1.4 (-4.5 - -1.3)	-1.3 ± 1.7 (-3.7 - +0.8)
$t_{1/2}$ (h)	14.4 ± 4.4 (8.7 – 19.6)	10.5 ± 2.7 (7.1 – 13.9)	+3.9 ± 7.3 (-5.6 - +13.7)	+2.1 ± 3.3 (-2.2 - +5.4)
t_{max} (h)	*1 (1 – 1.5)*	*1 (1 – 1.5)*	*2 (1.5 – 3)**	*2.0 (1.5 – 2)**

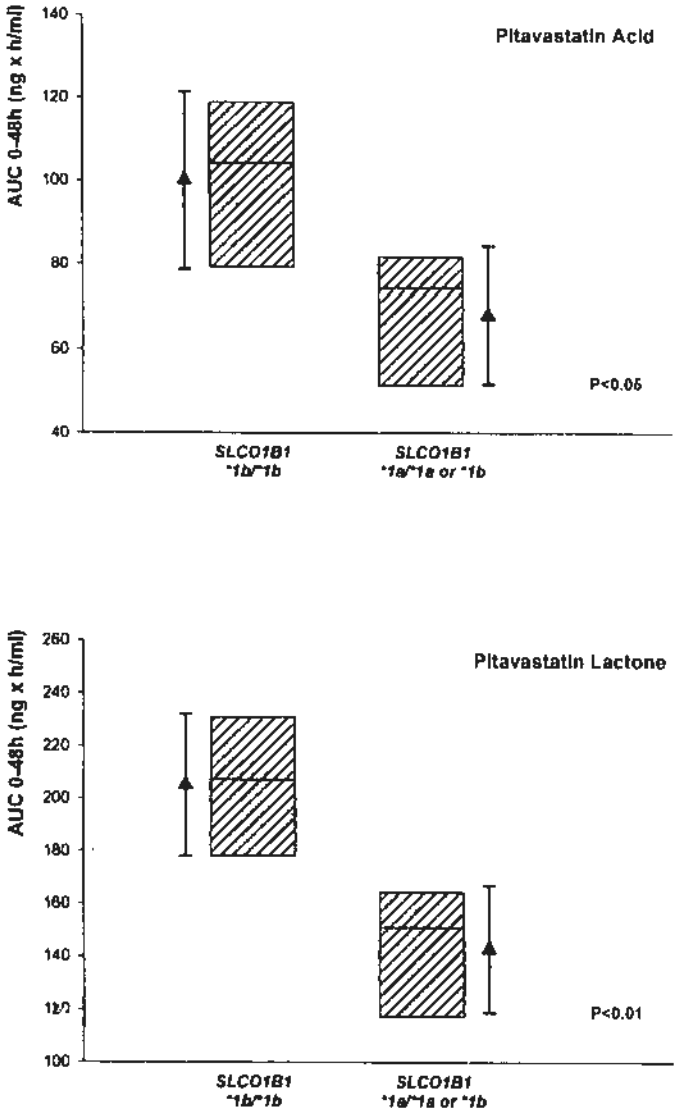
Data are given as mean±SD and range, statistical comparison (*GG vs AG*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. # P<0.05 and §P<0.01 are ssignificant. * t_{max} data are as recorded as administered under GFJ.

Graph 4.9: C_{max} as administered with water of pitavastatin acid and lactone on *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT - c.388AA/AG)* genotypes. Mean ± SD with boxplots showing median

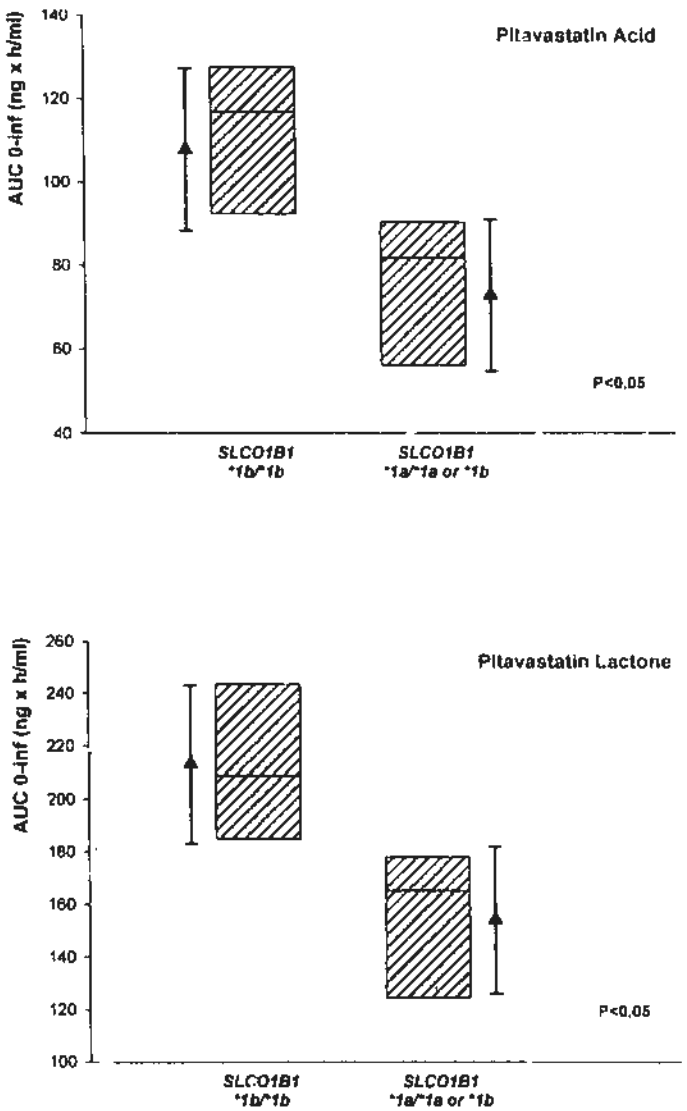




Graph 4.10: AUC $_{0\text{-}48h}$ as administered with water of pitavastatin acid and lactone on *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT - c.388AA/AG)* genotypes. Mean ± SD with boxplots showing median



Graph 4.11: AUC $_{0-\infty}$ as administered with water of pitavastatin acid and lactone on *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT - c.388AA/AG)* genotypes. Mean ± SD with boxplots showing median



4.5.3. Genetic influence from *CYP3A5*3 (c.6986A>G)*

Homozygous *CYP3A5 *3/*3* subjects tended to have higher C_{max} and AUCs than heterozygous **1/*3* individuals (Table 4.8), but differences were of no statistical significance; similar results were obtained when compared against those with one copy of the **1* wild-type allele (Table 4.9). No gene-food interaction was noted as evaluated by absence of significant differences among genotype groups in changes of pharmacokinetic parameters after GFJ.

Table 4.8: Pharmacokinetic parameters according to *CYP3A5 *1* and **3* genotypes, and the changes after administration with Grapefruit Juice (GFJ)

Genotype	**1/*1* (n=1)	**1/*3* (n=4)	**3/*3* (n=7)	**1/*1* (n=1)	**1/*3* (n=4)	**3/*3* (n=7)
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	50.7	32.3±11.6 (19.5–47.7)	40.2±15.5 (21.1–63.5)	-13.0	-5.0±12.4 (-18.2-+10.8)	-4.1±11.0 (-24.6-+6.8)
AUC_{0-24h} (ng×h/ml)	97.6	77.0±21.5 (49.2–99.7)	87.7±24.9 (53.5–125.5)	+17.8	+3.1 ± 10.7 (-5.7-+16.6)	+13.9±15.3 (-8.0-+42.1)
AUC_{0-48h} (ng×h/ml)	97.6	80.1±24.6 (49.2–107.7)	89.8±25.5 (53.5–127.5)	+27.8	+3.7 ± 15.8 (-11.2-+24.6)	+17.2±18.5 (-7.2-+52.1)
AUC_{0-t} (ng×h/ml)	87.4	76.9±24.7 (45.0–103.7)	85.9±26.7 (46.9–125.5)	+34.0	+3.2±14.1 (-9.2-+22.2)	+15.9±17.8 (-8.0-+48.5)
$AUC_{0-\infty}$ (ng×h/ml)	110.0	83.4±25.7 (49.7–110.9)	97.3±25.1 (58.3–129.8)	+20.5	+4.0±16.8 (-11.5-+26.6)	+15.0±18.3 (-4.6-+52.9)
Apparent oral clearance (L/h)	18.2	26.2±9.8 (18.0–40.2)	21.9±6.4 (15.4–34.3)	-2.9	-1.3±3.8 (-4.8-+2.1)	-2.6±2.2 (-5.5-+0.8)
$t_{1/2}$ (h)	9.2	8.2±2.5 (4.7–10.0)	9.6±4.2 (4.6–16.9)	+3.4	-0.0±3.6 (-3.2-+5.1)	-0.3±3.7 (-7.8-+2.9)
t_{max} (h)	*0.5*	*1 (0.5–1)*	*1 (0.5–1)*	*1**	*1.5 (1–2)**	*1 (1–1)**
Pitavastatin Lactone						
C_{max} (ng/ml)	25.5	24.6±10.4 (17.1–39.6)	24.7±7.4 (13.0 – 31.2)	-2.6	-4.5±6.1 (-11.9-+3.1)	-3.0±4.0 (-9.3-+2.6)
AUC_{0-24h} (ng×h/ml)	132.9	145.3±45.3 (99.1–207.4)	163.8±36.4 (104.0–209.6)	+13.2	+7.4±19.9 (-22.5-+18.6)	+23.8±30.7 (-17.0-+80.4)
AUC_{0-48h} (ng×h/ml)	146.5	168.0±48.8 (118.7–234.6)	186.1±41.3 (116.0–238.8)	+22.8	+11.6±20.1 (-18.5-+23.0)	+34.2±41.4 (-17.4-+114.0)
AUC_{0-t} (ng×h/ml)	140.1	165.8±51.9 (109.9–234.6)	183.7±42.4 (111.2–238.8)	+19.6	+13.8±22.1 (-18.5-+31.8)	+35.4±43.8 (-17.4-+120.4)
$AUC_{0-\infty}$ (ng×h/ml)	149.5	180.1±49.6 (130.1–248.2)	194.9±43.4 (118.7–250.0)	+30.6	+18.0±23.7 (-15.6-+38.3)	+39.3±47.4 (-19.6-+130.2)
Apparent oral clearance (L/h)	13.4	11.7±3.0 (8.1–15.4)	10.8±2.9 (8.0–16.9)	-2.3	-1.5±1.7 (-3.5-+0.5)	-1.7±1.8 (-4.5-+0.8)
$t_{1/2}$ (h)	8.1	15.2±3.2 (12.8–19.6)	11.3±3.7 (7.1–18.0)	+3.7	+3.6±8.2 (-5.6-+13.7)	+2.3±3.3 (-2.2-+6.2)
t_{max} (h)	*0.5*	*1 (1–1.5)*	*1 (1–1.5)*	*1.5**	*2 (1.5 –3)**	*2 (1.5–2)* #*

Data are given as mean±SD and range, statistical comparison (**1/*3 vs *3/*3*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. # $P<0.05$ is statistically significant. * t_{max} data were as recorded as administered under GFJ. Apparent oral clearance is calculated by Dose / $AUC_{0-\infty}$

Table 4.9: Pharmacokinetic parameters according to *CYP3A5 *1* and **3* genotypes, and the changes after administration with Grapefruit Juice (GFJ)

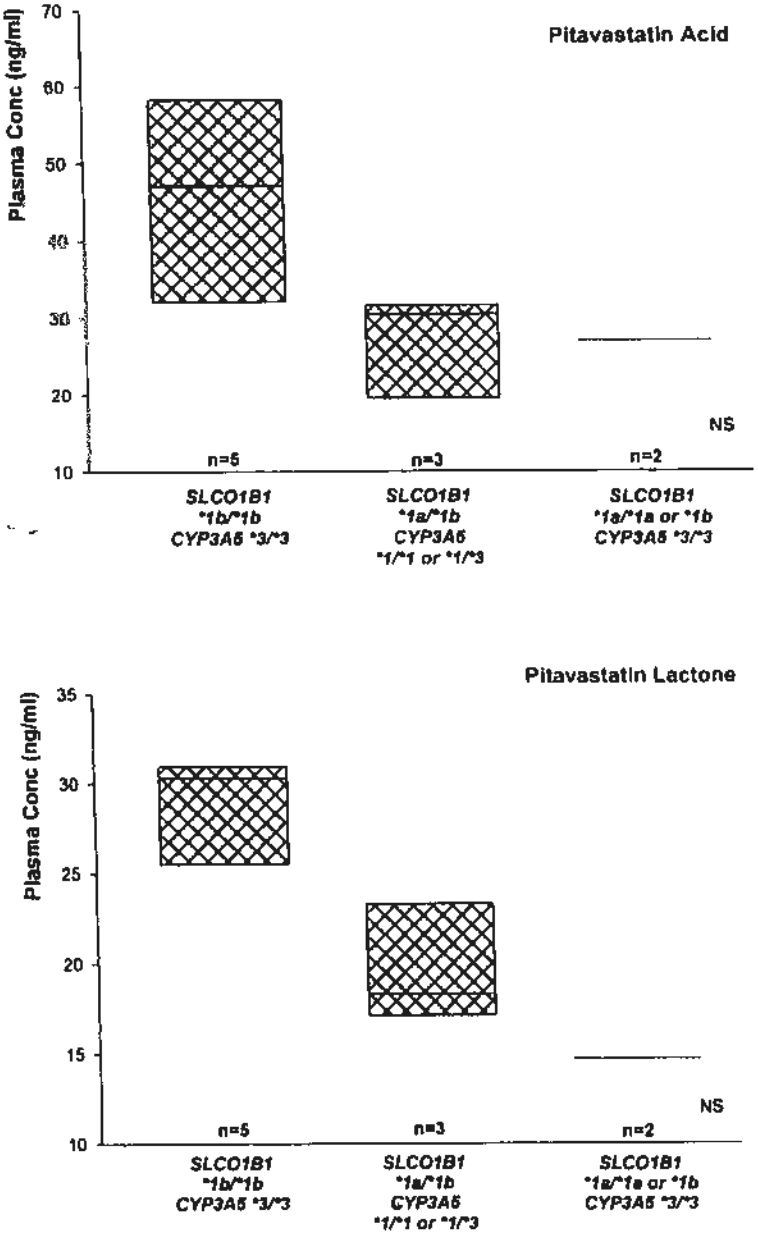
Genotype	**1/*1 or*1/*3 (n=5)*	**3/*3 (n=7)*	**1/*1 or*1/*3 (n=5)*	**3/*3 (n=7)*
Administration			*Changes after consumption with GFJ*	
Pitavastatin Acid				
C_{max} (ng/ml)	36.0 ± 13.0 (19.5 – 50.7)	40.2 ± 15.5 (21.1 – 63.5)	-6.8 ± 11.3 (-18.2 - +10.8)	-4.1 ± 11.0 (-24.6 - +6.8)
AUC_{0-24h} (ng×h/ml)	81.1 ± 20.8 (49.2 – 99.7)	87.7 ± 24.9 (53.5 – 125.5)	+6.1 ± 11.3 (-5.7 - +17.8)	+13.9 ± 15.3 (-8.0 - +42.1)
AUC_{0-48h} (ng×h/ml)	83.6 ± 22.7 (49.2 – 107.7)	89.8 ± 25.5 (53.5 – 127.5)	+8.5 ± 17.4 (-11.2 - +27.8)	+17.2 ± 18.5 (-7.2 - +52.1)
AUC_{0-t} (ng×h/ml)	79.0 ± 21.9 (45.0 – 103.7)	85.9 ± 26.7 (46.9 – 125.5)	+9.4 ± 18.4 (-9.2 - +34.0)	+15.9 ± 17.8 (-8.0 - +48.5)
$AUC_{0-\infty}$ (ng×h/ml)	88.7 ± 25.3 (49.7 – 110.9)	97.3 ± 25.1 (58.3 – 129.8)	+7.3 ± 16.3 (-11.5 - +26.6)	+15.0 ± 18.3 (-4.6 - +52.9)
Apparent oral clearance (L/h)	24.6 ± 9.2 (18.0 – 40.2)	21.9 ± 6.4 (15.4 – 34.3)	-1.6 ± 3.4 (-4.8 - +2.1)	-2.6 ± 2.2 (-5.5 - +0.8)
$t_{1/2}$ (h)	8.4 ± 2.2 (4.7 – 10.0)	9.6 ± 4.2 (4.6 – 16.9)	+0.7 ± 3.5 (-3.2 - +5.1)	-0.3 ± 3.7 (-7.8 - +2.9)
t_{max} (h)	*1 (0.5 – 1)*	*1 (0.5 – 1)*	*1.5 (1 – 2)**	*1 (1 – 1)**
Pitavastatin Lactone				
C_{max} (ng/ml)	24.8 ± 9.0 (17.1 – 39.6)	24.7 ± 7.4 (13.0 – 31.2)	-4.1 ± 5.4 (-11.9 - +3.1)	-3.0 ± 4.0 (-9.3 - +2.6)
AUC_{0-24h} (ng×h/ml)	142.8 ± 39.6 (99.1 – 207.4)	163.8 ± 36.4 (104.0 – 209.6)	+8.5 ± 17.4 (-22.5 - +18.6)	+23.8 ± 30.7 (-17.0 - +80.4)
AUC_{0-48h} (ng×h/ml)	163.7 ± 43.4 (118.7 – 234.6)	186.1 ± 41.3 (116.0 – 238.8)	+13.9 ± 18.1 (-18.5 - +23.0)	+34.2 ± 41.4 (-17.4 - +114.0)
AUC_{0-t} (ng×h/ml)	160.7 ± 46.4 (109.9 – 234.6)	183.7 ± 42.4 (111.2 – 238.8)	+15.0 ± 19.4 (-18.5 - +31.8)	+35.4 ± 43.8 (-17.4 - +120.4)
$AUC_{0-\infty}$ (ng×h/ml)	174.0 ± 45.1 (130.1 – 248.2)	194.9 ± 43.4 (118.7 – 250.0)	+20.5 ± 21.2 (-15.6 - +38.3)	+39.3 ± 47.4 (-19.6 - +130.2)
Apparent oral clearance (L/h)	12.0 ± 2.7 (8.1 – 15.4)	10.8 ± 2.9 (8.0 – 16.9)	-1.6 ± 1.5 (-3.5 - +0.5)	-1.7 ± 1.8 (-4.5 - +0.8)
$t_{1/2}$ (h)	13.8 ± 4.2 (8.1 – 19.6)	11.3 ± 3.7 (7.1 – 18.0)	+3.6 ± 7.1 (-5.6 - +13.7)	+2.3 ± 3.3 (-2.2 - +6.2)
t_{max} (h)	*1 (1 – 1.5)*	*1 (1 – 1.5)*	*2 (1.5 – 3)**	*2 (1.5 – 2)* #*

Data are given as mean±SD and range, statistical comparison (**1/*3 vs *3/*3*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. # $P<0.05$ is statistically significant.* t_{max} data were as recorded as administered under GFJ. Apparent oral clearance is calculated by Dose / $AUC_{0-\infty}$

To compare the possible gene-gene interaction, Graph 4.12 and 4.13 showed comparisons of C_{max} and $AUC_{0\text{-}48h}$ of plasma pitavastatin acid and lactone levels among groups combining *SLCO1B1 *1a* or **1b* and *CYP3A5 *1* or **3* genotypes. The group of subjects with *SLCO1B1 *1b/*1b* and *CYP3A5 *3/*3* had statistically higher $AUC_{0\text{-}48h}$ pitavastatin lactone values than subjects with *SLCO1B1 *1a/*1b* and *CYP3A5 *1* (Graph 4.13).

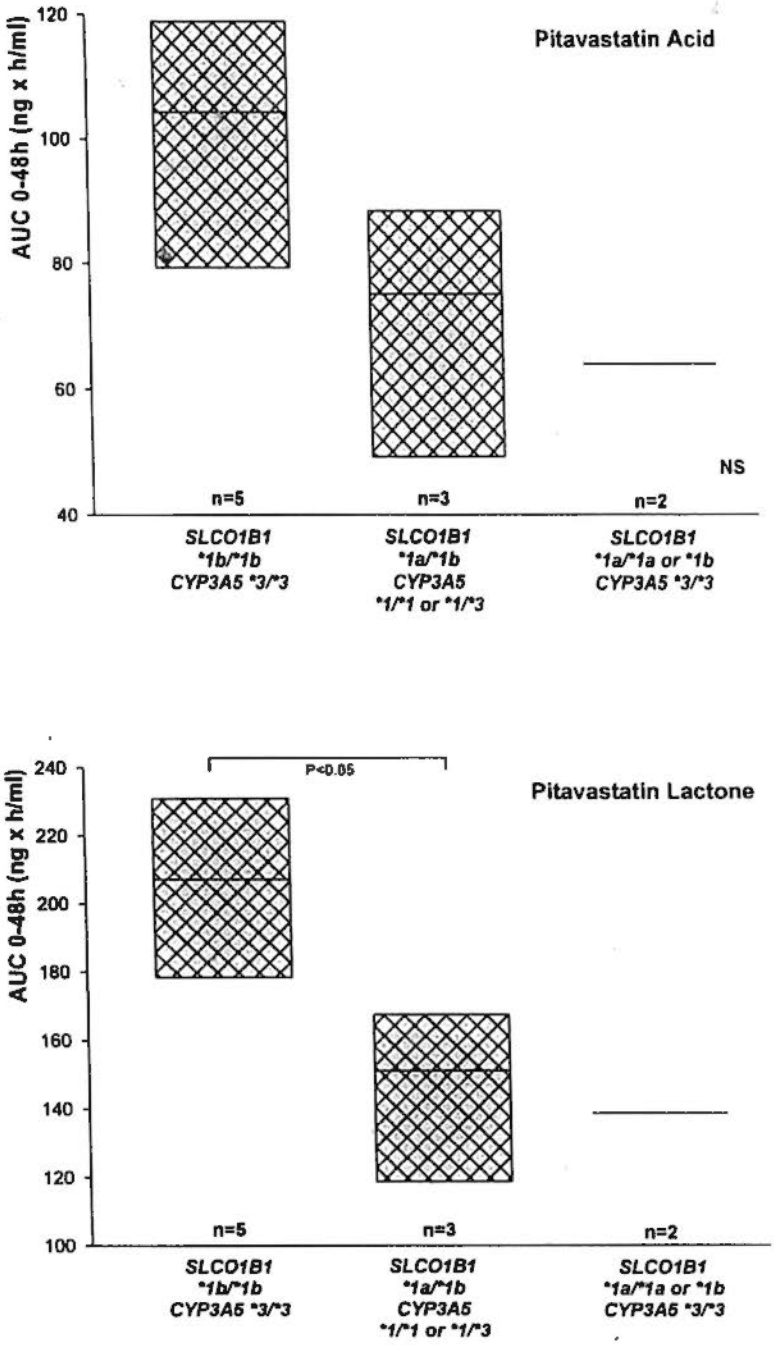
C_{max} and AUCs plots of pitavastatin acid and lactone between administration with water or GFJ on individual subjects were shown in Graph 4.14-4.17, grouped by *CYP3A5 *1* or **3/*3* showing their respective *SLCO1B1 *1a, *1b* or **15* haplotypes. Administration with GFJ generally reduced C_{max} and increased AUCs, but no observable differences were seen between the two *CYP3A5* genotype groups. Both *SLCO1B1 *15* individuals were of *CYP3A5 *1* genotype. The C_{max} and AUCs values administered with water or GFJ, were generally higher for both acid and lactone than the rest of *CYP3A5 *1* and non-**15* individuals. In the group of *CYP3A5 *3/*3* subjects, *SLCO1B1 *1b/*1b* carriers had higher C_{max} and $AUC_{0\text{-}48h}$ values than most **1a* carriers for both pitavastatin acid and lactone. That was confirmed by statistical comparison of AUCs values for pitavastatin acid and lactone between sub-groups of subjects with *CYP3A5*1* and *SLCO1B1*1a* carriers comparing to those of *CYP3A5*3/*3* and *SLCO1B1*1b/*1b* genotypes (Table 4.10).

Graph 4.12: C_{max} as administered with water of pitavastatin acid and lactone according to *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into *CYP3A5 *1* or **3* haplotypes. Boxplots showing median



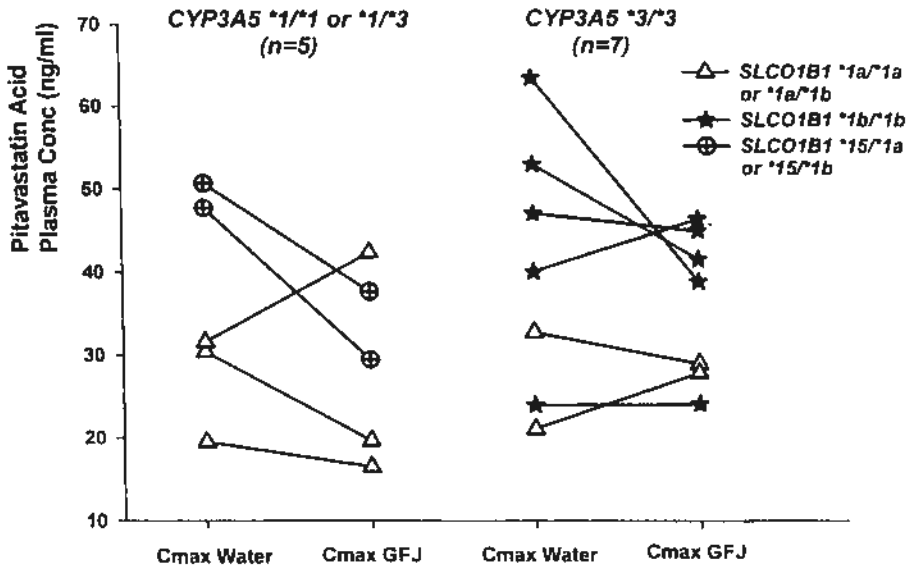
Statistical comparison by Mann-Whitney U test on first group. No statistical significance found.

Graph 4.13: AUC $_{0\text{-}48h}$ as administered with water of pitavastatin acid and lactone according to *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into *CYP3A5 *1* or **3* haplotypes. Boxplots showing median

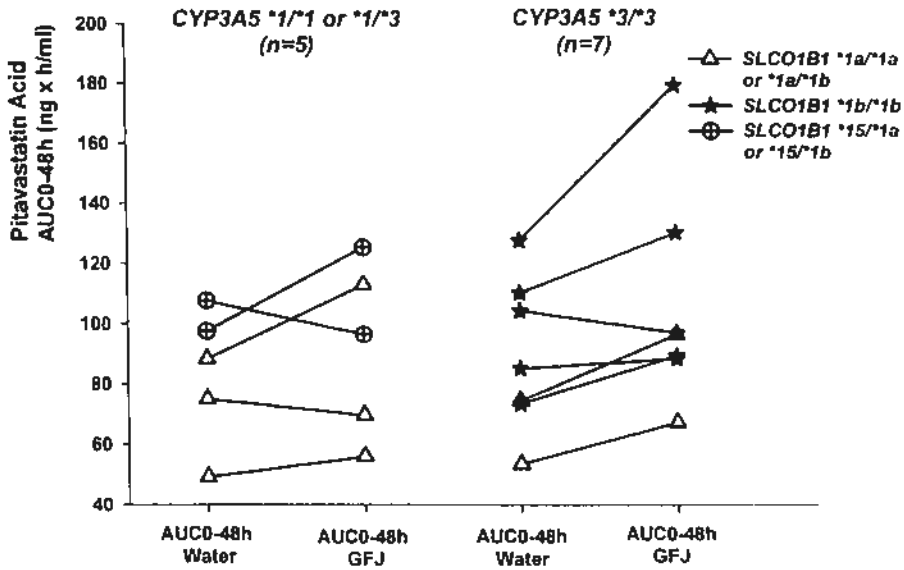


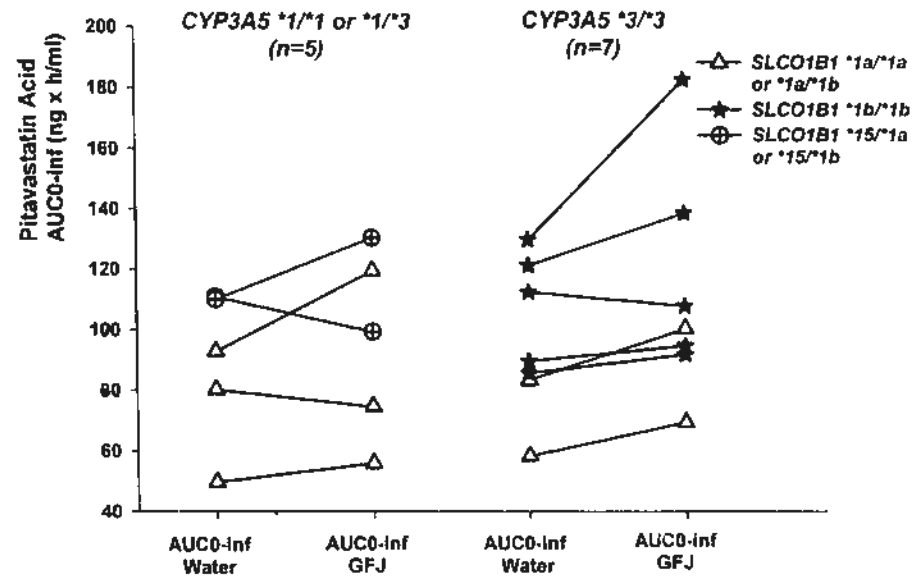
Statistical comparison by Mann-Whitney U test on first group. P<0.05 is statistical significant. No statistical significance found with Pitavastatin Acid.

Graph 4.14: Individual C_{max} of pitavastatin acid comparison between administration with water and GFJ according to *CYP3A5 *1* or **3*, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b*, and **15* haplotypes

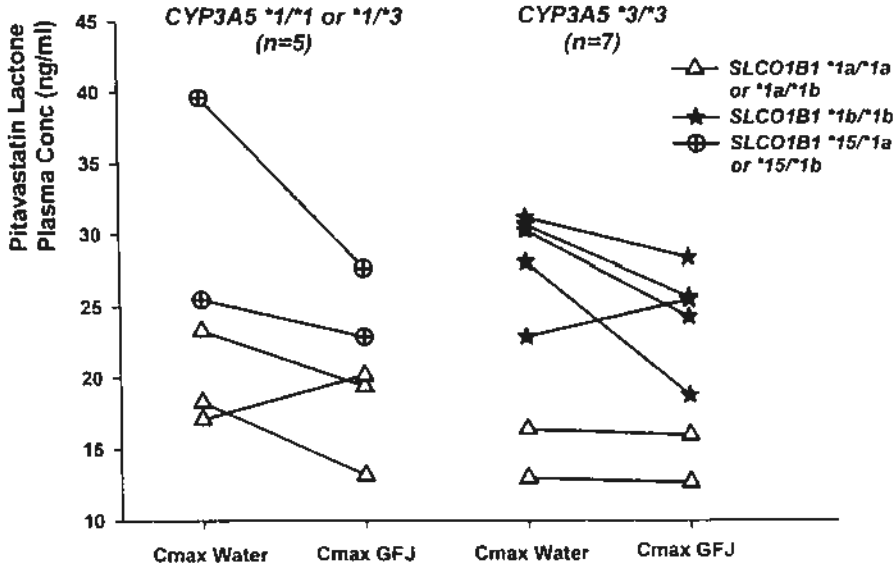


Graph 4.15: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of pitavastatin acid comparison between administration with water and GFJ according to *CYP3A5 *1* or **3*, identified by *SLCO1B1 *1b/*1b*, **1a/*1a* or **1b*, and **15* haplotypes

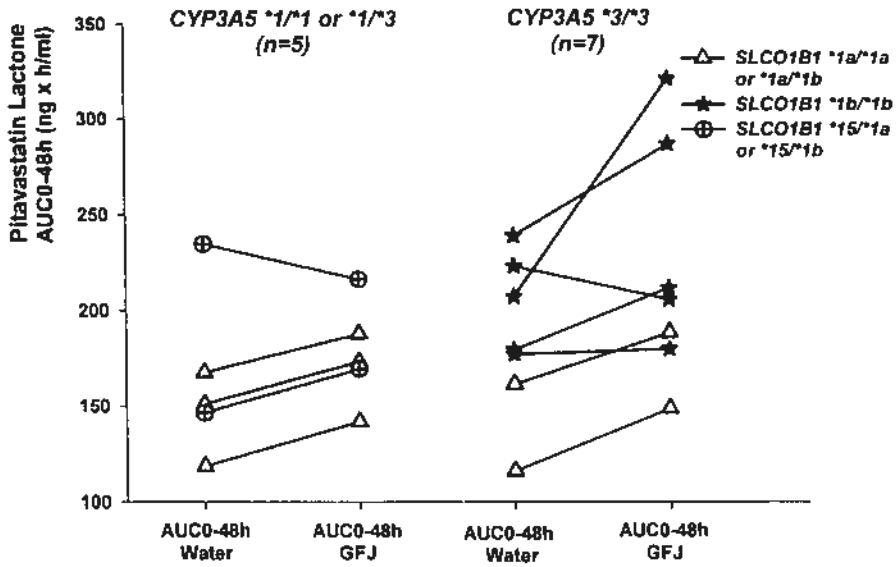




Graph 4.16: Individual C_{max} of pitavastatin lactone comparison between administration with water and GFJ according to *CYP3A5 *1* or **3*, identified by *SLCO1B1 *1b/*1b*, **1a/*1a* or **1b*, and **15* haplotypes



Graph 4.17: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of pitavastatin lactone comparison between administration with water and GFJ according to *CYP3A5 *1* or **3*, identified by *SLCO1B1 *1b/*1b*, **1a/*1a* or **1b*, and **15* haplotypes



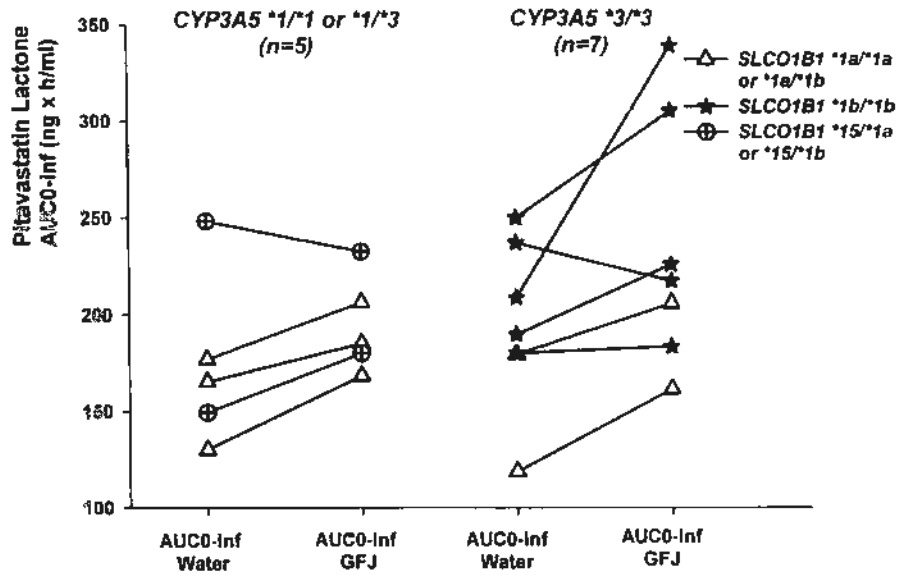


Table 4.10: Pharmacokinetic parameters comparison between subjects with *CYP3A5 *3/*3* and *SLCO1B1 *1b/*1b*, and with at least one *CYP3A5 *1* and *SLCO1B1 *1a* (excluding **15*), after administration with water or GFJ

Genotype Group	*CYP3A5 *3/*3* and *SLCO1B1 *1b/*1b (n=5)*	*CYP3A5 *1* and *SLCO1B1 *1a (n=3)*	*CYP3A5 *3/*3* and *SLCO1B1 *1a (n=2)*	*CYP3A5 *3/*3* and *SLCO1B1 *1b/*1b (n=5)*	*CYP3A5 *1* and *SLCO1B1 *1a (n=3)*	*CYP3A5 *3/*3* and *SLCO1B1 *1a (n=2)*
Administration	*Water*			*GFJ (Grapefruit Juice)*		
Pitavastatin Acid						
C_{max} (ng/ml)	47.1 (24.0 – 63.5)	30.4 (19.5 – 31.6)	27.0 (21.1 – 32.8)	41.6 (24.1 – 46.4)	19.7 (16.5 – 42.4)	28.5 (27.9 – 29.0)
AUC_{0-24h} (ng×h/ml)	101.1 (70.2 – 125.5)	73.1 (49.2 – 86.0)	62.4 (53.5 – 71.2)	93.1 (87.0–167.6)	67.4 (56.1–102.5)	77.6 (67.4– 87.8)
AUC_{0-48h} (ng×h/ml)	104.3 (73.4 – 127.5)	75.1 (49.2 – 88.4)	64.0 (53.5 – 74.4)	97.1 (88.5–179.6)	69.8 (56.1–112.9)	82.0 (67.4– 96.6)
AUC_{0-t} (ng×h/ml)	101.1 (70.2 – 125.5)	73.1 (45.0 – 86.0)	59.1 (46.9 – 71.2)	93.1 (80.1– 174.0)	67.4 (50.7–108.1)	76.4 (60.2– 92.6)
$AUC_{0-\infty}$ (ng×h/ml)	112.5 (85.7 – 129.8)	80.3 (49.7 – 92.9)	70.9 (58.3 – 83.5)	107.9 (91.9– 182.7)	74.7 (56.0–119.4)	84.9 (69.5–100.4)
CL/F (L/h)	17.8 (15.4 – 23.3)	24.9 (21.5 – 40.2)	29.1 (24.0 – 34.3)	18.5 (11.0 – 21.8)	26.8 (16.7 – 35.7)	24.4 (19.9 – 28.8)
$t_{1/2}$ (h)	9.9 (4.6–16.9)	8.0 (4.7–10.0)	8.9 (7.2–10.6)	9.1 (5.9–15.2)	8.5 (4.1–13.1)	8.1 (5.4–10.9)
t_{max} *(h)*	0.5 (0.5 – 1)	1 (0.5 – 1)	0.75(0.5 – 1)	1 (1 – 1)	1.5 (1 – 2)	1 (1 – 1)
Pitavastatin Lactone						
C_{max} (ng/ml)	30.3 (22.9 – 31.2)	18.3 (17.1 – 23.3)	14.7 (13.0 – 16.4)	25.5 (18.8 – 28.4)	19.4 (13.2 – 20.2)	14.4 (12.7 – 16.0)
AUC_{0-24h} (ng×h/ml)	189.1 (155.8–209.6)	132.8 # (99.1–141.9)	119.1 (104.0–34.1)	184.8 (162.0–270.8)	148.3 # (117.7–159.6)	140.8 (122.7–158.9)
AUC_{0-48h} (ng×h/ml)	207.1 (177.0–238.8)	151.2 # (118.7–167.5)	138.7 (116.0–161.3)	211.7 (179.9–321.2)	173.1 (141.7–187.6)	168.6 (148.7–188.5)
AUC_{0-t} (ng×h/ml)	200.7 (171.4–238.8)	151.2 # (109.9–167.5)	136.3 (111.2–161.3)	211.7 (171.6 – 321.2)	173.1 (141.7 – 187.6)	168.6 (148.7 – 188.5)
$AUC_{0-\infty}$ (ng×h/ml)	208.9 (180.1–250.0)	165.3 # (130.1–176.9)	149.1 (118.7–179.5)	226.0 (183.5 – 339.1)	185.1 (168.5 – 206.4)	183.8 (161.6 – 206 0)
CL/F (L/h)	9.6 (8.0 – 11.1)	12.1 # (11.3 – 15.4)	14.0 (11.1 – 16.9)	8.8 (5.9 – 10.9)	10.8 (9.7 – 11.9)	11.0 (9.7 – 12.4)
$t_{1/2}$ (h)	11.0 (7.1–13.9)	12.9(12.8–19.6)	13.3(8.7–18.0)	12.5 (8.3–16.5)	18.6 (14.0–26.5)	16.1 (14.9–17.4)
t_{max} *(h)*	1 (1 – 1.5)	1 (1 – 1)	1 (1 – 1)	2 (1.5 – 2)	2 (1.5 – 3)	2 (2 – 2)

Data are given as median and range, statistical comparison by Mann-Whitney U Test vs the group of *CYP3A5 *3/*3 and SLCO1B1 *1b/*1b*. # P<0.05 are statistically significant.

4.5.4. Genetic influence from *ABCB1 c.1236C>T, c.2677G/A>T, c.3435C>T,* and *CGC* and *TTT* haplotype

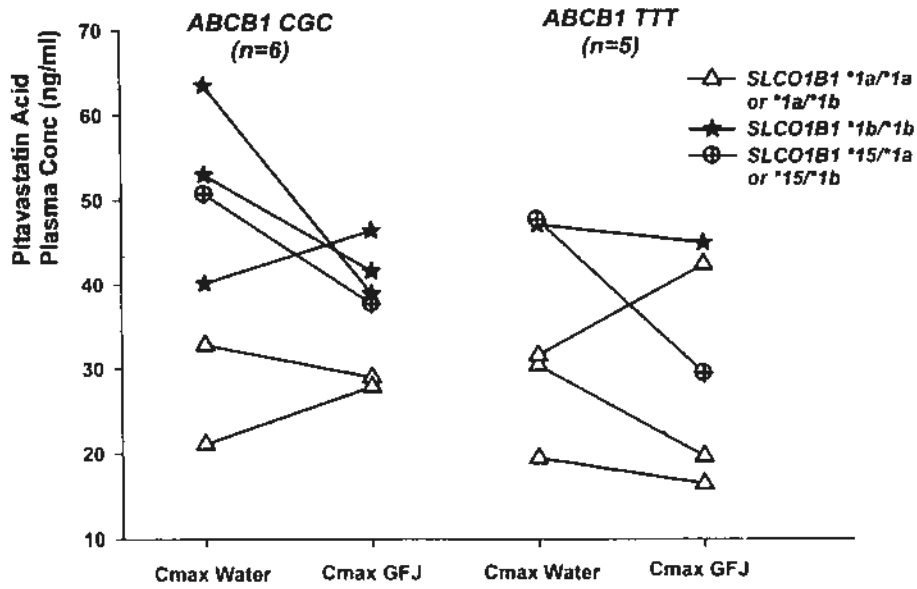
There were no statistically significant differences in the comparison of pharmacokinetic parameters between *ABCB1* haplotypes *c.1236:2677:3435 TTT* carriers and *CGC* carriers (Table 4.11). Individual variabilities of pharmacokinetic parameters for either pitavastatin acid or lactone did not seem to have group-wise differences between subjects having *ABCB1 CGC* or *TTT* haplotypes upon administration with water or GFJ (Graph 4.18-4.21).

Table 4.11: Pharmacokinetic parameters comparison of *ABCB1* haplotypes *c.1236C-c.2677G-c.3435C (CGC)* or *c.1236T-c.2677T-c.3435T (TTT)*, and changes with GFJ

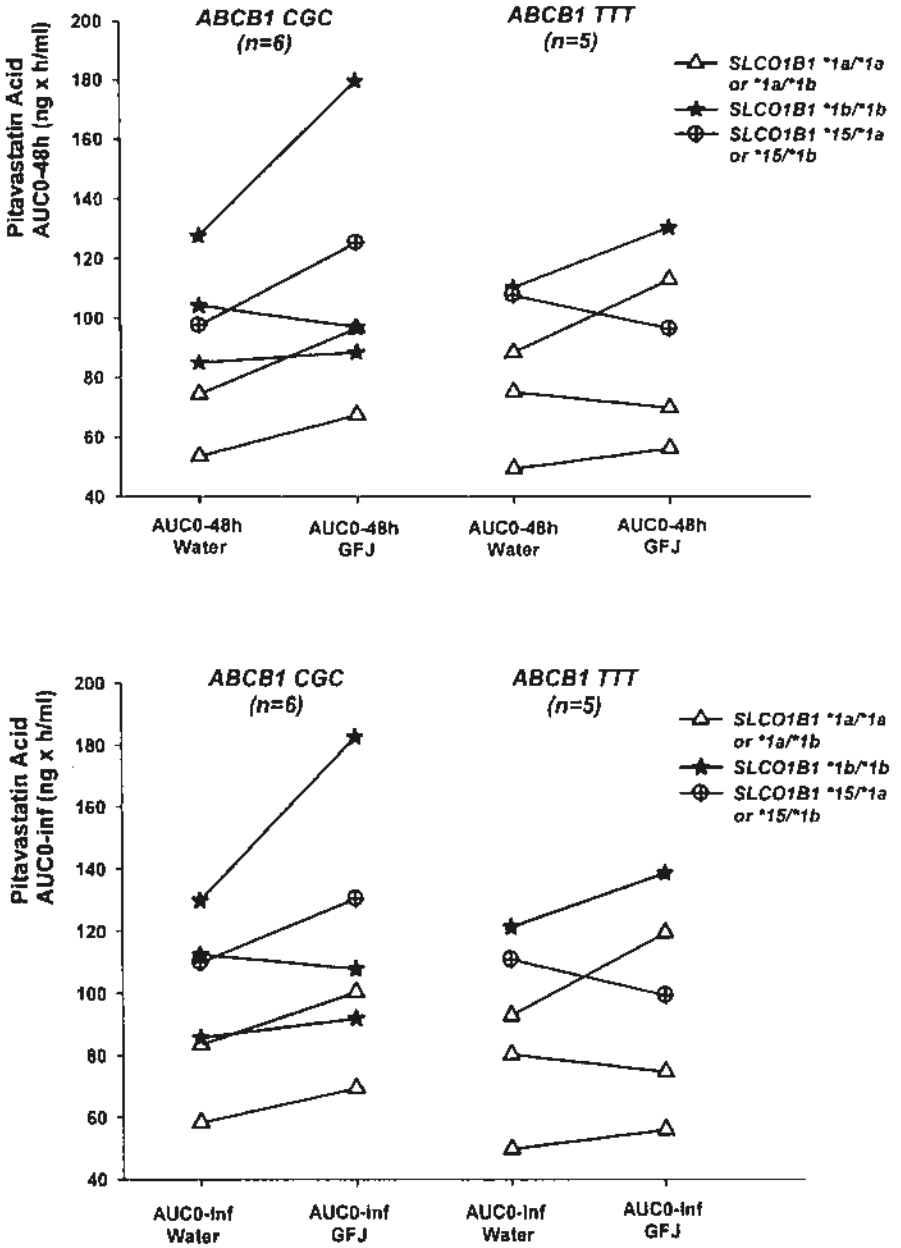
Genotype Group	*CGC (n=6)*	*TTT (n=5)*	*others (n=1)*	*CGC (n=6)*	*TTT (n=5)*	*others (n=1)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	43.5±15.3 (21.1–63.5)	35.3±12.0 (19.5–47.4)	24.0	-6.6±12.2 (-24.6-+6.8)	-4.6±10.8 (-18.2-+10.8)	+0.1
AUC_{0-24h} (ng×h/ml)	89.0±25.1 (53.5–125.5)	83.0±22.9 (49.2–107.0)	70.2	+14.3±16.8 (-8.0-+42.1)	+5.0±10.1 (-5.7-+16.6)	+16.8
AUC_{0-48h} (ng×h/ml)	90.4±25.6 (53.5–127.5)	86.1±25.2 (49.2–110.2)	73.4	+18.7±20.7 (-7.2-+52.1)	+7.0±15.5 (-11.2-+24.6)	+16.0
AUC_{0-t} (ng×h/ml)	85.2±26.8 (46.9–125.5)	83.0±25.3 (45.0–107.3)	70.2	+18.3±20.9 (-8.0-+48.5)	+6.2±13.9 (-9.2-+22.2)	+16.8
$AUC_{0-\infty}$ (ng×h/ml)	96.6±25.7 (58.3–129.8)	91.0±28.0 (49.7–121.3)	89.7	+17.2±19.6 (-4.6-+52.9)	+6.6±15.7 (-11.5-+26.6)	+5.2
Apparent oral clearance (L/h)	22.2±6.8 (15.4–34.3)	24.2±9.5 (16.5–40.2)	22.3	-2.9±2.3 (-5.5-+0.8)	-1.5±3.3 (-4.8-+2.1)	-1.2
$t_{1/2}$ (h)	7.9±2.4 (4.6–10.6)	9.0±2.9 (4.7–12.4)	16.9	+1.0±1.9 (-1.8-+3.4)	+0.5±3.4 (-3.2-+5.1)	-7.8
t_{max} (h)	*0.5 (0.5–1)*	*1 (0.5–1)*	*1*	*1 (1–1)**	*1.5 (1–2)**	*1**
Pitavastatin Lactone						
C_{max} (ng/ml)	24.2±7.7 (13.0–31.2)	25.7±9.3 (17.1–39.6)	22.9	-3.4±3.4 (-9.3- -0.3)	-4.8±5.4 (-11.9-+3.1)	+2.6
AUC_{0-24h} (ng×h/ml)	152.3±34.5 (104.0–190.3)	158.2±48.7 (99.1–209.6)	155.8	+19.8±33.3 (-17.0-+80.4)	+12.3±20.6 (-22.5-+32.4)	+29.0
AUC_{0-48h} (ng×h/ml)	171.8±39.4 (116.0–223.1)	182.2±52.8 (118.7–238.8)	179.4	+30.3±45.0 (-17.4-+114.0)	+18.9±23.8 (-18.5-+48.0)	+32.3
AUC_{0-t} (ng×h/ml)	168.0±40.4 (111.2–223.1)	180.4±55.6 (110.0–238.8)	179.4	+31.2±47.9 (-17.4-+120.4)	+20.7±24.5 (-18.5-+48.0)	+32.3
$AUC_{0-\infty}$ (ng×h/ml)	179.0±41.9 (118.7–237.2)	194.1±53.1 (130.1–250.0)	189.7	+35.7±51.4 (-19.6-+130.2)	+25.5±26.4 (-15.6-+55.4)	+36.3
Apparent oral clearance (L/h)	11.7±3.0 (8.4–16.9)	11.0±3.1 (8.0–15.4)	10.5	-1.9±2.0 (-4.5-+0.8)	1.5±1.4 (-3.5-+0.5)	-1.7
$t_{1/2}$ (h)	10.7±4.3 (7.1–18.0)	14.4±3.3 (11.0–19.6)	11.9	+2.0±3.5 (-2.2-+6.2)	+3.5±7.1 (-5.6-+13.7)	+4.6
t_{max} (h)	*1 (1–1)*	*1 (1–1.5)*	*1*	*1.75 (1.5–2)**	*2 (1.5–3)**	*2**

Data as mean±SD and range, statistical comparison (*CGC vs TTT*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. No statistical significance found.
* t_{max} data were as recorded as administered under GFJ.

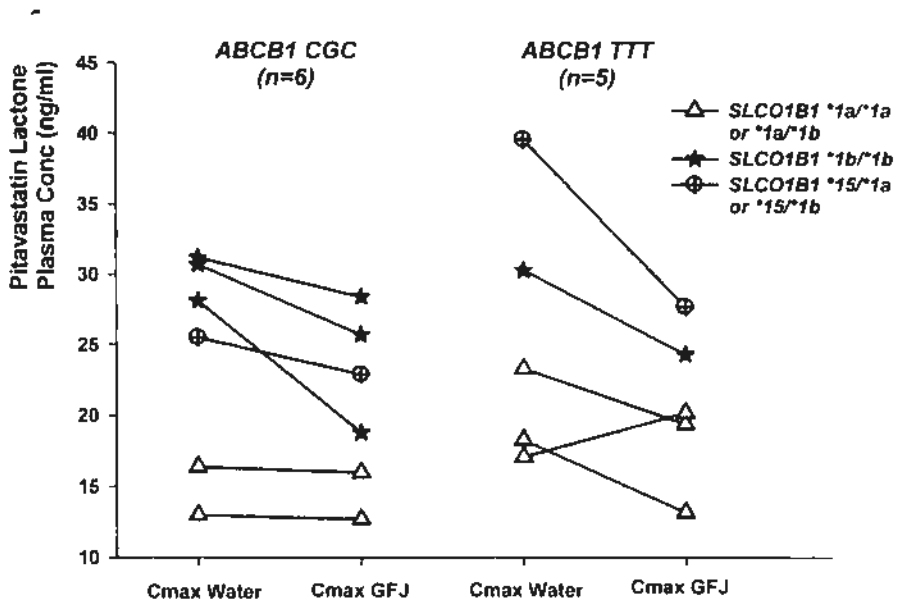
Graph 4.18: Individual C_{max} of pitavastatin acid comparison between administration with water and GFJ on *ABCB1 CGC* or *TTT* carriers, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b*, and **15* haplotypes



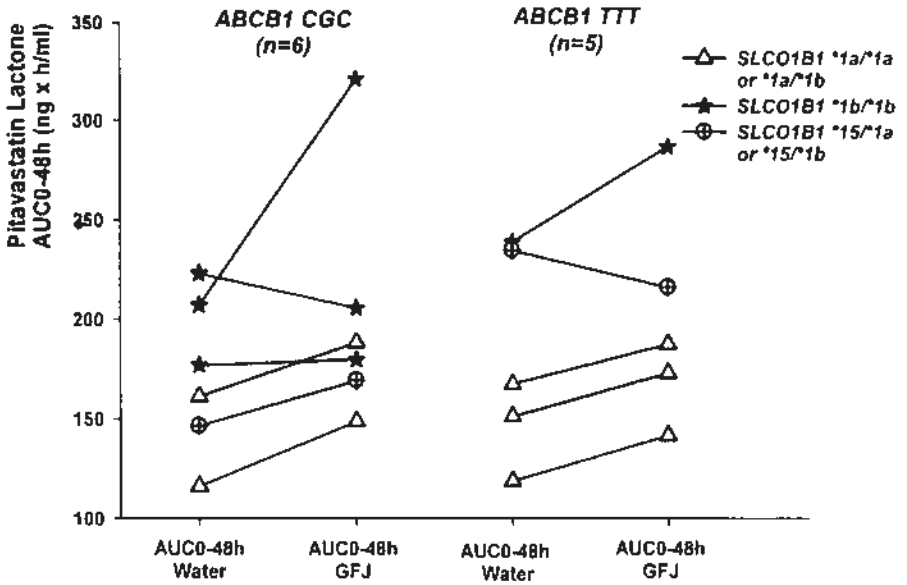
Graph 4.19: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of pitavastatin acid comparison between administration with water and GFJ on *ABCB1 CGC* or *TTT* carriers, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b*, and **15* haplotypes

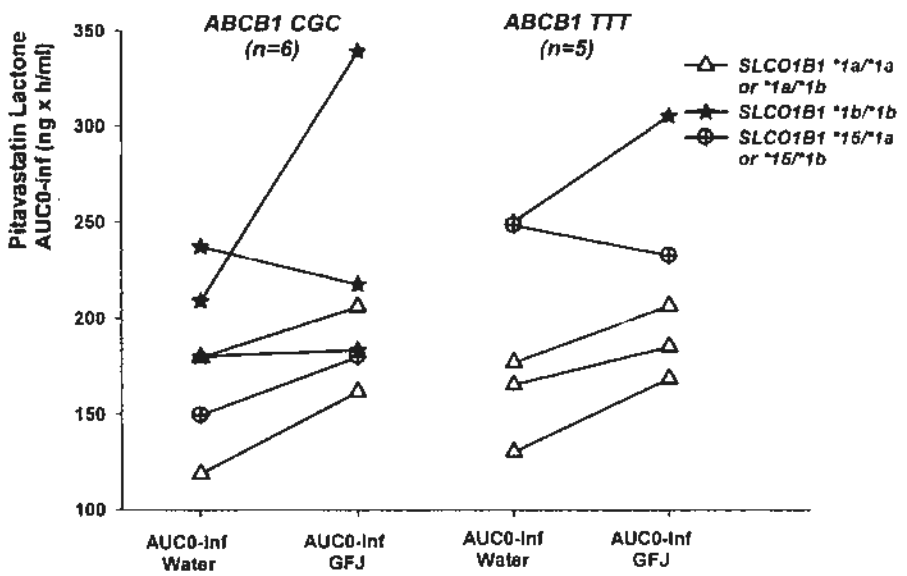


Graph 4.20: Individual C_{max} of pitavastatin lactone comparison between administration with water and GFJ on *ABCB1 CGC* or *TTT* carriers, identified by *SLCO1B1 *1b/*1b*, **1a/*1a* or **1b*, and **15* haplotypes



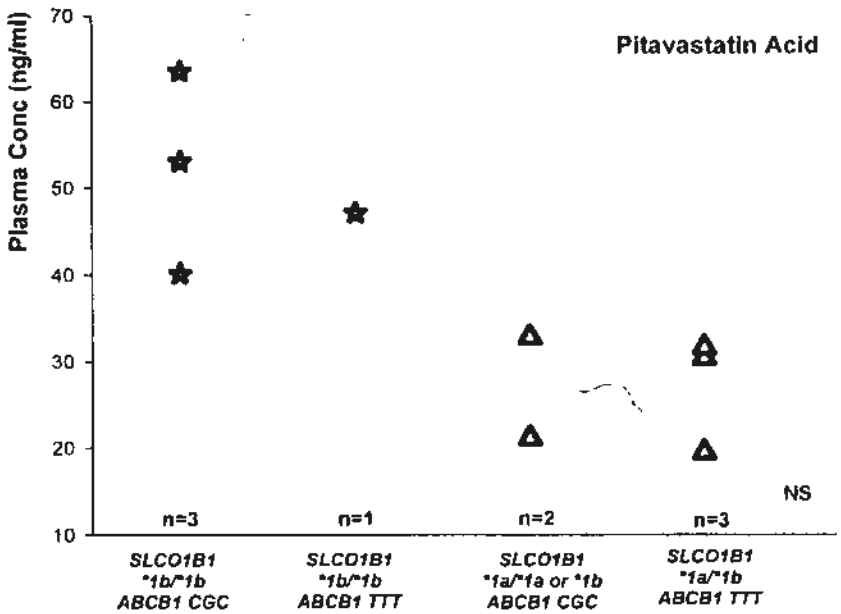
Graph 4.21: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of pitavastatin lactone comparison between administration with water and GFJ on *ABCB1 CGC* or *TTT* carriers, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b*, and **15* haplotypes

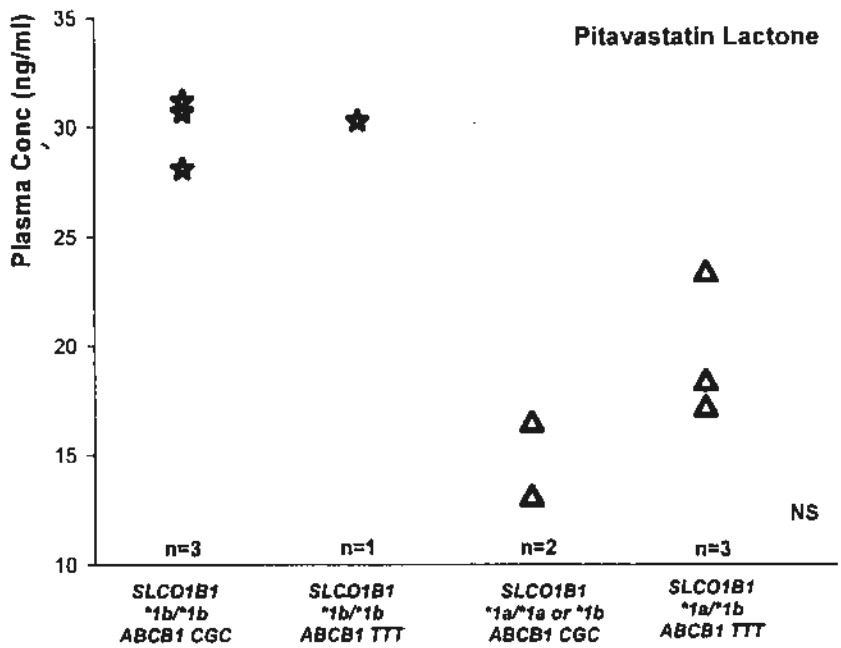




Gene-gene interaction plots with *SLCO1B1* haplotypes on C_{max} and $AUC_{0\text{-}48h}$ when administered with water did not have significant comparisons (Graph 4.22 and 4.23). Further analysis on parameters after administration in water and respective changes after GFJ did not show significant differences between individual SNPs *ABCB1 c.1236C>T, c.2677G/A>T* and *c.3435C>T* (Table 4.12-4.14).

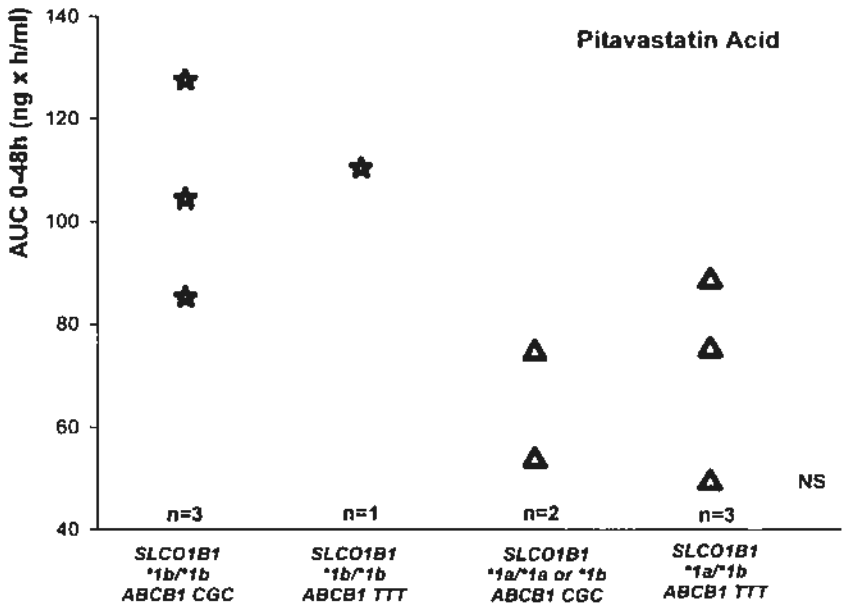
Graph 4.22: Individual C_{max} as administered with water of pitavastatin acid and lactone on *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into those with at least one copy of *ABCB1 CGC* or *TTT* haplotypes

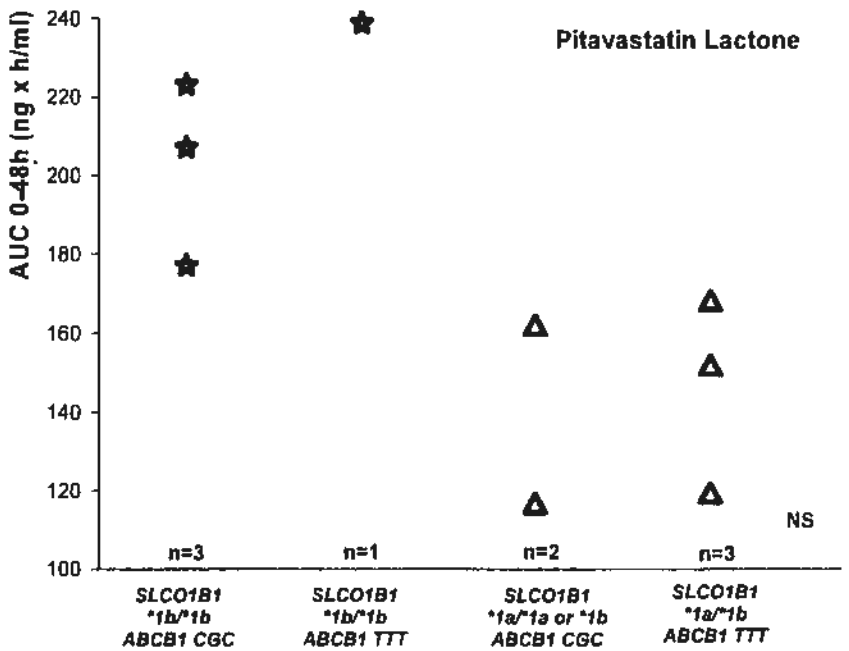




Statistical comparison by Kruskal-Wallis test. No statistical significance found.

Graph 4.23: Individual AUC $_{0\text{-}48h}$ as administered with water of pitavastatin acid and lactone on *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into those with at least one copy of *ABCB1 CGC* or *TTT* haplotypes





Statistical comparison by Kruskal-Wallis test. No statistical significance found.

Table 4.12: Pharmacokinetic parameters comparison of *ABCB1 c.1236C>T* genotypes and changes after administration with Grapefruit Juice (GFJ)

Genotype Group	*c.1236CC (n=2)*	*c.1236CT (n=4)*	*c.1236TT (n=6)*	*c.1236CC (n=2)*	*c.1236CT (n=4)*	*c.1236TT (n=6)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	36.5 (32.8, 40.1)	47.1±18.2 (21.1–63.5)	33.4±11.7 (19.5–47.7)	+1.3 (-3.8, +6.3)	-10.6±13.0 (-24.6-+6.8)	-3.9±9.9 (-18.2-+10.8)
AUC_{0-24h} (ng×h/ml)	86.2 (71.2,101.1)	90.4±29.8 (53.5–125.5)	80.9±21.2 (49.2–107.0)	+4.3 (-8.0,+16.6)	+19.2±16.4 (+3.3-+42.1)	+7.0±10.3 (-5.7-+16.8)
AUC_{0-48h} (ng×h/ml)	89.4 (74.4,104.3)	90.9±30.6 (53.5–127.5)	84.0±23.1 (49.2–110.2)	+7.5 (-7.2,+22.2)	+24.2±21.1 (+3.3-+52.1)	+8.5±14.3 (-11.2-+24.6)
AUC_{0-t} (ng×h/ml)	86.2 (71.2,101.1)	84.7±32.3 (46.9–125.5)	80.9±23.2 (45.0–107.3)	+6.7 (-8.0,+21.4)	+24.1±21.1 (+0.9-+48.5)	+8.0±13.2 (-9.2-+22.2)
$AUC_{0-\infty}$ (ng×h/ml)	98.0 (83.5,112.5)	96.0±30.9 (58.3–129.8)	90.8±25.0 (49.7–121.3)	+6.2 (-4.6,+17.0)	+22.7±21.0 (+6.2-+52.9)	+6.4±14.1 (-11.5-+26.6)
Apparent oral clearance (L/h)	20.9 (17.8, 24.0)	22.8±8.3 (15.4–34.3)	23.9±8.5 (16.5–40.2)	-1.6 (-4.1, +0.8)	-3.6±1.7 (-5.5- -1.6)	-1.4±3.0 (-4.8-+2.1)
$t_{1/2}$ (h)	10.2 (9.9, 10.6)	6.7±2.0 (4.6–9.2)	10.3±4.1 (4.7–16.9)	+0.3 (+0.3, +0.4)	+1.4±2.3 (-1.8-+3.4)	-0.8±4.5 (-7.8-+5.1)
t_{max} (h)	*0.5 (0.5, 0.5)*	*0.5 (0.5–1)*	*1 (0.5–1)*	*1 (1, 1)**	*1 (1–1)**	*1.25 (1–2)**
Pitavastatin Lactone						
C_{max} (ng/ml)	22.3 (16.4, 28.1)	25.1±8.5 (13.0–31.2)	25.3±8.4 (17.1–39.6)	-4.9 (-9.3, -0.4)	-2.7±1.9 (-5.0- -0.3)	-3.5±5.7 (-11.9-+3.1)
AUC_{0-24h} (ng×h/ml)	161.6 (134.1,189.1)	147.7±37.4 (104.0–190.3)	157.8±43.5 (99.1–209.6)	+3.9 (-17.0,+24.8)	+27.7±36.2 (-1.4-+80.4)	+15.1±19.6 (-22.5-+32.4)
AUC_{0-48h} (ng×h/ml)	192.2 (161.3,223.1)	161.7±39.2 (116.0–207.1)	181.7±47.3 (118.7–238.8)	+4.9 (-17.4,+27.2)	+43.1±48.9 (+2.8-+114.0)	+21.1±22.0 (-18.5-+48.0)
AUC_{0-t} (ng×h/ml)	192.2 (161.3,223.1)	155.9±38.7 (111.2–200.7)	180.2±49.7 (109.9–238.8)	+4.9 (-17.4,+27.2)	+44.4±52.9 (+0.2-+120.4)	+22.6±22.4 (-18.5-+48.0)
$AUC_{0-\infty}$ (ng×h/ml)	208.3 (179.5,237.2)	164.3±38.9 (118.7–208.9)	193.4±47.5 (130.1–250.0)	+3.4 (-19.6,+26.5)	+51.8±54.9 (+3.4-+130.2)	+27.3±24.1 (-15.6-+55.4)
Apparent oral clearance (L/h)	9.8 (8.4, 11.1)	12.7±3.2 (9.6–16.9)	10.9±2.8 (8.0–15.4)	-0.3 (-1.4, +0.8)	-2.7±1.9 (-4.5- -0.2)	-1.5±1.3 (-3.5-+0.5)
$t_{1/2}$ (h)	15.9 (13.9, 18.0)	8.1±0.7# (7.1–8.7)	14.0±3.1 (11.0–19.6)	-1.4 (-2.2, -0.6)	+3.7±2.9 (-0.3-+6.2)	+3.7±6.4 (-5.6-+13.7)
t_{max} (h)	*1 (1, 1)*	*1 (1–1)*	*1 (1–1.5)*	*1.75 (1.5,2)**	*1.75 (1.5–2)**	*2 (1.5–3)**

Data as mean±SD and range, statistical comparison (*CT vs TT* and *CC/CT vs TT*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. # P<0.05 is statistically significant vs *TT*. * t_{max} data were as recorded as administered under GFJ.

Table 4.13: Pharmacokinetic parameters comparison of *ABCB1 c.2677G/A>T* genotypes and changes after administration with Grapefruit Juice (GFJ)

Genotype Group	*c.2677GG/GA (n=7)*	*c.2677GT (n=3)*	*c.2677TT (n=2)*	*c.2677GG/GA (n=7)*	*c.2677GT (n=3)*	*c.2677TT (n=2)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	40.7±15.8 (21.1–63.5)	32.3±13.9 (19.5–47.1)	39.7 (31.6,47.7)	-5.7±11.4 (-24.6-+6.8)	-5.3±4.7 (-10.7- -2.1)	-3.7 (-18.2,+10.8)
AUC $_{0\text{-}24h}$ (ng×h/ml)	86.3±24.0 (53.5–125.5)	76.4±29.0 (49.2–107.0)	92.8 (86.0,99.7)	+14.6±15.3 (-8.0-+42.1)	+4.6±9.3 (-5.7-+12.5)	+5.7 (-5.2,+16.6)
AUC $_{0\text{-}48h}$ (ng×h/ml)	88.0±24.2 (53.5–127.5)	78.2±30.6 (49.2–110.2)	98.0 (88.4,107.7)	+18.3±18.9 (-7.2-+52.1)	+7.2±12.7 (-5.3-+20.1)	+6.7 (-11.2,+24.6)
AUC $_{0\text{-}t}$ (ng×h/ml)	83.1±25.1 (46.9–125.5)	75.1±31.2 (45.0–107.3)	94.8 (86.0,103.7)	+18.1±19.1 (-8.0-+48.5)	+6.1±11.9 (-5.7-+18.2)	+6.5 (-9.2,+22.2)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	95.6±23.6 (58.3–129.8)	83.8±35.9 (49.7–121.3)	101.9 (92.9,110.9)	+15.4±18.4 (-4.6-+52.9)	+6.0±11.5 (-5.5-+17.4)	+7.5 (-11.5,+26.6)
Apparent oral clearance (L/h)	22.2±6.2 (15.4–34.3)	27.2±12.0 (16.5–40.2)	19.8 (18.0,21.5)	-2.7±2.2 (-5.5-+0.8)	-1.6±3.2 (-4.5-+1.9)	-1.4 (-4.8, +2.1)
$t_{1/2}$ (h)	9.2±4.0 (4.6–16.9)	9.0±3.9 (4.7–12.4)	9.0 (8.0, 10.0)	-0.2±3.7 (-7.8-+3.4)	+0.3±2.3 (-1.6-+2.9)	+1.0 (-3.2, +5.1)
t_{max} (h)	*0.5 (0.5–1)*	*1 (0.5–1)*	*1 (1, 1)*	*1 (1–1)**	*1.5 (1–2)**	*1.25 (1,1.5)**
Pitavastatin Lactone						
C_{max} (ng/ml)	24.0±7.0 (13.0–31.2)	24.0±6.0 (18.3–30.3)	28.4 (17.1,39.6)	-2.5±3.8 (-9.3-+2.6)	+5.0±1.1 (-6.0- -3.9)	-4.4 (-11.9,+3.1)
AUC $_{0\text{-}24h}$ (ng×h/ml)	152.8±31.6 (104.0–190.3)	147.2±56.7 (99.1–209.6)	174.7 (141.9,207.4)	+21.1±30.6 (-17.0-+80.4)	+22.2±9.0 (+15.5-+32.4)	-2.4 (-22.5,+17.7)
AUC $_{0\text{-}48h}$ (ng×h/ml)	172.9±36.1 (116.0–223.1)	169.6±62.1 (118.7–238.8)	201.1 (167.5,234.6)	+30.6±41.1 (-17.4-+114.0)	+31.0±14.8 (+21.9-+48.0)	+0.8 (-18.5,+20.1)
AUC $_{0\text{-}t}$ (ng×h/ml)	169.6±37.1 (111.2–223.1)	166.6±65.8 (109.9–238.8)	201.1 (167.5,234.6)	+31.4±43.7 (-17.4-+120.4)	+33.9±13.2 (+21.9-+48.0)	+0.8 (-18.5,+20.1)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	180.5±38.5 (118.7–237.2)	181.8±61.6 (130.1–250.0)	212.5 (176.9,248.2)	+35.8±46.9 (-19.6-+130.2)	+37.9±17.8 (+19.9-+55.4)	+7.0 (-15.6,+29.5)
Apparent oral clearance (L/h)	11.6±2.8 (8.4–16.9)	11.8±3.7 (8.0–15.4)	9.7 (8.1, 11.3)	-1.9±1.8 (-4.5-+0.8)	-2.1±1.2 (-3.5- -1.3)	-0.5 (-1.6, +0.5)
$t_{1/2}$ (h)	10.9±3.9 (7.1–18.0)	14.4±4.5 (11.0–19.6)	14.3 (12.9,15.7)	+2.4±3.4 (-2.2-+6.2)	+3.8±9.7 (-5.6-+13.7)	+3.1 (+0.6, +5.7)
t_{max} (h)	*1 (1–1)*	*1 (1–1.5)*	*1.25 (1, 1.5)*	*2 (1.5–2)**	*2 (2–3)**	*1.75 (1.5,2)**

Data as mean±SD and range, statistical comparison (*GG/GA vs GT* and *GG/GA vs GT/TT*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. No statistical significance found. * t_{max} data were as recorded as administered under GFJ.

Table 4.14: Pharmacokinetic parameters comparison of *ABCB1 c.3435C>T* genotypes and changes after administration with Grapefruit Juice (GFJ)

Genotype Group	*c.3435CC* *(n=6)*	*c.3435CT* *(n=4)*	*c.3435TT* *(n=2)*	*c.3435CC* *(n=6)*	*c.3435CT* *(n=4)*	*c.3435TT* *(n=2)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	38.7±16.3 (21.1–63.5)	37.5±15.3 (19.5–53.0)	39.7 (31.6,47.7)	-4.7±12.2 (-24.6-+6.8)	-6.8±4.9 (-11.4- -2.1)	-3.7 (-18.2,+10.8)
AUC_{0-24h} (ng×h/ml)	79.8±18.2 (53.5–101.1)	88.7±34.1 (49.2–125.5)	92.8 (86.0,99.7)	+10.0±10.3 (-8.0-+17.8)	+13.9±20.2 (-5.7-+42.1)	+5.7 (-5.2,+16.6)
AUC_{0-48h} (ng×h/ml)	81.4±18.4 (53.5–104.3)	90.5±35.1 (49.2–127.5)	98.0 (88.4,107.7)	+12.6±12.7 (-7.2-+27.8)	+18.4±24.7 (-5.3-+52.1)	+6.7 (-11.2,+24.6)
AUC_{0-t} (ng×h/ml)	76.0±18.3 (46.9–101.1)	87.7±35.8 (45.0–125.5)	94.8 (86.0,103.7)	+13.0±14.9 (-8.0-+34.0)	+16.7±23.3 (-5.7-+48.5)	+6.5 (-9.2, +22.2)
$AUC_{0-\infty}$ (ng×h/ml)	89.9±19.8 (58.3–112.5)	95.3±37.3 (49.7–129.8)	101.9 (92.9,110.9)	+9.2±9.0 (-4.6-+20.5)	+17.7±25.2 (-5.5-+52.9)	+7.5 (-11.5,+26.6)
Apparent oral clearance (L/h)	23.3±6.0 (17.8–34.3)	24.3±11.5 (15.4–40.2)	19.8 (18.0,21.5)	-2.4±2.2 (-5.5-+0.8)	-2.3±3.0 (-4.5-+1.9)	-1.4 (-4.8, +2.1)
$t_{1/2}$ (h)	9.7±4.1 (4.6–16.9)	8.2±3.6 (4.7–12.4)	9.0 (8.0, 10.0)	-0.7±3.9 (-7.8-+3.4)	+0.8±2.3 (-1.6-+2.9)	+1.0 (-3.2, +5.1)
t_{max} (h)	*0.5 (0.5–1)*	*0.75 (0.5–1)*	*1 (1, 1)*	*1 (1–1)**	*1.25 (1–2)**	*1.25 (1,1.5)**
Pitavastatin Lactone						
C_{max} (ng/ml)	22.8±6.9 (13.0–30.7)	25.8±6.1 (18.3–31.2)	28.4 (17.1,39.6)	-2.5±4.2 (-9.3-+2.6)	+4.5±1.4 (-6.0- -2.8)	-4.4 (-11 9, +3.1)
AUC_{0-24h} (ng×h/ml)	146.6±29.4 (104.0–189.1)	157.9±51.1 (99.1–209.6)	174.7 (141.9,207.4)	+11.2±17.4 (-17.0-+29.0)	+36.7±30.0 (+15.5-+80.4)	-2.4 (-22.5,+17.7)
AUC_{0-48h} (ng×h/ml)	167.2±35.9 (116.0–223.1)	178.9±54.1 (118.7–238.8)	201.1 (167.5,234.6)	+16.7±20.0 (-17.4-+32.7)	+51.7±43.2 (+21.9-+114.0)	+0.8 (-18.5,+20.1)
AUC_{0-t} (ng×h/ml)	164.4±37.8 (111.2–223.1)	175.1±56.4 (109.9–238.8)	201.1 (167.5, 234.6)	+16.5±21.1 (-17.4-+37.5)	+55.5±44.6 (+21.9-+120.4)	+0.8 (-18.5,+20.1)
$AUC_{0-\infty}$ (ng×h/ml)	175.8±39.9 (118.7–237.2)	188.6±52.1 (130.1–250.0)	212.5 (176.9,248.2)	+20.0±23.6 (-19.6-+42.9)	+61.0±48.4 (+19.9-+130.2)	+7.0 (-15.6,+29.5)
Apparent oral clearance (L/h)	11.9±2.9 (8.4–16.9)	11.3±3.2 (8.0–15.4)	9.7 (8.1, 11.3)	-1.6±1.8 (-4.5-+0.8)	-2.5±1.3 (-3.7- -1.3)	-0.5 (-1.6, +0.5)
$t_{1/2}$ (h)	11.5±3.9 (8.1–18.0)	12.6±5.2 (7.1–19.6)	14.3 (12.9,15.7)	+1.9±3.4 (-2.2-+6.2)	+4.2±7.9 (-5.6-+13.7)	+3.1 (+0.6, +5.7)
t_{max} (h)	*1 (1–1)*	*1 (1–1.5)*	*1.25 (1, 1.5)*	*1.75(1.5–2)**	*2 (2–3)**	*1.75 (1.5,2)**

Data as mean±SD and range, statistical comparison (*CC vs CT* and *CC vs CT/TT*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. No statistical significance found. * t_{max} data were as recorded as administered under GFJ.

4.5.5. Genetic influence from *ABCG2 c.421C>A*

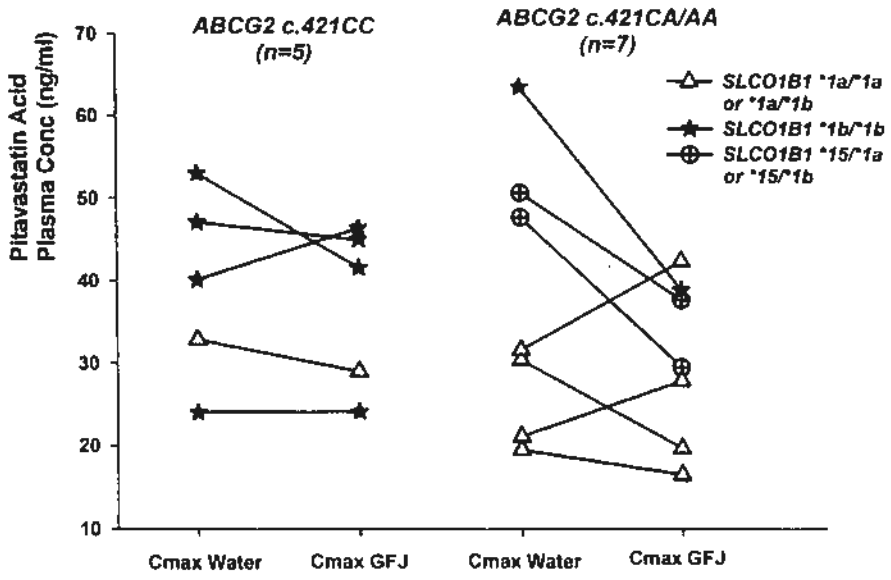
Comparison of pharmacokinetic parameters among genotype groups in *ABCG2 c.421C>A* did not have statistical significance (Table 4.15). Graph 4.24-4.27 were C_{max} and AUCs on individual changes between administration with water and GFJ on pitavastatin acid and lactone as grouped by *c.421CC* and *c.421CA/AA* genotypes and identified by *SLCO1B1* haplotypes. AUCs of both pitavastatin acid and lactone were generally higher when administered with GFJ. Carriers of *SLCO1B1 *1b/*1b* with *ABCG2 c.421CC* had higher AUCs with acid and lactone. Gene-gene interaction plots on *SLCO1B1 *1a, *1b* or **15* haplotypes and *ABCG2 c.421C>A* for C_{max} and AUC_{0-48h} of pitavastatin acid after administration with water did not show significant difference (Graph 4.28). The values of AUC_{0-48h} on pitavastatin lactone were higher with individuals of *SLCO1B1 *1b/*1b* and *ABCG2 c.421CA* compared to those with *SLCO1B1 *1a* and *ABCG2 c.421A* carriers, and were of statistical significance ($P<0.05$) (Graph 4.29).

Table 4.15: Pharmacokinetic parameters comparison of *ABCG2 c.421C>A* genotypes and changes after administration with Grapefruit Juice (GFJ)

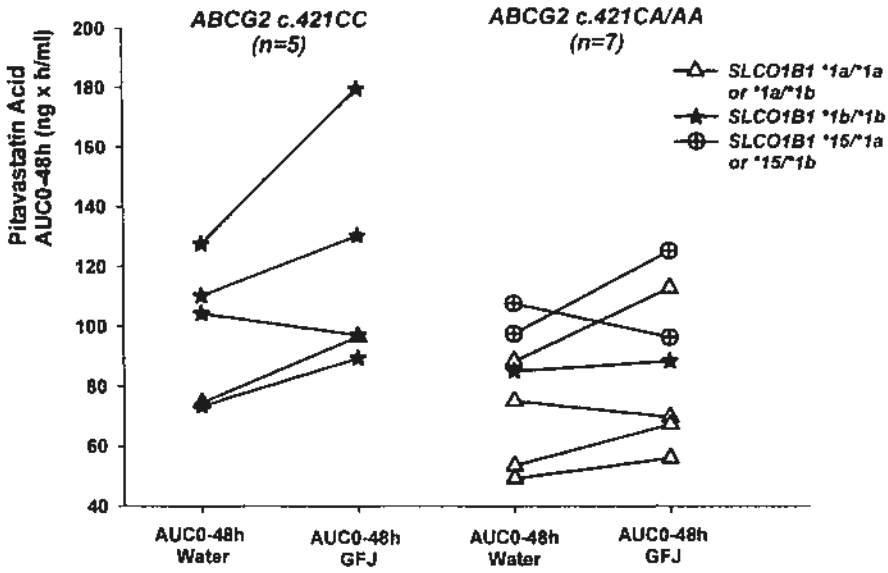
Genotype Group	*c.421CC* *(n=5)*	*c.421CA* *(n=5)*	*c.421AA* *(n=2)*	*c.421CC* *(n=5)*	*c.421CA* *(n=5)*	*c.421AA* *(n=2)*
Administration				*Changes after consumption with GFJ*		
Pitavastatin Acid						
C_{max} (ng/ml)	39.4±11.5 (24.0–53.0)	42.4±17.4 (19.5–63.5)	26.4 (21.1,31.6)	-2.2±6.4 (-11.4-+6.3)	-13.9±8.1 (-24.6- -3.0)	+8.8 (+6.8,+10.8)
AUC $_{0\text{-}24h}$ (ng×h/ml)	95.0±23.9 (70.2–125.5)	80.9±20.7 (49.2–99.7)	69.7 (53.5,86.0)	+16.0±17.8 (-8.0-+42.1)	+3.4±9.7 (-5.7-+17.8)	+15.2 (+13.8,+16.6)
AUC $_{0\text{-}48h}$ (ng×h/ml)	98.0±23.6 (73.4–127.5)	82.9±22.5 (49.2–107.7)	70.9 (53.5,88.4)	+20.6±21.1 (-7.2-+52.1)	+4.3±14.9 (-11.2-+27.8)	+19.2 (+13.8,+24.6)
AUC $_{0\text{-}t}$ (ng×h/ml)	95.1±24.0 (70.2–125.5)	77.7±21.6 (45.0–103.7)	66.4 (46.9,86.0)	+19.4±20.1 (-8.0-+48.5)	+5.1±17.1 (-9.2-+34.0)	+17.7 (+13.2,+22.2)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	107.3±20.1 (83.5–129.8)	87.3±25.2 (49.7–110.9)	75.6 (58.3,92.9)	+17.5±21.7 (-4.6-+52.9)	+3.2±12.3 (-11.5-+20.5)	+18.9 (+11.1,+26.6)
Apparent oral clearance (L/h)	19.2±3.7 (15.4–24.0)	24.9±9.1 (18.0–40.2)	27.9 (21.5,34.3)	-2.2±2.2 (-4.5-+0.8)	-1.0±2.9 (-4.5-+2.1)	-5.1 (-5.5,-4.8)
$t_{1/2}$ (h)	11.1±4.0 (6.0–16.9)	7.7±2.8 (4.6–10.0)	7.6 (7.2, 8.0)	-0.3±4.3 (-7.8-+2.9)	-0.1±2.5 (-3.2-+3.4)	+1.7 (-1.8, +5.1)
t_{max} (h)	*0.5 (0.5–1)*	*0.5 (0.5–1)*	*1 (1, 1)*	*1 (1–1)**	*1.5 (1–2)**	*1 (1, 1)**
Pitavastatin Lactone						
C_{max} (ng/ml)	25.8±6.2 (16.4–31.2)	27.5±8.1 (18.3–39.6)	15.1 (13.0,17.1)	-3.2±4.7 (-9.3-+2.6)	-5.7±3.6 (-11.9- -2.6)	+1.4 (-0.3, +3.1)
AUC $_{0\text{-}24h}$ (ng×h/ml)	175.8±30.3 (134.1–209.6)	147.1±40.7 (99.1–207.4)	123.0 (104.0,141.9)	+29.9±34.6 (-17.0-+80.4)	+4.7±17.0 (-22.5-+18.6)	+18.2 (+17.7,+18.7)
AUC $_{0\text{-}48h}$ (ng×h/ml)	202.0±31.6 (161.3–238.8)	165.6±43.8 (118.7–234.6)	141.8 (116.0,167.5)	+40.8±47.6 (-17.4-+114.0)	+10.4±18.3 (-18.5-+23.0)	+26.4 (+20.1,+32.7)
AUC $_{0\text{-}t}$ (ng×h/ml)	200.7±31.5 (161.3–238.8)	161.4±46.6 (109.9–234.6)	139.4 (111.2,167.5)	+42.1±50.1 (-17.4-+120.4)	+11.0±20.1 (-18.5-+31.8)	+28.8 (+20.1,+37.5)
AUC $_{0\text{-}\infty}$ (ng×h/ml)	213.1±30.1 (179.5–250.0)	174.6±45.1 (130.1–248.2)	147.8 (118.7,176.9)	+45.8±54.7 (-19.6-+130.2)	+15.3±21.7 (-15.6-+38.3)	+36.2 (+29.5,+42.9)
Apparent oral clearance (L/h)	9.5±1.3 (8.0–11.1)	12.0±2.7 (8.1–15.4)	14.1 (11.3,16.9)	-1.5±1.6 (-3.7-+0.8)	-1.3±1.6 (-3.5- -0.5)	-3.0 (-4.5, -1.6)
$t_{1/2}$ (h)	12.4±4.0 (7.1–18.0)	12.9±4.8 (8.1–19.6)	10.8 (8.7,12.9)	+2.1±3.3 (-2.2-+5.4)	+2.4±7.2 (-5.6-+13.7)	+5.9 (+5.7, +6.2)
t_{max} (h)	*1 (1–1.5)*	*1 (1–1.5)*	*1 (1, 1)*	*2 (1.5–2)**	*2 (1.5–3)**	*1.75 (1.5, 2)**

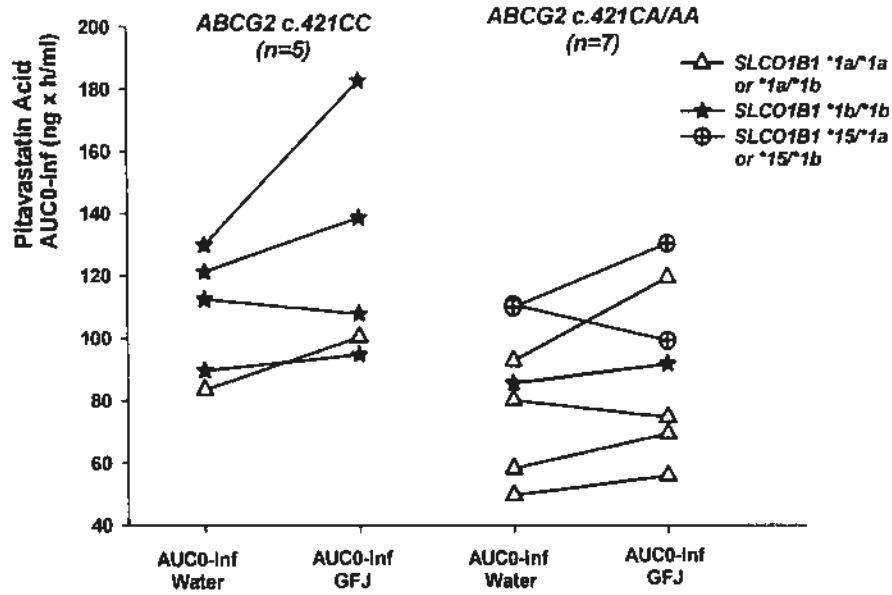
Data as mean±SD and range, statistical comparison (*CC vs CA*, and *CC vs CA/AA*) by Student's t-test, except t_{max} data as median (range) and compared by Mann-Whitney U Test. No statistical significance found. * t_{max} data were as recorded as administered under GFJ.

Graph 4.24: Individual C_{max} of pitavastatin acid comparison between administration with water and GFJ on *ABCG2 c.421C>A*, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b*, and **15* haplotypes

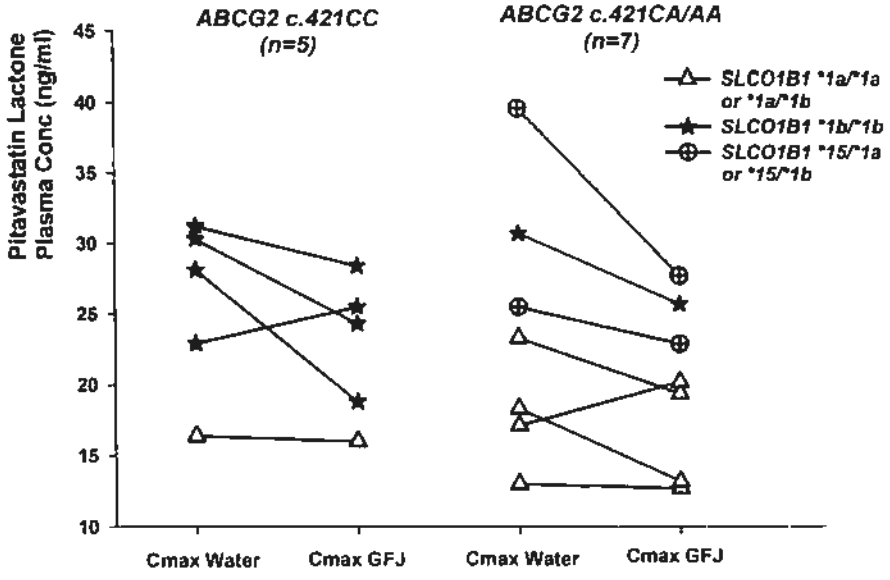


Graph 4.25: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of pitavastatin acid comparison between administration with water and GFJ on *ABCG2 c.421C>A*, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b,* and **15* haplotypes

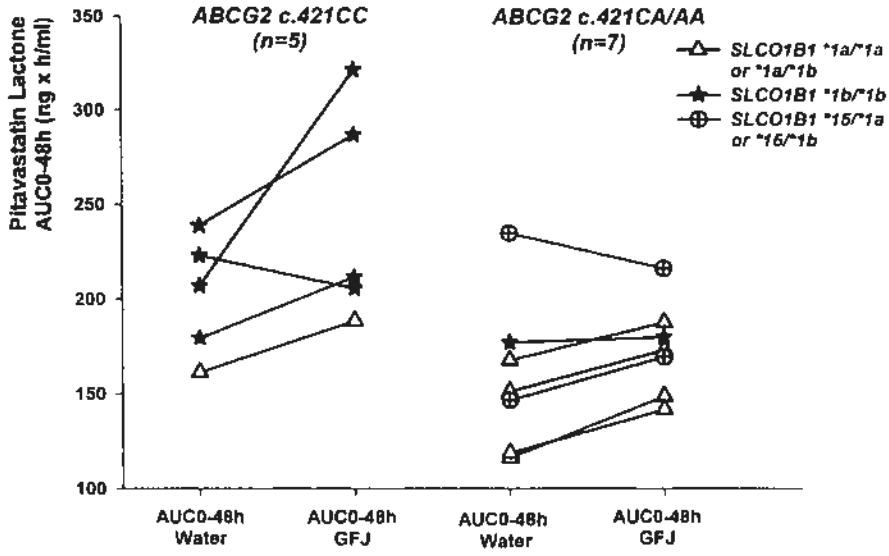


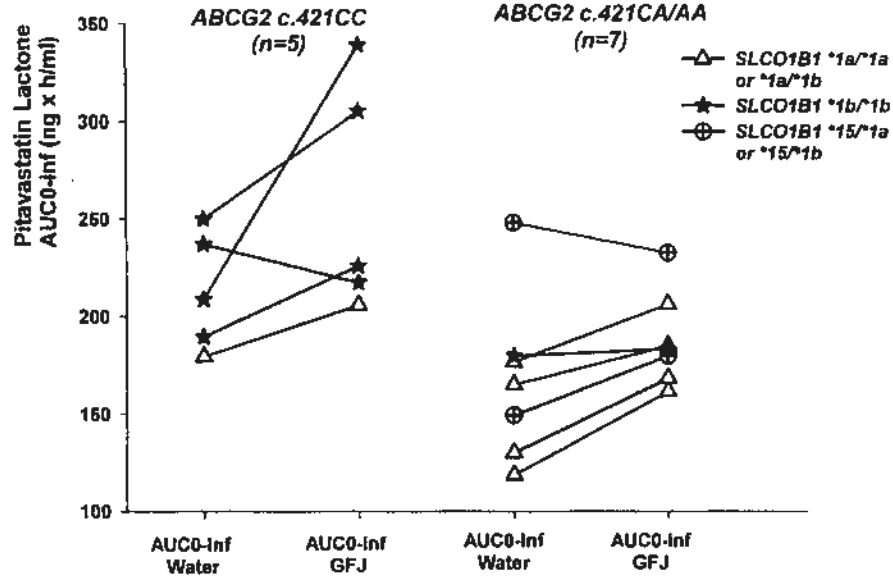


Graph 4.26: Individual C_{max} of Pitavastatin Lactone comparison between administration with water and GFJ on *ABCG2 c.421C>A*, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or *1b*, and *15* haplotypes



Graph 4.27: Individual AUC $_{0\text{-}48h}$ and AUC $_{0\text{-}\infty}$ of Pitavastatin Lactone comparison between administration with water and GFJ on *ABCG2 c.421C>A*, identified by *SLCO1B1 *1b/*1b, *1a/*1a* or **1b,* and **15* haplotypes





Statistical analysis between sub-groups of subjects with *SLCO1B1 *1b/*1b* and *ABCG2 c.421CC*, with those of *SLCO1B1 *1a* and *ABCG2 c.421A* carriers have confirmed higher pitavastatin lactone AUCs (median $AUC_{0\text{-}48h}$ water: 215.1 vs. 134.9 ng×h/ml; GFJ: 249.3 vs. 160.9 ng×h/ml) and lower CL/F (median water: 9.0 vs. 13.7 L/h; GFJ: 7.7 vs. 11.3 L/h) and were of statistical significance (Table 4.16).

Table 4.16: Pharmacokinetic parameters comparison between subjects with *ABCG2 c.421CC* and *SLCO1B1 *1b/*1b*, and those with at least one *ABCG2 c.421A* and *SLCO1B1 *1a* genotype, after administration with Water or GFJ (excluding *SLCO1B1 *15* carriers)

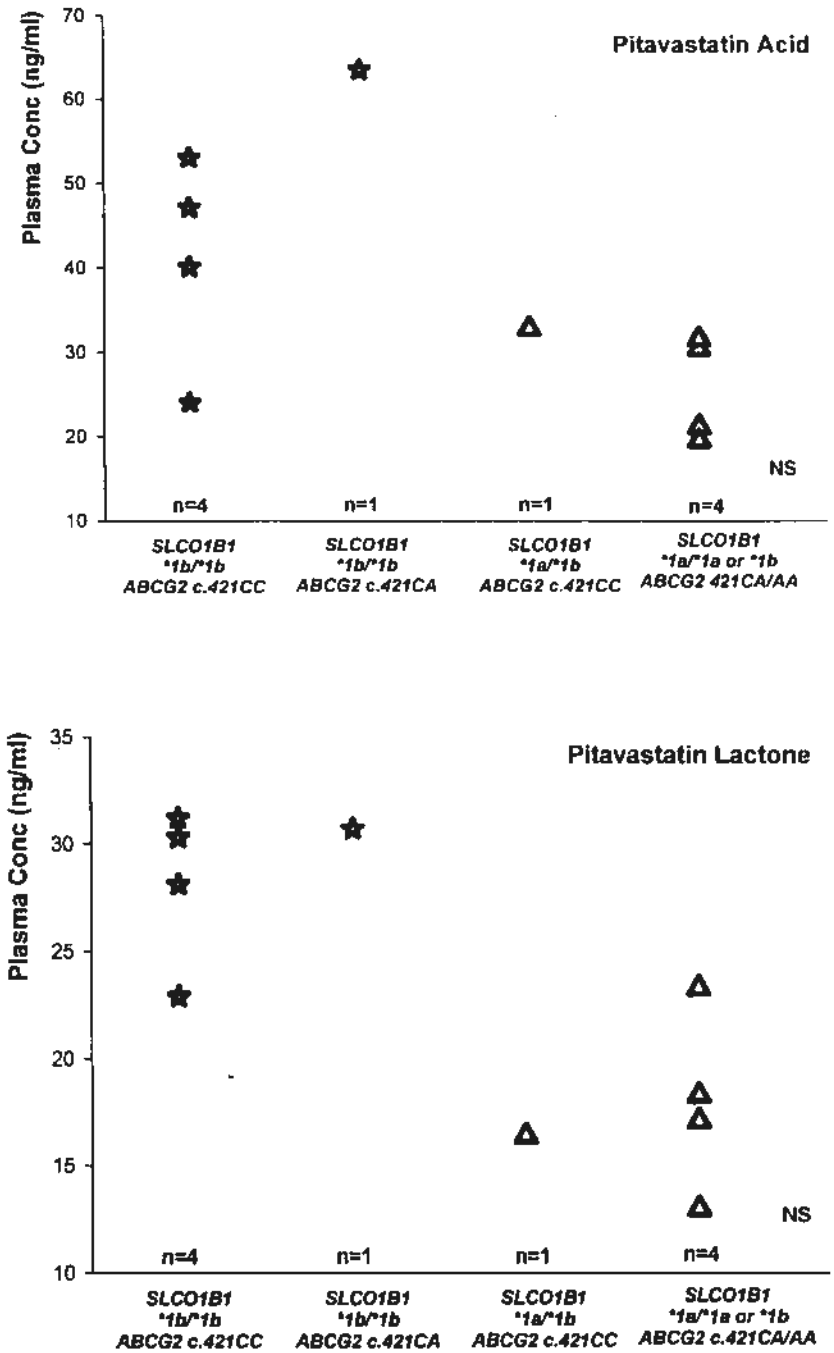
Genotype Groups	*ABCG2 c.421CC* and*SLCO1B1 *1b/*1b (n=4)*	*ABCG2 c.421A* and *SLCO1B1 *1a (n=4)*	*ABCG2 c.421CC* and *SLCO1B1 *1b/*1b (n=4)*	*ABCG2 c.421A* and *SLCO1B1 *1a (n=4)*
Administration	*Water*		*GFJ (Grapefruit Juice)*	
Pitavastatin Acid				
C_{max} (ng/ml)	43.6 (24.0 – 53.0)	25.8 (19.5 – 31.6)	43.3 (24.1 – 46.4)	23.8 (16.5 – 42.4)
AUC_{0-24h} (ng×h/ml)	104.1 (70.2 – 125.5)	63.3 (49.2 – 86.0)	106.3 (87.0 – 167.6)	67.4 (56.1 – 102.5)
AUC_{0-48h} (ng×h/ml)	107.3 (73.4 – 127.5)	64.3 (49.2 – 88.4)	113.7 (89.4 – 179.6)	68.6 (56.1 – 112.9)
AUC_{0-t} (ng×h/ml)	104.2 (70.2 – 125.5)	60.0 (45.0 – 86.0)	109.3 (87.0 – 174.0)	63.8 (50.7 – 108.1)
$AUC_{0-\infty}$ (ng×h/ml)	116.9 (89.7 – 129.8)	69.3 (49.7 – 92.9)	123.3 (94.8 – 182.7)	72.1 (-56.0 – 119.4)
Apparent oral clearance (L/h)	17.1 (15.4 – 22.3)	29.6 (21.5 – 40.2)	16.5 (11.0 – 21.1)	27.8 (16.7 – 35.7)
$t_{1/2}$ (h)	11.1 (6.0 – 16.9)	7.6 (4.7 – 10.0)	9.7 (8.6 – 15.2)	6.9 (4.1 – 13.1)
t_{max} (h)	*0.75 (0.5 – 1)*	*1 (0.5 – 1)*	*1 (1 – 1)*	*1 (1 – 2)*
Pitavastatin Lactone				
C_{max} (ng/ml)	29.2 (22.9 – 31.2)	17.7 (13.0 – 23.3)	24.9 (18.8 – 28.4)	16.3 (12.7 – 20.2)
AUC_{0-24h} (ng×h/ml)	189.7 (155.8 – 209.6)	118.4 # (99.1 – 141.9)	213.4 (172.1 – 270.8)	135.5 # (117.7 – 159.6)
AUC_{0-48h} (ng×h/ml)	215.1 (179.4 – 238.8)	134.9 # (116.0 – 167.5)	249.3 (205.7 – 321.2)	160.9 # (141.7 – 187.6)
AUC_{0-t} (ng×h/ml)	211.9 (179.4 – 238.8)	131.2 # (109.9 – 167.5)	249.3 (205.7 – 321.2)	160.9 # (141.7 – 187.6)
$AUC_{0-\infty}$ (ng×h/ml)	223.0 (189.7 – 250.0)	147.7 # (118.7 – 176.9)	265.7 (217.5 – 339.1)	176.8 # 161.6 – 206.4)
Apparent oral clearance (L/h)	9.0 (8.0 – 10.5)	13.7 (11.3 – 16.9) #	7.7 (5.9 – 9.2)	11.3 (9.7 – 12.4) #
$t_{1/2}$ (h)	11.5 (7.1 – 13.9)	12.9 (8.7 – 19.6)	13.4 (11.7 – 16.5)	16.7 (14.0 – 26.5)
t_{max} (h)	*1 (1 – 1.5)*	*1 (1 – 1)*	*2 (1.5 – 2)*	*2 (1.5 – 3)*

Data are given as median and range, statistical comparison by Mann-Whitney U Test.

# $P<0.05$ are statistically significant. Apparent oral clearance is calculated by Dose / $AUC_{0-\infty}$

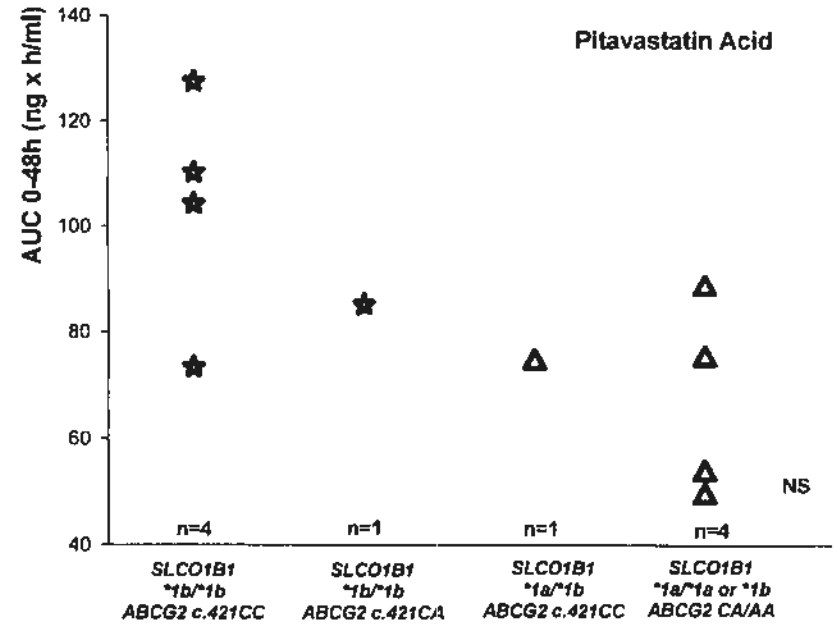
Gene-gene interaction plots according to *SLCO1B1 *1a, *1b* or **15* haplotypes and *ABCG2 c.421C>A* for C_{max} and $AUC_{0\text{-}48h}$ of pitavastatin acid after administration with water did not show any significant difference (Graph 4.28). The values of $AUC_{0\text{-}48h}$ for pitavastatin lactone were higher with individuals of *SLCO1B1 *1b/*1b* and *ABCG2 c.421CA* compared to those with *SLCO1B1 *1a* and *ABCG2 c.421A* carriers, and were of statistical significance ($P<0.05$) (Graph 4.29).

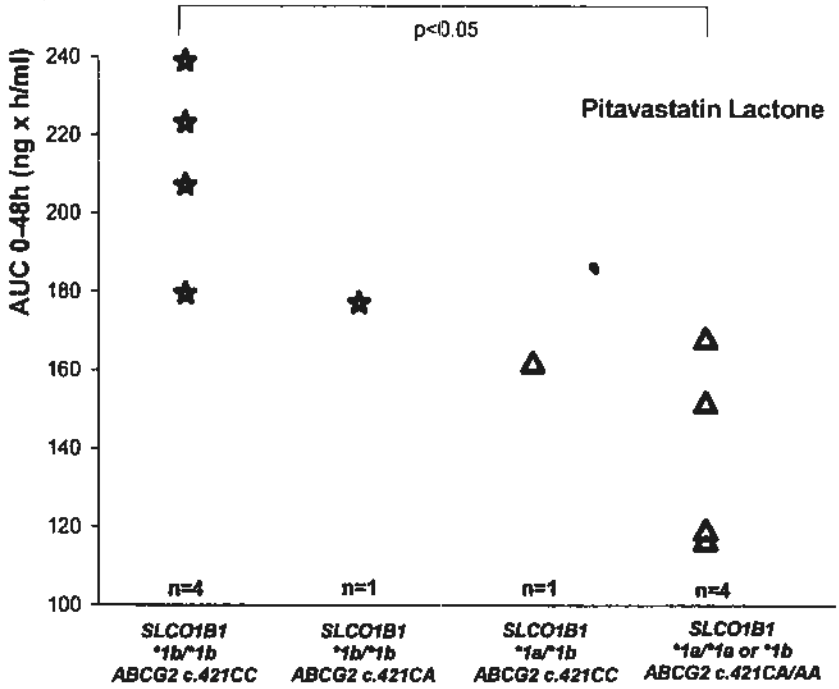
Graph 4.28: Individual C_{max} as administered with water of pitavastatin acid and lactone according to *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into those with at least one copy of *ABCG2 c.421CC* and *c.421CA or AA*



Statistical comparison by Mann-Whitney U test between first and last groups. No statistical significance found.

Graph 4.29: Individual AUC $_{0\text{-}48h}$ as administered with water of pitavastatin acid and lactone according to *SLCO1B1 *1b/*1b (c.521TT-c.388GG)* and **1a/*1a or *1b (c.521TT-c.388AA/AG)* genotypes, subclasses into those with at least one copy of *ABCG2 c.421CC* and *c.421CA or AA*





Statistical comparison by Mann-Whitney U test between first and last groups. P<0.05 is statistical significant. No statistical significance found with pitavastatin acid.

4.6. Discussion

Pharmacogenetic analyses of statin pharmacokinetics, efficacy and safety have received much attention recently, in particularly in relation to drug transporters (Hu et al., 2011b; Romaine et al., 2010). One particular area is the effect of ethnicity which may influence the overall pharmacokinetics of statins (Tirona, 2005). It is well recognized that systemic exposure to rosuvastatin is approximately 2-fold higher in Japanese and Chinese subjects compared to Caucasians and the *c.521T>C* and *c.388A>G* polymorphisms in *SLCO1B1* did not account for this difference in a study in Singapore (Lee et al., 2005). The main genetic factor identified to date to explain the increased plasma levels of rosuvastatin in East Asian populations is the *c.421C>A* polymorphism in the *ABCG2* gene encoding the efflux transporter ABCG2 (Keskitalo et al., 2009b; Zhang et al., 2006) which also influences the lipid response (Tomlinson et al., 2010). It has also been reported that there was greater atorvastatin exposure and a trend for greater simvastatin exposure in Chinese and Japanese compared to Caucasians with ratios (90% CI) of C_{max} of 1.29 (1.03–1.61) and AUC_{0-t} of 1.23 (0.96 - 1.58) in Chinese compared to Caucasians for simvastatin lactone (Birmingham et al., 2008).

The objective of the study was to evaluate the effect of candidate SNPs on the pharmacokinetics of pitavastatin, and to examine gene-gene interactions or gene-food interactions with grapefruit juice. From this analysis, it was noted that the *SLCO1B1 c.388A>G* SNP which affected the efficacy of the encoded uptake transporter OATP1B1 had shown higher C_{max} and AUCs, and lower CL/F values, suggesting higher systemic exposure of both pitavastatin acid and the lactone metabolite. There were no significant changes in the half-life ($t_{1/2}$), which further

confirmed the possible effect would be through the uptake and absorption phase, instead of the elimination phase of metabolism.

Pitavastatin is a substrate of influx transporter OATP1B1 (gene *SLCO1B1*), which is responsible for the extraction of hydrophilic molecules from the blood circulation into hepatocytes. The SNPs of *SLCO1B1 c.388A>G* and *c.521T>C* are significant due to their prominence in affecting transporter efficacy and the respective higher frequencies across populations (Tomlinson et al., 2008). There were studies on *c.521T>C* regarding statins pharmacokinetic and pharmacodynamic effects, attributing to reduced transporter efficacy with the mutant allele. Chung et al. had suggested that the *c.521T>C* SNP was related to decreased uptake of pitavastatin (Chung et al., 2005). In our study, since there were only two *c.521TC* subjects and no *c.521CC* subject, we were not able to achieve any realistic analysis on this SNP.

Our results suggested that the *c.388A>G* polymorphism may have a noticeable effect on pitavastatin disposition, This will have implications considering the significant frequency of this allele among Asians. The subjects with the *c.388G* allele were found to have apparently higher C_{max} and AUC values for both pitavastatin acid and lactone metabolite, a trend as previously reported by Wen et al. (Wen and Xiong, 2010a). Consequently, the SNP would be associated with less effective uptake of pitavastatin into the hepatocytes, possibly reduced pharmacodynamic effects and larger systemic exposure with the risk of myotoxicity. Previous pharmacokinetic studies with rosuvastatin failed to note any significant trend between haplotypes **1a* and **1b* (Wen and Xiong, 2010a). The similar insignificant difference was also reported by an earlier in-vitro study (Choi et al., 2008; Lee et al., 2005). Their suggestion regarding higher OATP1B1 transporter activity with the *c.388G* allele had been supported by the in-vitro

study from Kameyama et al. (Tirona et al., 2001). Furthermore, the transporter activity with the **1b* (*c.388G-c.521T*) haplotype was noted to be increased as demonstrated by lowered C_{max} and AUC values with repalginide (Narumiya et al., 2004) and with a number of drugs including pravastatin (Kalliokoski et al., 2008). The genomewide association study on simvastatin has identified a slightly greater cholesterol-lowering effect with the *c.388G* allele (Hirano et al., 2006; Mwinyi et al., 2004). All these studies suggested the *c.388G* allele might lead to increased influx of statins into hepatocytes. Hence, the present results could not support these observations regarding the effect of the SNP *SLCO1B1 c.388A>G* on related pharmacokinetic disposition.

Cytochrome P450 enzyme mediated pathways have been the focus of drug-drug interactions and adverse drug reactions, but it has been recognized that interactions also occur through transporters. An example is the interaction between gemfibrozil and statins which was thought to be due to CYP3A4 inhibition, but is probably more through inhibition of OATP1B1 (Backman et al., 2000; Backman et al., 2002; Hu et al., 2009a; Kyrklund et al., 2001). In our study, the polymorphism with *CYP3A5*3* did not seem to affect the pharmacokinetics of pitavastatin, hence the loss of effect of CYP3A5 enzyme system may not affect the bioavailability or metabolism of the drug. In the gene-gene interaction analysis with *CYP3A5*3* polymorphism and among wild-type *SLCO1B1*1a* subjects, there were statistically significant differences of pitavastatin lactone when comparing between sub-groups of subjects with *CYP3A5*1* and *SLCO1B1*1a* to those of *CYP3A5*3/*3* and *SLCO1B1*1b/*1b*. Whether that was the result from gene-gene interaction would require a larger sample size to confirm. Apparently, the influence from SNP *SLCO1B1 c.388A>G* and hence **1b* haplotype could be the dominant factor.

There was no statistically significant genetic influence from *ABCB1* on the pharmacokinetics of pitavastatin. Analysis performed on individual SNPs or the haplotype of *TTT* did not draw significant conclusions. The *TTT* haplotype had been reported to alter the pharmacokinetics of digoxin leading to increased drug exposure, but not the individual SNPs or diplotypes (Wang et al., 2008). Pitavastatin metabolism seemed not being influenced by the alteration of transporter efficacy of p-glycoprotein resulting from polymorphic changes. Intuitively, inhibition of p-glycoprotein would be unlikely as the major factor influencing the effect of grapefruit juice on the pharmacokinetics of pitavastatin.

The lack of significant findings from the *ABCG2 c.421C>A* analysis concurred with the report by Ieiri et al. on pitavastatin in Japanese subjects (Ieiri et al., 2007). There were other studies that reported this SNP to be associated with increased systemic exposure of atorvastatin, rosuvastatin, fluvaststin and simvastatin (Keskitalo et al., 2009a; Keskitalo et al., 2009b; Zhang et al., 2006). However, it has also been suggested that the role of BCRP contributing to biliary elimination of rosuvastatin would be more significant than that of pravastatin (Kitamura et al., 2008). Therefore, our observations being different from those for other statins could be explained by substrate specificity of BCRP or pathway differences of pitavastatin. Ieiri et al. suggested multiple organic transporters along the canalicular membrane to be involved in the biliary excretion of pitavastatin. An *in vitro* study establishing a vectorial basal-to-apical transport of pitavastatin across double transfectants involving pairings between OATP1B1/*SLCO1B1* with BCRP/*ABCG2* or MDR1/*ABCB1* or MRP2/*ABCC2* (Hirano et al., 2005). Comparison of drug transportation among the three pairings did not have significant differences. The existence of alternative pathways may definitely offset the dependence on a single pathway. Our analysis on gene-gene interaction has found significantly higher AUCs with pitavastain lactone metabolite in carriers of

*SLCO1B1 *1b/*1b* and *ABCG2 c.421CC* than those with *SLCO1B1 *1a* and *ABCG2 c.421A*. The findings could represent a gene-gene interaction between the two SNPs, or could be due to the overwhelming effects from *SLCO1B1 c.388A>G* in **1b* and **1a* haplotype, since the trend would be contrary to expectations if *ABCG2 c.421C>A* would lead to a reduced transporter efficacy. Due to limitation in population size, such observation would be inconclusive.

The concomitant administration of grapefruit juice (GFJ) with pitavastatin has increased its bioavailability by a modest 15-16% increase of $AUC_{0\text{-}48h}$ for both pitavastatin acid and lactone ($P<0.05$), but plasma half-life was not affected. This would confirm that the absorption of pitavastatin should go through one of the pathways that would be affected by administration of GFJ. This effect was observed consistently with all trial individuals. The lack of changes in terms of half-life would suggest the absence of action through interaction at the metabolic or elimination pathway.

In their work on drug-drug interaction between rosuvastatin and gemfibrozil, Bergman et al. (Bergman et al., 2010) suggested evaluation of interaction could be under-estimated upon repeated dosing because of accumulation of metabolites or inhibitory capacity of the inhibitor. The authors further suggested the mathematical differences of observed to predicted inhibition by gemfibrozil on pitavastatin were due to presence of multiple transporters involved, including OATP2B1 which could have a minute contribution . That could be extrapolated to the repeated dosing of GFJ. The significance of transporter based interaction has duly been recognized by regulatory authorities including the US FDA as a possible source of serious adverse events (Hirano et al., 2005). Further study is warranted to evaluate different transporters roles in pitavastatin metabolic pathway and drug-drug interactions.

Pitavastatin is mostly eliminated from the systemic circulation through hepatic uptake routes as unchanged drug (Hirano et al., 2004), so inhibition or reduced efficacy at the site of hepatic transporter should have particular pharmacokinetic implication. Our study further looked but failed to note significant differences among individual genotype groups of candidate SNPs and their respective changes after administration with GFJ. The predominant effect of GFJ in pharmacodynamics, pharmacokinetics and systemic exposure of coadministered drugs are expected to be due to interaction at the site of intestinal mucosal inhibition of CYP3A4 first-pass metabolism (Hu et al., 2009a), or alternatively from inhibition of the efflux transporter P-glycoprotein, and the uptake transporters OATP1A2 (Bailey et al., 1998; Tomlinson and Chow, 2006; Vaquero et al., 2010). Therefore the SNPs of interest in gene-food interaction study for pitavastatin and GFJ would be *CYP3A5*3 (c.6986A>G)* and *ABCB1 (c.1236C>T, c.2677G/A>T, c.3435C>T)*. Absence of GFJ-genotype interactions with pitavastatin suggested GFJ could have inhibitory actions of multiple transporters and CYP3A4/5 pathways, leading to compensatory mechanisms to lessen the counter-effects from a particular genetic influence on related protein efficacy. In an earlier study, Hirano et al. has also suggested OATP1B3 as a minor contributor to pitavastatin hepatic transport (Hirano et al., 2006; Lau et al., 2007).

In summary, this study was performed primarily to examine the effect of GFJ on pitavastatin pharmacokinetics. The retrospective pharmacogenetic analysis does identify some small subgroups which are too small to draw conclusions which can be generalized. To clearly elucidate the mechanisms of GFJ and the genetic interaction on pitavastatin pharmacokinetics would need further prospective study with selected genotype groups of sufficient number of subjects.

4.7. Conclusion

This study looked at pitavastatin pharmacokinetic profiles and changes in relation to *SLCO1B1 c.388A>G* polymorphsim which encodes the hepatic uptake transporter OATP1B1. This particular SNP apparently resulted in higher C_{max} and AUC levels, suggesting less uptake of pitavastatin into hepatocytes and greater systemic exposure. The effects had been similar with both active pitavastatin acid and the inactive lactone metabolite. Co-administration of grapefruit juice would affect the pharmacokinetic profiles to a modest extent. Lack of influence from *CYP3A5, ABCB1* or *ABCG2* polymorphism on pitavastatin pharmacokinetics suggested these enzymes and transporters might not be significant in pitavastatin metabolism and disposition. Previous studies on atorvastatin pharmacokinetics attributed increased systemic exposure to acid and lactone metabolites were related to the events of myopathy, but no relationship could be established with polymorphisms in *SLCO1B1, ABCB1* and *CYP3A5* (Hermann et al., 2006). Our findings of increased systemic exposure of pitavastatin with the SNP *SLCO1B1 c.388A>G* and the coadministration of grapefruit juice are worthy of further study on their clinical implications.

5. Study of the genetic and phenotypic influence in predicting elevated levels of high-sensitivity C-reactive protein (hsCRP) in Chinese patients on simvastatin therapy

5.1. Introduction

Circulating plasma concentrations of high-sensitivity C-reactive protein (hsCRP) had been identified as good predictors of coronary events in patients with stable or unstable angina (Haverkate et al., 1997). Aggressive statin therapy could lead to intensive reduction of plasma low-density lipoprotein cholesterol (LDL-C) levels and is associated with greater reduction of cardiovascular events (Baigent et al., 2005). Clinical evidence suggest that patients with lower hsCRP levels after statin therapy have better clinical outcomes, which were independent of plasma LDL-C levels (Ridker et al., 2005). Reduction in progression of atherosclerosis examined by ultrasonography was noted to relate to greater reductions in hsCRP levels among patients with coronary artery disease after statin treatment (Nissen et al., 2005). Common genetic variations involved in metabolic and inflammatory regulation were suggested to have significant effects on hsCRP levels (Ridker et al., 2008b). Other demographic and phenotypic factors have also been noted to contribute to interindividual variability in hsCRP levels (Ford et al., 2004; Shen and Ordovas, 2009). We studied the genetic and phenotypic influences on hsCRP levels in a group of Chinese patients after at least four weeks of treatment with simvastatin 40 mg daily.

5.2. High-sensitivity C-reactive protein (hsCRP)

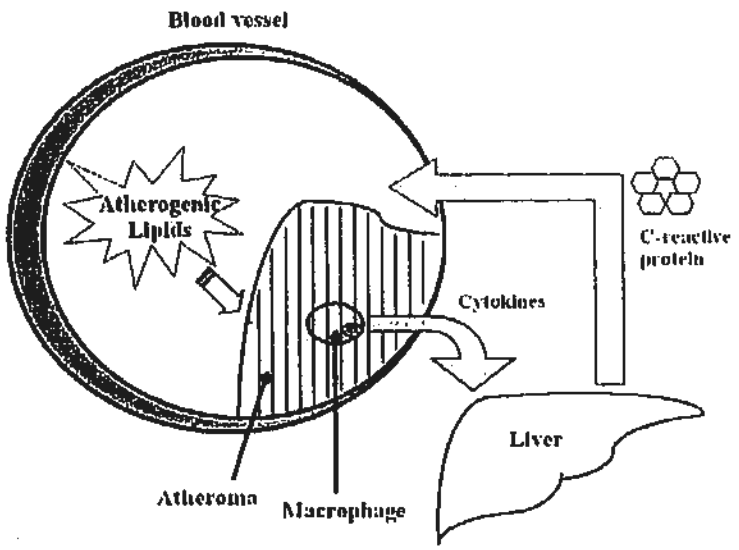
5.2.1 Atherosclerosis, hsCRP and cardiovascular risk

Atherosclerosis is the major causative factor in cardiovascular events. The atherosclerotic process begins with LDL-C accumulating in the arterial intima, leading to a series of inflammatory responses (Hansson et al., 2006). Inflammation is a major determining factor in atherosclerosis (Libby, 2002), leading to the formation, progression (Pepys and Hirschfield, 2003) and finally rupture of atherosclerotic plaques (Libby et al., 2010).

Plasma hsCRP is an important biomarker for atherosclerotic vascular disease because of its stability, availability of reference standards and assay techniques of acceptable precision (Blake and Ridker, 2001). It may not be a causual factor towards cardiovascular disease as suggested by a recent Mendelian randomization meta-analysis study (Wensley et al., 2011). Even in other cardiovascular conditions such as heart failure, hsCRP may have predictive value as patients having hsCRP levels above the median of 3.23mg/L, had features of more severe heart failure than those with hsCRP levels below the median in one study (Anand et al., 2005). CRP is an acute-phase response protein of the pentraxins family that is synthesized and released by hepatocytes upon stimulation by inflammatory cytokines and hormones. The accumulation of LDL-C and triglycerides (TG) in arterial walls leads to building up of atheroma and triggers an inflammatory response and release of immunomodulating or messenger cytokines (e.g. tumour necrosis factor-α, interleukin-6 etc.), which in turn will set off the hepatic synthesis of CRP and release into the circulation (Pearson et al., 2003) (Figure 5.1 ; Ref :(Nordestgaard, 2009). In the general population, elevated plasma hsCRP levels in the absence of acute illness were suggested to relate to long term risk of

cardiovascular events (Casas et al., 2008; Lowe, 2005; Pai et al., 2004; Ridker et al., 1997). The combination of demographic and phenotypic factors all contributed substantially to the interindividual variability of hsCRP levels (Pankow et al., 2001).

Figure 5.1: Schematic representation of the release of C-reactive protein upon inflammatory response from LDL-C and triglycerides accumulation in the atheroma



(Adapted from Nordestgaard BG, Current Opinion of Lipidology 2009)

A large scale epidemiological study found the odds ratio for coronary heart disease was 1.45 (95% confidence interval, 1.25 to 1.68) for subjects with plasma hsCRP levels in the top third of the population compared to those in the bottom third after adjustment for several phenotypic and environmental factors (Danesh et al., 2004). The importance of hsCRP in predicting vascular effects among diabetic patients has also been strongly suggested (Pfutzner et al., 2010). It has been suggested that adding hsCRP levels to the usual risk factors, such as the Framingham Risk Score, can improve the risk classification as with the Reynolds Risk Score and hsCRP values of <1mg/L, 1-3mg/L and >3mg/L denote lower,

average, and higher relative risk for future vascular events (Ridker and Silvertown, 2008). It had been proposed that individuals with plasma LDL-C levels <2.0 mmol/L and hsCRP values <2 mg/L would have reduced cardiovascular risk (Genest, 2010). Hence, it has been suggested that a therapeutic target for reduction of both LDL-C and hsCRP levels is important to manage cardiovascular risk (Quist-Paulsen, 2010). There have been discussions suggesting that various lipid lowering agents, antihypertensive agents, antidiabetic agents, anti-inflammatory, antioxidants and antiplatelet agents might reduce plasma hsCRP levels (Prasad, 2006).

5.2.2. Anti-inflammatory effects from statins and hsCRP values

The anti-inflammatory effects of statins may be related to effects in modulation of kinase phosphorylation and protein prenylation (Montecucco and Mach, 2009). An early study on lovastatin reported 14.8% reduction in plasma hsCRP levels, which was independent of the changes in lipid parameters (Ridker et al., 2001). Another study with 3-months treatment with simvastatin 10 mg daily showed 74% reduction in hsCRP values to 0.3 mg/L (Kostakou et al., 2010). In the Measuring Effects on intima media Thickness: an Evaluation Of Rosuvastatin (METEOR) study, subjects with median baseline hsCRP of 1.4mg/L had treatment with rosuvastatin 40 mg for 2-years. The mean on-treatment CRP levels was lowered by 36% and this change was not related to changes in LDL-C (Peters et al., 2010b). Similar results were seen in hypercholesterolaemic patients in whom correlations between hsCRP values and LCL-C levels on simvastatin monotherapy were weak and not significant (Pearson et al., 2009). Another trial in a Chinese population reported 24% and 40% reduction in hsCRP values after 12-weeks treatment with atorvastatin and rosuvastatin, respectively (Qu et al., 2009). Significant reductions in hsCRP values in patients with carotid stenosis were also noted with different dosages (10-80 mg daily) of atorvastatin (Kadoglou

et al., 2010), and in a dose dependent manner with atorvastatin 10 to 80 mg in high-risk subjects previously not on statins (Gensini et al., 2010). The new statin pitavastatin in a 2 mg daily dose was also shown to produce significant reductions in hsCRP after 3-months treatment (Yoshika et al., 2010). High dose simvastatin 80 mg daily treatment for 4-weeks resulted in similar hsCRP reductions (30% reduction with simvastatin alone from a median of 4.21 to 2.96 mg/L) compared to those with the combination of simvastatin 10 mg and ezetimibe 10 mg (23% reduction with combination from a median of 3.64 to 2.81 mg/L) in high risk subjects (Araujo et al., 2010).

Nevertheless, there were also reports of lack of improvement in hsCRP values with simvastatin 40 mg monotherapy compared to the benefits with different combinations of rosuvastatin (5-20 mg) and fenofibric acid 135 mg (Roth et al., 2010), or when fluvastatin 80 mg daily was compared with placebo (Ostadal et al., 2010). Changes in hsCRP values were comparable after the combination of simvastatin 20 mg with ezetimibe 10 mg treatment or in triple combination with extended-release niacin 2 gm over the 64-weeks trial period (Fazio et al., 2010). These results suggest the direct influence of statins on hsCRP values may not be affected by co-medication or by other factors that might affect the lipid profiles. Another study in hypercholesterolaemic patients eligible for carotid endarterectomy randomised into 3 groups receiving atorvastatin 10 mg/day, atorvastatin 80 mg/day or cholestyramine 8 g/day plus sitosterol 2.5 g/day for 3 months failed to show statistically significant reductions of hsCRP values with any of the treatments (Puato et al., 2010).

Various randomized trials suggested that short-term administration of high-dose statin before coronary intervention procedures improved clinical outcome in patients with acute coronary syndromes and/or high hsCRP levels (Mega et al.,

2010). In fact, randomised clinical trials with statins aiming at either primary or second prevention have reported significant reductions in myocardial infarction, stroke and vascular events (Scandinavian-4S-Group, 1994; Shepherd et al., 1995). However, a clinical trial with rosuvastatin in patients undergoing dialysis did not come to a similar conclusion (Fellstrom et al., 2009). Likewise, the anti-inflammatory benefits from statins among patients with chronic rheumatoid arthritis have yet to be confirmed (Peters et al., 2010a). In summary, potent statins that are able to have greater percentage reductions in plasma LDL-C and hsCRP levels were likely to achieve more reduction in cardiovascular risk (Ridker et al., 2008a; 2009). Targeting statin treatment based on plasma LDL-C levels alone may not provide an optimal risk reduction for all individuals (Stewart, 2009). Drawing reference to a retrospective analysis from the CORONA study, treatment with rosuvastatin 10 mg daily in patients over 60-years old with chronic systolic heart failure during long term follow-up suggested a better cardiovascular outcome in the group with higher baseline hsCRP values (≥2mg/L, median 5.5mg/L; relative hazard ratio 0.87, 95% CI 0.77 to 0.98 vs. placebo group), while the plasma LDL-C levels were comparable in the groups with higher or lower baseline hsCRP (McMurray et al., 2009). It has been suggested that the use of statin treatment would be beneficial to reduce cardiovascular risk for those with low plasma LDL-C levels and high hsCRP values (Ridker, 2010).

In the landmark JUPITER study, the authors evaluated the relationship of cardiovascular and all-cause mortality with treatment with rosuvastatin 20 mg daily in patients with elevated plasma hsCRP and average cholesterol levels (Cushman et al., 2010). This study was designed to assess the benefits in apparently healthy people (with LDL-C<3.4 mmol/L) but who had above average hsCRP values (≥2mg/L) (Ridker et al., 2008a). The trial population was noted to have an increase in the absolute vascular event risk with increasing baseline

hsCRP values (Ridker et al., 2010c). After follow-up for a median of 1.9 years, there were 50% and 37% reductions in plasma LDL-C and hsCRP levels, respectively, leading to an observed 44% decrease in cardiovascular disease and 20% decrease in total mortality. The primary prevention effect was consistent in sub-group analysis (Everett et al., 2010), including those patients with moderate renal impairment (Ridker et al., 2010b), of either gender (Mora et al., 2010), in older and younger subgroups with elevated hsCRP values but normal plasma LDL-C levels (Glynn et al., 2010). However, in a subsequent analysis the effect of plasma high-density lipoprotein cholesterol (HDL-C) levels was not predictive of residual vascular risk in patients treated with rosuvastatin achieving very low concentrations of LDL-C (Ridker et al., 2010a). The conclusion from this study suggested the benefits of statins in primary prevention were related in part to decreased inflammation as shown by reduction in hsCRP.

5.2.3. Genetic and other factors influencing hsCRP values

There are a number of clinical features and genetic factors that could affect plasma hsCRP levels. Higher hsCRP levels tend to correlate with factors like aging, gender, body mass index, smoking, diabetes, plasma LDL-C, HDL-C and TG levels (Ray et al., 2005). In this study, it was suggested that an average hsCRP level would be 1.2% higher per year of age, and 47% higher in female patients not receiving hormone replacement therapy compared to male subjects. These would all be regarded as factors for evaluation of cardiovascular risk (Acharjee et al., 2010). Plasma hsCRP levels may be lowered by statins, as well as diet, exercise and smoking cessation, which were found to relate to lower vascular event rates in terms of clinical outcome (Conen and Ridker, 2007). In a genomewide association study from data collected in 10 years, five polymorphisms were found to associate with plasma hsCRP levels, namely *rs6700896* in *LEPR* gene locus, *rs4537545* in *IL6R*, *rs7553007* in *CRP*, *rs1183910* in *HNF1 α* (*HNF1A*), and *rs4420638* in

APOE cluster. In the subsequent meta-analysis, *rs4537545* in *IL6R* was associated lower risk, and *rs6700896* in *LEPR* and *rs4420638* in *APOE* cluster were associated with higher risk of coronary heart disease. Variants in the *CRP* locus showed no such association (Elliott et al., 2009).

We have studied a group of Chinese patients undergoing treatment with simvastatin 40 mg daily, relating their on-treatment hsCRP levels to a number of genetic and phenotypic factors. The selection of these factors included probable uncontrolled cardiovascular risk factors which may be associated with metabolic or inflammatory disorders. After undergoing statistical analytical procedures, factors showing significant independent correlations ($P<0.05$) with hsCRP values were incorporated into a reference model to standardize the adjustment of phenotypic factors in our evaluation of genetic influence. Lastly, we examined the influence of these genotypic factors on the relative cardiovascular risk exposure by instituting a threshold hsCRP value of 1 mg/L, which was expected to be higher than previously reported median hsCRP values of 0.55 mg/L for a Chinese population (Zhao et al., 2010), and likely to infer medium risk of inflammation (Sabanayagam et al., 2010). Referring to the JUPITER and CORONA trials, a threshold of 2 mg/L for hsCRP values was adopted upon recognition of its possible role in defining cardiovascular risk assessment (Ridker, 2010). As a matter of fact, the exact threshold of hsCRP for increased risk of cardiovascular events is debatable (Brugts and Deckers, 2010).

5.3. Materials and Methods

5.3.1. Method

The study was performed in the Prince of Wales Hospital. Hyperlipidaemic patients were recruited from the Lipid Clinic and other outpatient clinics for treatment with simvastatin 40 mg daily for at least 4-weeks (median duration 6-weeks). Some of the patients had been started on simvastatin recently with no previous treatment and some had been switched from other treatments such as rosuvastatin. Patients were assessed at the baseline visit for medical history, physical body measurements (including body weight, waist circumference, hip circumference, and percentage of total body fat using an impedance device), biochemical parameters (including lipid profiles and plasma enzyme values) and concomitant medications. Biochemical parameters and hsCRP values were reassessed after completion of at least 4 weeks treatment with the simvastatin therapy. All laboratory serum measurements were done in the Chemical Pathology Laboratory at the Prince of Wales Hospital. A separate sample of 10 ml blood was collected in ethylenediaminetetraacetic acid (EDTA) for DNA extraction and genotyping. A total of 15 single nucleotide polymorphisms (SNPs) were chosen for genotyping and further analysis based on possible interference with statin pharmacokinetics or clinical responses.

Individuals with uncontrolled diabetes, hypertension or thyroid disease, significant renal impairment, hepatic dysfunction, unexplained high (>3 upper limit of normal [ULN]) serum creatine kinase (CK) or who had experienced a cardiovascular event within 3 months before recruitment were excluded. Treatment compliance was checked by self-reporting and tablet counting during consultation sessions. The study protocol was approved by the local Clinical

Research Ethics Committee, and all participants gave written informed consent before any study procedures were undertaken.

5.3.2. Laboratory assessments

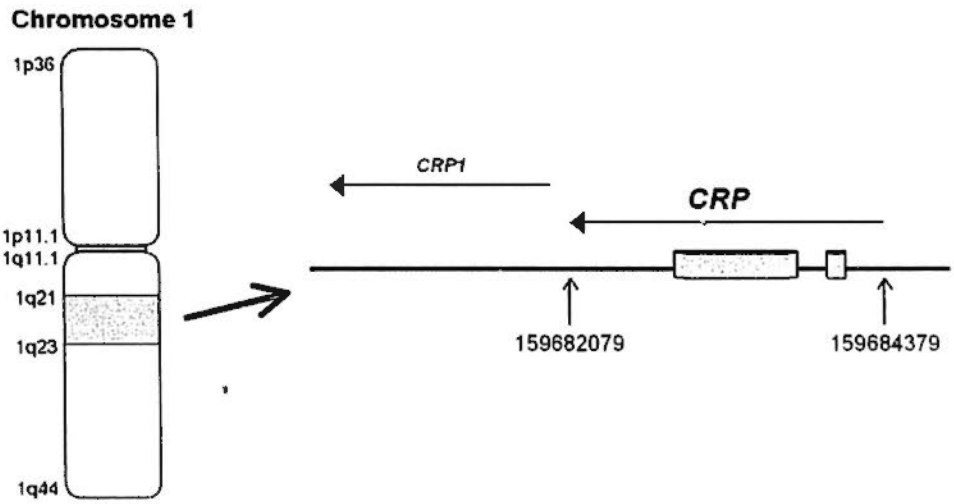
The plasma hsCRP concentration was determined by an immunonephelometric method (Siemens Dade Behring CardioPhase hsCRP assay) on Siemens BN ProSpec® System. The calibration of the assay was traceable to Certified Reference Material 470. The detection limit was 0.175 mg/L, and the measurement range was 0.175 –9.35 mg/L and at higher levels the plasma sample was diluted and remeasured automatically. The inter-assay coefficients of variation (CV) were 2.5, 3.8 and 2.1% at hsCRP concentrations of 0.5, 1.3 and 2.1 mg/L, respectively. The performance of this assay was monitored with commercial controls at different hsCRP levels (Siemens Dade Behring Apolipoprotien Control Serum CHD, N/T Rheumatology Controls SL/1 and SL2). To minimize the variation of the assay, all hsCRP measurements were performed with the same batch of reagent and within the same calibration. As turbidity and particles in the sample may interfere with the determination of hsCRP, all frozen samples were thawed once and centrifuged prior to testing. Lipaemic or turbid samples were clarified by centrifugation for 10 minutes at 15000 rpm before analysis. The lipid and laboratory safety parameters were measured by routine methods.

5.3.3. Single nucleotide polymorphism selection and genotyping

A total of 15 single nucleotide polymorphisms (SNP) or haplotypes from 11 candidate genes/loci were analysed for the study. The selected SNPs were of potential significance in terms of allelic frequencies among Han Chinese, and each had more than 5% frequency in our patient group. The list included two common SNPs in the *CRP* gene *c.3872G>A* [*rs1205*] and *c.5237A>G* [*rs2808630*]

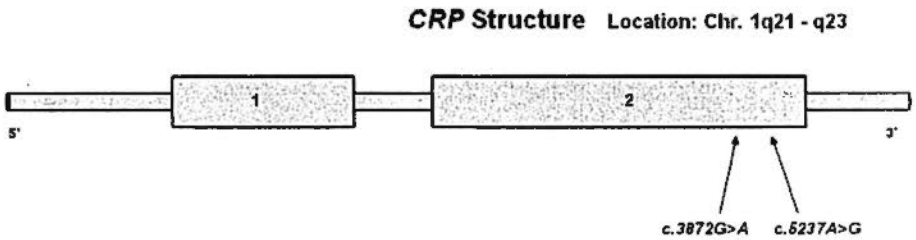
(Figure 5.2 and 5.3) based on the findings in a prior study among Framingham Heart Study participants (Kathiresan et al., 2006) and significant findings among subjects on rosuvastatin (Hu et al., 2010a). Other selections included SNPs in candidate genes related to metabolic conditions and inflammatory pathways. These included the *HNF1 α* (*HNF1A*) gene Ile27Leu (*c.79A>C, rs1169288*) (Figure 5.4 and 5.5) (Ridker et al., 2008b), *APOE* gene Cys112Arg (*c.334T>C, rs429358*) and Cys158Arg (*c.472C>T, rs7412*) (Chasman et al., 2006; Eiriksdottir et al., 2006; Hubacek et al., 2010), *APOE-CI-CII* gene cluster *rs4420638 A>G* (Elliott et al., 2009) and *LEPR* gene Gln223Arg (*c.668A>G, s1137101*) (De Rosa et al., 2009; Takahashi-Yasuno et al., 2003). In addition, 8 common polymorphisms or haplotypes among 6 genes potentially related to the pharmacokinetics of statins were also selected (Hu et al., 2009a), including those involved in cytochrome P450 (CYP) metabolic enzymes: *CYP2D6 *5* [gene deletion] and **10* [*c.100C>T, rs1065852*]; *CYP3A4 *1G* [*c.20230G>A, rs2242480*] (Gao et al., 2008); *CYP3A5 *3* [*c.6986A>G, rs776746*]; or in cellular transporters: *ABCB1* haplotype *c.1236/2677/3435 CGC>TTT* [*rs1128503 / rs2032582 / rs1045642*]; *ABCG2 c.34G>A* [*rs2231137*] and *c.421C>A* [*rs2231142*]; *SLCO1B1 c.388A>G* [*rs2306283*] and *c.521T>C* [*rs4149056*]. Genotyping were performed in the Genome Research Centre, University of Hong Kong using the mass-spectroscopy based, high-throughput MassARRAY iPLEXTM platform (Sequenom, San Diego, CA). All SNPs genotyped were in Hardy-Weinberg equilibrium (χ^2 test P>0.05).

Figure 5.2: Gene map of chromosome 1 and *CRP* with base no.



Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

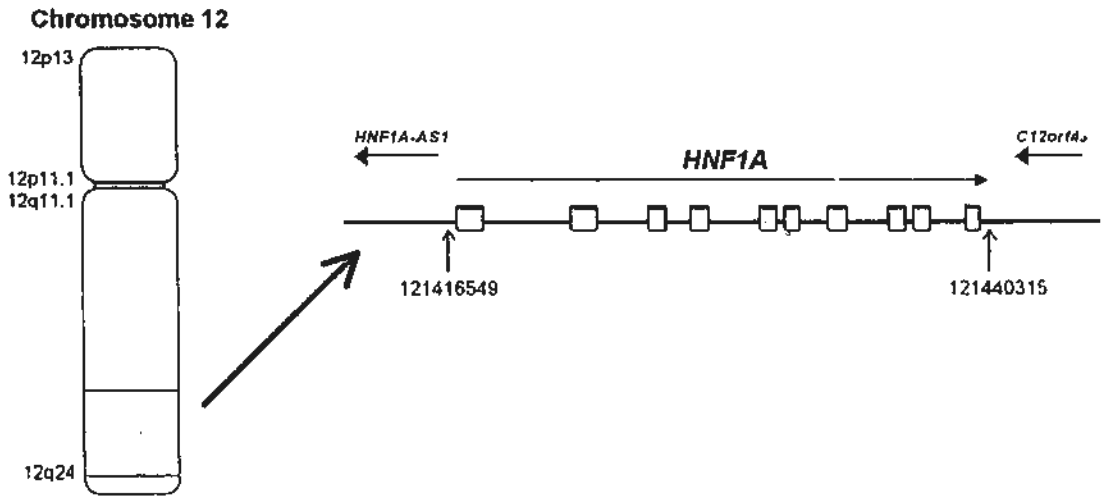
Figure 5.3: Schematic presentation of *CRP*



Allele	Frequency distribution		
	Whites	Blacks	Asians
c.3872G>A	25.0-34.1%	13.6%	46.3-70.3%
c.5237A>G	20.5-36.4%	6.2-10.0%	5.6-24.4%

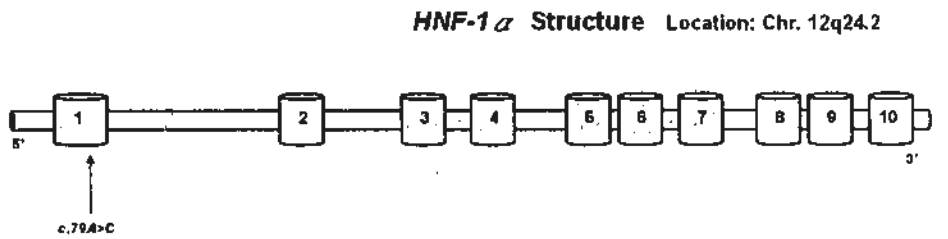
Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

Figure 5.4: Gene map of chromosome 12 and *HNF1-α* with base no.



Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

Figure 5.5: Schematic presentation of *HNF1-α*



Allele	Frequency distribution		
	Whites	Blacks	Asians
c.79A>C (Ile27Leu)	71.7-79.5%	83.3%	38.6-64.6%

Ref: www.ncbi.nlm.nlh.gov by National Center for Biotechnology Information

5.3.4. Statistical analysis

Statistical analysis was performed in stages to establish a reference model to set the platform of adjustment factors for the subsequent evaluation and comparison of genetic influence on hsCRP levels.

The first stage was to organize the data and perform a preliminary correlation analysis of individual genotypes, environmental and phenotypic factors against hsCRP values. Subjects with hsCRP values less than 10 mg/L after simvastatin treatment were included in the analysis. Those hsCRP values above this level were likely to be due to concurrent acute illness (Nordestgaard, 2009). Subjects with hsCRP levels below the limit of detection (i.e. 0.175 mg/L) were assigned the value of 0.1 mg/L. hsCRP values were tested for normality of distribution. They were transformed by natural logarithm to ln-hsCRP values for analysis to fulfill the model assumption of residual normality. Clinical characteristics of the subjects were mapped. Phenotypic and environmental factors examined in the study were gender, age, body weight, body height, body mass index (BMI), waist circumference, hip circumference, waist-to-hip ratio (WHR), systolic and diastolic blood pressure, drinking and smoking status, history of familial hypercholesterolaemia (FH), diabetes, hypertension, cardiovascular event or coronary heart disease (CHD), rheumatoid arthritis, co-medications, and biochemical parameters at baseline and on simvastatin treatment, including plasma total cholesterol, LDL-C, HDL-C, TG and fasting glucose levels. Selected SNPs or haplotypes were analyzed for comparison of on-treatment hsCRP values among genotype groups.

The second stage was to build a linear regression model of changes in hsCRP levels on individual phenotypic and environmental factors. Univariate regression

analysis on scale variables and Student's t-test on categorical variables were applied to identify their statistical significance in relation to ln-hsCRP values. Those factors of statistical significant correlations ($P<0.05$) were first applied to determine their contributions to the variance of ln-hsCRP by means of multivariate forward selection stepwise linear regression analysis. Collinearity tolerance was tested to avoid over-adjustment. The resultant linear regression factors identified were subsequently refined through another phase of backward selection linear regression analysis by removal of related factors to improve collinearity tolerance. Gender, age and plasma HDL-C changes (per 0.1 mmol/L) were factors added related to suggestions from previous publications to develop a final regression equation (Hu et al., 2010a).

In recognition of the effects of demographic and phenotypic factors on the genetic influence of hsCRP levels, the third stage of analysis employed the model as previously published (Hu et al., 2010a). Factors that were found of statistical significance in our analysis were also included. The same sets of SNPs and haplotypes as in last section were used in the analysis.

The last stage was to deliver an inflammatory risk prediction model on a threshold level of hsCRP $\geq$1 mg/L as the biomarker. The same set of adjusting factors being worked on in previous stage were entered with individual SNPs or haplotypes into logistic regression analysis to evaluate the adjusted odds ratio for prediction of hsCRP $\geq$1 mg/L. Three SNPs of particular interest from recent citations in relevant studies: *ABCG2 c.421C>A*, *CRP c.3872G>A* and *HNF1A c.79A>C* were also evaluated on a dominant model (i.e. carriers with at least one mutant SNP) basis before and after removal of outliers to test for possible skewed data that could affect the statistical procedures.

Statistical significance was set at $P<0.05$ for screening. Bonferroni adjustment for multiple testing among the 15 SNPs or haplotypes was set at experiment-wide significance level of $0.05/15 = 0.0033$. All analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

5.4. Results

5.4.1. Clinical characteristics of participants

229 subjects completed the trial with good compliance to simvastatin and having plasma hsCRP values less than 10 mg/L. Their clinical characteristics were shown in Table 5.1. There were 97 male participants and the median age of the whole population was 56 years. The median hsCRP value was 0.90 mg/L (range 0.10-8.90 mg/L), including 14 subjects with plasma hsCRP values less than the limit of detection of 0.175 mg/L. There were no significant differences between the two gender groups on age, BMI and ln-hsCRP values. The median body weight, height, waist circumference, WHR, systolic and diastolic blood pressures were higher in male subjects and these were of statistical significance ($P<0.05$). About half of the subjects were diagnosed as having familial hypercholeseterolaemia (53.3%) or hypertension (48.0%), whereas only 18.8% had diabetes and 14.4% had history of atherosclerotic CVD with evidence of coronary artery disease or cerbrovascular disease. 12.7% of subjects were current smokers and 8.3% were drinkers, and these were found mainly among male subjects ($P<0.05$). Plasma lipid profiles of the participants were listed in Table 5.2. Median total cholesterol and LDL-C levels before treatment were 7.6 mmol/L and 5.2 mmol/L, and after treatment were 4.7 mmol/L and 2.6 mmol/L respectively. After treatment, median changes of total cholesterol and LDL-C were -36.1% and -48.0% respectively, and there was no difference between genders. There were statistical differences in total cholesterol and HDL-C levels between the two gender groups before and after treatment. However, the other values were mostly comparable.

Table 5.1: Clinical characteristics in study participants with hsCRP<10 mg/L

Characteristics	Total (n=229)	Male (n=97)	Female (n=132)	P-value
Age (years)	56 (21-79)	55 (29-79)	58 (21-78)	0.685
Body Weight (kg)	62.9 (39.2-113.0)	71.9 (48.9-113.0)	57.5 (39.2-96.6)	<0.001
Body Height (m)	1.60 (1.40-1.81)	1.68 (1.51-1.81)	1.55 (1.40-1.69)	<0.001
BMI (kg/m^2)	24.6 (16.6-42.3)	25.5 (16.6-36.7)	24.0 (16.6-42.3)	0.125
Waist circumference (cm)	85.0 (60.5-123.8)	88.6 (68.0-123.8)	82.0 (60.5-121.0)	<0.001
Hip circumference (cm)	96.1 (83.0-129.0)	97.5 (86.8-121.6)	95.5 (83.0-129.0)	0.156
WHR	0.88 (0.69-1.11)	0.91 (0.71-1.11)	0.85 (0.69-1.07)	<0.001
SBP (mmHg)	123 (84-187)	127 (94-187)	121 (84-177)	<0.050
DBP (mmHg)	73 (48-144)	78 (64-144)	70 (48-96)	<0.001
FH, n (%)	122 (53.3)	45 (46.4)	77 (58.3)	0.074
Diabetes, n (%)	43 (18.8)	25 (25.8)	18 (13.6)	<0.050
Hypertension, n (%)	110 (48.0)	53 (54.6)	57 (43.2)	0.086
History of CVD, n (%)	33 (14.4)	18 (18.6)	15 (11.4)	0.126
RA, n (%)	14 (6.1)	4 (4.1)	10 (7.6)	0.281
Current drinker, n(%)	19 (8.3)	17 (17.5)	2 (1.5)	<0.001
Current smoker, n (%)	29 (12.7)	26 (26.8)	3 (2.3)	<0.001
Baseline Glucose (mmol/L)	5.4 (3.9-18.9)	5.4 (4.5-18.9)	5.3 (3.9-11.7)	<0.050[#]
hsCRP (mg/L)	0.90 (0.10-8.90)	0.83 (0.10-5.38)	0.91 (0.10-8.90)	0.370[#]

Abbreviations:

BMI = body mass index; CVD = cardiovascular disease; DBP = diastolic blood pressure; FH = familial hypercholesterolemia; RA = rheumatoid arthritis; SBP = systolic blood pressure; WHR = waist-hip ratio.

Data were expressed as median (range) or n (%) and compared by t-test for continuous variables or chi-square test for categorical variables.

[#] Data were natural logarithmic transformed for data normality before comparison.

P<0.05 is statistically significant.

Table 5.2: Plasma lipid profiles in study participants with hsCRP<10 mg/L

Characteristics	Total (n=229)	Male (n=97)	Female (n=132)	P-value
Lipid Profile Before Treatment				
TC (mmol/L)	7.6 (4.1-14.5)	7.3 (4.1-13.5)	7.8 (4.4-14.5)	<0.050
LDL-C (mmol/L)	5.2 (2.3-12.0)	4.8 (2.3-10.8)	5.4 (2.3-12.0)	0.074
HDL-C (mmol/L)	1.5 (0.8-2.9)	1.3 (0.8-2.4)	1.6 (0.9-2.9)	<0.001
TG (mmol/L)	1.6 (0.4-15.8)	1.8 (0.5-7.2)	1.4 (0.4-15.8)	0.264
Lipid Profile After Treatment				
TC (mmol/L)	4.7 (2.9-8.8)	4.6 (2.9-8.1)	4.8 (3.0-8.8)	<0.050
LDL-C (mmol/L)	2.6 (0.9-6.3)	2.5 (0.9-6.3)	2.7 (1.3-6.3)	0.191
HDL-C (mmol/L)	1.4 (0.8-2.5)	1.3 (0.8-2.2)	1.5 (0.9-2.5)	<0.001
TG (mmol/L)	1.2 (0.3-5.3)	1.4 (0.4-4.1)	1.2 (0.3-5.3)	<0.050
Lipid Profile Changes				
TC (%)	-36.1 (-59.8 - 0.0)	-36.0 (-58.4 - -15.1)	-36.1 (-59.8 - 0.0)	0.995 *
LDL-C (%)	-48.0 (-80.4 - +37.0)	-47.5 (-80.4 - +37.0)	-48.6 (-73.3 - 0.0)	0.909 *
HDL-C (%)	-3.2 (-34.3 - +55.6)	-2.8 (-29.2 - +55.6)	-4.0 (-34.3 - +38.5)	0.770 *
TG (%)	-20.0 (-74.7 - +200)	-20.0 (-66.4 - +200)	-18.2 (-74.7 - +90.0)	0.832 *

Abbreviations:

TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol;

HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.

Data were expressed as median (range) and compared by t-test for continuous variables or by * Mann-Whitney U test for ranked variables.

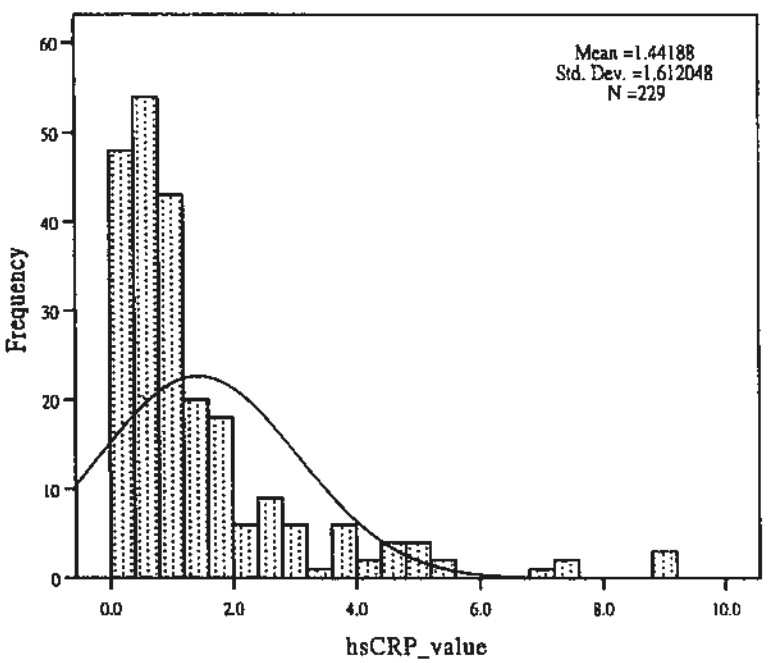
P<0.05 is statistically significant.

5.4.2. Analysis on hsCRP values with genetic, phenotypic and environmental factors

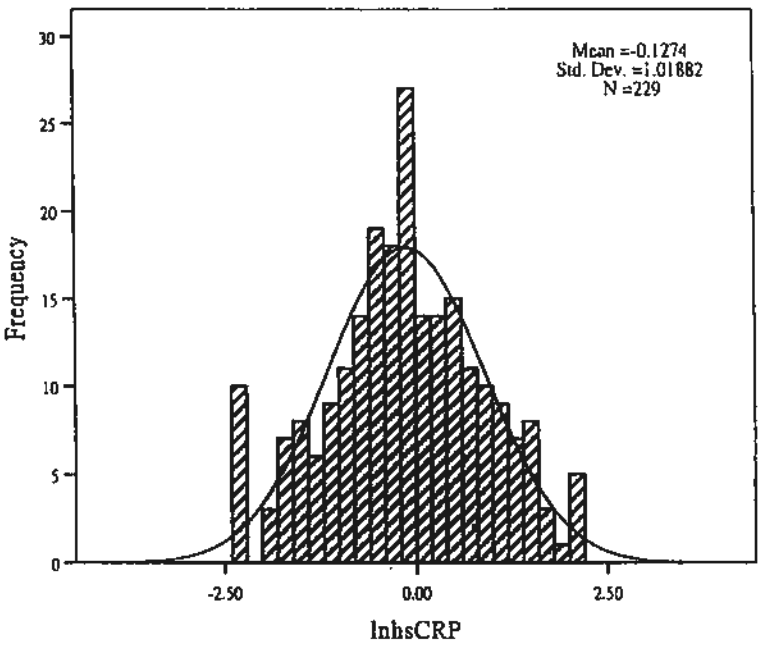
There were 229 subjects with hsCRP levels below 10 mg/L. The distribution was mapped in Graph 5.1a and shown to be skewed. Natural logarithmic values (ln-hsCRP) were obtained to fulfill the assumption of residual normality (Graph 5.1b).

Graph 5.1: High sensitivity C-Reactive Protein (hsCRP) values distribution characteristics, for population with hsCRP<10 mg/L (n=229)

a) Linear plot: Mean: 1.442 mg/L; SD: 1.612
Median: 0.90 mg/L; Range: 0.10 – 8.90 mg/L

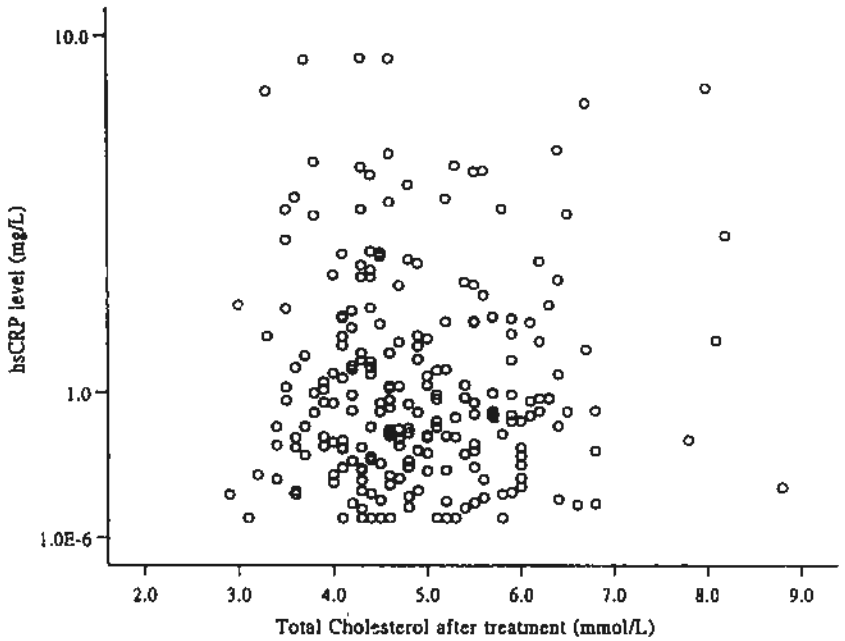


b) Natural logarithmic plot (Ln): Geometric Mean: 0.880 mg/L; Median: 0.900 mg/L; 25% quartile: 0.481 mg/L; 75% quartile: 1.790 mg/L

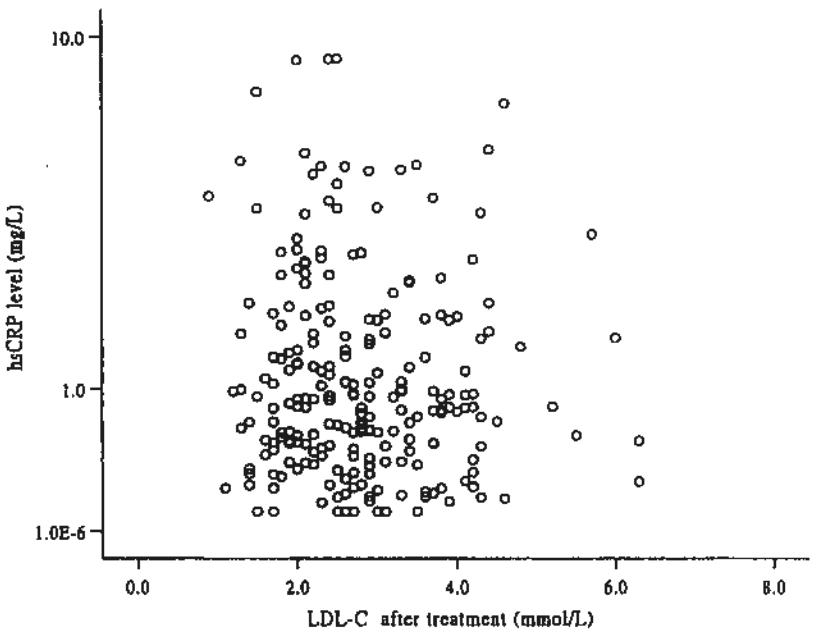


There were no statistically significant correlations or observed relationships of plasma hsCRP values with total cholesterol or LDL-C levels after treatment, both of which are key parameters in evaluating statin therapy Graph 5.2 and 5.3.

Graph 5.2: Scatterplot of plasma total cholesterol levels vs. hsCRP values after treatment with simvastatin 40mg daily



Graph 5.3: Scatterplot of plasma LDL-C levels vs. hsCRP values after treatment with simvastatin 40mg daily



The 15 SNPs or haplotypes analysed were listed in Table 5.3, and were in HWE (Chi-square test P>0.05). Geometric means and 95% CI of hsCRP values between genotype groups were listed in Table 5.4. Comparison of ln-hsCRP values showed no statistically significant difference on multiple testing principle (P>0.0033).

Table 5.3: List of 15 single nucleotide polymorphisms (SNP) or haplotypes from the 11 candidate genes/loci examined

a) Genes encoding metabolic enzymes or cellular transporters

Locus	Candidate Genes / Nearby Genes	Symbol	Polymorphism (Amino acid changes)	dbSNP
7q21.1	ATP-binding cassette, subfamily B, member 1	*ABCB1*	Haplotype *c.1236/2677/3435 C>T/G>T/C>T*	rs1128503 rs2032582 rs1045642
4q22	ATP-binding cassette, subfamily G, member 2	*ABCG2*	*c.34G>A* (Val-12Met)	rs2231137
4q22	ATP-binding cassette, subfamily G, member 2	*ABCG2*	*c.421C>A* (Gln141Lys)	rs2231142
22q13.1	Cytochrome P450 family 2, subfamily D, polypeptide 6	*CYP2D6*	**10, c.100C>T* **5* gene deletion	rs1065852
7q22.1	Cytochrome P450 family 3, subfamily A, polypeptide 4	*CYP3A4*	**1G, c.20230G>A*	rs2242480
7q22.1	Cytochrome P450 family 3, subfamily A, polypeptide 5	*CYP3A5*	**3, c.6986A>G*	rs776746
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.388A>G* (Asn130Asp)	rs2306283
12p12	Solute carrier organic anion transporter family, member 1B1	*SLCO1B1*	*c.521T>C* (Val174Ala)	rs4149056

b) Genes encoding cellular receptors relating to clinical responses

Locus	Candidate Genes / Nearby Genes	Symbol	Polymorphism (Amino acid changes)	dbSNP
19q13.2	Apolipoprotein E	*APOE*	*c.388T>C* (Cys112Arg)	rs429358
19q13.2	Apolipoprotein E	*APOE*	*c.472C>T* (Cys158Arg)	rs7412
19q13	Apolipoprotein E/C-I/C-IV/C-II (APOE cluster)	*APOE/C1 /C4/C2*	*A>G*	rs4420638
1q21	C-reactive protein, pentraxin-related	*CRP*	*c.3872G>A*	rs1205
1q21	C-reactive protein, pentraxin-related	*CRP*	*c.5237A>G*	rs2808630
12q24.2	HNF1 homeobox A	*HNF1A*	*c.79A>C* (Ile27Leu)	rs1169288
19p13.3	Low density lipoprotein receptor	*LDLR*	*c.1866C>T* (Asn591Asn)	rs688
19p13.3	Low density lipoprotein receptor	*LDLR*	*c.2052T>C* (Val653Val)	rs5925
1p31	Leptin receptor	*LEPR*	*c.668A>G* (Gln223Arg)	rs1137101

Table 5.4: Association of hsCRP values and selected genetic factors after treatment with simvastatin 40 mg daily (for subjects with hsCRP<10 mg/L; n=229)

Gene	SNP	Genotype	n	Geometric mean (mg/L)	95% CI	P-value
ABCB1	Haplotype	*CGC/CGC*	23	1.088	0.712, 1.663	
		CGC/TTT	32	1.118	0.808, 1.547	
		TTT/TTT	22	1.255	0.748, 2.105	
		Others	152	0.770	0.655, 0.905	0.044
ABCG2	*c.34G>A*	*GG*	116	0.950	0.789, 1.143	
	(Val-12Met)	*GA*	95	0.805	0.652, 0.994	
		AA	18	0.867	0.518, 1.450	0.504
ABCG2	*c.421C>A*	*CC*	98	0.804	0.643, 1.006	
	(Gln141Lys)	*CA*	90	0.856	0.701, 1.046	
		AA	41	1.162	0.881, 1.532	0.144
CYP2D6	**10*	**1*1, *2*2*	27	1.027	0.695, 1.518	
	c.100C>T	*hetero *10*	86	0.869	0.711, 1.061	
	**5* gene	**10*10*	63	0.944	0.722, 1.233	
	deletion	**5 carriers*	25	0.813	0.522, 1.266	0.807
CYP3A4	**1G*	*GG*	121	0.879	0.740, 1.045	
	c.20230G>A	*GA*	92	0.867	0.696, 1.078	
		AA	14	1.002	0.451, 2.225	0.885
CYP3A5	**3*	**1*1*	13	0.888	0.443, 1.777	
	c.6986A>G	**1*3*	98	0.939	0.767, 1.150	
		**3*3*	118	0.834	0.693, 1.004	0.695
SLCO1B1	*c.388A>G*	*AA*	11	0.924	0.432, 1.979	
	(Asn130Asp)	*AG*	44	0.838	0.620, 1.134	
		GG	123	0.853	0.703, 1.036	0.964

Gene	SNP	Genotype	n	Geometric mean (mg/L)	95% CI	P-value
SLCO1B1	*c.521T>C*	*TT*	173	0.861	0.744, 0.995	
	(Val174Ala)	*TC*	52	0.929	0.666, 1.297	
		CC	4	1.164	0.400, 3.381	0.768
APOE	*c.388T>C*	*TT*	177	0.924	0.800, 1.067	
	(Cys112Arg)	*TC*	48	0.770	0.544, 1.089	
		CC	4	0.516	0.205, 1.299	0.312
APOE	*c.472C>T*	*CC*	214	0.890	0.776, 1.022	
	(Cys158Arg)	*CT*	15	0.751	0.437, 1.290	0.532
APOE	*A>G*	*AA*	173	0.887	0.767, 1.025	
cluster		*AG*	44	0.887	0.613, 1.282	
rs4420638		*GG*	4	0.516	0.205, 1.299	0.573
CRP	*c.3872G>A*	*GG*	38	1.155	0.824, 1.617	
		GA	123	0.825	0.688, 0.990	
		AA	65	0.827	0.651, 1.049	0.174
CRP	*c.5237A>G*	*AA*	143	0.938	0.794, 1.108	
		AG	71	0.789	0.622, 1.000	
		GG	8	0.833	0.350, 1.983	0.489
HNF1A	*c.79A>C*	*AA*	95	0.992	0.817, 1.204	
	(Ile27Leu)	*AC*	97	0.856	0.700, 1.048	
		CC	37	0.697	0.468, 1.039	0.192
LEPR	*c.668A>G*	*AA*	2	0.694	0.172, 2.808	
	(Gln223Arg)	*AG*	37	1.079	0.795, 1.465	
		GG	187	0.854	0.735, 0.993	0.421

Data presented as geometric means and 95% confidence intervals (95%CI). Statistical analysis on natural logarithmic transformed data by Student's t-test (for 2-groups) or ANOVA (for 3-groups). P<0.0033 is statistically significant on multiple testing principle.

Correlation coefficients of ln-hsCRP values and individual phenotypic factors were listed in Table 5.5. The list of standardized correlation coefficients compared the relative weighing of each factor on their influence towards ln-hsCRP values. The most influential factors were waist circumference (per 10cm; 0.395), BMI (0.387), WHR (0.340), on-treatment plasma TG levels (0.340) and hip circumference (per 10cm; 0.303) (all $P<0.05$). Other notable factors included body weight, on-treatment plasma HDL-C, baseline HDL-C and TG and height (all $P<0.05$). Table 5.6 and 5.7 show the lists of categorical phenotypic factors and co-medications, evaluating the effects on ln-hsCRP values of their presence in the subjects. Higher geometric means of hsCRP values were also noted in patients if not diagnosed with familial hypercholeseterolaemia (FH) (+36%), having a history of diabetes (+64%) or hypertension (+31%) (all $P<0.05$). Patient groups on co-medications with antihypertensives (+32%), beta-blockers (+37%), anti-diabetics (+67%) and sulphonylureas (+73%) were also found to have higher geometric mean hsCRP values (all $P<0.05$), which may be related to their underlying conditions.

Table 5.5: Correlations between phenotypic factors and hsCRP values (natural logarithmic transformed) in subjects with hsCRP < 10 mg/L and after treatment with simvastatin 40 mg daily (n=229)

Phenotypic Factors	Correlation Coefficient		
	Unstandardized	Standardized	P-value
Age (years)	0.007	0.074	0.264
Body Weight (kg)	0.018	0.236	<0.001
Height (m)	-1.904	-0.159	<0.050
BMI (kg/m^2)	0.094	0.387	<0.001
Waist circumference (per 10cm)	0.353	0.395	<0.001
Hip circumference (per 10cm)	0.404	0.303	<0.001
WHR	4.595	0.340	<0.001
SBP (mmHg)	0.004	0.077	0.245
DBP (mmHg)	0.008	0.090	0.175
Baseline Plasma Parameters			
TC (mmol/L)	-0.049	-0.081	0.223
LDL-C (mmol/L)	-0.062	-0.101	0.127
HDL-C (mmol/L)	-0.523	-0.195	<0.010
TG (mmol/L)	0.147	0.220	<0.010
Fasting Glucose (mmol/L)*	0.835	0.176	<0.010
Plasma Parameters after Treatment			
TC (mmol/L)	0.004	0.004	0.956
LDL-C (mmol/L)	-0.054	-0.053	0.426
HDL-C (mmol/L)	-0.687	-0.248	<0.001
TG (mmol/L)	0.434	0.340	<0.001
Fasting Glucose (mmol/L)*	0.581	0.122	0.068

* Plasma fasting glucose values were natural logarithmic transformed before analysis. Data were tested by linear regression. $P<0.05$ is statistically significant.

Table 5.6: Correlations between phenotypic factors and hsCRP values (natural logarithmic transformed) in subjects with hsCRP < 10 mg/L and after treatment with simvastatin 40 mg daily (n=229)

Phenotypic Factors	n (%)	Presence	Absence	P-value
Gender (Male)	97(42.4%)	0.820 (0.675,0.997)	0.927 (0.773,1.112)	0.370
FH	122(53.3%)	0.762 (0.633,0.918)	1.038 (0.861,1.252)	<0.050
Diabetes	43(18.8%)	1.316 (0.964,1.797)	0.802 (0.694,0.927)	<0.010
Hypertension	110(48.0%)	1.015 (0.846,1.219)	0.772 (0.638,0.934)	<0.050
History of CVD	33(14.4%)	1.051 (0.717,1.541)	0.855 (0.741,0.985)	0.281
RA	14(6.1%)	1.204 (0.696,2.085)	0.863 (0.752,0.990)	0.236
Current drinker	19(8.3%)	0.899 (0.596,1.357)	0.879 (0.763,1.011)	0.926
Current smoker	29(12.7%)	1.176 (0.866,1.597)	0.844 (0.730,0.976)	0.102

Geometric means and 95% confidence intervals of each group were shown.
For each phenotypic factor, population expressed as n (%) of Presence.
Statistical comparison by Student's t-test on natural logarithmic transformed data.
$P<0.05$ is statistically significant.

Table 5.7: Correlations between co-medications groups and hsCRP values (natural logarithmic transformed) in subjects with hsCRP < 10 mg/L and after treatment with simvastatin 40 mg daily (n=213)

Co-medicated therapeutic class	n (%)	Presence	Absence	P-value
Antihypertensives	108(50.7%)	0.989 (0.821,1.191)	0.747 (0.611,0.914)	<0.050
Calcium Antagonists	64(30.0%)	1.015 (0.786,1.310)	0.802 (0.682,0.945)	0.122
Beta-Blockers	57(26.8%)	1.082 (0.858,1.366)	0.792 (0.671,0.936)	<0.050
ACE Inhibitors	45(21.1%)	1.101 (0.817,1.483)	0.806 (0.691,0.941)	0.068
Diuretics	35(16.4%)	1.000 (0.691,1.447)	0.836 (0.721,0.970)	0.342
Anti-Diabetics	31(14.6%)	1.333 (0.941,1.888)	0.799 (0.690,0.927)	<0.010
Aspirin	29(13.6%)	0.920 (0.686,1.233)	0.852 (0.731,0.993)	0.707
Metformin	23(10.8%)	1.195 (0.813,1.756)	0.828 (0.715,0.958)	0.102
Hydrochlorothiazide	22(10.3%)	0.973 (0.589,1.608)	0.849 (0.736,0.980)	0.553
Alpha-Blockers	21(9.9%)	1.033 (0.687,1.552)	0.844 (0.729,0.977)	0.390
Sulphonylureas	20(9.4%)	1.417 (0.864,2.325)	0.818 (0.710,0.943)	<0.050
ARBs	13(6.1%)	0.816 (0.425,1.567)	0.864 (0.750,0.995)	0.884

Abbreviation:
ARBs = angiotensin receptor blockers.

A total of 213 patients had history of co-medications available.
Only therapeutic class co-medicated with >5% population were analyzed and reported.
Geometric means and 95% confidence intervals of each group were shown.
For each therapeutic class, population expressed as n (%) of Presence.
Statistical comparison by Student's t-test on natural logarithmic transformed data.
P<0.05 is statistically significant.

Summarising the findings above, factors of statistical significance (P<0.05) were entered into the second stage of statistical analysis of univariate linear regression analysis to look for interactions. By working through forward selection logics, the final results shown in Table 5.8 suggested a regression model between ln-hsCRP and the most significant factors which were BMI (P<0.010), on-treatment plasma TG levels (P<0.005), body weight (P<0.005) and waist circumference per 10cm (P<0.050). The collinearity tolerance was tested and the acceptance level was rather low at 0.186 since three of the four factors (except on-treatment plasma TG levels) were probably related due to their association with the metabolic syndrome.

Table 5.9 is the refined regression model (by backward selection) upon retaining adjusting factors of on-treatment TG levels (regression coefficient: 0.316; P<0.001) and BMI (regression coefficient: 0.076; P<0.001) to improve collinearity tolerance to 0.869 and gender was added in this refined model (Male: regression coefficient: -0.262; P=0.037). The other two factors found in the previous model were dropped because of lower regression coefficients. Age was tested as the additional factor, but did not reach statistical significance (P=0.555).

Table 5.8: Multiple regression analysis (by forward selection) on phenotypic factors with hsCRP values (natural logarithmic transformed) in subjects with hsCRP < 10 mg/L and after treatment with simvastatin 40 mg daily (n=221)

Factors analyzed	Regression Coefficient (95% CI)	P-value	R-Square Change	Collinearity Tolerance
(Constant)	-3.568 (-4.608, -2.527)	<0.001		
BMI (Body Mass Index)	0.092 (0.027, 0.157)	<0.010	0.178	0.208
Plasma TG after treatment	0.289 (0.117, 0.461)	<0.005	0.055	0.831
Body Weight	-0.034 (-0.053, -0.015)	<0.005	0.027	0.249
Waist Circumference (per 10cm)	0.336 (0.078, 0.594)	<0.050	0.025	0.186

Excluded variables:	Regression Coefficient	P-value	Collinearity Tolerance
Body Height	0.094	0.788	0.032
Hip Circumference (per 10cm)	0.108	0.428	0.205
Waist-Hip Ratio	-0.090	0.560	0.161
Plasma HDL-C at baseline	-0.015	0.840	0.717
Plasma TG at baseline	-0.032	0.695	0.564
Baseline Fasting Plasma Glucose (ln)	0.069	0.294	0.885
Plasma HDL-C after treatment	-0.036	0.632	0.662
Familial Hypercholesterolaemia	0.088	0.189	0.850
Diabetes	0.079	0.224	0.905
Hypertension	-0.083	0.235	0.783
Antihypertensive co-medication	-0.129	0.066	0.774
Beta-Blockers co-medication	-0.081	0.226	0.867
Anti-Diabetes co-medication	0.074	0.259	0.905
Sulphonylureas co-medication	0.070	0.279	0.919

Demographic or phenotypic factors selected were of statistical significance (P<0.05) when analyzed individually as per Table 5.5, 5.6 and 5.7. P<0.05 is statistically significant.

Table 5.9: Refined multiple regression analysis (by backward selection) with hsCRP values (natural logarithmic transformed) in subjects with hsCRP < 10 mg/L on selected phenotypic factors per Table 5.8 (n=225)

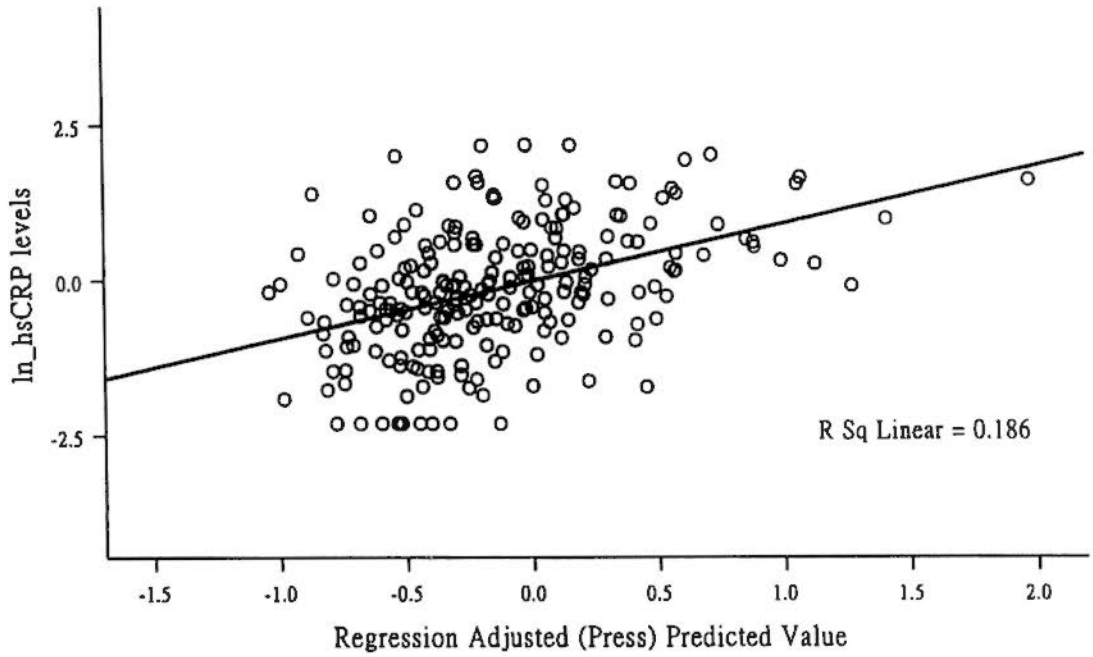
The final regression equation:
ln hsCRP = -2.400 + 0.076(BMI) + 0.316(TG after treatment) – 0.262(Gender: Male)

Factors analyzed	Regression Coefficient (95% CI)	P-value	R-Square Change	Collinearity Tolerance
(Constant)	-2.400 (-3.130, -1.670)	<0.001		
BMI (Body Mass Index)	0.076 (0.046, 0.107)	<0.001	0.150	0.875
Plasma TG after treatment	0.316 (0.154, 0.478)	<0.001	0.047	0.869
Gender (for Male gender)	-0.262 (-0.508, -0.016)	0.037	0.016	0.978

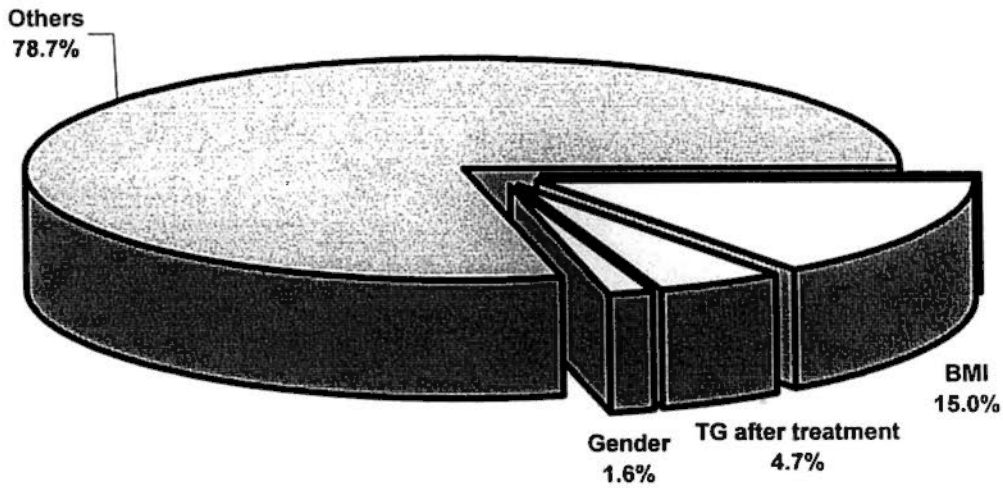
Excluded variables:	Regression Coefficient	P-value	Collinearity Tolerance
Age	-0.036	0.555	0.940
Plasma HDL-C changes after treatment (per 0.1 mmol/L)	0.005	0.933	0.968

Graph 5.4 showed the multiple regression plot with a low R square linear value of 0.186 representing a weak association with ln-hsCRP values. Graph 5.5 showed the contribution of the three factors totally of 21.3% to the variance of ln-hsCRP. BMI was the biggest single contributor at 15.0%, followed by on-treatment plasma TG levels at 4.7% and gender (male) at 1.6%. Demographic or phenotypic factors selected were as of Table 5.9 and of statistical significance (P<0.05). Body weight and Waist circumference (per 10cm) were not involved in analysis due to collinearity. Gender, Age and Plasma HDL-C changes after treatment (per 0.1 mmol/L) were included from literature suggestion.

Graph 5.4: Multiple regression plot on model as described under Table 5.9
Natural logarithmic (ln) hsCRP values vs. Regression adjusted predicted values
Adjusted factors: BMI (body mass index), TG (plasma triglycerides level after treatment), and Gender (Male). Best fit line as shown with R-square value of 0.186.



Graph 5.5: Final multiple regression model as described under Table 5.9
Percentage contribution of the four significant factors in terms of percentage variance to natural logarithmic hsCRP values.



By incorporating the adjusting factors of the reference publications with the factors found in our analysis, a total of 17 adjusting factors were included in the univariate comparison of hsCRP values on genetic influence: Age, Gender, BMI (threshold level of 25 kg/m^2), WC (per 10 cm), HC (per 10 cm), plasma lipid levels after treatment (LDL-C, and TG), plasma HDL-C changes after treatment (per 0.1 mmol/L), history of FH, diabetes, hypertension, CVD, current smoker, comedication with antihypertensives, beta-blockers, anti-diabetics and sulphonylureas. Table 5.10 listed the ANOVA comparison of ln-hsCRP values with the selected genetic factors upon adjustment of the selected demographic and phenotypic factors mentioned. No significant comparison was found at Bonferroni multiple testing principle (P>0.0033).

Table 5.10: Univariate comparison of hsCRP values after treatment with simvastatin 40 mg daily, with selected genetic factors upon adjustment on selected demographic and phenotypic factors (for subjects with hsCRP <10 mg/L, n=203)

Gene	SNP	Genotype	n	Adjusted Geometric Mean (mg/L)	95% CI	P-value
ABCB1	Haplotype	*CGC/CGC*	20	1.186	0.780, 1.802	
		CGC/TTT	27	1.102	0.780, 1.556	
		TTT/TTT	17	0.878	0.560, 1.376	
		Others	139	0.754	0.647, 0.876	0.082
ABCG2	*c.34G>A*	*GG*	101	0.890	0.743, 1.065	
	(Val-12Met)	*GA*	89	0.791	0.653, 0.959	
		AA	13	0.796	0.475, 1.334	0.675
ABCG2	*c.421C>A*	*CC*	85	0.760	0.626, 0.923	
	(Gln141Lys)	*CA*	86	0.835	0.689, 1.013	
		AA	32	1.105	0.803, 1.522	0.152
CYP2D6	**10*	**1*1, *2*2*	25	0.944	0.655, 1.359	
	c.100C>T	*hetero *10*	78	0.810	0.658, 0.997	
	**5* gene	**10*10*	56	0.882	0.691, 1.126	
	deletion	**5 carriers*	22	0.883	0.603, 1.294	0.896

Gene	SNP	Genotype	n	Adjusted Geometric Mean (mg/L)	95% CI	P-value
CYP3A4	**1G*	*GG*	111	0.854	0.717, 1.016	
	c.20230G>A	*GA*	77	0.818	0.664, 1.008	
		AA	13	0.850	0.507, 1.426	0.952
CYP3A5	**3*	**1*1*	11	0.920	0.528, 1.606	
	c.6986A>G	**1*3*	86	0.867	0.710, 1.058	
		**3*3*	106	0.810	0.676, 0.969	0.842
SLCO1B1	*c.388A>G*	*AA*	10	0.773	0.416, 1.438	
	(Asn130Asp)	*AG*	40	0.809	0.598, 1.093	
		GG	109	0.816	0.682, 0.977	0.986
SLCO1B1	*c.521T>C*	*TT*	155	0.803	0.695, 0.926	
	(Val174Ala)	*TC*	45	0.942	0.717, 1.236	
		CC	3	1.513	0.517, 4.424	0.335
APOE	*c.388T>C*	*TT*	152	0.867	0.749, 1.003	
	(Cys112Arg)	*TC*	47	0.748	0.572, 0.975	
		CC	4	0.967	0.382, 2.442	0.604
APOE	*c.472C>T*	*CC*	188	0.838	0.736, 0.953	
	(Cys158Arg)	*CT*	15	0.860	0.540, 1.369	0.916
APOE	A>G	*AA*	149	0.840	0.725, 0.973	
cluster		*AG*	42	0.811	0.610, 1.077	
rs4420638		*GG*	4	0.916	0.361, 2.323	0.956
CRP	*c.3872G>A*	*GG*	33	1.079	0.788, 1.477	
		GA	110	0.806	0.679, 0.955	
		AA	57	0.760	0.600, 0.964	0.189
CRP	*c.5237A>G*	*AA*	123	0.855	0.728, 1.004	
		AG	66	0.840	0.672, 1.051	
		GG	7	0.639	0.321, 1.271	0.716

Gene	SNP	Genotype	n	Adjusted Geometric Mean (mg/L)	95% CI	P-value
HNF1A	*c.79A>C*	*AA*	83	0.946	0.775, 1.154	
	(Ile27Leu)	*AC*	86	0.817	0.674, 0.993	
		CC	34	0.671	0.489, 0.919	0.195
LEPR	*c.668A>G*	*AA*	1	0.438	0.072, 2.683	
	(Gln223Arg)	*AG*	33	1.048	0.760, 1.446	
		GG	166	0.812	0.707, 0.933	0.282

Data were shown as adjusted geometric means and 95% confidence intervals (95%CI).

Statistical analysis on natural logarithmic transformed data by multivariable-adjustment model. Demographic or phenotypic factors selected were as of Tables 5.5, 5.6 and 5.7. Selection was based statistical significance ($P<0.05$) or as suggested by literature. List of factors included: Age, Gender, BMI, WC (per 10 cm), HC (per 10 cm), plasma lipid levels after treatment (LDL-C, HDL-C, TG), history of FH, diabetes, hypertension, CVD, current smoker, comedication with antihypertensives, beta-blockers, anti-diabetics and sulphonylureas. $P<0.0033$ is statistically significant on Bonferroni multiple testing principle.

5.4.3. Phenotypic and demographic influence on risk prediction model of hsCRP values

The final stage of analysis was to evaluate the inflammatory risk prediction model on a threshold value of hsCRP ≥1 mg/L. Table 5.11a listed the clinical characteristics of the participants. Subjects with elevated hsCRP levels apparently had higher body weight, BMI, waist and hip circumference ($P<0.001$), all of which are parameters closely associated with metabolic syndrome. Evidently, the higher risk group has 27.8% of participants with diabetes comparing to 12.1% of the lower risk group ($P<0.010$). Table 5.11b showed that the high risk group (hsCRP ≥1 mg/L) of participants had lower on-treatment HDL-C, and higher TG levels ($P<0.001$).

Adjusting factors that were entered in the previous ANOVA analysis on genetic factors and hsCRP values were reassessed in the risk prediction model. Statistical analysis was performed via binary logistic backward selection methodology

Table 5.12 listed results from the logistic regression analysis by multivariate adjustment of the selected factors. Body mass index was analyzed by categorizing on the 25 kg/m^2 level as a marker for overweight. Incremental changes of on-treatment plasma HDL-C, and after treatment LDL-C levels were added as covariance. Male patients tended to have lower risk (AOR=0.448, 95% CI: 0.210, 1.000; P=0.050), while incremental increase of 10 cm waist circumference (AOR=1.912, 95% CI: 1.053, 3.472; P=0.033) and increase of plasma TG levels (AOR=1.830, 95% CI: 1.097, 3.053; P=0.021) were of higher risk to have hsCRP ≥1 mg/L. Subjects with BMI ≥25 kg/m^2 tended to have higher risk of hsCRP above the threshold level (AOR=2.099, 95% CI: 0.816, 5.400; P=0.124).

Table 5.11: Clinical characteristics in study participants with hsCRP<10 mg/L based on high risk prediction on threshold hsCRP value of 1 mg/L

a) Demographic and phenotypic factors

Characteristics	Total (n=229)	hsCRP≥1mg/L (n=97)	hsCRP<1mg/L (n=132)	P-value
hsCRP (mg/L)	*0.90 (0.10-8.90)*	*1.95 (1.03-8.90)*	*0.54 (0.10-0.99)*	-
Gender–Male, n (%)	97 (42.3)	41 (42.3)	56 (42.4)	0.981
Age (years)	56 (21-79)	57 (21-79)	55 (22-78)	0.676
Body Weight (kg)	62.9(39.2-113.0)	68.5(44.4-112.1)	59.7(39.2-113.0)	<0.001
Body Height (m)	1.60 (1.40-1.81)	1.58 (1.41-1.81)	1.61 (1.40-1.80)	0.433
BMI (kg/m^2)	24.6 (16.6-42.3)	26.7 (17.5-42.3)	23.5 (16.6-36.7)	<0.001
Waist circumference (cm)	85.0 (60.5-123.8)	91.0 (67.5-123.8)	82.0 (60.5-117.0)	<0.001
Hip circumference (cm)	96.1 (83.0-129.0)	99.8 (84.5-129.0)	94.0 (83.0-115.0)	<0.001
WHR	0.88 (0.69-1.11)	0.90 (0.73-1.11)	0.87 (0.69-1.07)	<0.001
SBP (mmHg)	123 (84-187)	124 (84-187)	123 (84-167)	0.448
DBP (mmHg)	73 (48-144)	75 (49-144)	72 (48-99)	0.117
FH, n (%)	122 (53.3)	45 (46.4)	77 (58.3)	0.074
Diabetes, n (%)	43 (18.8)	27 (27.8)	16 (12.1)	<0.010
Hypertension, n (%)	110 (48.0)	51 (52.6)	59 (44.7)	0.238
History of CVD, n (%)	33 (14.4)	17 (17.5)	16 (12.1)	0.250
RA, n (%)	14 (6.1)	6 (6.2)	8 (6.1)	0.969
Current drinker, n (%)	19 (8.3)	9 (9.3)	10 (7.6)	0.644
Current smoker, n (%)	29 (12.7)	16 (16.5)	13 (9.8)	0.135

Data were expressed as median (range) or n (%) and compared by Student's t-test for continuous or chi-square test for categorical variable. P<0.05 is statistically significant.

b) Plasma lipid parameters and fasting glucose

Characteristics	Total (n=229)	hsCRP ≥1 mg/L (n=97)	hsCRP<1 mg/L (n=132)	P-value
Baseline Plasma Parameters				
TC (mmol/L)	7.6 (4.1-14.5)	7.4 (4.1-13.5)	7.8 (4.4-14.5)	0.145
LDL-C (mmol/L)	5.2 (2.3-12.0)	4.9 (2.3-10.8)	5.4 (2.3-12.0)	0.102
HDL-C (mmol/L)	1.5 (0.8-2.9)	1.4 (0.9-2.5)	1.6 (0.8-2.9)	<0.010
TG (mmol/L)	1.6 (0.4-15.8)	2.0 (0.5-15.8)	1.3 (0.4-5.6)	<0.005
Fasting Glucose (mmol/L)	5.4 (3.9-18.9)	5.5 (4.5-18.9)	5.2 (3.9-14.3)	<0.010#
Plasma Parameters After Treatment				
TC (mmol/L)	4.7 (2.9-8.8)	4.6 (3.0-8.2)	4.8 (2.9-8.8)	0.429
LDL-C (mmol/L)	2.6 (0.9-6.3)	2.4 (0.9-6.0)	2.8 (1.1-6.3)	0.275
HDL-C (mmol/L)	1.4 (0.8-2.5)	1.3 (0.8-2.4)	1.5 (0.9-2.5)	<0.001
TG (mmol/L)	1.2 (0.3-5.3)	1.5 (0.4-4.9)	1.1 (0.3-5.3)	<0.001
Fasting Glucose (mmol/L)	5.2 (4.1-18.0)	5.3 (4.2-11.4)	5.1 (4.1-18.0)	<0.050#

Data were expressed as median (range) and compared by Student's t-test
Data were log transformed for comparison. P<0.05 is statistically significant.

Table 5.12: Logistic regression analysis by multivariate adjustment of selected demographic and phenotypic factors to predict high risk hsCRP level ≥1 mg/L in subjects with hsCRP < 10 mg/L (n=205)

Adjustment factors	Adjusted Odds Ratio	95% CI	P-value
Age (years)	0.970	0.934, 1.006	0.103
Gender Female (n=117)	1.000		
Male (n=88)	0.448	0.201, 1.000	0.050
Body mass index <25 kg/m^2 (n=107)	1.000		
=>25 kg/m^2 (n=98)	2.099	0.816, 5.400	0.124
Waist circumference (per 10 cm)	1.912	1.053, 3.472	0.033
Hip circumference (per 10 cm)	0.952	0.441, 2.059	0.901
Plasma LDL-C after treatment (mmol/L)	0.952	0.614, 1.478	0.827
Plasma TG after treatment (mmol/L)	1.830	1.097, 3.053	0.021
Plasma HDL-C changes after treatment (per 0.1 mmol/L)	0.901	0.774, 1.049	0.179
Familial Hypercholesterolaemia (n=118)	1.113	0.451, 2.751	0.816
Diabetes mellitus (n=38)	1.678	0.369, 7.638	0.503
Hypertension (n=100)	1.055	0.210, 5.308	0.948
History of cardiovascular disease (n=32)	2.031	0.728, 5.666	0.176
Current smoker (n=26)	1.211	0.419, 3.497	0.724
Antihypertensive comedication (n=103)	0.845	0.147, 4.850	0.850
Beta-Blocker comedication (n=54)	0.508	0.186, 1.390	0.187
Anti-Diabetics comedication (n=28)	1.635	0.182, 14.718	0.661
Sulphonylureas comedication (n=19)	1.008	0.125, 8.127	0.994

Demographic or phenotypic factors selected were as of Table 5.5, 5.6 and 5.7. Selection was based statistical significance (P<0.05) or as suggested by literature.
P<0.05 is statistically significant.

5.4.4. Genotypic influence on risk prediction model on hsCRP values

The following observations were made with the whole population on carriers with *ABCG2 c.421AA* (AOR = 2.58; 95% CI: 1.16, 5.97; P = 0.027); *CRP c.3872GA* (AOR = 0.34; 95% CI: 0.14, 0.82; P = 0.017) and *CRP c.3872AA* (AOR = 0.30; 95% CI: 0.11, 0.80; P = 0.017), but were not of statistical significance on correcting for multiple testing (P>0.0033). Another observation was noted with the whole population group with SNP *ABCG2 c.34GA* (AOR = 0.51; 95% CI: 0.27, 0.96; P = 0.037). However, *ABCG2 c.34AA* carriers did not have similar consistency. Among the sub-population groups, there were findings with the female subjects group on SNPs *ABCG2 c.34GA* (AOR = 0.41; 95% CI: 0.18, 0.96; P = 0.041) and *c.421AA* (AOR = 3.54; 95% CI: 1.20, 10.50; P = 0.022), but these were not of statistical significance on correcting for multiple testing (P>0.0033). There were no similar findings with other testing in the whole population or individual gender groups.

The final stage of analysis was to evaluate genotypic interaction on the cut off threshold for hsCRP ≥1 mg/L through adjustment by the reference model obtained in the previous section. Results were tabulated in Table 5.13 as categorized between genotypes. Statistical analysis was performed to calculate AOR with 95% confidence intervals (95% CI) of the individual genotype groups in comparison with their respective homozygous wild-type genotypes. Experiment-wide statistical significance level was set at P<0.0033 on the Bonferroni multiple testing principle.

Table 5.13: Logistic regression analysis to predict high risk hsCRP level ≥1 mg/L in subjects with hsCRP <10 mg/L upon multivariate adjustment on selected demographic and phenotypic factors (adjusted odds ratio AOR and 95% CI)

Gene	SNP	Genotype	n	Adjusted Odds Ratio	95% CI	P-value
ABCB1	Haplotype	*CGC/ CGC*	20	1.000		
		CGC/ TTT	28	1.294	0.324, 5.164	0.715
		TTT/ TTT	18	2.082	0.416, 10.419	0.372
		Others	139	1.037	0.336, 3.204	0.950
ABCG2	*c.34G>A*	*GG*	102	1.000		
	(Val-12Met)	*GA*	90	0.691	0.347, 1.373	0.291
		AA	13	0.555	0.123, 2.497	0.443
ABCG2	*c.421C>A*	*CC*	86	1.000		
	(Gln141Lys)	*CA*	87	1.025	0.487, 2.161	0.947
		AA	32	2.095	0.787, 5.581	0.139
		CA/AA	***119***	***1.249***	***0.626, 2.493***	***0.528***
CYP2D6	**10* *c.100C>T* **5* gene deletion	**1*1, *2*2*	25	1.000		
		*hetero *10*	79	1.084	0.353, 3.329	0.888
		**10*10*	57	1.211	0.382, 3.842	0.745
		**5 carriers*	22	1.529	0.387, 6.044	0.545
CYP3A4	**1G*	*GG*	112	1.000		
	c.20230G>A	*GA*	78	1.175	0.576, 2.399	0.657
		AA	13	1.224	0.307, 4.880	0.775
CYP3A5	**3*	**1*1*	11	1.000		
	c.6968A>G	**1*3*	86	1.075	0.245, 4.704	0.924
		**3*3*	108	0.816	0.183, 3.634	0.790

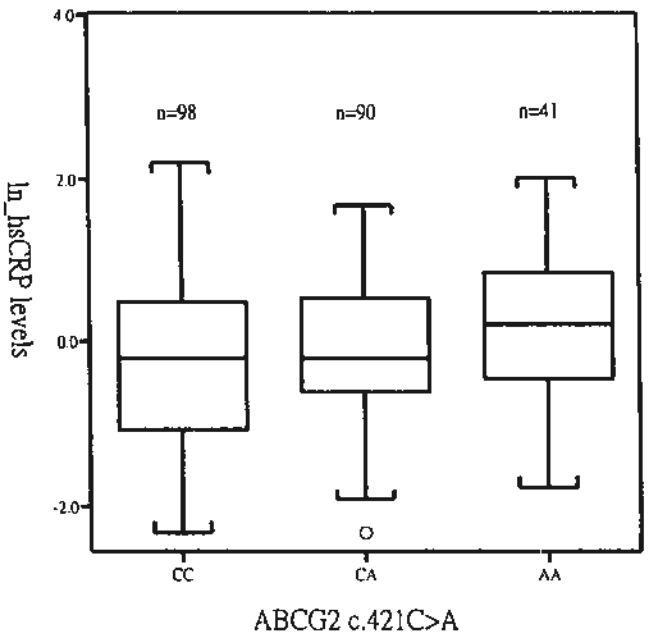
Gene	SNP	Genotype	n	Adjusted Odds Ratio	95% CI	P-value
SLCO1B1	*c.388A>G*	*AA*	10	1.000		
	(Asn130Asp)	*AG*	40	0.960	0.165, 5.590	0.964
		GG	109	0.934	0.175, 4.996	0.936
SLCO1B1	*c.521T>C*	*TT*	156	1.000		
	(Val174Ala)	*TC/CC*	49	1.333	0.613, 2.900	0.468
APOE	*c.334T>C*	*TT*	154	1.000		
	(Cys112Arg)	*TC/CC*	51	1.041	0.474, 2.284	0.920
APOE	*c.472C>T*	*CC*	190	1.000		
	(Cys158Arg)	*CT*	15	1.293	0.386, 4.336	0.677
APOE cluster	*A>G*	*AA*	151	1.000		
rs4420638		*AG/GG*	46	0.991	0.429, 2.290	0.984
CRP	*c.3872G>A*	*GG*	33	1.000		
		GA	112	0.289	0.102, 0.819	0.020
		AA	57	0.288	0.096, 0.864	0.026
		GA/AA	***169***	***0.289***	***0.107, 0.778***	***0.014***
CRP	*c.5237A>G*	*AA*	125	1.000		
		AG/GG	73	0.869	0.423, 1.783	0.701
HNF1A	*c.79A>C*	*AA*	83	1.000		
	(Ile27Leu)	*AC*	86	0.572	0.269, 1.214	0.146
		CC	36	0.625	0.232, 1.682	0.352
		AC/CC	***122***	***0.587***	***0.294, 1.173***	***0.131***
LEPR	*c.668A>G*	*AA/AG*	34	1.000		
	(Gln223Arg)	*GG*	168	0.673	0.269, 1.685	0.398

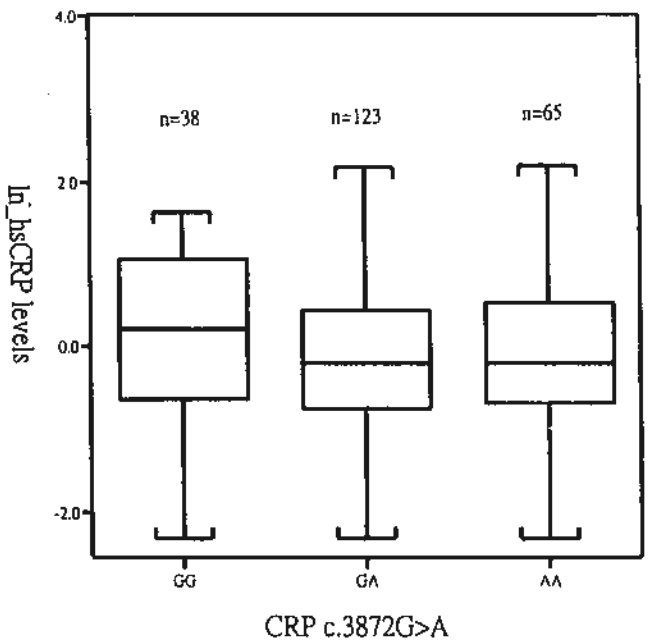
Data were shown as adjusted odds ratio and 95% CI. Statistical analysis by binary logistics regression model. Demographic or phenotypic factors selected were those applied in Table 5.10. List of factors included: Age, Gender, BMI (threshold level of 25 kg/m^2), WC (per 10 cm), HC (per 10 cm), plasma lipid levels after treatment (LDL-C, and TG), plasma HDL-C changes after treatment (per 0.1 mmol/L), history of FH, diabetes, hypertension, CVD, current smoker, comedication with antihypertensives, beta-blockers, anti-diabetics and sulphonylureas. P<0.0033 for statistical significance.

Genotype groups occurring in less than 5% of the population were merged with the heterozygous group for analysis. P<0.0033 is statistically significant on the Bonferroni multiple testing principle. No statistically significant findings were noted. The following observations were made with SNPs of interest: *ABCG2 c.421AA* (AOR = 2.095; 95% CI: 0.787, 5.581; P = 0.139); *HNF1 α c.79AC* (AOR = 0.572; 95% CI: 0.269, 1.214; P = 0.146), *CRP c.3872GA* (AOR = 0.289; 95% CI: 0.102, 0.819; P = 0.020) and *CRP c.3872AA* (AOR = 0.288; 95% CI: 0.096, 0.864; P = 0.026). Testing with the dominant genotype model suggested *CRP c.3872GA/AA* had similar significance (AOR = 0.289; 95% CI: 0.107, 0.778; P = 0.014).

From the captioned SNPs, we re-analyzed to eliminate outliers of ln-hsCRP data points to avoid possible skewed data that could affect the statistical analysis. Graph 5.6 showed the boxplots with medians of ln-hsCRP levels against individual genotype groups. Outliers, as defined as those falling beyond 1.5 times of the boxlength (i.e. 1.5 x difference between 25th and 75th percentile), were identified in the genotype groups of *ABCG2 c.421CA* and *HNF1A c.79AA*. All outliers were attributed to hsCRP values falling below the detection limit, i.e. those assigned with a value of 0.1 mg/L. There were no outliers noted in the boxplot for *CRP c.3872G>A*.

Graph 5.6: Boxplots (with medians) of natural logarithmic hsCRP levels (ln hsCRP levels) vs selected SNPs with statistical significance to predict high risk level (hsCRP level ≥1 mg/L) in subjects with hsCRP<10 mg/L: *ABCG2 c.421C>A, HNF1A c.79A>C, CRP c.3872G>A* (outliers identified in circles)





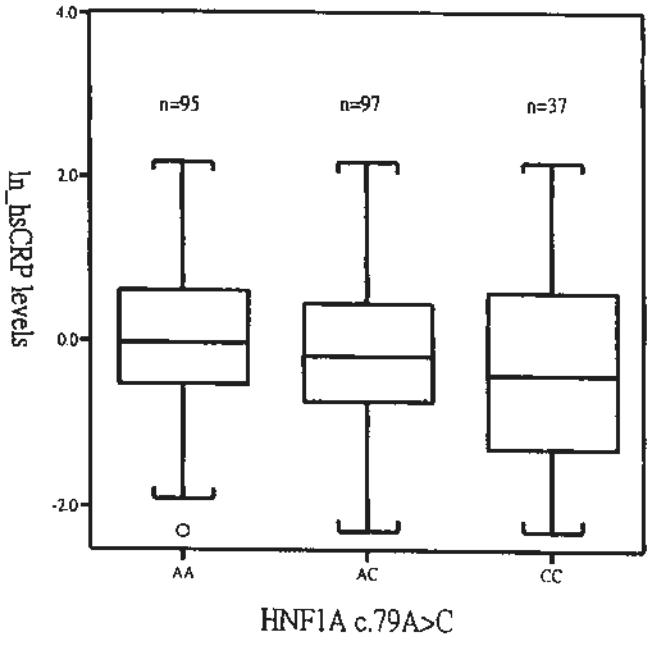


Table 5.14 showed the subsequent logistic regression analysis after elimination of outliers. Similar results were noted, genetic interaction with *CRP c.3872G>A* had been the most significant in predicting a higher hsCRP threshold level.

Table 5.14: Logistic regression analysis to predict high risk hsCRP level ≥1 mg/L in subjects with hsCRP<10 mg/L upon multivariate adjustment on selected demographic and phenotypic factors, and with selected SNPs. Data points identified as outliers were excluded in analysis

Gene	SNP	Genotype	n	Adjusted Odds Ratio	95% CI	P-value
ABCG2	*c.421C>A*	*CC*	86	1.000		
	(Gln141Lys)	*CA*	82	1.124	0.529, 2.387	0.761
		AA	32	2.093	0.787, 5.564	0.139
		CA/AA	***114***	***1.345***	***0.671, 2.696***	***0.404***
CRP	*c.3872G>A*	*GG*	33	1.000		
		GA	112	0.289	0.102, 0.819	0.020
		AA	57	0.288	0.096, 0.864	0.026
		GA/AA	***169***	***0.289***	***0.107, 0.778***	***0.014***
HNF1A	*c.79A>C*	*AA*	81	1.000		
	(Ile27Leu)	*AC*	86	0.561	0.264, 1.192	0.133
		CC	36	0.611	0.227, 1.643	0.329
		AC/CC	***122***	***0.575***	***0.288, 1.151***	***0.118***

Data were shown as adjusted odds ratio and 95% CI. List of factors included: Age, Gender, BMI (threshold level of 25 kg/m^2), WC (per 10 cm), HC (per 10 cm), plasma lipid levels after treatment (LDL-C, and TG), plasma HDL-C changes after treatment (per 0.1 mmol/L), history of FH, diabetes, hypertension, CVD, current smoker, comedication with antihypertensives, beta-blockers, anti-diabetics and sulphonylureas. P<0.0033 is statistically significant on Bonferroni multiple testing principle.

5.5. Discussion

The present study recruited patients attending the Lipid Clinic and other clinics with the primary aim for lipid control. Study subjects received simvastatin 40 mg daily therapy for at least 4-weeks. The objective of the study was to investigate the correlation of genetic factors and on-treatment hsCRP levels, an inflammatory biomarker that would suggest the individual being at risk of chronic inflammatory conditions, atherlosclerosis and hence elevated cardiovascular risk.

We have adopted the methodology similar to an earlier publication described for cohort analysis among participants from the Framingham Offspring Study, an extension to the original Framingham Heart Study (Kannel et al., 1979), for genetic and environmental interaction on plasma CRP levels. In this study, twelve clinical covariates were found to be able to explain 26% of the variance, whereas BMI was the single biggest contributor of 15% (Kathiresan et al., 2006). In our correlation analysis, we have identified 17 factors with statistically significant correlations individually. With our linear regression analysis on their interaction, the final model accounted for 21.3% of the variance of ln-hsCRP values, with BMI as the single factor for 15.0%, on-treatment plasma triglyceride levels for 4.7% and gender (male) for 1.6%. There were significant findings on independent correlation analysis with co-medication with antihypertensives, beta-blockers, anti-diabetics and sulphonylureas. Aspirin was suggested to have independent plasma CRP levels lowering effects (Ikonomidis et al., 1999). ACE inhibitors were also noted to have such direct effects (Soriano et al., 2007). However, our data did not reveal significant differences in the presence of these two co-medications.

Our methodology was also similar to the post-hoc analysis in the PROVE IT-TIMI 22 trial, evaluating the correlation of uncontrolled risk factors to on-treatment CRP levels after standard or intensified statins (Ray et al., 2005). This study suggested the effects of statins in lowering CRP levels, as well as the importance

of individual risk factors that may directly affecting CRP levels, including BMI, age and gender. Hence, for correction of statistical comparison in our analysis, we employed the same set of adjusting factors from a previous publication on rosuvastatin and its relationship with on-treatment hsCRP levels (Hu et al., 2010a). Furthermore, we have added adjusting factors that were found to be of significance in the correlation analysis. Individual SNPs or haplotypes were added to evaluate their genetic interaction. Unfortunately, the limitation of our study was only measurements of on-treatment hsCRP levels were available. We were not able to draw references to statin related hsCRP levels modifications during treatment.

In the process to identify candidate genes and the respective SNPs for gene-environment interaction on hsCRP analysis, we have taken the conventional approach of Mendelian randomization in naturally occurring genetic variation resulting from independent gene assortment (Davey Smith and Ebrahim, 2003). Candidate genes selected including those encoding metabolic enzymes or cellular transporters which may be involved in statin pharmacokinetics, and those encoding cellular receptors that may be involved in related clinical expression towards hsCRP levels.

Plasma CRP concentration is a heritable complex trait. Genetic factors in combination with environmental or phenotypic factors might explain the variations (Hage and Szalai, 2009). The *CRP* gene is highly polymorphic. The role of *CRP* polymorphisms and its gene expression had been intensively studied for their relationship with inherited disease states, but without an outright conclusion (Hage and Szalai, 2007). Differences in allele frequencies of concerned SNPs among ethnic groups may be the underlying explanation to the distribution in plasma CRP levels among population groups (Kelley-Hedgepeth et al., 2008). Asian populations were noted to have lower CRP levels than Caucasians (Lakoski et al., 2005; Saito et al., 2007). In studies on healthy subjects and patients with carotid artery stenosis, *CRP c.1444C>T* genotype was associated with higher hsCRP values after adjustment for clinical covariates

(Jones et al., 2009; Liu et al., 2009). This particular SNP was suggested to be associated with elevated chance of ischaemic or coronary events in high risk patients (Arenillas et al., 2009; Casas et al., 2006). Conversely, in a retrospective study pooling 4,600 cases, the authors reported lack of association between this SNP and coronary heart disease, even though there was positive correlation with plasma CRP levels (Lawlor et al., 2008). Another author identified nine *CRP* SNPs, including *c.3872G>A* and *c.5237A>G*, having statistically significant associations with plasma CRP levels (Kathiresan et al., 2006). A study in patients with metabolic syndrome on fenofibrate treatment suggested the polymorphic *CRP* gene accounted for the differences in baseline CRP levels (Shen et al., 2008). In a Danish cohort study, four *CRP* SNPs were found to be good predictors of high plasma CRP levels, but the association with increased risk of ischaemic vascular disease remained unclear (Zacho et al., 2008). A recent study with rosuvastatin associated SNPs *CRP c.3872G>A* and *c.5237A>G* with lower on-treatment hsCRP levels and the corresponding multivariate-adjusted odds ratios in predicting elevated hsCRP levels over 1 mg/L (Hu et al., 2010a). Our study had similar findings suggested *CRP c.3872G>A* and *c.5237A>G* were associated with lower hsCRP levels (P>0.05). The alleles of *c.3872GA* and *c.3872AA* were found to be significant in predicting a smaller chance to have hsCRP levels exceeding 1 mg/L with geometric means on adjusted odds ratio (AOR) 0.289 and 0.288 (P = 0.020 and 0.026, respectively), but these were not of statistical significance after adjustment for multiple testing (P>0.0033). In our study, the allele frequency of *CRP c.3872G>A* was 56%, similar to the 64% observed frequency among Asian populations in another multi-ethnic study, who also had lower plasma CRP levels compared to homozygous wild-type carriers (Lee et al., 2009). The frequency of this polymorphism is understood to fluctuate among ethnic group, which may help to explain the ethnic difference of CRP concentration (Shen and Ordovas, 2009). This polymorphism would likely play an important role in hsCRP level prediction among the Chinese population.

Hepatic nuclear factor 1- α (HNF1- α) is a homeodomain-containing transcription factor and is known to regulate the expression of various liver genes,

including the *CRP* gene linking to the plasma CRP levels (Armendariz and Krauss, 2009; Ridker et al., 2008b). It was suggested that HNF1-α regulates the synthesis of CRP by binding to the promoter region of the *CRP* gene (Li and Goldman, 1996). Genome-wide association analysis suggested a particular locus in *HNF1-α* was associated with plasma CRP levels (Okada et al., 2011). Several SNPs within *HNF1-α* were suggested to relate to plasma CRP levels in a population cohort study (Kleber et al., 2010) and among genomewide association studies (Armendariz and Krauss, 2009; Reiner et al., 2008).

The SNP *c.79A>C* (Ile27Leu) was suggested to be associated with lower hsCRP levels while on-treatment with rosuvastatin (Hu et al., 2010a). Our study showed a similar trend but this was not of statistical significance. Carriers of the *c.79AC* allele were associated with a smaller chance to have hsCRP levels ≥1 mg/L (AOR 0.572, P = 0.146). The evolving evidence of HNF1-α in association with CRP levels and other anti-inflammatory markers would warrant further investigation.

Obesity is associated with metabolic syndrome and widely regarded as an inflammatory condition. CRP levels rise with increasing proportion of visceral adipose tissue and are related to factors involved in the metabolic syndrome, including adiponectin (Libby et al., 2010). Upregulation of cytokines including interleukin-6 and tumour necrosis factor-α by obesity has been suggested as a contributing factor (Kelley-Hedgepeth et al., 2008). Our study has suggested an incremental increase of 10 cm in waist circumference was related to 2-fold increased risk of having hsCRP ≥1 mg/L. Circulating adiponectin levels were suggested to correlate with coronary plaque regression during aggressive statin therapy in high risk patients (Ohashi et al., 2010). Leptin, an adipocyte derived protein, was also suggested to be associated with obesity, appetite regulation and plasma CRP levels (Shamsuzzaman et al., 2004), even though the genetic association with individual SNPs may not be as conclusive (Zhang et al., 2007). Our study with *LEPR c.668A>G* did not have any significant findings in relationship with hsCRP levels.

Previous studies have identified LDL-C responses to statins were associated with polymorphisms in genes encoding target enzymes and proteins involved in lipid metabolism including apolipoprotein E (gene *APOE*) (Zintzaras et al., 2009). The associations between the *APOE* gene and plasma CRP levels have been suggested even though the mechanism remained unclear (Chasman et al., 2006). A recent study has associated *APOE e4* carriers encoding the isoform of E4 of lowest median hsCRP levels at 0.72 mg/L, and the significance was not affected by adjustment for phenotypic status of individuals (Hubacek et al., 2010), even though it was previously suggested in meta-analysis as related to slightly higher risk of coronary heart disease (Bennet et al., 2007). We have incorporated the two SNPs of *APOE c.388T>C* and *c.472C>T* which define *APOE e4* (*c.388C/c.472C*) in the analysis. A genomewide association study had also suggested the minor allele *rs4420638* in the *APOE* cluster to be associated with plasma CRP levels (Elliott et al., 2009). All these SNPs were included in our analysis but without significant findings.

Breast cancer resistance protein (BCRP, gene *ABCG2*) belongs to the family of ATP-binding cassette (ABC) efflux transporters, and is suggested to have a significant involvement towards statin disposition (Hu et al., 2011b). *ABCG2* is expressed in multiple tissues, including hepatocytes and biliary canaliculi as part of the elimination pathways. BCRP is important for the disposition of a wide variety of drugs including statins (Cusatis and Sparreboom, 2008). The nonsynonymous SNP *ABCG2 c.421C>A* is known to affect gene expression and transportation efficiency (Imai et al., 2002; Robey et al., 2009; Tamura et al., 2006).

This polymorphism is important to the Chinese population because of its high frequency of the mutant allele (approx. 35%) (Hu et al., 2009a). The influence of the *ABCG2 c.421C>A* SNP on substrate drugs, and in particular for statins, has been reported in studies in healthy volunteers and patients (Bailey et al., 2010; Hu et al., 2010b; Keskitalo et al., 2009a; Keskitalo et al., 2009b; Niemi, 2010; Tomlinson et al., 2010). The hydrophilic statin rosuvastatin has been well tested

by *in vitro* and animal studies confirming the role of ABCG2 in its disposition (Huang et al., 2006; Kitamura et al., 2008). A clinical study suggested that *ABCG2 c.421AA* carriers were associated with a 6.9% increase in LDL-C lowering response with rosuvastatin 10 mg after 4-weeks of treatment compared with *c.421CC* subjects (Tomlinson et al., 2010). Another non-synonymous SNP *ABCG2 c.34G>A* did not have the same degree of impact on protein expression or function (Cusatis and Sparreboom, 2008; Kondo et al., 2004; Robey et al., 2009). Our results suggested *c.34GA* and *c.34AA* carriers tended to have lower chance of hsCRP ≥1 mg/L (AOR = 0.691 and 0.555, P = 0.291 and 0.443, respectively). The fact that *ABCG2 c.34A>G* is associated with the *c.421C>A* SNP due to its weak linkage disequilibrium might explain the observation (Hu et al., 2011b). Carriers of *ABCG2 c.421AA* alleles had a higher chance to be in the high risk group of hsCRP ≥1 mg/L after multivariate adjustment (AOR = 2.093, P = 0.139). The pharmacokinetics of simvastatin lactone, but not simvastatin acid, have been reported to be affected by the SNP *ABCG2 c.421C>A*, where the exposure of individuals who are *c.421AA* carriers may be 111% larger than those of the *c.421CC* wild-type carriers (Keskitalo et al., 2009a). Our findings may suggest a gene-drug interaction of simvastatin treatment with *ABCG2 c.421C>A* in the resultant higher risk in terms of hsCRP ≥1 mg/L.

The current study only looked at hsCRP values after simvastatin treatment as a biomarker for the evaluation of relatively higher exposure to cardiovascular risk. Our limitation without the starting hsCRP values would not allow us to evaluate whether simvastatin has an effect over the course of treatment. The CORONA study suggested patients on rosuvastatin 10 mg daily and with higher baseline hsCRP levels (≥2 mg/L) had greater reduction (-33.3%) of cardiovascular risk compared to the placebo group (-11.1%) after 3-months of treatment (McMurray et al., 2009). Another Japanese study in hypertensive hyperlipidaemic patients with comedicated angiotensin receptor antagonists suggested pitavastatin 2 mg daily treatment might independently lower hsCRP levels by 50% after 3-months of therapy in this high risk group (Yoshika et al., 2010). The topic of statins with direct pleiotropic effect on plasma CRP levels should be noted for future

investigation.

The threshold hsCRP value ≥ 1mg/L was adopted relating to the PROVE IT-TIMI 22 and JUPITER study which looked at thresholds of 1mg/L and 2mg/L for hsCRP (Ridker et al., 2005; Ridker et al., 2009). Furthermore, we have taken into account the lower hsCRP in ethnic Chinese populations. In our study, the median adjusted hsCRP levels was relatively low at 0.9 mg/L, and there were not many with levels above 2mg/L, so the threshold of 1mg/L was chosen. A multiethnic study on CRP concentrations suggested a median of 0.7 mg/L (interquartile range 0.4-1.6 mg/L) for a Chinese population, and BMI was attributed to account for much of the ethnic difference (Kelley-Hedgepeth et al., 2008). In our study, the median hsCRP level was 0.9 mg/L, with BMI and on-treatment plasma TG levels identified as the key adjusting factors, both of which are important criteria in metabolic syndrome. Gender was added because of prior research findings. Hence we believe our study subjects may be representative of a typical Chinese population in some aspects, but they are all at increased cardiovascular risk. Subsequent gene interaction studies suggested SNPs *CRP c.3872G>A* would lead to lower risk of having hsCRP ≥1 mg/L, compared to the wild-type genotype ($P<0.05$), but became non-significant after adjusting for multiple testing ($P = 0.0033$).

The revised multivariate logistic regression model as demonstrated in a recent publication with rosuvastatin served as a good reference model to our study (Hu et al., 2010a). In this study, on-treatment rosuvastatin was found to have significant association with higher risk of hsCRP ≥1 mg/L with CRP polymorphisms, but not found with our simvastatin model. Further study is warranted for confirmation. The limitation of our study in terms of small population size and undetermined pre-treatment hsCRP levels would not be satisfactory to draw conclusions on the influence from the selection of statins.

5.6. Conclusion

We believe the methodology developed in this study can serve for qualitative evaluation for statins on direct pleiotropic effects independent from the lipid lowering capability and the study of pharmacogenetic evaluations on independent single nucleotide polymorphisms. Whether these data can be translated as an indicator to hazard ratio to increase risk of cardiovascular disease is debatable (Ridker et al., 2010c; Shah et al., 2009; Zacho et al., 2008), and may be beyond our scope due to their long term epidemiological nature. A recent genome wide association study identified a number of SNPs that were strongly associated with plasma CRP levels, however a subsequent Mendelian randomization study on *CRP* genetic loci did not find a relationship with risk in coronary heart disease (Elliott et al., 2009). Our study results may be better interpreted in terms of pharmacogenetic significance towards predicting higher hsCRP values, and would be more conclusive with growing data on a larger study population.

6. Conclusion

6.1. Summary of findings

Statins are widely used in clinical practice for lowering low-density lipoprotein cholesterol (LDL-C) and reducing cardiovascular risk. Evidence on clinical benefits leading to reduction in cardiovascular events has been reported. Statins such as simvastatin undergo metabolism and distribution through complex metabolic pathways mediated by a number of cell membrane transporters and cytochrome P450 (CYP) enzymes. Their encoding genes are polymorphic, and hence are subject to enhanced or reduced efficacy depending on the degree of gene expression and activity of the gene product. Pharmacogenetic studies of statins have been conducted on possible genetic variation leading to effects on statin efficacy and probably susceptibility to statin-induced adverse reactions.

This thesis describes research performed on genetic polymorphisms that may lead to effects on pharmacokinetics and pharmacodynamics of some statins.

The CYP3A enzymes, and their genetic polymorphisms have been examined in relation to changes in simvastatin pharmacodynamics. The CYP3A enzymes are the major enzymes because of their abundance in hepatocytes. Simvastatin is known to undergo metabolism via the CYP3A4/3A5 pathway. In the studies of candidate single nucleotide polymorphisms (SNPs) of *CYP3A* genes, *CYP3A4*1G*, *CYP3AP1*3* and *CYP3A5*3* were noted to result in altered CYP3A4/3A5 enzyme expression and/or activities. However, no statistically significant relationship was found between these SNPs and a potential phenotypic marker of CYP3A activity, the non-invasive urinary 6β-OHC/C concentration ratio.

The key pharmacodynamic parameter to assess the response to simvastatin therapy is the percentage LDL-C reduction. Analysis was performed to relate the lipid lowering responses with individual SNPs or a combination of SNPs, gene-gender interaction and with the urinary 6β-OHC/C concentration ratio. There are no statistically significant findings. However, among the gene-disease

interactions analysis, patients with familial hypercholesterolaemia (FH) were found to have significant variations in LDL-C lowering in relation to the candidate SNPs (P<0.05). *CYP3A5*3/*3* and *CYP3AP1*3/*3* subjects were found to have reduced LDL-C and total cholesterol lowering responses compared to the wild-type individuals. However, the homozygous *CYP3A4*1G/*1G* individuals were found to have the contrary results of greater lipid-lowering responses than the wild-type individuals. These findings are difficult to interpret and the statistical significance may be related to performing multiple tests in multiple subgroups.

Although CYP3A4/A5 enzymes are known to play the predominant role in the metabolism of simvastatin and lovastatin, polymorphisms in *CYP2D6* were reported to be associated with the cholesterol-lowering effect and/or tolerability of simvastatin. The second study examined whether the *CYP2D6*10* polymorphism, which is common in Chinese populations, affected the pharmacokinetics of lovastatin and simvastatin in Chinese healthy subjects in two separate single-dose pharmacokinetic studies. Plasma concentrations of lovastatin, simvastatin and their active acid metabolites were determined after single oral doses of 40 mg of the statins. The analysis compared the results against the *CYP2D6 wt/wt* group, and the area under plasma concentration-time curve ($AUC_{0-\infty}$) values for lovastatin lactone increased (P<0.001) on average (95% CI) by ratios of 1.68 (0.28-3.08), 2.15 (0.75-3.55), 2.62 (0.91-4.33), 5.80 (3.86-7.74) in the *wt/*10*, **10/*10*, **10/*5* and **5/*5* groups and the values of lovastatin plasma clearance (CL/F) were reduced by 32.5%, 52.2%, 62.1% and 84.4% in these genotype groups, respectively The pharmacokinetics of lovastatin acid was not statistically different among the genotype groups. The *CYP2D6*10* polymorphism did not significantly affect the pharmacokinetics of simvastatin, although subjects with **10/*10* genotype tended to have an increased systemic exposure to simvastatin lactone with $AUC_{0-\infty}$ increased by 50.7% (-47.6% to +149%) compared to *wt/wt*, but this was not seen with the active acid metabolite. In conclusion, it was suggested that the *CYP2D6*5* variant influenced the disposition of lovastatin lactone whereas the **10* variant did not show a significant effect in these small

groups of subjects, but a minor effect of *CYP2D6*10* on the disposition of lactones of lovastatin and simvastatin in Chinese populations cannot be ruled out.

Statins exert their primary pharmacological effects in the liver. Various uptake and efflux transporters assist the disposition of statins, and especially the hydrophilic entities. Polymorphic changes in genes encoding these proteins may affect their efficacy. We examined pitavastatin, being a new hydrophilic statin administered as the active hydroxy acid and its pharmacokinetic changes relating to polymorphisms in genes encoding cellular transporters. The hepatic uptake of pitavastatin is aided by organic anion transporting polypeptide (OATP) 1B1 (gene *SLCO1B1*) and its biliary elimination by efflux transporter breast cancer resistance protein (BCRP, gene *ABCG2*). Our study looked at the influence from polymorphisms in these and other related genes, their corresponding gene-gene interactions, as well as gene-food interaction from grapefruit juice (GFJ). Our results suggested co-administration of GFJ moderately increased $AUC_{0\text{-}48h}$ by 15-16% and hence the bioavailability of pitavastatin for both the acid and lactone ($P<0.05$). Polymorphic changes at *SLCO1B1 c.388A>G* would lead to higher C_{max} and AUC levels, suggesting less uptake of pitavastatin into hepatocytes and greater systemic exposure. Mean C_{max} for pitavastatin acid was higher by 68%, $AUC_{0\text{-}48h}$ by 47% and CL/F was lower by 34% in *c.388GG* subjects compared to *c.388AA/AG* subjects ($P<0.05$). A similar comparison was noted for pitavastatin lactone with mean C_{max} being higher by 63%, $AUC_{0\text{-}48h}$ higher by 44% ($P<0.01$), $AUC_{0\text{-}\infty}$ by 38% ($P<0.05$) and CL/F lower by 29% ($P<0.05$). No significant gene-food interaction was noted. Lack of influence from SNPs analyzed in genes *CYP3A5, ABCB1* or *ABCG2*, suggested a minimal role for these proteins in pitavastatin metabolism and disposition. Further studies would be required to confirm if there are any clinical implications from polymorphisms in *SLCO1B1* and pitavastatin exposure or efficacy.

Clinical evidence has been reported that after statin therapy, patients with lower plasma high sensitivity C-reactive protein (hsCRP) levels could have better clinical outcomes. The findings suggested the benefits would be independent of

plasma LDL-C levels. Since the hsCRP level may be an important biomarker for chronic inflammatory conditions such as atherosclerosis and therefore a good predictor of cardiovascular risk, we further looked into probable genetic and phenotypic factors which might influence hsCRP in our subjects after simvastatin 40 mg daily treatment over at least 4 weeks, to evaluate possible influences among ethnic Chinese. We developed a reference model by incorporating the same set of covariance factors for investigation of gene-environment interaction. A total of 15 SNPs or haplotypes from 11 candidate genes were analyzed. These results did not reveal any significant findings when comparing hsCRP levels among genotype groups with or without covariance adjustments. To further categorize individuals as high or medium risk, we set a threshold for hsCRP levels of 1 mg/L as the cutoff point for evaluation. The *CRP c.3872 G>A* SNP appeared to lead to lower risk compared to the wild-type genotype ($P<0.05$). The limitation of this study in terms of the small population size and the lack of pre-treatment hsCRP levels made it difficult to draw further conclusions on the direct pleiotropic effects of statins and gene interaction. Further investigation is warranted.

6.2. Future research

In summary, the studies described here have examined various different perspectives of the pharmacogenetics of statins. From the clinical viewpoint, we have identified possible gene-disease interaction among FH patients in the lipid lowering responses with simvastatin treatment. Working on the genetic influence on CRP levels did not provide any significant findings to associate gene-drug interaction for possible pleiotropic effects from statins. On the other hand, the role of GFJ and the polymorphisms in the *SLCO1B1* gene do appear to independently affect the pharmacokinetics of pitavastatin. Gene-gene or gene-food interactions were not noted. The study on a common SNP among the Chinese population of *CYP2D6*10* did not reveal significant effects on simvastatin metabolism.

The background of this research is to try to determine the optimal treatment with

statins for individual patients within the ethnic Chinese population of Hong Kong. Although the most obvious candidate SNPs were chosen for these analyses the results have been largely negative or have shown a relatively small effect. In fact, the same has been true for much larger studies involving large groups of patients from some of the major clinical studies with statins and looking at multiple polymorphisms. Even the genome wide association study to determine the genetic disposition to myopathy with a high dose of simvastatin only identified one important polymorphism, which was in the *SLCO1B1* gene. Whether this means that the lipid-lowering effect of statins is not really genetically determined or that alternative genes may be involved which have not been examined yet remains to be proven.

In clinical practice, it seems obvious that the response to statins is very much determined by adherence to medication and maintenance of appropriate diet. Examination for genetic influences is likely to be unrewarding if these two factors cannot be controlled. It should be easier to identify genetic effects by performing short term well controlled studies, for instance looking at the pharmacokinetics of the drugs and their major metabolites and looking at pharmacodynamic markers of short-term effects on cholesterol synthesis pathways and other pathways in lipid metabolism which may show rapid adaption in response to decreased cholesterol synthesis.

Furthermore, it will become important to consider combinations of lipid lowering drugs so that the best treatment can be found for individual patients. Adding other drugs such as ezetimibe, nicotinic acid or fibrates may have advantages in certain patients and currently the use of these combinations is largely empirical and based on an attempt to normalize the simple plasma lipid parameters so there is considerable scope for investigative studies to help perfect individualized lipid treatments.

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