# Pharmacogenetics of Rosuvastatin Therapy and Genetic Determinants of Some Cardiovascular Risk Factors in Chinese Patients

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## Abbreviations

| | |
|---|---|
| ABC | ATP-binding cassette |
| ABCA1 | ATP-binding cassette, subfamily A, member 1 |
| ABCG2 | ATP-binding cassette, subfamily G, member 2 |
| ACCESS | Atorvastatin Comparative Cholesterol Efficacy and Safety Study |
| ACE | Angiotensin converting enzyme |
| ACS | Acute coronary syndrome |
| ADAMTS1 | A disintegrin-like and metalloproteinase with thrombospondin motifs |
| ADIPOQ | Adiponectin |
| AFCAPS/TexCAPS | Air Force/Texas Coronary Atherosclerosis Prevention Study |
| ALP | Alkaline phosphatase |
| ALT | Alanine transaminase |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| APOA5 | Apolipoprotein A5 |
| APOB | Apolipoprotein B |
| APOE | Apolipoprotein E |
| ARBs | Angiotensin receptor blockers |
| ARIC | Atherosclerosis Risk in Communities |
| ASTEROID | A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden |
| AUC | Area under the plasma concentration-time curve |
| BCRP | Breast cancer resistance protein |
| BMI | Body mass index |
| BSEP | Bile salt exporting pump |
| CAD | Coronary artery disease |
| CAP | Cholesterol and Pharmacogenetics |
| CARE | Cholesterol and Recurrent Events |
| CE | Cholesteryl esters |
| CETP | Cholesteryl ester transfer protein gene |
| CHD | Coronary heart disease |
| CHS | Cardiovascular Health Study |
| CI | Confidence interval |
| CIMT | Carotid intima-media thickness |
| CK | Creatine kinase |

| | |
|---|---|
| CORONA | COntrolled ROsuvastatin MultiNAtional Trial in Heart Failure |
| CRP | C-reactive protein |
| CV | Coefficients of variation |
| CVD | Cardiovascular disease |
| CYP | Cytochrome P450 |
| CYP3AP1 | Cytochrome P450, family 3, subfamily A, polypeptide 5, pseudogene 1 |
| DDC | Drug Development Centre |
| EMs | Extensive metabolizers |
| EXCEL | Expanded Clinical Evaluation of Lovastatin |
| FH | Familial hypercholesterolaemia |
| FMO3 | Flavin containing monooxygenase 3 |
| FPG | Fasting plasma glucose |
| GCKR | Glucokinase (hexokinase 4) regulator |
| GLUT9 | Glucose transporter 9 |
| GREACE | GREek Atorvastatin and Coronary-heart-disease Evaluation |
| GWA | Genome-wide association |
| GWASs | Genome-wide association studies |
| HA | Hospital Authority |
| HDL-C | High-density lipoprotein cholesterol |
| HMG-CoA | 3-hydroxy-3-methylglutaryl coenzyme A |
| HMGCR | HMG-CoA reductase |
| HNF1A | Hepatic nuclear factor 1-alpha |
| HNF-4 | Hepatocyte nuclear factor-4 |
| HO-1 | Heme oxygenase-1 |
| hsCRP | High-sensitivity C-reactive protein |
| HSPG | Heparin sulphate proteoglycan |
| HWE | Hardy-Weinberg equilibrium |
| IL | Interleukin |
| IMs | Intermediate metabolizers |
| IQR | Interquartile range |
| JUPITER | Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin |
| KIF6 | Kinesin-like protein 6 |
| LCAT | Lecithin-cholesterol acyltransferase |
| LD | Linkage disequilibrium |
| LDL-C | Low-density lipoprotein cholesterol |
| LDLR | Low-density lipoprotein receptor |

| | |
|---|---|
| LEP | Leptin |
| LEPR | Leptin receptor |
| LFT | Liver function tests |
| LIPG/ACAA2 | Lipase, endothelial / Acetyl-Coenzyme Aacyltransferase 2 |
| LPL | Lipoprotein lipase |
| MAF | Minor allele frequency |
| MDR1 | Multidrug resistance protein 1 |
| METEOR | Measuring Effects on Intima-Media Thickness: an Evaluation of Rosuvastatin |
| MI | Myocardial infarction |
| MODY-3 | Maturity-onset diabetes of the young |
| MRP2 | Multidrug resistance-associated protein 2 |
| MSD | Membrane-spanning domain |
| NAT2 | N-acetyltransferase 2 |
| NBD | Nucleotide-binding domain |
| NCAN/CILP2/PBX4 | Neurocan / cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4 |
| NF-Y | Nuclear factor Y box-binding protein |
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| NTCP | Sodium-dependent taurocholate cotransporting polypeptide |
| OATP1B1 | Organic anion transporting polypeptide 1B1 |
| OR | Odds ratio |
| PBMC | Peripheral blood mononuclear cells |
| PCR | Polymerase chain reaction |
| PCR-RFLP | Polymerase chain reaction - restriction fragment length polymorphism |
| PCSK9 | Proprotein convertase subtilisin-like kexin type 9 |
| PMs | Poor metabolizers |
| POR | P450 oxidoreductase |
| PRINCE | Pravastatin Inflammation/CRP Evaluation |
| PROPER | Prospective Study of Pravastatin in the Elderly at Risk |
| PROVE-IT TIMI22 | Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22 |
| RA | Rheumatoid arthritis |
| RFT | Renal function tests |
| SEARCH | Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine |
| SLC | Solute carrier transporter |

| | |
|---|---|
| SLC2A9 | Solute carrier family 2, member 9 |
| SLCO | Solute carrier organic anion transporter family, member 1B1 |
| SNP | Single nucleotide polymorphism |
| SNPs | Single nucleotide polymorphisms |
| SPSS | Statistical Package for the Social Sciences |
| STELLAR | Statin Therapies for Elevated Lipid Levels compared Across doses to Rosuvastatin |
| STRENGTH | The statin response examined by genetic haplotype markers |
| t1/2 | Elimination half-life |
| TFPI-2 | Tissue factor pathway inhibitor-2 |
| TMAU | Trimethylaminuria |
| TNF | Tumor necrosis factor |
| TNT | Treating to New Target |
| UGTs | Uridine diphosphate glucuronosyltransferases |
| ULN | Upper limit of normal |
| UMs | Ultrarapid etabolizers |
| VDR | Vitamin D receptor |
| VKORC1 | Vitamin K epoxide reductase complex, subunit 1 |
| VSMC | Vascular smooth muscle cells |
| WC | Waist circumference |
| WGHS | Women's Genome Health Study |
| WHR | Waist to hip ratio |
| WOSCOPS | West of Scotland Coronary Prevention Study |
| WTCCC | Wellcome Trust Case Control Consortium |

## Publications

**Publications arising from this thesis**

1. B Tomlinson, M Hu, VWY Lee. **In vivo assessment of herb–drug interactions: Possible utility of a pharmacogenetic approach?** *Molecular Nutrition & Food Research*, 2008; 52(7): 799-809.

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

3. B Tomlinson, M Hu, VWY Lee, SSH. Lui, TTW Chu, EWM Poon, GTC Ko, L Baum, LS Tam, E K Li. **ABCG2 polymorphism is associated with the low-density lipoprotein cholesterol response to rosuvastatin.** *Clinical Pharmacology & Therapeutics*, 2010; 87(5): 558-562.

4. M Hu, MHK Lee, VWL Mak, B Tomlinson. **Effect of central obesity, low high density lipoprotein cholesterol and C-reactive protein polymorphisms on C-reactive protein levels during treatment with rosuvastatin 10 mg daily.** *American Journal of Cardiology*, 2010; (Submitted).

5. M Hu, SSH. Lui, VWL Mak, TTW Chu, VWY Lee, EWM Poon, TKC Tsui, GTC Ko, L Baum, LS Tam, E K Li. B Tomlinson. **Pharmacogenetic analysis of lipid responses to rosuvastatin in Chinese patients.** *Pharmacogenetics and Genomics*, 2010; (Submitted).

**Publications during preparation of the thesis**

1. M Hu, B Tomlinson. **Febuxostat in the management of hyperuricemia and chronic gout: a review.** *Therapeutics and Clinical Risk Management*, 2008; 4(6):1209-1220.

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1. M Hu, SSH Lui, TTW Chu, B Tomlinson. **Determinants of the HDL-cholesterol response to rosuvastatin in Chinese patients.** CUHK-Mayo Clinic-China Cardiovascular Summit 2007, Hong Kong, April 2007.

2. M Hu, SSH Lui, TTW Chu, E Poon, L Baum, GTC Ko, B Tomlinson. **The common polymorphism in CYP2D6 does not have a major effect on the lipid response to rosuvastatin in Chinese patients.** The 15th Annual Scientific Congress of the Hong Kong College of Cardiology, Hong Kong, May 2007.

3. B. Tomlinson, M. Hu, SSH. Lui, TTW. Chu. **Effects of dietary fats on serum lipids and the response to rosuvastatin in Hong Kong Chinese patients.** European Society of Cardiology, Congress 2007, Vienna, Austria, September 2007.

4. M Hu, SSH Lui, TTW Chu, B Tomlinson. **Comparison of safety and efficacy of rosuvastatin 10mg with simvastatin 40mg in Hong Kong Chinese patients.** 9th Hong Kong Diabetes and Cardiovascular Risk Factors: East Meets West Symposium, Hong Kong, Sept 30-Oct 1 2007.

5. M Hu, B Tomlinson, SSH Lui, TTW Chu, E Poon, L Baum, VWY Lee. **Pharmacogenetics of lipid responses to rosuvastatin in Chinese Patients: effects of polymorphisms in BCRP and CYP2D6.** International Symposium on Cardiovascular and Neurovascular Medicine (ISCNM) in conjunction with International Heart Failure Symposium – Hong Kong 2008, February 2008 International Journal of Cardiology, 2008; 125 Suppl.1: S41.

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7. M Hu, B Tomlinson, SSH Lui, GTC Ko. **Comparison of the efficacy and safety of Rosuvastatin with Atorvastatin for achieving lipid goals: the discovery - Hong Kong study.** The 77th EAS (European Atherosclerosis Society) Congress, Istanbul, Turkey, April, 2008. *Atherosclerosis* 2008; 9/1: 199.

8. M Hu, SSH Lui, TTW Chu, L Baum, B Tomlinson. **Common variants in CETP do not affect HDL-cholesterol levels or the HDL- cholesterol response to rosuvastatin.** The 16th Annual Scientific Congress of the Hong Kong College of Cardiology, Hong Kong, May, 2008. Journal of the Hong Kong college of Cardiology, 2008; 16(1): 17.

9. M Hu, TTW Chu, TKC Tsui, GTC Ko, VWY Lee, L Baum, B Tomlinson **Contribution of 18 Common Single-Nucleotide Polymorphisms (SNPs) in 10 Genes to the Lipid Responses to Rosuvastatin in Chinese Patients.** The 10th Hong Kong Diabetes & Cardiovascular Risk Factors – East Meets Wests Symposium, Hong Kong, September 2008 (***Young investigator Award***).

10. M Hu, VWL Mak, EWM Poon, TTW Chu, TKC Tsui, VWY Lee, L Baum, B Tomlinson. **Genetic determinants of HDL-cholesterol response to rosuvastatin in Chinese Patients with hypercholesterolaemia.** CUHK-Mayo Clinic-Asia Cardiovascular Summit (CMA) 2009, Hong Kong, April 2009.

11. M Hu, VWL Mak, EWM Poon, TTW Chu, TKC Tsui, B Tomlinson. **Genetic determinants of lipid response to rosuvastatin in Chinese patients with hypercholesterolaemia.** International Symposium of Atheroslerosis (ISA) 2009, Boston, USA, June 2009. Atherosclerosis Supplements 2009, Vol. 10, Issue 2. Abs P486 (***Young investigator Award by IAS; ISA 2009 Education Award by IAS Asia Pacific Federation***).

12. M Hu, VWL Mak, EWM Poon, TTW Chu, TKC Tsui, VWY Lee, L Baum, B Tomlinson. **Effects of polymorphisms in liver uptake transporter SLCO1B1 on the lipid response to rosuvastatin in Chinese patients with hypercholesterolaemia.** The 17$^{th}$ Annual Scientific Congress of the Hong Kong College of Cardiology, Hong Kong, Jun 2009. Journal of the Hong Kong College of Cardiology 2009; 17 (Suppl 1): 10. Abs 11.

13. M Hu, VWL Mak, TTW Chu, VWY Lee, L Baum, B Tomlinson. **The UGT1A1 211G>A polymorphism affects bilirubin levels in Chinese patients with hypercholesterolaemia.** 2009 International Conference on Personalized Medicine Hong Kong, September 2009.

14. M Hu, M Lee, VWL Mak, B Tomlinson. **Determinants of hsCRP levels during treatment with rosuvastatin.** The 11$^{th}$ Hong Kong Diabetes & Cardiovascular Risk Factors – East Meets Wests Symposium, Hong Kong, 30 Sep-1 Oct 2009

(***Young investigator Award***).

15. M Hu, VWL Mak, EWM Poon, TTW Chu, TKC Tsui, VWY Lee, L Baum, LS Tam, EK Li, B Tomlinson. **Pharmacogenetic analysis of LDL-cholesterol response to rosuvastatin.** International Congress of Cardiology. Hong Kong, February 2010. European Heart Journal Supplements, 2010; 12A: S3.

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17. M Hu, W Chu, D Yeung, L Shi, D Wang, B Tomlinson. **Influence of DGAT2 polymorphisms on triglyceride response to extended-release niacin in Chinese patients with dyslipidaemia.** The 6th IAS-sponsored Workshop on HDL, May 17-20, 2010, Whistler, Canada.

18. VWL Mak, M Hu, OQP Yin, MSS Chow, TTW Chu, TYK Chan, B Tomlinson. **Interaction of ABCG2 transporter polymorphism 421C>A and the effect of grapefruit juice on the pharmacokinetics of pitavastatin.** 2008 International Conference on Pharmacogenomics, Busan, Republic of Korea, April 2008.

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20. VWL Mak, M Hu, TTW Chu, B Tomlinson. Effects of Polymorphisms in Influx and Efflux Transporters SLCO1B1 and ABCG2 on the Pharmacokinetics of Pitavastatin in Healthy Chinese Subjects. **The 10$^{th}$ Hong Kong Diabetes & Cardiovascular Risk Factors – East Meets Wests Symposium, Hong Kong, September 2008.**

21. VWL Mak, M Hu, EWM Poon, TTW Chu, VWY Lee, TKC Tsui, B Tomlinson. **Influence of *APOE* Cluster Polymorphism on Baseline Lipids and Response to Simvastatin in Chinese Patients.** CUHK-Mayo Clinic-Asia Cardiovascular Submit (CMA) 2009, Hong Kong, April 2009.

22. VWL Mak, M Hu, TTW Chu, EWM Poon, TKC Tsui, B Tomlinson. **Effects of Common Single Nucleotide Polymorphisms and haplotypes of SLCO1B1 in Chinese on the plasma lipid responses to simvastatin.** International Symposium of Atheroslerosis (ISA) 2009, Boston, USA, June 2009. Atherosclerosis Supplements 2009, Vol. 10, Issue 2. Abs P487.

23. VWL Mak, M Hu, OQP Yin, TTW Chu, B Tomlinson. **Influence of ABCB1 Polymorphisms on Pharmacokinetics of Pitavastatin.** 2009 International Conference on Personalized Medicine, Hong Kong, September 2009.

## Abstract

Although the clinical efficacy of statins has been well established, there is a wide inter-individual variation in the lipid responses to statins. Pharmacogenetic studies have identified some genetic differences that contribute to the variation, but overall the results have been disappointing. The studies described in this thesis were performed to examine whether certain genetic variants predicted the lipid responses to rosuvastatin in Chinese patients. Over 400 Chinese patients with increased risk of cardiovascular disease (CVD) who were treated with rosuvastatin 10 mg daily for at least 4 weeks (more than 97% of patients had at least 6 weeks treatment) were studied, including 166 having familial hypercholesterolaemia (FH) and 36 having rheumatoid arthritis (RA). They were genotyped for 135 polymorphisms in 62 candidate genes/loci potentially related to pharmacokinetics or pharmacodynamics of statins and lipid metabolism. Associations between genetic polymorphisms and the lipid responses to rosuvastatin were analyzed in 386 patients with good compliance. The associations between genetic polymorphisms and some risk factors for CVD including baseline lipid levels, high-sensitivity C-reactive protein (hsCRP), uric acid and bilirubin levels were also analyzed.

The key findings of the study are as follows:

1. The polymorphisms most highly associated with the low-density lipoprotein cholesterol (LDL-C) response were 421C>A in the ATP-binding cassette G2 (*ABCG2*) gene ($P=9.2\times10^{-7}$), followed by 18281G>A (V257M) in the flavin-containing monooxygenase 3 (*FMO3*) gene (P=0.0002), 1421C>G in the lipoprotein lipase (*LPL*) gene (P=0.002), and rs4420638 in the apolipoprotein

E/C-I/C-IV/C-II (*APOE/C1/C4/C2*) gene cluster (P=0.004). These genetic polymorphisms and having FH totally explained 13.6% of the variance in percentage change in LDL-C in response to rosuvastatin. The greater percentage reduction in LDL-C in patients with the *ABCG2* 421AA genotype compared to those with the *ABCG2* 421CC genotype was equivalent to at least doubling the dose of rosuvastatin.

2. Three SNPs (glucokinase regulator [*GCKR*] rs1260326, apolipoprotein A5 [*APOA5*] -1131T>C and the solute carrier organic anion transporter 1B1 [*SLCO1B1*] 521T>C) tended to be associated with percentage changes in high-density lipoprotein cholesterol (HDL-C) (P<0.05), but none of these reached the overall significance level. In multivariate stepwise regression analysis, baseline HDL-C ($P=1.6\times10^{-6}$), having diabetes (P=0.0004) or RA (P=0.002) and the *SLCO1B1* 521T>C polymorphism (P=0.03) were determinants of HDL-C responses, contributing 9.9% of the variance in percentage change in HDL-C, but the genetic factors only contributed to 0.8% of the variance.
3. The triglyceride response to rosuvastatin was highly variable and was strongly related to baseline levels. The diacylglycerol acyltransferase-2 (*DGAT2*) rs10899113 C>T polymorphism tended to be associated with reduced triglyceride response in a gene-dose dependent manner. However, in multivariate stepwise regression analysis, baseline triglyceride level was the only factor that strongly related to the triglyceride response, explaining 14.4% of the variance.
4. This study has also analyzed relationships between on-treatment plasma hsCRP concentrations and cardiovascular risk factors and 14 single nucleotide polymorphisms in *CRP* and other candidate genes, which showed that central obesity, low HDL-C and *CRP* polymorphisms are major determinants of higher

hsCRP levels in Chinese patients on treatment with rosuvastatin.

5. The association between genetic polymorphisms and lipid traits were analyzed in FH and non-FH patients separately due to their different lipid profiles. The analysis has shown that there were different genetic predictors of lipid levels in patients with and without FH and that more genetic factors appeared to affect the baseline lipid levels in patients with FH compared to non-FH patients, suggesting complex interactions between genetic and environmental factors and plasma cholesterol levels in patients with and without FH.
6. The *SLC2A9* (solute carrier family 2, member 9) rs1014290 T>C was significantly associated with plasma uric acid levels in a gene-dose dependent manner ($P=1.0\times10^{-5}$) and the relationship was more pronounced in women or in patients without hypertension than in men or patients with hypertension. The *ABCG2* 421 C>A did not show a significant effect on uric acid levels.
7. The UGT1A1 (uridine diphosphate glucuronosyltransferases family, polypeptide A1) variants *28 ($P=1.5\times10^{-9}$) and *6 ($P=2.2\times10^{-7}$) were independently associated with increased baseline bilirubin levels. Polymorphisms in *SLCO1B1* did not appear to affect bilirubin levels in this study.

Some novel genetic determinants of the LDL-C response to rosuvastatin treatment have been identified in this study. The responses in HDL-C and triglycerides were related more closely to the baseline levels of these lipids than to any of the polymorphisms examined. Genetic associations with baseline lipid parameters, hsCRP, uric acid and bilirubin were identified and generally correspond with some of the previous reports of studies in Chinese and other ethnic groups.

# 中文摘要


盡管他汀類藥物的臨床療效已被廣泛認可，其降膽固醇的效果存在很大的個體差異。遺傳藥理學研究發現先天的遺傳變异可能對該藥物療效的個體差異有影響，但這些研究的結果總體而言並不理想。本論文中涉及的研究旨在考察是否某些遺傳變异會影響華人對瑞舒伐他汀（rosuvastatin）調脂作用的反應。在本研究中，超過 400 位帶有高心血管疾病風險的華人患者，包括 166 位患有家族遺傳性高膽固醇血症（FH）以及 36 位患有風濕性關節炎的患者，接受了爲期至少 4 個星期的每日 rosuvastatin10 毫克的治療（當中有 97%的患者接受了至少 6 個星期的 rosuvastatin 的藥物治療）。研究對這些患者的 62 個可能与他汀類藥物藥代動力學和藥效學以及脂代謝相關的基因或/基因位點的 135 個基因多態性的基因型進行了檢測，并進而對 386 位具有良好藥物順應性的患者對 rosuvastatin 的調脂作用的反應与基因多態性的關係進行了分析研究。另外，我們還對基因多態性与某些心血管疾病的危險因素例如基礎膽固醇，高敏感度 C-反應蛋白（hsCRP），尿酸以及膽紅素水準的關係進行了研究。

本研究的主要結論總結如下：

1. 三磷酸腺苷結合轉運體 G2 （ABCG2）421C>A 多態性与病人對 rosuvastatin 降低低密度脂蛋白胆固醇（LDL-C）作用的反應高度相關。另外，含黃素單氧化酶 3 (FMO3) 18281G>A，脂蛋白酯酶（LPL）1421C>G 以及位于脂蛋白元 E/C1/C4/C2 基因簇的 rs4420638 基因多態性也與 rosuvastatin 降低 LDL-C 的效果密切相關。這些基因多態性連同患有 FH 合共解釋病人對 rosuvastatin 降低 LDL-C 反應差異的 13.6%。帶有 ABCG2 421AA 基因型的患者與帶有 421CC 基因型的患者相比，其 LDL-C 降低的效果相當於服用了至少加倍劑量的 rosuvastatin。

2. 位于葡萄糖激胰調節基因 (GCKR1)的 rs1260326, 脂蛋白元 A5 (APOA5) -1131T>C 与溶質載體有機陰離子轉運多肽 1B1（SLCO1B1）521T>C 傾向於與 rosuvastatin 調節高密度脂蛋白膽固醇(HDL-C)的作用相關，但是這些相關性並沒有達到整體的顯著性水平。HDL-C 的基礎水平，患有 2 型糖尿病或風濕性關節炎以及 SLCO1B1 521T>C 基因多態性爲病人服用 rosuvastatin 后 HDL-C 變化的決定性因素。這些因素可決定 9.9%的變異，但 SLCO1B1 521T>C 基因多態性僅決定 0.8%的變異。
3. 病人服用 rosuvastatin 后其三酸甘油脂(TG)的變化有很大差異，并與 TG 基礎水準高度相關。位于二酰基甘油酰基轉移酶基因（DGAT2）的 rs10899113 C>T 基因多態性伴隨減退的降 TG 的效果，并呈基因型劑量相關性。但是多變量逐步回歸分析顯示 TG 基礎水準爲唯一的決定 TG 反應的因素，該因素可解釋 14.4%的變異。
4. 中央型肥胖，HDL-C 偏低以及 C-反應蛋白基因多態性爲中國人在接受 rosuvastatin 汀治療情況下 hsCRP 濃度偏高的主要決定因素。
5. FH 与非家族性高膽固醇血症(non-FH)患者的基礎血脂水平由不同的基因多態性所決定。與 non-FH 患者相比，有較多的遺傳因素與 FH 患者的血脂水平相關。這些結果顯示了遺傳因素、環境因素與血脂濃度之間的複雜的相互作用。
6. 溶質載體家族 2A9（SLC2A9）rs1014290 T>C 與血中尿酸濃度呈基因型劑量濃度相關性，這一相關性在女性患者或非高血壓患者中更爲顯著。而 ABCG2 421C>A 多態性對尿酸濃度有顯著影響。
7. 尿苷雙磷酸葡萄糖醛酸基轉移酶 1A1 (UGT1A1)基因多態性*28 和*6 與膽紅素高基礎濃度密切相關。而 SLCO1B1 基因多態性對膽紅素濃度沒有明顯影響。

本研究發現了對 rosuvastatin 降低 LDL-C 起到決定性作用的一些新的遺傳因

素，而用藥后 HDL-C 和 TG 的變化與其基礎水準密切相關。對基礎血脂水準，hsCRP，尿酸以及膽紅素濃度與遺傳基因多態性的關係的研究結果與早前在華人和其他種族中已報導的結果基本吻合。

## Table of Contents

## List of Tables

## List of Figures

# Chapter 1 Introduction

## 1 Overview of statin pharmacogenetics

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins are widely prescribed for the treatment of hypercholesterolaemia and the subsequent protection against coronary heart disease (CHD). Statins are administered orally, undergo absorption and some first-pass metabolism in the enterocytes, and then pass into the portal circulation for uptake into hepatocytes for competitive inhibition of HMG-CoA reductase (HMGCR), which is responsible for the rate-limiting step in cholesterol synthesis and this in turn results in up-regulation of expression of the low-density lipoprotein receptor (LDLR), and finally leading to lowering the plasma low-density lipoprotein cholesterol (LDL-C) concentration. Greater reductions in LDL-C appear to be associated with greater benefits in various large prospective clinical trials (Baigent C et al., 2005), but considerable inter-individual variation exists in the lipid-lowering response to statin therapy and/or clinical outcome, as well as in the incidence of the most serious side effect of statin-induced myopathy because of a combination of phenotypic and genotypic factors. It has been recognized that genetic factors contribute to inter-individual variability in the lipid-lowering response to statins, drug-interactions and the intolerance of statins (Hu M et al., 2009, Kajinami K et al., 2004e, Schmitz G et al., 2007).

Polymorphisms in the genes related to statin metabolism and disposition could affect drug exposure in the systemic circulation and the liver and consequently, clinical efficacy, and also the drug interaction with other substrate/inhibitor/inducer drugs.

The genes influencing cholesterol biosynthesis and metabolism are more likely to affect the pharmacodynamics of statins. Pharmacogenetic studies have investigated the relationship between common genetic variants and drug response and/or susceptibility to adverse effects with statin therapy, which could help to optimize statin therapy by identifying individuals with increased chance of risk or benefit.

## 1.1 Genetic influences on lipid-lowering effect of statins

### 1.1.1 Genes involved in statin pharmacokinetics

Lovastatin, pravastatin and simvastatin are fungal-derived inhibitors of HMG-CoA reductase, whereas atorvastatin, cerivastatin, fluvastatin, pravastatin, pitavastatin and rosuvastatin are fully synthetic compounds (Schachter M, 2005, Shitara Y and Sugiyama Y, 2006b). The chemical structures of the statins are shown in Figure **1-1**.

Although all statins share the same active pharmacophore which resembles HMG-CoA, the substrate of their target enzyme HMG-CoA reductase, the differences in their chemical structure define their different affinity for binding to the enzyme active site, different solubility properties of the drugs and therefore many of their pharmacokinetic properties. Among these statins, lovastatin and simvastatin possess a lactone ring in their structure and are transformed into the active open acid form in the body while other statins are administered as the biologically active open acid form (Schachter M, 2005, Shitara Y and Sugiyama Y, 2006b). Atorvastatin, fluvastatin, lovastatin and simvastatin are relatively lipophilic compounds, while pravastatin and rosuvastatin are more hydrophilic. These parameters and other physicochemical and pharmacokinetic properties are summarized in Table **1-1**.

**Figure 1-1. Chemical structures of the statins and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)**

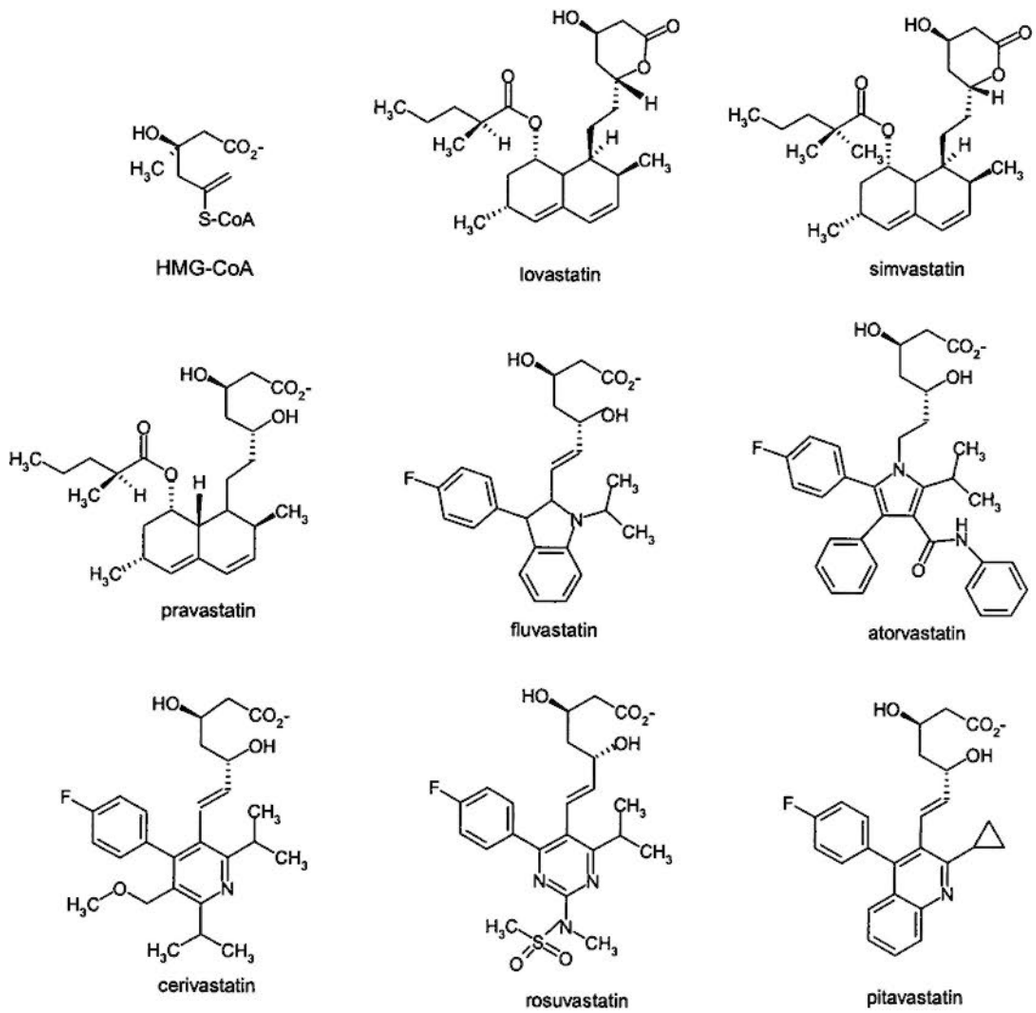


**Table 1-1. Pharmacokinetic properties of statins**

| | A | C | F | L | Pita | Pra | R | S |
|---|---|---|---|---|---|---|---|---|
| $IC_{50}$ (nM) † | 8.2 | 10.0 | 27.6 | N/A | NA | 44.1 | 5.4 | 11.2 |
| Lipophilicity ‡ | 1.0/1.25 | 1.50/1.75 | 1.0/1.25 | 3.91* | 1.5* | -0.75/-1.0 | -0.25/-0.5 | 4.4* |
| Absorption | 30% | >98% | 98% | 30% | 80% | 35% | 50% | 60-85% |
| Bioavailability | 12% | 60% | 24-30% | 5% | 60-80% | 18% | 20% | <5% |
| Hepatic extraction | 70% | 50-60% | ≥70% | ≥70% | NA | 45% | 63% | ≥80% |
| Renal excretion, | <5% | 30% | 6% | 10% | NA | 20% | 10% | 13% |
| Protein binding | >98% | >99% | >98% | >98% | 96% | 50% | 90% | >95% |
| Half-life, (h) | 7-20 | 1-3 | 1-3 | 2-5 | 10-13 | 1-3 | 20 | 2-5 |
| Metabolism | +++ | +++ | +++ | +++ | ++ | + | + | +++ |
| Metabolites, (n) | 2 | 2 | No | 3 | Minor | 2 | Minor | 3 |
| CYP | 3A4, 2C8 | 2C8, 3A4 | 2C9 | 3A4/5, 2C8 | 2C9 | 3A4 | 2C9, 2C19 | 3A4/5, 2C8 |
| UGTs | 1A1/1A3/ 2B7 | 1A1/1A3 | | 1A1/1A3 /2B7 | 1A3/ 2B7 | | | 1A1/1A3/ 2B7 |
| SLCO influx transporters | 1B1 | 1B1 | 1B1 | 1B1 | 1B1/ 1B3 | 1B1/2B1 | 1B1/1B3/ 2B1/1A2 | 1B1 |
| Other influx transporters | | | | MCT4 | | OAT3/ MCT1 | SLC10A1 | |
| ABC Efflux transporters | B1/G2 | B1/C2/ G2 | G2 | B1 | B1/C2/ G2 | B1/B11/ C2/G2 | B1/C2/G2 | B1 |

† $IC_{50}$ (inhibitor concentration to produce 50% inhibition) in purified human HMG-CoA reductase catalytic domain.

‡ Lipophilicity as log*D* at pH7.4 of administered form, except * at pH7.0.

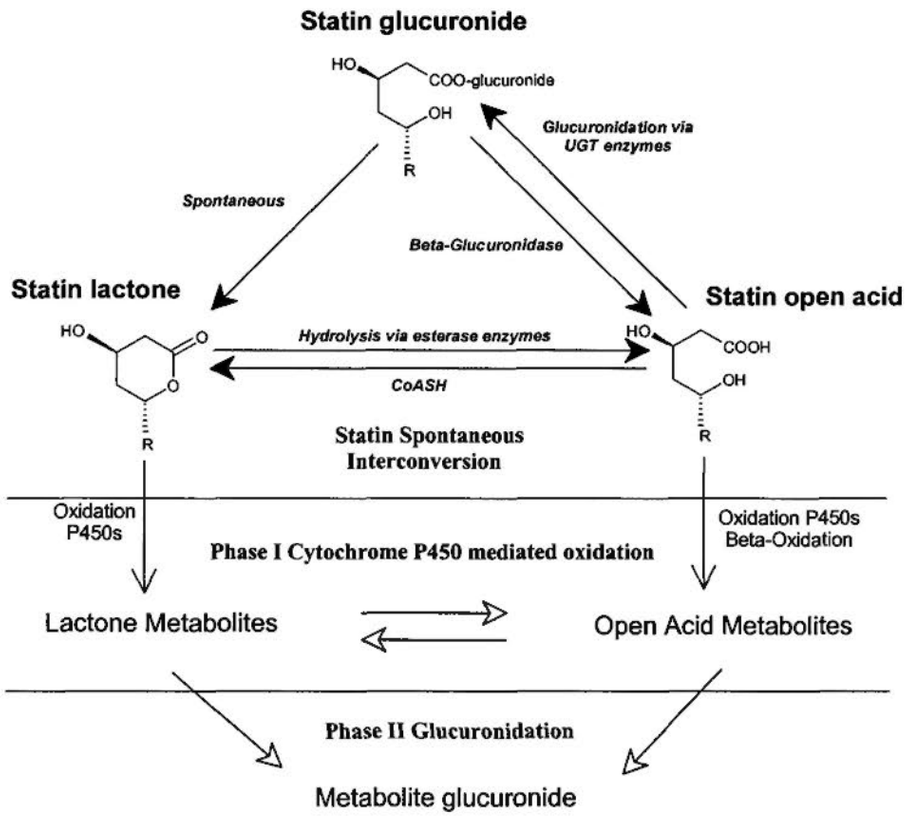
Abbreviations: A = atorvastatin; ABC = ATP-binding cassette; C = cerivastatin; CYP = cytochrome P450 enzymes; F = fluvastatin; L = lovastatin; Pita = pitavastatin; Pra = pravastatin; R = rosuvastatin; S = simvastatin; SLCO = solute carrier organic anion transporter family; UGTs = uridine diphosphate glucuronosyltransferases.

Adapted from (Hu M et al., 2009, Neuvonen PJ et al., 2006b, Shitara Y and Sugiyama Y, 2006b, Tirona RG, 2005)

The lipophilic statins are extensively metabolized, principally by cytochrome P450 (CYP) enzymes, whereas pravastatin, rosuvastatin, and pitavastatin are excreted mainly unchanged. Simvastatin, lovastatin and atorvastatin are mainly metabolized

by CYP3A, fluvastatin is chiefly metabolized by CYP2C9 isoenzyme, whereas cerivastatin is extensively metabolized by both CYP2C8 and CYP3A4 (Neuvonen PJ et al., 2006b). The lactone forms of all statins are metabolized by CYP enzymes more rapidly than their acid forms. In addition to oxidation by CYPs, statin acids can be converted to their lactones forms by a Co-enzyme A (CoASH)-dependent pathway (Prueksaritanont T et al., 2001) and by a pathway involving uridine diphosphate glucuronosyltransferases (UGTs), mainly UGT1A1 and UGT1A3, forming the acyl glucuronide conjugates, which undergo spontaneous cyclization to the lactone (Figure **1-2**) (Prueksaritanont T et al., 2002c). In humans, UGT-mediated lactonization of statin acids seems to only have a minor contribution to their total clearance (Prueksaritanont T et al., 2002c).

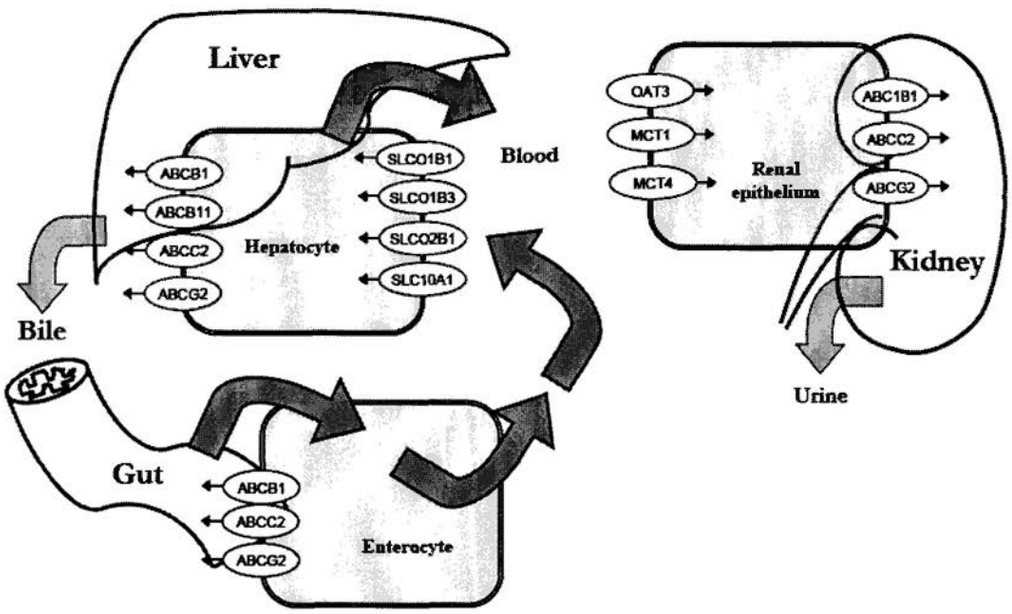
**Figure 1-2. Proposed metabolic pathway of a typical statin.**




Substrates for CYP3A enzymes are commonly also substrates for the efflux drug transporter p-glycoprotein, also know as multidrug resistance protein (MDR1, *ABCB1*). This is true for some of the statins, and other efflux transporters located in the enterocytes and at the canalicular membrane of the hepatocytes may also be involved. The list includes multidrug resistance-associated protein 2 (MRP2, *ABCC2*), breast cancer resistance protein (BCRP, *ABCG2*) and the bile salt exporting pump (BSEP, *ABCB11*), which may be involved in excretion of statins and their metabolites into the gut lumen or biliary system. Uptake of the more hydrophilic statin acids or lactones into hepatocytes is highly dependent on various drug influx

transporters including the solute carrier transporter (SLC) proteins, such as organic anion transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*) (Figure. **1-3**). All the statins appear to be substrates of OATP1B1 to a varying degree and the efficiency of this transport mechanism may be a major determinant for the ability of individual statins in the hydrophilic acid form to enter hepatocytes where their main pharmacological activity is exerted.

**Figure 1-3. Vectorial pathway for statin distribution from enterocytes to hepatocytes for their pharmacological action and metabolism and elimination via the bile and kidneys**




Listed across the compartment interfaces are efflux or uptake transporter proteins that may be involved.

Abbreviations: ABC = ATP-binding cassette; MCT= monocarboxylate transporter; OAT= organic anion transporter; SLC=solute solute carrier family; SLCO, solute carrier organic anion transporter family.

Genetic variations in these metabolizing enzymes and transporters may have a great

impact on the pharmacokinetics of statins, which may result in altered efficacy and toxicity of statins. The frequencies of some functional single nucleotide polymorphisms (SNPs) in these pharmacokinetic-related genes varied among populations, which may contribute to the ethnic differences in pharmacokinetics or pharmacodynamics of some statins. Most of the statins appear to have pharmacokinetic parameters that increase in proportion to the dose. However, doubling the dose of a statin typically only reduces the LDL-C by a further 6% from the original baseline (Roberts WC, 1997). The dose-response relationships for average changes in lipid parameters with different statins have been established in a number of large studies mainly performed in Caucasians (Jones P et al., 1998, Jones PH et al., 2003a). Thus it may be anticipated that any polymorphism in an enzyme or transporter, or a drug interaction, which increased the drug concentration in plasma or hepatocytes by 100% might only result in an additional 6% reduction in LDL-C.

### 1.1.2 Drug metabolizing enzymes affecting statin therapy

#### 1.1.2.1 CYP3A4 and CYP3A5

The CYP3A enzyme group constitutes the largest amount of CYP enzymes in the liver and enterocytes. The importance of both CYP3A4 and CYP3A5 in the metabolism of some of the statins in both the lactone and acid forms has been known for many years (Prueksaritanont T et al., 1997). Many drug interactions have been described between statins and potent inhibitors of CYP3A4 such as itraconazole, ketconazole and even grapefruit juice, which acts only on enterocyte CYP3A4 (Neuvonen PJ et al., 2006a). These combinations should generally be avoided because of the increased risk of myopathy and rhabdomyolysis. The enzyme activity of CYP3A4 may differ as much as 40-fold in both liver and small intestine, which is not currently explained by polymorphisms in *CYP3A4* (Xie HG et al., 2001). A

recent study suggested that the functional impairment SNP *28 (A503V) in the P450 oxidoreductase (POR), a key regulator of CYP450 enzymes contributed to the variability of total CYP3A activity in vivo and was a better genetic marker than genetic variants in *CYP3A* genes (Oneda B et al., 2009).

More than 40 SNPs of the *CYP3A4* gene have been identified on the Human Cytochrome P450 Allele Nomenclature Committee homepage (http:// www.imm.ki.se/CYPalleles), but most of these are not very frequent (Table **1-2**)

**Table 1-2. Common polymorphisms in *CYP3A4***

| Polymorphisms | Amino acid exchange | Enzyme activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| *1B (-392A>G) | | Uncertain | 3.9-4.2 | 53-69 | 0-4.7 |
| *1G (*18B, 20230G>A) | | Uncertain | <10 | 34-89 | 22-31 |
| *2 (15713T>C) | Ser222Pro | Substrate specific | 2.7 | 0 | 0 |
| *4 (352A>G, 13871A>G) | Ile118Val | Reduced | NA | NA | 3.3 |

Data are from (Hu M et al., 2009) or NCBI SNP website.

The frequency of the *CYP3A4* *1G variant allele is the highest of all the *CYP3A4* polymorphic alleles identified in Chinese, but the functional effect of this polymorphism remains unclear and previous studies have reported decreased or increased activity of CYP3A4 associated with the variant allele (Fukushima-Uesaka H et al., 2004, Hu YF et al., 2007, Zhang W et al., 2009). Similarly, the functional effect of *1B in *CYP3A4* examined in various studies is also not conclusive, although it has been suggested that the variant allele was assumed to be associated with enhanced CYP3A4 expression due to reduced binding of a transcriptional repressor (Amirimani B et al., 2003). The impact of these two SNPs on the pharmacokinetics and/or pharmacodynamics of substrate drugs was inconsistent. In one study with

atorvastatin 10 mg daily, patients who were homozygous for *CYP3A4* *1B had significantly higher post-treatment LDL-C levels, but there was no difference in absolute and percentage changes in LDL-C (Kajinami K et al., 2004b). A recent population-based cohort study in 1239 simvastatin and atorvastatin treated patients showed that the *CYP3A4* *1B polymorphism was associated with a lower incidence of dose decreases or switching during simvastatin and atorvastatin therapy due to an adverse drug reaction or to excessive reduction in cholesterol levels, which are most likely caused by elevated statin plasma levels, suggesting an increased metabolism rate in the *1B carriers (Gordon RY et al., 2009). The impact on statin therapy of the *CYP3A4* *4 polymorphism causing reduced activity of the enzyme has been reported with the *4 variant allele being associated with greater reductions in total cholesterol and/or triglyceride with simvastatin or atorvastatin treatment but not for LDL-C in Chinese hyperlipidaemic patients (Liu ZZ et al., 2008, Wang A et al., 2005). The infrequent *CYP3A4* *2 allele appeared to have substrate specific effects in a previous study using a baculovirus-directed cDNA expression system showing that the intrinsic clearance for nifedipine oxidation was decreased approximately six- to nine-fold with the variant enzyme compared with the wild-type enzyme, but there was no significant effect of the polymorphism on testosterone 6β-hydroxylation (Xie HG et al., 2001), but its effect on statins has not been reported.

The *CYP3A5*3* allele with decreased enzyme activity is relatively common in different ethnic groups and may contribute a substantial amount to inter-individual variability in metabolism of CYP3A substrates (Schmitz G and Langmann T, 2006) (Table **1-3**).

**Table 1-3. Common polymorphisms in *CYP3A5***

| Polymorphisms | Amino acid exchange | Enzyme activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| *1 | | Normal | 0-15 | 36-45 | 23-40 |
| *3 (6986A>G) | Premature stop | Severely decreased | 85-98 | 27-84 | 60-77 |
| *6 (14690G>A) | Premature stop | decreased | 0 | 8.7 | 1.6 |

Data are from (Hu M et al., 2009) or NCBI SNP website.

The *CYP3A5*3* polymorphism has been shown to be associated with increased systemic exposure to simvastatin in Korean healthy volunteers (Kim KA et al., 2007). The effect of the *CYP3A5*3* polymorphism on lipid responses to statins has been reported but the results are not consistent. In a study in subjects being treated with various statins (lovastatin, simvastatin and atorvastatin) metabolized through CYP3A, there were smaller lipid-lowering responses in the *CYP3A5*1* wild-type enzyme expressors compared to homozygous *CYP3A5*3* enzyme non-expressors, (Kivisto KT et al., 2004). However, some other studies failed to show a significant effect of the *CYP3A5*3* allele on lipid responses to simvastatin or atorvastatin (Fiegenbaum M et al., 2005b, Thompson JF et al., 2005). More recently, an *in vitro* study suggested that atorvastatin was preferentially metabolized by *CYP3A4* rather than by *CYP3A5* and as such, the genetic *CYP3A5* polymorphism might not be an important factor in the interindividual variation of atorvastatin disposition and pharmacodynamics in humans (Park JE et al., 2008).

Some statins, particularly simvastatin and atorvastatin, potentially interact with CYP3A4-dependent drugs through competitive inhibition of the enzyme, and furthermore, *in vitro* studies have suggested that statins themselves may also influence CYP3A expression (Willrich MA et al., 2009), but data on the effect of statins on CYP3A4 activity are limited and this remains controversial. Recently, an

open-label, randomized, 3-way crossover study which investigated the effect of simvastatin 10 mg, atorvastatin 10 mg, and pitavastatin 2 mg on the pharmacokinetics of midazolam in 11 Japanese healthy volunteers has shown that these statins had no effects on the pharmacokinetics of midazolam and its metabolite suggesting these statins can be used safely at least at low doses without influencing CAP3A4 enzyme activity (Yamasaki D et al., 2009).

#### 1.1.2.2 CYP2C9 and CYP2C19

CYP2C9 is responsible for the hydroxylation of a wide range of drugs, being particularly important for the narrow therapeutic range agents warfarin and phenytoin (Schwarz UI, 2003). The *CYP2C9* and *CYP2C19* genes are polymorphically expressed with 30 variant alleles for *CYP2C9* and 21 variant alleles for *CYP2C19* identified and many of these variants, the most common being *2 and *3, are associated with decreased or absent enzyme activities and decreased metabolism of the respective substrates. These two genes have marked inter-ethnic difference in the distributions of variant alleles with some minor alleles found only in some particular ethnic groups (Rosemary J and Adithan C, 2007). There is a relatively high frequency of *CYP2C19* poor metabolizers (PMs) in Chinese populations (Table **1-4**) and the *CYP2C19* *2 and *3 polymorphisms account for >99% of Oriental PMs in constrat to ~87% of Caucasian PMs being explained by these 2 SNPs (Rosemary J and Adithan C, 2007). However, the PM genotypes for *CYP2C9* in East Asians are very uncommon (Table **1-4**).

**Table 1-4. Common polymorphisms in *CYP2C9* and *CYP2C19***

| Polymorphisms | Amino acid exchange | Enzyme activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| **CYP2C9** | | | | | |
| *2 (430C>T) | Arg144Cys | Decreased | 10-15 | 2-4 | 0.1 |
| *3 (1075A>C) | Ile359Leu | Decreased | 5-10 | <2 | 1-4.9 |
| **CYP2C19** | | | | | |
| *2 (681G>A) | Val331Ile | Absent | 13-19 | 11-25 | 21-45 |
| *3 (636G>A) | Ile359Leu | Absent | 0-0.3 | 0-1.8 | 5-13 |

Data are from (Hu M et al., 2009) and (Rosemary J and Adithan C, 2007).

Fluvastatin is the only statin known to undergo a major degree of CYP2C9-mediated metabolism. The area under the plasma concentration-time curve (AUC) values for fluvastatin were found to be higher in Caucasian subjects with the *3 allele (Kirchheiner J et al., 2003). However, no correlation was found with the clinical effects in terms of lipid lowering efficacy in that study (Kirchheiner J et al., 2003). Similarly, a study involving 707 renal transplant patients on cyclosporine (ALERT study) found no significant effect of the *CYP2C9* *2 and *3 alleles on the LDL-C reduction with fluvastatin 40 mg (Singer JB et al., 2007). Pitavastatin, and to a lesser extent rosuvastatin, undergo a minor degree of metabolism by CYP2C9 and the latter is also metabolised by CYP2C19 (Igel M et al., 2002), but there are no reports of the effects of variants in these enzymes on the pharmacokinetics or pharmacodynamics of these statins.

### 1.1.2.3 CYP2D6

Although CYP2D6 accounts for only a small percentage of total hepatic CYP enzymes (~2 - 4%), it metabolizes ~25% of commonly used drugs and is the only non-inducible enzyme in the CYP450 family (Owen RP et al., 2009, Zhou SF, 2009). The *CYP2D6* gene is highly polymorphic with over 90 variants being identified and resulting in varied enzyme activity (Owen RP et al., 2009). Subjects can be assigned

into 4 categories based on their ability to metabolize CYP2D6 substrates: ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and PMs. The distribution of *CYP2D6* polymorphisms exhibits significant interethnic differences (Table **1-5**) resulting in variable percentages of PMs, IMs, EMs and UMs in different populations. In particular, IMs are located to a great extent in East Asia due to the high prevalence of the *CYP2D6* *10 allele, whereas, PMs are mainly found in Europe, and UMs are mainly found in North and Eastern African and Oceanian populations (Owen RP et al., 2009, Sistonen J et al., 2009).

**Table 1-5. Common polymorphisms in *CYP2D6***

| Polymorphisms/ haplotypes | Amino acid exchange | Enzyme activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| *1 | Wild-type | Normal | 33.4-83.8 | 27.8-90.4 | 22.7-49 |
| *2 (-1584C>G, 2850C>T, 4180G>C) | Wild-type | Normal | 32.4-35.3 | 9.9-40 | 8.0-13.4 |
| *3 (2549A>del) | 260 terminate | Inactive | 0-2.5 | 0-1 | 0 |
| *4 (100C>T, 974C>A, 984A>G, 1846G>A splice, 4180G>C) | 182 terminate | Inactive | 11%-29 | 0.9-9.3 | 0.2-0.8 |
| *5 | Gene deletion | No activity | 0.6-7.3 | 3.3-9 | 1.2-6.2 |
| *10 (100C>T, 4180G>C) | Pro34Ser, Ser486Thr | Decreased | 1.4-6.1 | 1-8.6 | 38-70 |
| *17 (1023C>T, 2850C>T, 4180G>C) | Thr107Ile, Arg296Cys, Ser486Thr | Decreased | 0-1.1 | 9-34 | 0 |
| *41 (-1584C>G, 2850C>T, 2988G>A, 4180G>C) | Arg296Cys, Ser486Thr | Decreased | 10-20 | 0 | 0 |
| *2 X N (N≥2) | Arg296Cys, Ser486Thr | Increased | 5-10 | 2-14 | 0.5 |

Data are from (Hu M et al., 2009).

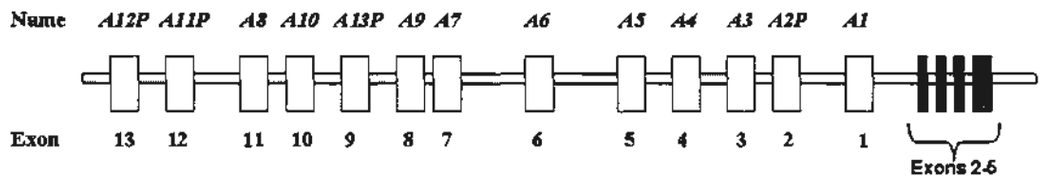
The involvement of the CYP2D6 enzyme in the metabolism of statins is more controversial than that of other enzymes mentioned above. CYP2D6 may not be directly involved in the metabolism of the statin lactone or hydroxy acid, but it may play a role in disposition of the downstream oxidative metabolites. There were

several studies reporting an increased cholesterol-lowering efficacy with simvastatin treatment in subjects with *CYP2D6* genotypes associated with decreased enzyme activities (Mulder AB et al., 2001, Nordin C et al., 1997, Zuccaro P et al., 2007), although an *in vitro* study suggested that *CYP2D6* was not involved in the metabolism of simvastatin (Prueksaritanont T et al., 2003).

#### 1.1.2.4 Uridine diphosphate glucuronosyltransferases

Glucuronidation mediated by UGT enzymes accounts for ~35% of all Phase II drug metabolism and plays an important role in the detoxification and excretion of numerous xenobiotics including therapeutic drugs and endogenous compounds (e.g. bilirubin) (Owens IS et al., 2005). The UGT superfamily can be subdivided into four subfamilies, UGT1, UGT2, UGT3, and UGT8, among which the enzymes of the UGT1 and UGT2 families are most efficient at using UDP glucuronic acid as the glycosyl donor (Mackenzie PI et al., 2005, Owens IS et al., 2005). The *UGT1A* locus is located on chromosome 2q37 and contains 13 individual promoters/first exons and a shared set of exons 2 to 5 (Figure **1-4**), which potentially generates 13 transcripts containing unique 5' ends and identical 3' ends with nine of them being functional (*UGT1A1*, *UGT1A3-1A10*) (Mackenzie PI et al., 2005). As each *UGT1A* gene shares four common exons, it was predicted that a deleterious defect in any common exon would inactivate the entire locus, whereas such a defect in a unique exon would affect a single UGT protein (Owens IS et al., 2005). There is tissue-specific expression of the UGT1A proteins with five of the nine functional UGT1A proteins being primarily expressed in hepatic tissues (UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9) (Tukey RH and Strassburg CP, 2000).

**Figure 1-4. The UGT1 family**




Each exon 1 is represented by a rectangle, labeled A1, A2, A3, etc. and its position relative to exons 2–5 is indicated. Exons 2–5, which are joined to each first exon in the mature transcript, are shown in grey. Adapted from (Mackenzie PI et al., 2005).

Unlike the UGT1 family, the UGT2 genes each comprise six exons that are not shared between the UGT2 family members except for *UGT2A1* and *UGT2A2* and are divided into UGT2A and 2B subfamilies (Mackenzie PI et al., 2005). In addition, the protein-protein interactions between human UGT2B7 and UGT1As or within UGT1As have been reported suggesting the complexities in glucuronidations in human liver (Fujiwara R et al., 2010, Fujiwara R et al., 2007).

Polymorphisms of UGTs causing absent or reduced enzyme activity have been found to be closely associated with altered drug clearance and/or drug response, hyperbilirubinaemia, Gilbert's syndrome, and Crigler-Najjar syndrome (Di YM et al., 2009). Common functional polymorphisms in *UGT1A1*, *1A3* and *2B7* are shown in Table **1-6.**

**Table 1-6. Common polymorphisms in *UGT1A1*, *1A3* and *2B7***

| Polymorphisms/ haplotypes | Amino acid exchange | Enzyme activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| **UGT1A1** | | | | | |
| *6 (211G>A) | Gly71Arg | Decreased | 0.7 | NA | 5-24.1 |
| *28 (-53(TA)$_{6>7}$) | | Decreased | 30-40 | 35-45 | 6.8-23 |
| *60(-3279T>G) | | Decreased | 44-55 | 85 | 17-33 |
| **UGT1A3** | | | | | |
| *1 | | | 35-54.4 | NA | 61-67.6 |
| *2 (31T>C / 81G>A / 140 T>C) | W11R / E27E / V47A | Substrate specific | 35.9-54.3 | NA | 10.4-12.5 |
| *3 (31T>C / 81G> A) | W11R / E27E | Substrate specific | 6-7 | NA | 10-12 |
| **UGT2B7** | | | | | |
| -327A>G | | No change | 39 | NA | 70.7-75.6 |
| -161T>C | | No change | 44-49 | 68 | ~75 |
| *71S (211G>T) | A71S | | 2 | 0 | 9-18.5 |
| *2 (802T>C) | H268Y | No change | 49-56 | 21-32 | 24.4-29.3 |

Data are from (Hu M et al., 2009) and multiple references: UGT1A3: (Caillier B et al., 2007, Chen Y et al., 2006); UGT1A6: (Innocenti F et al., 2008, Mehlotra RK et al., 2007, Saito K et al., 2006)

The UGT enzymes catalyze the conjugation of glucuronic acid to certain target substrates and they are involved in the inter-conversion of statin lactones and acids through a glucuronidation pathway (Prueksaritanont T et al., 2002a). Coadministration of gemfibrozil with certain statins was found to increase systemic exposure of statins to a different extent (from 1.2 to >5-fold) (Goosen TC et al., 2007), which may be due in part to the inhibition of statin glucuronidation by gemfibrozil (Prueksaritanont T et al., 2002c). A number of UGT enzymes were suggested to contribute to statin glucuronidation e.g. UGT1A3 and UGT2B7 (Fujino H et al., 2003, Goosen TC et al., 2007) and the number of alternative pathways may reduce the contribution of changes in individual UGT enzyme activities to result in altered pharmacokinetics of the statins. However, Riedmaier et al found that lactonization of atorvastatin is catalyzed mainly by UGT1A3 and that it is affected by common polymorphisms in the *UGT1A* locus, both in human liver microsomes and in healthy volunteers (Riedmaier S et al., 2009). Subjects with the *UGT1A3* *2

haplotype had an increase in atorvastatin lactonization as a result of increase in UTG1A3 expression. The atorvastatin lactone is a major metabolite that is pharmacologically inactive but is associated with toxicity (Hermann M et al., 2006, Skottheim IB et al., 2008). The result of this study suggested *UGT1A3* is a novel candidate gene that may influence the safety of atorvastatin.

### 1.1.3 Drug transporter proteins affecting statin disposition

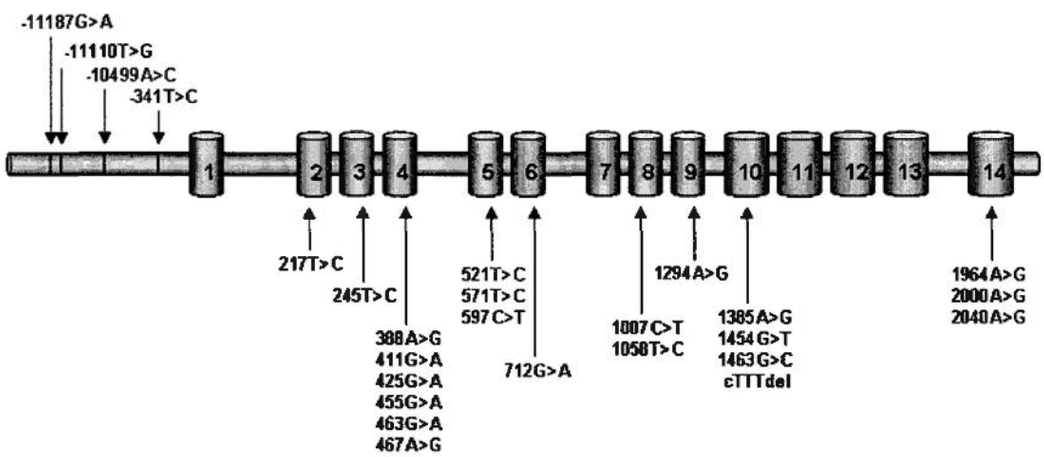
Recent pharmacogenomic/pharmacogenetic studies have disclosed important roles of drug transporters in the pharmacokinetic/pharmacodynamic profiles of some clinically relevant drugs (Ieiri I et al., 2009, Shitara Y and Sugiyama Y, 2006a). The uptake transporter OATP1B1 is specifically expressed in the liver and is considered important for statins, particularly as their pharmacological target organ is the liver. The ATP-binding cassette (ABC) efflux transporters like ABCG2 are expressed in various human tissues and are considered particularly important for reducing intestinal drug absorption and promoting hepatic drug elimination (Ieiri I et al., 2009, Shitara Y and Sugiyama Y, 2006a). Of the statins, pravastatin, rosuvastatin and pitavastatin are hydrophilic and are subject to relatively little metabolism, so drug transporters have a major effect on their disposition. Other statins or their active or inactive metabolites have also been shown to be substrates for some transporters. All statins appear to be substrates for OATP1B1 to different extents, but specificity for hepatobiliary (canalicular) efflux transporters differs among statins. Functional polymorphisms in genes encoding these drug transporters are likely to influence the pharmacokinetic profiles and the efficacy of transporter-dependent drugs. As with the CYP and UGT enzymes, the frequencies of polymorphisms in the genes for the OATP influx transporters and ABC efflux transporters vary between different ethnic groups, which may also contribute to some of the interethnic variability in the

pharmacokinetics and pharmacodynamics of drugs (Cascorbi I, 2006, Konig J et al., 2006).

### 1.1.3.1 SLCO1B1

The OATP1B1 (gene *SLCO1B1*), previously known as OATP-C or OATP2, is an uptake transporter expressed on the sinusoidal membrane of human hepatocytes, which is responsible for the hepatocellular uptake of a variety of endogenous and foreign chemicals (Niemi M, 2007). The *SLCO1B1* gene is highly polymorphic with different allele and haplotype distributions in different populations (Table **1-7**). Some of these polymorphisms are associated with reduced transport activity of OATP1B1. Two common SNPs, 388A>G (130Asn>Asp) and 521T>C (174Val>Ala), have been examined extensively due to marked consequences for transport activity (Figure **1-5**).

**Figure 1-5. Polymorphisms in the *SLCO1B1* gene**




The locations of the identified polymorphisms (arrows) are indicated in relation to the promoter and exon structure. The domain sizes are not to scale. Adapted from (Jada SR et al., 2007).

**Table 1-7. Common polymorphisms in *SLCO1B1***

| Polymorphisms/ haplotypes | Amino acid exchange | Transporter activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| *1a | | | 34-56 | 21-34 | 25-35 |
| -11187G>A | | Unknown | 6.6-7.4 | 0.5-10 | 13-15.3 |
| 388A>G | Asn130Asp | ? Increased | 30-46 | 70-80 | 60-90 |
| 463C>A | Pro155Thr | Unchanged | 13-16 | 2.2 | 0 |
| 521T>C | Val174Ala | Reduced | 14-20 | 1.0-2.5 | 11-16 |
| *1b (388G+521T) | Asn130Asp | ? Increased | 26-39 | 48-77 | 63 |
| *5 (388G+521C) | Val174Ala | Reduced | 0-2 | 0-2 | ~0 |
| *14 (388G + 463A) | Asn130Asp + Pro155Thr | Unchanged | 13.0-15.4 | NA | ~0 |
| *15 (388G + 521C) | Asn130Asp + Val174Ala | Reduced | 16-24 | 2-16 | 3.7-12 |
| *17 (-11187A + 388G +521C) | Asn130Asp + Val174Ala | Reduced | 6.9 | NA | 2.9-13.3 |

Data are from (Hu M et al., 2009) and (Jada SR et al., 2007, Kim SR et al., 2007, Mwinyi J et al., 2008, Pasanen MK et al., 2008b)

The frequency of 388A>G in East Asians (60-90%) was approximately two-fold higher than that in Caucasians (30-46%), whereas, the prevalence of 521T>C in East Asians (11-16%) was similar to values reported in Caucasians (14-20%). The functional effect of the 388A>G polymorphism remains controversial. Early *in vitro* studies have shown that the 388G variant was associated with reduced transport activity of OATP1B1 towards rifampicin, but not with other substrate drugs. *In vivo* pharmacokinetic studies have shown that the 388G variant was associated with reduced plasma pravastatin concentrations suggesting an increased activity of OATP1B1 (Maeda K et al., 2006, Mwinyi J et al., 2004). However, a very recent pharmacokinetic study in 18 Chinese healthy volunteers has shown that the 388G variant allele was associated with increased systemic exposure to pitavastatin suggesting a reduced enzyme activity associated with the variant allele (Wen J and

Xiong Y, 2010). These findings suggest that there may be differences in the effects of the *SLCO1B1* 388A>G polymorphism among different substrates and/or ethnic groups.

The 521T>C polymorphism has been consistently reported to be associated with reduced uptake activity of the OATP1B1 transporter and with a markedly reduced uptake of all statins, except for fluvastatin, from the bloodstream into hepatocytes, leading to remarkably increased plasma concentrations of statins in various populations (Hu M et al., 2009, Pasanen MK et al., 2008b). The effect of the *SLCO1B1* 521T>C polymorphism appears to be greatest with simvastatin with the AUC of active simvastatin acid being 221% greater in individuals with the 521CC genotype than that in those with the 521TT genotype (Niemi M, 2009). The AUCs of pitavastatin, atrovastatin, pravastatin and rosuvastatin in individuals with the 521CC genotype were shown to be 162–191%, 144%, 57–130% and 62–117%, respectively, greater than in those with the 521TT genotype (Niemi M, 2009). However, the *SLCO1B1* 521T>C polymorphism did not have a significant effect on the pharmacokinetics of fluvastatin (Niemi M et al., 2006b).

As statins exert their main cholesterol-lowering effects in hepatocytes, reduced OATP1B1-mediated uptake from the blood stream into the liver due to genetic variants may be associated with a reduced pharmacological efficacy, whereas, increased systemic exposure may lead to increased risk of side effects, such as myopathy. However, in contrast to the great impact of *SLCO1B1* polymorphisms on the pharmacokinetics of statins, few studies reported significant associations between these genetic variants and the lipid-lowering responses to statins. In the Heart Protection Study (HPS), subjects with the 521C variant had an increased risk of

developing myopathy with an odds ratio of 4.5 per cocy of the C variant allele and less reduction in LDL-C with simvastatin (-1.28% per C allele, $P<0.0001$) (Link E et al., 2008). In addition, several other studies have shown that subjects with the 521C variant allele had a smaller effect on cholesterol synthesis with pravastatin or less reduction in total cholesterol in response to pravastatin (Niemi M et al., 2005, Tachibana-Iimori R et al., 2004, Zhang W et al., 2007). The fingings of these studies are in line with the hypothesis that impaired hepatic uptake activity in those carrying the 521C allele results in decreased intracellular concentrations of pravastatin in hepatocytes, the primary site of action, although some other studies did not replicate the associations between the *SLCO1B1* 521T>C polymorphism and lipid responses to pravastatin (Thompson JF et al., 2005).

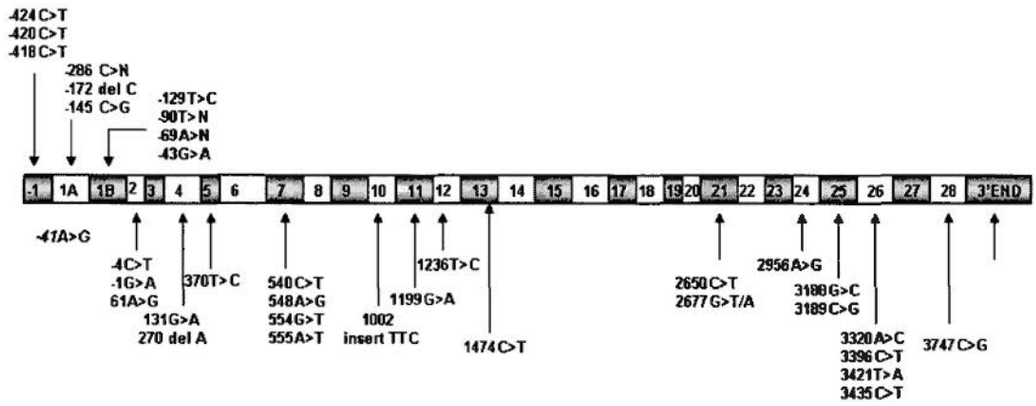
Couvert et al have recently reported that the variant allele of the non-synonymous SNP 463C>A (Pro155Thr) and the SLCO1B1*14 haplotype (388G and 463A) it defines were significantly associated with enhanced lipid-lowering efficacy of fluvastatin in 420 European elderly hypercholesterolaemic subjects (Couvert P et al., 2008). Subjects with the 463CC genotype (n = 294) exhibited significantly less LDL-C reduction than those with one or two copies of variant alleles (n = 111 and n = 15, respectively) (-31.5% : -36.2% : -41%, P<0.05). The effect of this polymorphism on pharmacokinetics and pharmacodynamics of other statins has not been reported.

#### 1.1.3.2 ABCB1 (multidrug resistance protein, P-glycoprotein)

The multidrug resistance protein (MDR1), P-glycoprotein (P-gp) or ABCB1 was the first ABC transporter identified. It is expressed in multiple organs in humans, including the small intestine, liver, kidney and brain in addition to being expressed in

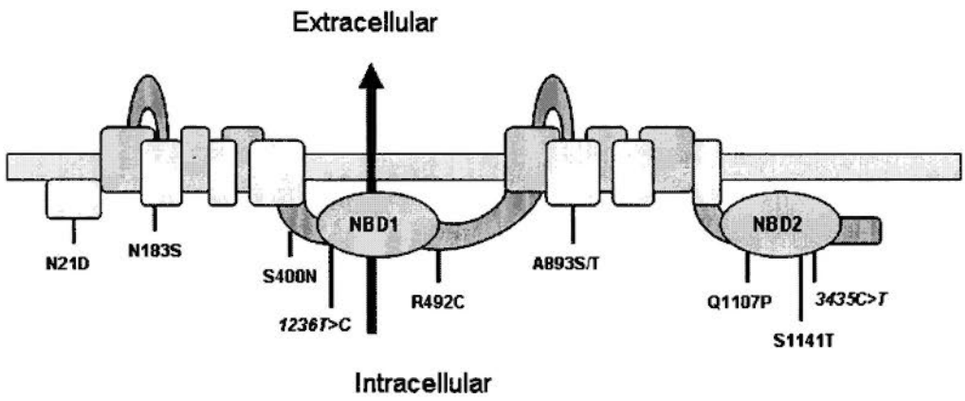
tumour cells. Although the expression of ABCB1 was found to be 7-fold higher in small-intestine enterocyte homogenates than in the liver (von Richter O et al., 2004), it contributes not only to reduce absorption of substrates from the gut but also to the excretion of substrates from the liver into the bile. There are more than 50 SNPs that have been identified in the human *ABCB1* coding region (Figure **1-6**), but the functional consequences of these reported SNPs are still uncertain and most of the known SNPs and haplotypes do not affect protein function *in vitro* (Fung KL and Gottesman MM, 2009). Three common SNPs are in strong linkage disequilibrium (LD), creating a common haplotype at positions 1236C>T (non-coding), 2677G>T (Ala893Ser) and 3435C>T (non-coding) (Figure **1-7**) (Choudhuri S and Klaassen CD, 2006). The frequencies of these SNPs and haplotypes are distributed in an ethnic-dependent manner (Table **1-8**).

**Figure 1-6. Polymorphisms in the *ABCB1* gene**




Schematic diagram showing relative positions of the SNP sites in the promoter and exons of the *ABCB1* gene. The domain sizes are not to scale. Adapted from (Leschziner GD et al., 2007).

**Figure 1-7. Two-dimensional structure of ABCB1 with locations of amino acid replacements and two synonymous frequent SNPs**




Adapted from (Cascorbi I, 2006). NBD = nucleotide-binding domain.

**Table 1-8. Common polymorphisms in *ABCB1***

| Polymorphisms/ Haplotypes | Amino acid exchange | Transporter activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| 1236C>T | Non-coding | Uncertain | 34.4-42 | 15 | 61.5-69.4 |
| 2677G>T | Ala893Ser | Decreased | 41.6-46 | 6.5-10 | 36-43.7 |
| 2677G>A | Ala893Thr | Decreased | 0-3.6 | 0-0.5 | 5.8-21.8 |
| 3435C>T | Non-coding | Decreased | 48-54 | 16-26 | 37-47 |
| 1236C-2677G-3435C | | Normal | 32-45 | 43.6-79 | 17-26 |
| 1236T-2677T-3435T | Ala893Ser | Decreased | 35-42 | 4.5-8.7 | 32.2-41.4 |
| -129T>C | Non-coding | Unknown | 5.9 | NA | 1.6-8.3 |
| 4036 A>G | Non-coding | Unknown | 20.5 | 22.7 | 26.1 |

Data are from (Hu M et al., 2009) and (Fung KL and Gottesman MM, 2009).

Although the results are not all consistent, the 3435C>T SNP was found to be a main functional polymorphism associated with several changes, from mRNA level, protein expression, protein folding to substrate specificity in cell lines and human samples but how this happens on a molecular level is not fully understood (Fung KL and Gottesman MM, 2009), but is probably due to altering the structure of substrate and

inhibitor interaction sites (Kimchi-Sarfaty C et al., 2007). A recent in vitro study has suggested that the effect of polymorphisms in *ABCB1* is substrate-dependent, so it may be difficult to predict the impact of *ABCB1* SNPs on the clinical effects of the substrate drugs (Gow JM et al., 2008).

Recent pharmacokinetic studies suggested that the *ABCB1* 1236T-2677T-3435T haplotype was associated with 60% greater $AUC_{(0\text{-}12h)}$ of simvastatin acid and 55% larger $AUC_{(0\text{-}infinity)}$ of atorvastatin acid than those in subjects with the wild-type alleles (1236C-2677G-3435C), but there was no effect on the pharmacokinetics of lactones of these two statins (Keskitalo JE et al., 2008) or on the pharmacokinetics of the other statins (fluvastatin, pravastatin, lovastatin, and rosuvastatin) (Keskitalo JE et al., 2009a). It has been shown that patients with one or two copies of the *ABCB1* 1236T variant allele had greater reductions in total cholesterol and LDL-C with simvastatin treatment than those homozygous wild-type alleles (total cholesterol -29.0% [95% confidence interval (CI), -25.9 to -32.5] vs. -24.2% [-19.0 to -29.3], and LDL-C -39.6% [-35.8 to -44.0] vs. -33.8% [-27.4 to -40.2], respectively, P = 0.042 for both) and similar results were also observed for the 2677G>A/T polymorphism and the haplotypes these SNPs defined (Fiegenbaum M et al., 2005c). Other studies have also identified the association between polymorphisms or haplotypes of the *ABCB1* gene and the LDL-C response to atorvastatin or fluvastatin (Bercovich D et al., 2006, Kajinami K et al., 2004d, Thompson JF et al., 2005), although fluvastatin is not thought to be a substrate for ABCB1-mediated transport (Bercovich D et al., 2006).

However, some statins or their metabolites, such as atorvastatin, lovastatin and simvastatin lactone, were found to induce the mRNA expression of MDR1 and

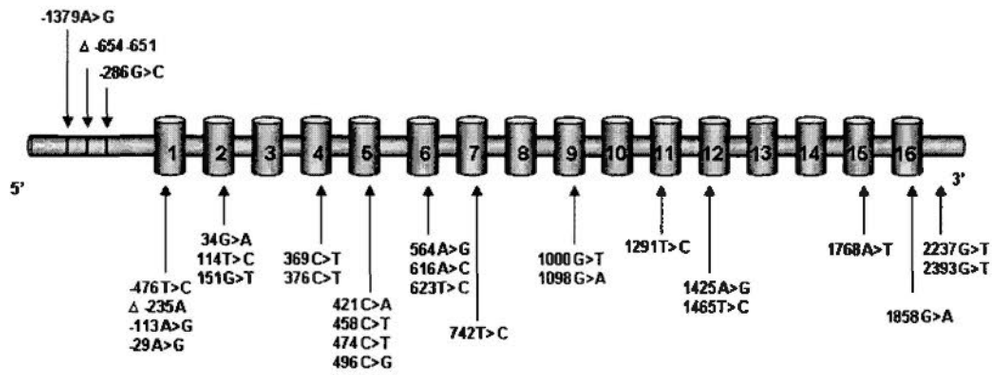
CYP3A in a concentration-dependent manner in cell lines (Bogman K et al., 2001, Yamasaki D et al., 2009). In addition, it has also been reported that ABCB1 may play a role in cholesterol homeostasis and several studies have demonstrated that polymorphisms in ABCB1 were associated with altered baseline cholesterol levels in hypercholesterolaemic patients and healthy subjects (Jeannesson E et al., 2009, Rodrigues AC et al., 2005), although the findings of thses studies are not consistent. Furthermore, cholesterol concentration can also regulate ABCB1 expression and activity with elevated cellular cholesterol levels markedly increasing P-gp activity (Troost J et al., 2004). These factors may influence the associations between *ABCB1* polymorphisms and statin responses. Indeed, Rebecchi et al has recently reported that atorvastatin significantly reduced ABCB1 and ABCC1 mRNA levels in peripheral blood mononuclear cells (PBMC) and the *ABCB1* 2677 G>T/A polymorphism was associated with reduced expression of ABCB1. In this study, lower basal ABCB1 mRNA levels were associated with smaller reductions for LDL-C and apoB in response to atorvastatin, suggesting atorvastatin was more effective in reducing lipid levels in ABCB1 high-expressors than in low-expressors (Rebecchi IM et al., 2009).

#### 1.1.3.3 ABCG2

The ABCG2 transporter, also known as BCRP, is highly expressed in the placenta, colon, liver and small intestine and may play a role in absorption, distribution, and elimination of drugs that are BCRP substrates (Mao Q and Unadkat JD, 2005). ABCG2 is a half-transporter that is composed of a single nucleotide-binding domain (NBD) followed by one membrane-spanning domain (MSD), therefore homodimerization may be essential for it to act as a drug transporter (Ieiri I et al., 2009, Mao Q and Unadkat JD, 2005). Among over 80 naturally occurring sequence variations identified in the *ABCG2* gene (Figure **1-8**), the nonsynonymous 421C>A

SNP reducing the transport function of ABCG2 due to a change of glutamine to lysine at codon 141 has been studied most extensively (Robey RW et al., 2009). This polymorphism was most prevalent in both the Japanese and Chinese populations with an allele frequency of ~35%, but was less common in Caucasian (9-15%) and almost absent in Blacks (Table **1-9**).

**Figure 1-8. Polymorphisms in the *ABCG2* gene**




Schematic diagram showing relative positions of the SNP sites in the promoter and exons of the *ABCG2* gene. The domain sizes are not to scale. Adapted from (Yanase K et al., 2006).

**Table 1-9. Common polymorphisms in *ABCG2***

| Polymorphisms | Amino acid exchange | Transporter activity | Allele frequency (%) | | |
|---|---|---|---|---|---|
| | | | White | Black | East Asian |
| 34G>A | Val12Met | Uncertain | 2-10.3 | 4 | 15-36 |
| 421C>T | Gln141Lys | Reduced | 9-15 | 0-5 | 25-35 |

Data are from (Hu M et al., 2009) and (Niemi M, 2009)

ABCG2 contributes to the disposition of a wide variety of endogenic substances and drugs including some statins (Cusatis G and Sparreboom A, 2008). The plasma concentrations of rosuvstatin, atorvastatin, simvastatin lactone and fluvastatin have been markedly increased in individuals with the 421A variant alelle in various

pharmacokinetic studies (Keskitalo JE et al., 2009b, Keskitalo JE et al., 2009c, Zhang W et al., 2006a), but not for pitavastatin, pravastatin and simvastatin acid (Ho RH et al., 2007, Ieiri I et al., 2007, Keskitalo JE et al., 2009b). The effect is strongest on rosuvastatin with subjects with 421AA genotype having about 2-fold higher systemic exposure to rosuvastatin compared to those with 421CC genotype (Keskitalo JE et al., 2009c, Zhang W et al., 2006a). The higher prevalence of this polymorphism in East Asians than in Caucasians may contribute to the ethnic difference in rosuvastatin pharmacokinetics. Although pitavastatin and pravastatin has been demostrated to be substrates of ABCG2, the 421C>A polymorphism did not alter their pharmacokinetics, which may be attributed to the contributions of other ABC transporters e.g. ABCC2 in the pharmacokinetics of pitavastatin and pravastatin (Ieiri I et al., 2007, Kivisto KT and Niemi M, 2007). The effect of the *ABCG2* polymorphisms on the lipid response to statins has not been reported.

#### 1.1.3.4 Other transporters

The ATP-binding cassette transporter ABCC2 or MRP2, encoded by the *ABCC2* gene plays an important role in drug excretion processes. Impaired function of MRP2 may result in increased gastrointestinal absorption and decreased biliary and/or urinary excretion of its substrates. This efflux transporter is thought to be one of the major transporters involved in the pharmacokinetics of pravastatin in humans (Kivisto KT and Niemi M, 2007). More than 40 SNPs in the *ABCC2* gene have been identified, but little is known about the effect of genetic variations in *ABCC2* on the pharmacokinetics of drugs in humans. Niemi et al. reported a significant association between a synonymous SNP in *ABCC2* (1446C>G) and the pharmacokinetics of pravastatin (Niemi M et al., 2006a). The 1446G variant allele was associated with increased hepatic expression of *ABCC2* mRNA and reduced plasma concentrations

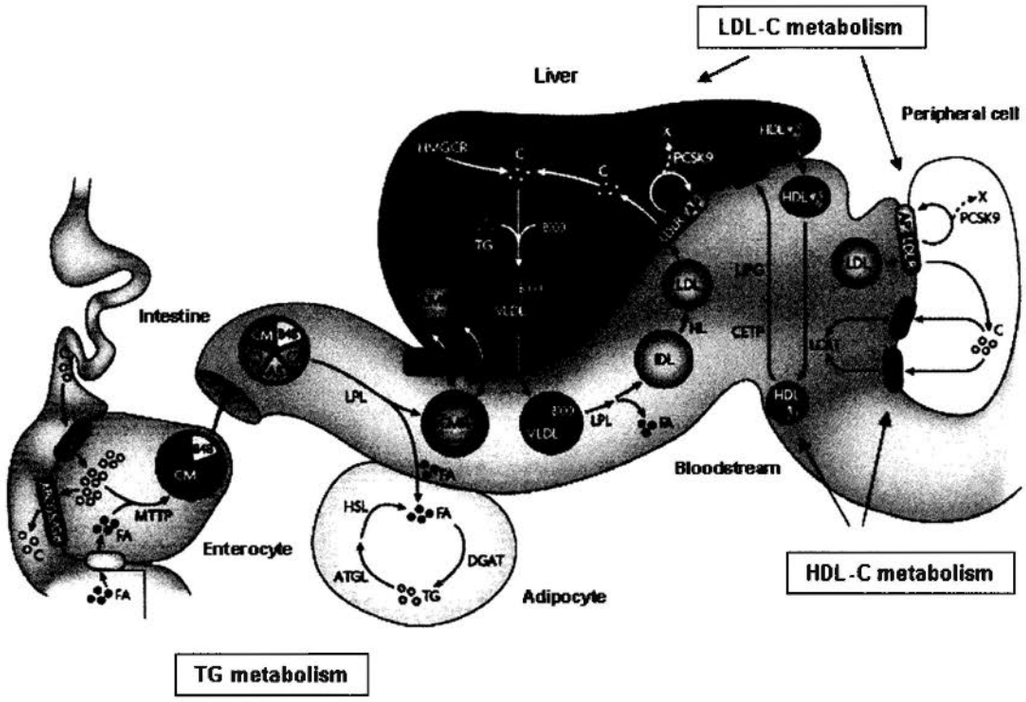
of pravastatin although the mechanism by which this synonymous SNP increases MRP2 expression is still unclear (Niemi M et al., 2006a). Recently, another study failed to find a relationship between 3 commonly occurring non-synonymous polymorphisms in *ABCC2* (1249G>A, 3563T>A and 4544G>A) and the pharmacokinetics of pravastatin (Ho RH et al., 2007).

Several *in vitro* studies have suggested that other transporters including SLCO2B1 (OATP2B1), SLC22A8 (OAT3), ABCB11 (BSEP), and sodium-dependent taurocholate cotransporting polypeptide (NTCP) may also play a role in the disposition of some statins (Hirano M et al., 2005, Ho RH et al., 2006, Kivisto KT and Niemi M, 2007). The possible effects of polymorphisms in these genes on statin pharmacokinetics have not been thoroughly addressed.

### 1.1.4 Genes involved in cholesterol synthesis and lipoprotein metabolism affecting statin responses

In contrast to the different effects of genetic polymorphisms on the pharmacokinetics of different statins, polymorphisms affecting pharmacodynamics are likely to have similar effects to all statins. A number of candidate genes involved in lipid metabolism pathways (Figure **1-9**) that may affect the pharmacodynamic lipid-regulating effect of statins have been studied (Hu M et al., 2009). To date, the most extensively examined genes related to lipid metabolism pathways and statin effects are the LDLR, apolipoprotein E (APOE), the cholesteryl ester transfer protein gene (CETP) and the target enzyme for statins, HMGCR.

**Figure 1-9. Overview of lipoprotein metabolism**




Abbreviations: ABCG5/G8 = ATP-binding cassette transporter G5/G8; AP = adaptor protein; APOB = apolipoprotein B; ATGL = adipose TG lipase; C = cholesterol; CETP = cholesterol ester transfer protein; CMRs = chylomicron remnants; CMs = chylomicrons; DGAT = acyl CoA:diacylglycerol acyltransferase; FA = fatty acid; HDL = high-density lipoprotein; HL = hepatic lipase; HMGCR = 3-hydroxy-3-methylglutaryl coenzyme A reductase; HSL = hormone sensitive lipase; IDL = intermediate-density lipoprotein (VLDL remnants); LCAT = lecithin-cholesterol acyltransferase; LDL = low-density lipoprotein; LDLR = LDL receptor; LIPG = endothelial lipase; LPL = lipoprotein lipase; LRP1 = LDLR-related protein-1; MTTP = microsomal TG-transfer protein; NPC1L1 = Niemann-Pick C1-like 1; SRB1 = scavenger receptor class B type I; TG = triglyceride; VLDL = very low-density lipoprotein. Adapted from (Hegele RA, 2009)

### 1.1.4.1 HMG-CoA reductase

The HMGCR is the rate-limiting enzyme in cholesterol synthesis and the direct enzymatic target of statins. Unlike other well known determinants of cholesterol homeostasis, e.g. LDLR or APOE, associations between polymorphisms in HMGCR and baseline LDL-C concentrations have been discovered in the recent genome-wide

association studies (GWASs) (Kathiresan S et al., 2009). Variants in this gene were associated with multiple lipid/lipoprotein traits with different impact among different ethnicities (Burkhardt R et al., 2008, Chen YC et al., 2009, Kathiresan S et al., 2009).

The two tightly linked SNPs, SNP12 (rs17244841) in intron 5 and SNP29 (rs17238540) in intron 18 have been reported to be associated with reduced lipid response in the PRINCE (Pravastatin Inflammation/CRP Evaluation) subjects with pravastatin treatment (Chasman DI et al., 2004), a black cohort in CAP (Cholesterol and Pharmacogenetics) study with simvastatin (Krauss RM et al., 2008) and a large population-based cohort of patients with diabetes with various statins (67.2% on simvastatin, 21% on atorvastatin, 5.4% on fluvastatin, and 4.8% on pravastatin) (Donnelly LA et al., 2008). However, there were several large studies that did not replicate associations between *HMGCR* polymorphisms and lipid responses to atorvastatin in a European cohort in ACCESS (Atorvastatin Comparative Cholesterol Efficacy and Safety Study) (Thompson JF et al., 2005), to fluvastatin in a cohort of renal transplant recipients (Singer JB et al., 2007) or to pravastatin in a large cohort of PROSPER (Prospective Study of Pravastatin in the Elderly at Risk) participants aged 70–82 (Polisecki E et al., 2008). These discrepancies may be due to differences in the magnitude of the pharmacogenetic associations for different statins, different frequency of the variant allele among populations, or different LD patterns with the other causal variants among populations (Krauss RM et al., 2008).

#### 1.1.4.2 Low-density lipoprotein receptor

The LDLR in the liver plays a key role in LDL particle uptake and catabolism. The lack of the LDLR or dysfunctional receptors reduces the clearance of LDL particles, resulting in an increase in the plasma LDL-C levels. To date, more than 1,000

mutations in the LDLR have been described causing familial hypercholesterolaemia (FH) (Abifadel M et al., 2009, Garg A and Simha V, 2007, Soutar AK and Naoumova RP, 2007). Mutations in the *LDLR* gene have been divided into five classes. Class I and II mutations are known as receptor negative mutations, and Class III - V mutations are receptor defective mutations.

Some previous studies have reported that different types of *LDLR* mutations were associated with different response to statins in FH. In some studies, patients with mutations having a mild effect or with receptor defective mutations showed a better response in terms of LDL-C reduction than that in patients with severe or receptor negative mutations (Table 1-10), but other studies failed to find these associations (Couture P et al., 1998, Sijbrands EJ et al., 1998, Sun XM et al., 1998). In addition, there was no single study to compare lipid responses to statins in patients with heterozygous FH and those without.

**Table 1-10. Associations between mutations in *LDLR* and lipid response to statins**

| Polymorphisms | Statins | Effects | References |
|---|---|---|---|
| C660X, D147H, 652delGGT | F | Smaller reduction in LDL-C in patients with "Sephardic" and "Lithuanian" mutations | (Leitersdorf E et al., 1993) |
| D206E, V408M | S | Greater reduction in TC and LDL-C in patients with FH2 | (Jeenah M et al., 1993) |
| Del 6kb-ex15 P664L | Pra with other LLD | Greater reduction in LDL-C in patients with $FH_{Kanazawa-2}$ | (Kajinami K et al., 1998) |
| W66G; Del 15kb-ex1, C646Y | S | Smaller reduction in LDL-C in those with W66G mutation than those with the deletion>15 kb and the C646Y mutation | (Couture P et al., 1998) |
| Mild, Severe | S | Fewer achieved LDL-C goals with severe LDLR mutation compared to those with mild mutations | (Heath KE et al., 1999) |
| Null mutation Defective mutation | S | Smaller reduction in TC and LDL-C in patients with null mutations than those with defective mutations | (Chaves FJ et al., 2001) |
| R-negative, R-defective | S | Smaller reduction in LDL-C in those with receptor-negative mutation than those with receptor-defective mutation | (Vohl MC et al., 2002) |
| AvaII | Pra | Greater reduction in LDL-C in patients with AvaII mutations | (Lahoz C et al., 2005b) |
| C44857T, A44964G | Pra | The 44857T variant allele was associated with greater LDL-C lowering response to pravastatin and a lower risk for CHD. | (Polisecki E et al., 2008) |

Abbreviations: F=fluvastatin; LLD=lipid-lowering drugs; Pra=pravastatin; S=simvastatin; TC=total cholesterol.

### 1.1.4.3 Proprotein convertase subtilisin- like kexin type 9

PCSK9 is a newly discovered serine protease that plays a key role in regulating plasma LDL-C levels. Some rare gain-of-function mutations cause hypercholesterolaemia by decreasing the number of LDLR, which account for a much smaller percentage of dominant hypercholesterolaemia than mutations in *LDLR* and apolipoprotein B (*APOB*); whereas some loss-of-function variants were

found to be associated with a reduction of LDL-C levels and a decreased risk of CHD (Abifadel M et al., 2009, Soutar AK and Naoumova RP, 2007). The frequencies of those functional mutations in *PCSK9* are relatively low at about 2%, while other SNPs have no clinical impact and are generally more frequent in all populations (Abifadel M et al., 2009).

It has been reported that D374Y mutation in the *PCSK9* gene was associated with a severe clinical phenotype and attenuated statin response compared to FH patients with severe *LDLR* mutations (Naoumova RP et al., 2005). However, reports on the effects of *PCSK9* mutations on the responses to treatment with simvastatin or atorvastatin have been inconclusive (Berge KE et al., 2006, Bertolini S et al., 2004). A recent compreshensive analysis in 5745 TNT (Treating to New Target) participants with European ancestry revealed that a rare SNP in *PCSK9* rs11591147 with a minor allele frequency of 0.65% in the population was associated with greater LDL-C reduction with pravastatin treatment (Thompson JF et al., 2009). Considering the low frequency of this polymorphism in all ethnic groups, it is unlikely to significantly affect statin responses at a population level.

#### 1.1.4.4 Apolipoprotein E

Apoliporotein E (apo E) is a 299 amino-acid protein that is synthesized and secreted primarily by hepatocytes and is mainly expressed in the brain and liver (Mahley RW et al., 2009). The primary functional role of apo E is to transport and deliver lipids mainly through the LDLR pathway or the heparin sulphate proteoglycan (HSPG)/LDLR related protein pathway. Apo E acts as a ligand for several cell receptors and controls the removal of apo E-(triglyceride)-rich lipoproteins by the LDLR, thereby influencing the metabolism of plasma lipoproteins and cholesterol

(Jofre-Monseny L et al., 2008, Mahley RW et al., 2009). The human *APOE* gene is polymorphic, encoding one of 3 common epsilon ( ε ) alleles ( ε 2 [rs7412], ε 3, and ε 4 [rs429358]), resulting in 3 major isoforms of human apo E (E2, E3, and E4). Apo E isoforms differ in amino acid residues at positions 112 and 158, which leads to protein structure differences resulting in different functions in lipid metabolism and certain diseases (Table **1-11**) (Jofre-Monseny L et al., 2008, Mahley RW et al., 2009).

**Table 1-11. Structure, action and frequency of apo E isoforms**

| Isoform | SNP ID | Residues | | LDLR binding affinity | Lipoprotein affinity | Associated lipid changes |
|---|---|---|---|---|---|---|
| | | 112 | 158 | | | |
| apo E2 | rs7412 | Cys | Cys | <2% | HDL | ↓ TC and LDL-C; ↑ TG in some homozygous E2 |
| apo E3 | | Cys | Arg | High | HDL | Normal |
| apo E4 | rs429358 | Arg | Cys | High | VLDL, CM | ↑ LDL-C |
| | | | | Frequency of isoforms | | |
| | | | | European-Caucasians | African-American | Chinese |
| apo E2 | | | | 4.1-8.2 | 11.7 | 5.6-10.2 |
| apo E3 | | | | 71.9-85 | 68.2 | 82.6-87.9 |
| apo E4 | | | | 9-22.7 | 20.2 | 6.3-8.6 |

↓ = decrease; ↑ = increase. Adapted from (Jofre-Monseny L et al., 2008, Liang S et al., 2009, Mahley RW et al., 2009)

The effect of polymorphisms in the *APOE* gene on the plasma lipid response to statins has been addressed in many studies (Table **1-12**), in particular regarding e2/e3/e4 polymrophisms (Hu M et al., 2009, Nieminen T et al., 2008). Most studies suggest that subjects with the e2 allele had greater lipid responses whereas e4 carriers had the poorest response although some studies either included patients with FH or

were underpowered to fail to show a significant interaction between *APOE* polymorphisms and the lipid response to statins (Hu M et al., 2009, Nieminen T et al., 2008). A meta-analysis of 24 studies has shown that there were no significant effects of *APOE* genotypes on lipid responses to statins (Zintzaras E et al., 2009). However, recent pharmacogenetic analysis in three large trials (TNT, PROVE-IT TIMI22 [Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22] and STRENGTH [The statin response examined by genetic haplotype markers]) have consistently reported that among all SNPs examined the rs7412 (E2) polymorphism had the most significant impact on the LDL-C response to various statins with E2 carriers having greater LDL-C reduction than those with E3 or E4, which highlights the importance of polymorphisms in *APOE* on lipid response to statins (Mega JL et al., 2009, Thompson JF et al., 2009, Voora D et al., 2008). Few studies have investigated the interaction between the *APOE* e2/e3/e4 polymorphisms and statin therapy in relation to the course of CHD and the results of these studies are inconsistent and so far inconclusive (Nieminen T et al., 2008).

**Table 1-12. Associations between *APOE* polymorphisms and lipid response to statins**

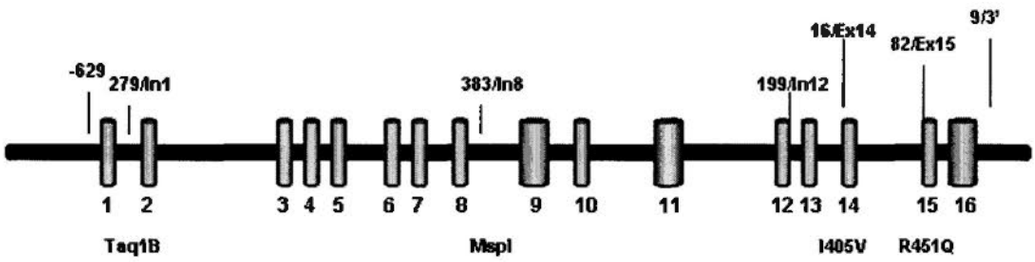
| Polymorphisms | Statins | Effects | References |
|---|---|---|---|
| E2, E3, E4 | L | Smaller reduction in LDL-C, but greater increase in HDL-C in male E4 carriers | (Carmena R et al., 1993) |
| E2, E3, E4 | A | E4 allele more frequent in good responders | (O'Neill FH et al., 2001) |
| E2, E3, E4 | Pra | Greater LDL-C response with E2 | (Ordovas JM et al., 1995) |
| E2, E3, E4 | S | Greatest response in those with E2 | (Nestel P et al., 1997) |
| E2, E3, E4 | F | Less reduction in LDL-C in E4 carriers but similar benefit in terms of progression. | (Ballantyne CM et al., 2000) |
| E2, E3, E4 | A | Greater LDL-C and TG response in males with E2 | (Pedro-Botet J et al., 2001) |
| -491A/T | A | Greater LDL-C-lowering effect with -491T allele carriers | (Garcia-Otin AL et al., 2002) |
| E2, E3, E4 | A | Greater HDL-C response in E2 carriers than those homozygous for E3 | (Thompson JF et al., 2005) |
| E2, E3, E4 | Pra | E2 carriers had largest improvement of HDL-C and LDL/HDL ratios, compared with E3 and E4 carriers, but not with regard to angiographic parameters. | (Maitland-van der Zee AH et al., 2006) |
| E2, E3, E4 | Various statins | Greater HDL-C response in E2 carriers | (Zuccaro P et al., 2007) |
| E2, E3, E4 | S, L | Greater LDL-C response in E4 carries than those with E2 | (Tavintharan S et al., 2007) |
| E2, E3 | A,S, Pra | Greater LDL-C response in E2 carriers | (Voora D et al., 2008) |
| E2, E3, E4 | A, Pra | Greater LDL-C response in E2 carries than those with E4 | (Mega JL et al., 2009) |
| E2, E3, E4, SNP17 | A | Greater LDL-C response in patients with E2 or the minor allele of SNP17 but less LDL-C response in E4 compared to those with E3 | (Thompson JF et al., 2009) |

Abbreviations: A=atorvastatin; F=fluvastatin; L=lovastatin; Pra=pravastatin; S=simvastatin; TG= triglycerides.

### 1.1.4.5 Cholesteryl ester transfer protein gene

The CETP facilitates the transfer of cholesteryl esters (CE) from HDL to apolipoprotein B-containing particles in exchange for triglycerides, thus increased CETP activity is associated with lower HDL-C and higher LDL-C levels. In addition, it has also been suggested that CETP may be antiatherogenic by enhancing the rate of reverse cholesterol transport - the mechanism by which cholesterol in peripheral tissues is transported to the liver for elimination (Boekholdt SM et al., 2004). Genetic mutations in the *CETP* gene are the main cause of CETP deficiency, which is associated with higher plasma HDL-C concentrations, but its association with CVD is still controversial. The two common SNPs in *CETP*, -629C>A and the TaqIB (Figure **1-10)** have been shown to be associated with HDL-C levels in many populations (McCaskie PA et al., 2007, Tai ES et al., 2003, Thompson JF et al., 2003).

**Figure 1-10. The *CETP* gene structure, and the location of some common polymorphisms**




The location of some of the common polymorphisms are shown in the context of the *CETP* gene structure. Adapted from (Thompson JF et al., 2003).

Some previous studies have shown that *CETP* polymorphisms were associated with a lower risk of CVD, which was independent of the effect on HDL-C (Blankenberg S et al., 2003), but others did not find this association (de Grooth GJ et al., 2004,

McCaskie PA et al., 2007, Tsai MY et al., 2008). A recent meta-analysis in about 200,000 subjects from 138 available prospective studies has demostrated that the three common (TaqIB, I405V, and -629C>A) polymorphisms in CETP associated with moderate reduction of CETP activity (and, therefore, modestly higher HDL-C levels) were weakly inversely related to coronary risk and the odds ratios for coronary disease were compatible with the expected reductions in risk for equivalent increases in HDL-C concentration (Thompson A et al., 2008). The effects of common polymorphisms in *CETP* on lipid responses or clinical outcome with statin treatment in different groups of patients are also inconsistent (Table **1-13**).

**Table 1-13. Associations between *CETP* polymorphisms and lipid response to statins**

| Polymorphisms | Statins | Effects | References |
|---|---|---|---|
| Taq1B (B1, B2) | Pra | Pravastatin slowed progression of coronary disease in B1B1 carriers but not in B2B2 carriers | (Kuivenhoven JA et al., 1998) |
| Taq1B (B1, B2) | A | Patients with B1B1 or 629CC genotype had a greater increase in HDL-C and a larger reduction in TG | (van Venrooij FV et al., 2003) |
| Haplotype | A, C, Pra | SNPs1-6 associated with increase in HDL-C and SNPs4-9 with decrease in TG | (Winkelmann BR et al., 2003) |
| Taq1B (B1, B2) | S | Patients with B2B2 had greater improvement in HDL-C than B1carriers | (Fiegenbaum M et al., 2005a) |
| Taq1B (B1, B2) | A, Pra, S | Patients with the B2B2 had a higher CVD risk than the B1 carriers. | (Mohrschladt MF et al., 2005) |
| Haplotype: -2600, -867, -525, Taq1B, I405V | F | CETP-H13 was associated with greater reduction in LDL-C. CETP-H5 was associated with decreased TG and HDL-C response. | (Bercovich D et al., 2006) |

Abbreviations: A=atorvastatin; CVD=cardiovascular disease; C=cerivastatin; F=fluvastatin; L=lovastatin; Pra=pravastatin; S=simvastatin; TG=triglycerides.

### 1.1.4.6 Others

Polymorphisms in some other genes related to cholesterol synthesis and lipoprotein synthesis have also been reported to be associated with lipid response to statins but replication of such results has been sporadic, which may be due to the small sample sizes frequently used, lack of true effect or confounding factors. Some of the positive effects of polymorphisms in these candiate genes on the lipid responses to statins and clinical outcomes in previous studies are summarized in Table **1-14.**

In addition to well-established lipid regulators, recent large GWASs have identified some new loci associated with lipid concentrations and/or the risk of CHD. (Kathiresan S et al., 2008a, Kathiresan S et al., 2009, Kooner JS et al., 2008, Sandhu MS et al., 2008, Willer CJ et al., 2008a). These newly identified genetic markers of the lipid traits may also contribute to the variation in lipid response to statins and may be promising targets for further investigation.

**Table 1-14. Positive effects of polymorphisms in various genes on the lipid response to statin therapy**

| Genes | Polymorphisms | Statins | Effects | References |
|---|---|---|---|---|
| **LPL** | Haplotype: 12 SNPs | L | Several haplotypes related to HDL-C and TG response and graft progression protection | (Goodarzi MO et al., 2007) |
| **LIPC** | C-514T | L with other LLD | Subjects with the CC genotype had the greatest improvement in LDL density and HDL(2)-C and the greatest angiographic improvement | (Zambon A et al., 2001) |
| | C-514T | Pra | Greater increase in HDL-C in those T carriers than those with CC genotype | (Lahoz C et al., 2005a) |
| **APOB** | XbaI, ins/del, EcoRI | F | Greater reduction in LDL-C in ins homozygotes | (Guzman EC et al., 2000) |
| **PON** | R192Q M55L | Pra | R192Q polymorphism modulates HDL-C and apo AI responses | (Malin R et al., 2001) |
| | -107C>T, 192Q>R | A, S | Greater HDL-C in subjects with -107CT/TT or 192QR/RR genotype. | (Himbergen TM et al., 2005) |
| **APOA1** | G-75A | Pra | Greater increase in HDL-C in non-smoking male GG carriers | (Lahoz C et al., 2003) |
| | G-75A, +83 | A | Gender-difference in HDL-C increase in +83 variant carriers | (Kajinami K et al., 2005a) |
| **ABCG5/G8** | Q604E, D19H, Y54C, T400K, and A632V | A | D19H variant associated with greater reduction in LDL-C | (Kajinami K et al., 2004a) |
| **CYP7A1** | A-204C | A | A-204C promoter variant associated with poor response of LDL-C reduction | (Kajinami K et al., 2004c, Kajinami K et al., 2005b) |
| **ABCA1** | C-477T, -419C, G-320C | F | -477C/T variants associated with the severity of coronary atherosclerosis. | (Lutucuta S et al., 2001) |
| **PPAR** | Haplotype | F | PPARδ haplotype 2 determine TG and apoC-III response and coronary lesions, PPARγ haplotypes 7 associated with reduced mean lumen diameter. | (Chen S et al., 2004) |
| **LEPR** | Arg223Gln | S | Subjects with Arg/Arg had less reduction in TC than those Gln carriers | (Takahashi-Yasuno A et al., 2003) |

Abbreviations: A=atorvastatin; ABCA1 = ATP-binding cassette transporter A1; ABCG5/G8 = ATP-binding cassette transporter G5/G8; AP = adaptor protein; APOA1 = apolipoprotein

A-I; APOB = apolipoprotein B; C=cerivastatin; CYP7A1 = cholesterol 7α-hydroxylase; F=fluvastatin; L=lovastatin; LEPR = leptin receptor; LIPC = hepatic lipase; LPL = lipoprotein lipase; LLD=lipid-lowering drugs; Pi=pitavastatin; PON = paraoxonase; PPAR = peroxisome proliferator-activated receptor; Pra=pravastatin; R=rosuvastatin; S=simvastatin.

### 1.2 Genetic influences on pleiotropic effects of statins

Evolving evidence suggest that statins possess several anti-inflammatory and antioxidant activities resulting in the beneficial reduction of atherosclerotic processes and cardiovascular risk beyond their effect on the lipid profile. The mechanisms of the anti-atherosclerotic effects of statins have been extensively investigated and appear to involve effects on numerous factors modulating endothelial function, inflammatory and atherothrombotic process, e.g. nitric oxide enhancement, inhibition of vascular inflammation, immune modulation, and a possible effect on thrombogenicity (Blum A and Shamburek R, 2009, Lahera V et al., 2007). Polymorphisms in these modulating genes could not only alter the progression of atherosclerosis and the risk of cardiovascular events, but also influence the beneficial effect of statins on clinical outcomes.

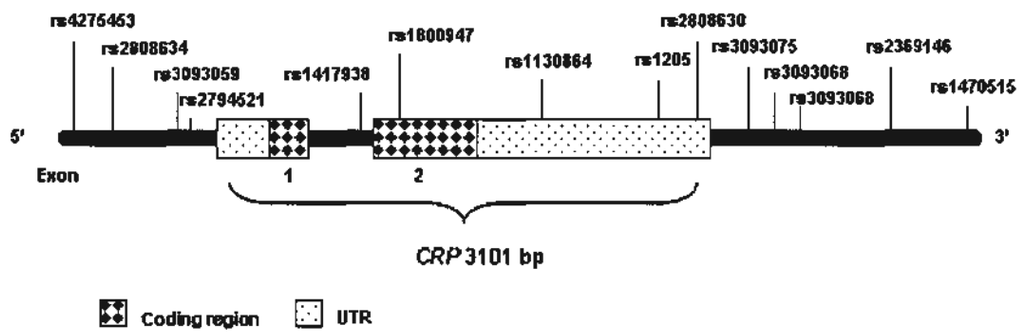
#### 1.2.1 C-reactive protein

Convincing evidence supports that inflammation plays a pivotal role in the formation and activation of atherosclerotic plaques (Hansson GK and Libby P, 2006, Ross R, 1999). C-reactive protein (CRP), the classical acute phase protein, is the most extensively studied systemic marker of inflammation for its possible role in the pathogenesis of CVD (Casas JP et al., 2008, Hingorani AD et al., 2009). High-sensitivity C-reactive protein (hsCRP) levels in the absence of acute illness have been shown to be consistently associated with various cardiovascular endpoints as well as with high-risk vascular phenotypes such as high blood pressure and

metabolic syndrome in more than 30 prospective observational studies (Casas JP et al., 2008, Danesh J et al., 2004, Hingorani AD et al., 2009, Sakkinen P et al., 2002, Ye X et al., 2007), although neither the normal functions of human CRP nor its role in disease states have been fully determined.

CRP concentration is a heritable trait and genetic factors could explain about as much as 50% of the variance of baseline hsCRP concentrations, which might be largely attributable to non-coding polymorphisms in the *CRP* gene (Figure 1-11) (Casas JP et al., 2008, Shen J and Ordovas JM, 2009). Statins, depending upon the doses, reduce hsCRP from 13 to 50% in a wide range of populations and the CRP-lowering effect of atrovastatin has been shown to be dose-dependent (Prasad K, 2006). Whether genetic polymorphism could influence the CRP-lowering effect of statins and thus cardiovascular outcomes has not so far been reported.

**Figure 1-11. The *CRP* gene structure, and the location of some polymorphisms**




Adapted from (Lee CC et al., 2009).

### 1.2.2 Kinesin-like protein 6

Kinesin-like protein 6 (KIF6) is a member of the superfamily of molecular motors

that are responsible for many of the major microtubule-dependent transport pathways in neuronal and non-neuronal cells (Schnapp BJ, 2003). Kinesins share a conserved motor domain that interacts with microtubules and a nonconserved tail domain that interacts with a specific cargo either directly or through an adaptor protein (Schnapp BJ, 2003). KIF6 is expressed in many tissues and cell types, including vascular cells (Su AI et al., 2002). A Trp719Arg polymorphism in *KIF6* has been found to be associated with CHD incidence in both males and females in various large population based studies (Morrison AC et al., 2007, Shiffman D et al., 2008), although the role of kinesins in CHD and, more specifically, the roles of *KIF6* and the Trp719Arg variant in CHD are not understood. This polymorphism has also been shown to influence the response to statin treatment in regard to clinical event benefit. Carriers of the KIF6 risk variant 719Arg received a substantially greater benefit from statin therapy resulting in significantly fewer serious fatal or nonfatal coronary events in multiple prospective studies than noncarriers (Iakoubova OA et al., 2008a, Iakoubova OA et al., 2008b). In the PROVE IT-TIMI 22 study, although carriers of 719Arg had significantly greater benefit from intensive statin therapy than noncarriers (absolute risk reduction: 10.0% in carriers vs. 0.8% in noncarriers), carriers and non-carriers did not differ in median LDL-C, triglyceride, or CRP levels at baseline or during the study, suggesting the superior benefit is due to a mechanism distinct from lipid or CRP lowering (Iakoubova OA et al., 2008a).

### 1.2.3 A disintegrin-like and metalloproteinase with thrombospondin motifs

The ADAMTS1 (a disintegrin-like and metalloproteinase with thrombospondin motifs) matrix metalloproteinase is a protein that has been shown to cleave versican, a key proteoglycan that regulates vascular smooth muscle cell migration and contributes to the structural integrity of the fibrous cap in atherosclerotic lesions

(Apte SS, 2009, Hu M et al., 2009). It has been reported that ADAMTS1 may promote atherogenesis by cleaving extracellular matrix proteins, promoting vascular smooth muscle cell (VSMC) migration (Jonsson-Rylander AC et al., 2005) and modifying the extracellular location of the matrix inhibitor tissue factor pathway inhibitor-2 (TFPI-2) to disrupt the remodeling machinery (Torres-Collado AX et al., 2006).

Recently, the variant allele of Ala227Pro polymorphism in the *ADAMTS1* gene has been found to be associated with increased CHD risk and greater benefit with pravastatin therapy in reducing fatal CHD or nonfatal myocardial infarction (MI) in 2421 male subjects from the CARE (Cholesterol and Recurrent Events) study and in 1565 male male subjects from the WOSCOPS (West of Scotland Coronary Prevention Study) (Sabatine MS et al., 2008). In men not on pravastatin, those homozygous for the 227Pro allele of *ADAMTS1* have a nearly 2-fold increased risk of CHD events compared with noncarriers. In this high-risk group, treatment with pravastatin is highly efficacious, reducing the odds of fatal coronary disease or nonfatal MI by approximately 75%, as compared with 25% in noncarriers or heterozygotes (Sabatine MS et al., 2008). It is possible that the ADAMTS1 227Pro allele leads to increased cleavage of versican, promotion of atherosclerosis, and plaque instability, and hence greater risk of CHD. In individuals homozygous for the 227Pro allele, the greater benefit of statins may be either due to enhanced inhibition of ADAMTS-1 via statin-mediated pathways, or simply because in such individuals the effect of statins on plaque stabilization is particularly important.

### 1.2.4 Others

More and more genetic variants have recently been identified to be associated with

cardiovascular risk and various manifestations of atherosclerosis (Humphries SE et al., 2008, Jang Y et al., 2008, Remmler C and Cascorbi I, 2008, Trompet S et al., 2007), which may influence the beneficial effects from treatment with statins through various pathways and may be worthy of more attention in future studies.

A growing body of data indicates that vitamin D deficiency is associated with coronary risk factors and adverse cardiovascular outcomes. Vitamin D deficiency activates the renin-angiotensin-aldosterone system and can predispose to hypertension and left ventricular hypertrophy (Judd SE and Tangpricha V, 2009, Lee JH et al., 2008). In addition, vitamin D deficiency causes an increase in parathyroid hormone, which increases insulin resistance and is associated with diabetes, hypertension, inflammation, and increased cardiovascular risk (Judd SE and Tangpricha V, 2009, Lee JH et al., 2008). Vitamin D and cholesterol have the same pre-cursor, 7-dehydrocholesterol. Statins inhibit synthesis of 7-dehydrocholesterol by inhibiting HMGCR, therefore a reduction in Vitamin D synthesis with statin treatment may be expected. However, no study has been reported a vitamin D lowering effect of statins, in contrast, recent studies have shown that some potent statins increase the vitamin D level in the blood, which may contribute to the beneficial effect of statins in the prevention of atherosclerosis and coronary artery disease (CAD), although the exact mechanism of this action is still unclear (Grimes DS, 2009, Perez-Castrillon JL et al., 2007, Yavuz B et al., 2009). Vitamin D receptor (VDR) is present in most tissues and polymorphisms in this gene may affect the association between vitamin D level, CHD risk and beneficial effects of statins.

### 1.3 Genetic influences on statin-induced adverse effects

The statins have an excellent safety profile in large clinical trials, but some patients

may be more susceptible to serious adverse drug reactions, especially when high doses are used (Rosenson RS, 2004). The withdrawal of cerivastatin due to the increased risk of rhabdomyolysis compared to other statins highlighted concerns about the myotoxic potential of this class of drugs (Furberg CD and Pitt B, 2001). Rhabdomyolysis is generally defined as creatine kinase (CK) elevations greater than 10 times the upper limit of normal (ULN) accompanied by muscle symptoms and the presence of urinary myoglobin indicated by brown-colored urine (Pasternak RC et al., 2002). Less serious muscle symptoms such as muscle pain and weakness, with or without CK elevation, are more common than rhabdomyolysis and may affect 1% to 5% of patients (Thompson PD et al., 2003). The risk for myopathy is increased by certain predisposing factors, such as advanced age, female gender, complex medical problems, including hypothyroidism or impaired renal function and certain genetic factors (Joy TR and Hegele RA, 2009, Thompson PD et al., 2003).

The reason for statin-related myopathy in some patients is largely unknown, although various mechanisms have been proposed to explain statin myotoxicity including altered pharmacokinetics due to drug metabolism or drug-drug interactions, physiochemical properties of the drugs, effects on metabolic end products such as coenzyme Q10, and interference with metabolic pathways regulating muscle repair (Ruano G et al., 2007). Polymorphisms in candidate genes related to these mechanisms including genes involved in the pharmacokinetics of statins, metabolism of coenzyme Q10 and serotonin pain receptors were inconsistently associated with statin-induced myotoxicity (Table **1-15**) (Joy TR and Hegele RA, 2009, Vladutiu GD, 2008).

**Table 1-15. Association of genetic polymorphisms with statin-induced myopathy**

| Polymorphism | Statins | Outcomess | References |
|---|---|---|---|
| ***Statin pharmacokinetics related genes*** | | | |
| CYP3A5 (*3) | A | Patients with homozygous *3 had greater serum CK levels than heterozygous for *3 | (Veenstra DL et al., 2005). |
| CYP2D6 (*4) | A | The frequency of the *4 allele was higher in cases than that in controls (50% vs. 28%, P<0.05) | (Frudakis TN et al., 2007) |
| ABCB1 (1236C>T, 2677G>A/T, 3435C>T) | S | The T-non-G-T haplotype was less frequent in patients with myalgia than those without ADR to simvstatin (20% vs. 41.4%, P=0.03) | (Fiegenbaum M et al., 2005b) |
| SLCO1B1 (521T>C) | S | More than 60% of the myopathy cases could be attributed to the C variant. | (Link E et al., 2008) |
| | A, S, Pra | The C variant allele was associated with mild statin-induced side effects | (Voora D et al., 2009) |
| ***Metabolic muscle disease related genes*** | | | |
| COQ2 (SNP1, SNP2) | Various | Increased risk of statin intolerance among homozygotes for the rare alleles of SNP1 and SNP2 genotypes, and the 2-SNP haplotype. | (Oh J et al., 2007) |
| CPT2 (S113L) PYGM (R50X) AMPD1 (Q12X, P48L) | Various | Frequencies of heterozygotes or homozygotes for mutations were 10% and 3% in patients with myopathy and without (P=0.04) | (Vladutiu GD et al., 2006) |
| ***Others*** | | | |
| HTR3B(rs2276307) HTR7 (rs1935349) | Various | The 2 variant alleles were significantly associated with the myalgia score. | (Ruano G et al., 2007) |

Abbreviations: ABCB1 = ATP-binding cassette B1; ADR = adverse drug reactions AMPD1 = adenosine monophosphate deaminase 1; COQ2 = coenzyme Q2 homolog; CPT2 = carnitine palmitoyltransferase II; CYP = cytochrome P450 enzyme; HTR = 5a-hydroxytryptamine receptor; PYGM = phosphorylase, glycogen, muscle (muscle glycogen phosphorylase); SLCO1B1 = Solute carrier organic anion transporter family, member 1B1.

However, a recent genome-wide scan from the SEARCH (Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine) participants taking the 80 mg dose of simvastatin and replicated in the HPS study with the 40 mg dose has

identified a single genetic marker associated with simvastatin induced myopathy, which was a non-coding SNP in *SLCO1B1* (Link E et al., 2008) that is in strong LD with the functional SNP 521T>C. More than 60% of the myopathy cases could be attributed to the 521C variant in the study and this finding is likely to apply to other statins since *SLCO1B1* polymorphisms affect the blood levels of most of statins. Most recently, a pharmacogenetic study examined whether common genetic polymorphisms that lead to reduced function in drug metabolizing enzymes or transporters would be associated with mild statin-induced side effects, particularly those without CK elevations in STRENGTH participants who were randomly assigned to 8 weeks of 10 mg/day atorvastatin, 20 mg/day simvastatin, or 10 mg/day pravastatin followed by 8 weeks of 80 mg/day atorvastatin, 80 mg/day simvastatin, and 40 mg/day pravastatin, respectively during the trial (Voora D et al., 2009). The *SLCO1B1* 521T>C polymorphism was associated with statin-induced side effects in a gene-dose dependent manner, and the carriers of the 521C variant allele were at a 2-fold relative risk of mild statin induced side effects, the majority of which had normal CK levels. Furthermore, this study also identified that the risk of the side effects was greatest in those 521C carriers assigned to simvastatin and negligible in those assigned to pravastatin, which was consistent with the pharmacokinetic observation in the study that the acid metabolite concentration of the drug was elevated in carriers who received simvastatin but not in subjects receiving pravastatin (Voora D et al., 2009). The results of this study expand the findings of the SEARCH trial and emphasize the crucial role of the elevated systemic exposures to statins and/or their active metabolites in statin-induced myopathy.

# Chapter 2 Aim of the study

Although the clinical efficacy of statins has been well established, there is a wide inter-individual variation in the lipid responses to statins, and pharmacogenetic studies have identified some genetic differences that contribute to the variation, but overall the results have been disappointing (Hu M et al., 2009, Kajinami K et al., 2004e). As more intensive reduction of LDL-C with statins is associated with greater reductions in cardiovascular events (Baigent C et al., 2005), it may be anticipated that optimizing the choice of statin and the dose for an individual will have favorable effects.

Rosuvastatin is one of most potent statins and it was recommended to use lower starting doses of this drug in 'Asian' patients due to evidence of higher systemic drug exposure in Asians compared to non-Asians (Po AL, 2007), but ethnic differences in efficacy have not been described. The functional SNP (421C>A, Lys141Gln) in *ABCG2* resulted in increased plasma concentrations of rosuvastatin (Keskitalo JE et al., 2009d, Zhang W et al., 2006b), and this is likely to play a major role in the ethnic differences observed in rosuvastatin pharmacokinetics because the 421A variant allele has a frequency of about 35% in Chinese and other East Asian subjects compared to 14% in Caucasians. However, the effect of this polymorphism on lipid response to rosuvastatin has not been reported. Previous studies have also identified polymorphisms in *HMGCR* (Chasman DI et al., 2004, Krauss RM et al., 2008) or *APOE* (Zintzaras E et al., 2009), being associated with LDL-C responses, but results have been inconsistent or the variants are uncommon in some ethnic groups. Non-genetic confounding factors such as drug adherence and changes in diet are also

likely to play an important role.

The present study was conducted to examine the effect of the *ABCG2* 421C>A SNP and other common polymorphisms in genes potentially related to the pharmacokinetics of statins or loci in the lipid metabolism pathways on the LDL-C response to rosuvastatin in Chinese patients with increased risk of CVD including some with FH. Attempts were made to minimize the influence of non-genetic factors by using a single drug dose for a relatively short time and excluding results from patients who reported poor adherence to therapy. Assessed phenotypic variables included age, gender, body mass index (BMI), waist circumference (WC), percentage of total body fat, baseline values of lipid parameters, smoking status (current smoker/nonsmoker), FH and other comorbidities. The primary endpoint was prospectively defined as the percentage reduction in LDL-cholesterol compared to a baseline level when on no lipid-lowering treatment, which could largely reduce the influence of the baseline levels. The genetic determinants of the percent change in total cholesterol, HDL-C, triglyceride and non-HDL-C were also assessed.

In addition, a secondary analysis was performed to examine the genetic determinants of some cardiovascular risk factors e.g. hsCRP, uric acid, baseline lipid and bilirubin levels in study patients.

# Chapter 3 Methodology

## 3.1 Patient recruitment

This study was approved by the Joint Clinical Research Ethics Committee of The Chinese University of Hong Kong and New Territories East Cluster (CUHK-NTEC) and all participants gave written informed consent before any study procedures were undertaken.

### 3.1.1 Inclusion criteria

Subjects aged ≥18 years with baseline low-density lipoprotein cholesterol (LDL-C) >2.6 mmol/L were eligible for inclusion if they were considered at increased risk of cardiovascular disease (CVD) because of a history of coronary heart disease (CHD), other clinical evidence of atherosclerosis, diabetes mellitus, calculated 10-year CHD risk score >20%, or having familial hypercholesterolaemia (FH). The diagnosis of FH was made on clinical criteria considered appropriate for Hong Kong Chinese patients (Mak YT et al., 1998).

### 3.1.2 Exclusion criteria

Patients in which baseline lipid levels were not available and who were unwilling to stop current lipid-lowering treatment or in whom interruption of treatment may be considered unsafe were not eligible to enter this study. Individuals with uncontrolled diabetes, hypertension or thyroid disease, or a history of hypersensitivity to statins, significant renal impairment, hepatic dysfunction, unexplained high (>3 ULN) serum creatine kinase (CK) or who had experienced a cardiovascular event within the 3 months before recruitment or who were unwilling to give additional blood samples

taken for DNA tests or who were those taking other drugs known to modify plasma lipids or to have an interaction with rosuvastatin e.g. corticosteroids, cyclosporine etc were excluded from this study.

### 3.1.3 Patients with hypercholesterolaemia

Patients involved in the study were mainly recruited from the Lipid Clinic of the Prince of Wales Hospital and the Drug Development Centre (DDC) of the Chinese University of Hong Kong. They were recruited if they fulfilled the above criteria and were being treated or eligible to be treated with rosuvastatin 10 mg daily according to the local Hospital Authority (HA) guideline. For patients who had previously received lipid-lowering treatment they were asked to stop their lipid-lowering medication for at least 4 weeks to get the baseline lipid levels examined before taking rousvastatin 10 mg. For those patients in whom suspending lipid treatment were not appropriate, they were invited to join the study only if the baseline lipid levels on no treatment for at least 4 weeks could be obtained from previous medical records. All patients were advised to continue with their usual diet and other aspects of lifestyle during the study and education on the reasons for taking lipid lowering treatment and importance of maintaining treatment was given to improve drug compliance.

Participants were interviewed at the DDC at a scheduled follow-up visit after at least 4 weeks treatment with rosuvastatin (more than 97% of patients had at least 6 weeks treatment with rosuvastatin). Subjects were asked to fast for 12 hours before their visit. Routine assessments, anthropometric measurement, drug compliance assessment, collection of fasting blood samples for routine blood tests and DNA extraction were performed at the visit. At this time aliquots of heparin plasma and

spot urine samples were stored for future analysis. Routine assessments included sitting blood pressure and heart rate which were measured in triplicate after 10 minutes resting. Anthropometric measurements included body weight, body height, waist circumference (WC), hip circumference, and percentage of total body fat. Using an electronic scale, participants were weighed to the nearest 0.1 kg after removing their shoes and outdoor clothing. Height was measured to the nearest 0.1 cm. The body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Hip and waist circumference were also obtained using standardized protocols. Percentage of total body fat was measured using an impedance device (TANITA Body Composition Analyzer BF-350, TANITA Corporation, Tokyo, Japan). Adherence to therapy was assessed by asking patients about their medication-taking behavior in a non-judgmental manner and tablet counting and subjects with poor compliance with therapy (if they took <80% or >120% of the prescribed number of tablets) were excluded unless they agreed to improve compliance and had a further assessment after another 4 weeks treatment.

Some subjects were recruited from the Alice Ho Mui Ling Nethersole Hospital at Tai Po. All these subjects were patients with primary hypercholesterolaemia and high cardiovascular risk, who had been involved in a 12-week, randomized, open-label, parallel-group study to assess the efficacy and safety of starting doses of rosuvastatin and atorvastatin in Chinese patients in Hong Kong (Zhu JR et al., 2007).

### 3.1.4 Patients with rheumatoid arthritis

A small group of patients (n=36) with rheumatoid arthritis (RA) who had participated in a double-blind, placebo-controlled, randomized study to assess the effect of rosuvastatin 10 mg on carotid intima-media thickness (CIMT) and arterial stiffness

(ClinicalTrials.gov ID: NCT00555230) were recruited from the Rheumatology Clinic of the Prince of Wales Hospital. These patients were clinically diagnosed to have RA for at least 6 months before enrollment and had baseline total cholesterol levels of ≤6.2 mmol/L.

### 3.2 Routine biochemistry tests

All routine biochemistry tests e.g. lipid profiles, renal and liver function tests etc were performed in the Department of Chemical Pathology laboratory at the Prince of Wales Hospital, which has international laboratory accreditation.

#### 3.2.1 Lipids

Total cholesterol level was measured by the enzymatic method (Centrichem chemistry System, Baker Instruments Co. Allentown). Esterified cholesterol is hydrolyzed by cholesterol esterase to release free cholesterol that is further oxidized by cholesterol oxidase to release hydrogen peroxide, which then reacts with a chromogen to give a measurable color change at 540nm. High-density lipoprotein cholesterol (HDL-C) level was determined by using the fractional precipitation of dextran sulphate with manganous ion. Triglycerides levels were measured by the glyceryl dehydrogenase reaction following the hydrolysis of the triglyceride (Centrichem Chemistry System, Baker Instruments Co., Allentown). LDL-C level was estimated by using the Friedewald's formula (Friedewald WT et al., 1972) or directly measured if triglycerides levels were greater than 4.5mmol/L.

#### 3.2.2 Fasting plasma glucose

Fasting plasma glucose (FPG) level was measured using a standard glucose oxidase method.

### 3.2.3 Laboratory safety parameters

Renal function tests (RFT) included sodium, potassium, urea, creatinine. Plasma electrolytes were measured by ion-selective electrodes on a parallel multichannel analyzer (American Monitor, IND, USA). Creatinine was measured using the Jaffe method on a Beckman Astra-8 Chemistry analyzer (Beckman, Brea, CA). The CK levels and the liver function tests (LFT) including total protein, albumin, total bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT) were measured by standard methods.

### 3.2.4 Uric acid

Plasma uric acid was measured using the uricase method on the Dade Dimension clinical chemistry system (Dade International Inc, DE, USA).

## 3.3 High-sensitive CRP measurement

The plasma high-sensitivity C-reactive protein (hsCRP) on-treatment concentration was determined by an immunonephelometric method (Siemens Dade Behring CardioPhase hsCRP assay) on Siemens BN ProSpec® System with the help of another PhD student, Michael Lee. The detection limit was 0.146 mg/L, and the measurement range was 0.146 –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.

## 3.4 Selection of genetic polymorphisms and genotyping

### 3.4.1 Selection of genetic polymorphisms

With extensive literature searching, a total of 135 common polymorphisms in 62 candidate genes/loci potentially related to the pharmacokinetics and pharmacodynamics of statin, lipid metabolism and metabolic disorders were selected based on previous publications (Table **3-1**). Two single nucleotide polymorphisms (SNPs) failed for genotyping, 1 SNP was not in Hardy-Weinberg equilibrium (HWE) and 7 SNPs with a minor allele frequency (MAF) less than 1% were excluded from the analysis as shown in Table **3-1**. In addition, another 5 SNPs in three genes related to uric acid transport were also selected due to special interest (Table **3-2**).

**Table 3-1. List of 135 SNPs examined in the study**

| Locus | Candidate Genes / Nearby Genes | Symbol | Polymorphism (Amino acid changes) | dbSNP |
|---|---|---|---|---|
| ***Genes potentially involved in statin pharmacokinetics*** | | | | |
| 7q21.1 | ATP-binding cassette, sub-family B, member 1 | *ABCB1* | 2677G>T | rs2032582 |
| 7q21.1 | ATP-binding cassette, sub-family B, member 1 | *ABCB1* | 3435C>T | rs1045642 |
| 7q21.1 | ATP-binding cassette, sub-family B, member 1 | *ABCB1* | 1236 C>T | rs1128503 |
| 10q24 | ATP-binding cassette, sub-family C, member 2 | *ABCC2* | 4544G>A (Cys1515Tyr) | rs8187710* |
| 10q24 | ATP-binding cassette, sub-family C, member 2 | *ABCC2* | 1446C>G (Thr482Thr) * | |
| 10q24 | ATP-binding cassette, sub-family C, member 2 | *ABCC2* | 1249G>A (Val417Ile) | rs2273697 |
| 10q24 | ATP-binding cassette, sub-family C, member 2 | *ABCC2* | 3563T>A (Val1188Glu) | rs8187694* |
| 4q22 | ATP-binding cassette, subfamily G, member 2 | *ABCG2* | 421C>A (Gln141Lys) | rs2231142 |
| 4q22 | ATP-binding cassette, subfamily G, member 2 | *ABCG2* | 34G>A | rs2231137 |
| 10q24 | Cytochrome P450 family 2, subfamily C, polypeptide 19 | *CYP2C19* | *3, 636G>A | |
| 10q24 | Cytochrome P450 family 2, subfamily C, polypeptide 19 | *CYP2C19* | *2, 681G>A | |
| 10q24 | Cytochrome P450 family 2, subfamily C, polypeptide 9 | *CYP2C9* | *3, 1075A>C | rs1057910 |
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | 188C>T | |
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | 1934G>A | |
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | 1846G>A/T | |
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | 2938C>T | |

| | | | | |
|---|---|---|---|---|
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | 4268G>C | |
| 22q13.1 | Cytochrome P450 family 2, subfamily D, polypeptide 6 | *CYP2D6* | *5, deletion | |
| 7q22.1 | Cytochrome P450 family 3, subfamily A, polypeptide 4 | *CYP3A4* | *1G, 20230G>A | rs2242480 |
| 7q22.1 | Cytochrome P450 family 3, subfamily A, polypeptide 5 | *CYP3A5* | *3 | rs776746 |
| 7q22.1 | Cytochrome P450, family 3, subfamily A, polypeptide 5, pseudogene 1 | *CYP3AP1* | *3, -44G>A | |
| 1q23-q25 | Flavin containing monooxygenase 3 | *FMO3* | Glu158Lys | rs2266782 |
| 1q23-q25 | Flavin containing monooxygenase 3 | *FMO3* | Val 257Met | rs1736557 |
| 1q23-q25 | Flavin containing monooxygenase 3 | *FMO3* | Glu 308Gly | rs2266780 |
| 8p22 | N-acetyltransferase 2 | *NAT2* | *6,590G>A (Arg197Gln) | rs1799930 |
| 8p22 | N-acetyltransferase 2 | *NAT2* | *7,857G>A (Gly286Glu) | rs1799931 |
| 14q24.1 | Solute carrier family 10, member 1 | *SLC10A1 (NTCP)* | *2,800C>T (Ser267Phe) | rs2296651 |
| 6q26 | Solute carrier family 22 (organic cation transporter), member 1 | *SLC22A1 (OCT1)* | 480C>G (Phe160Leu) | rs683369 |
| 6q26 | Solute carrier family 22 (organic cation transporter), member 1 | *SLC22A1 (OCT1)* | 1022C>T (Pro341Leu) | rs2282143 |
| 6q26 | Solute carrier family 22 (organic cation transporter), member 1 | *SLC22A1 (OCT1)* | 1222A>G (Met480Val) | rs628031 |
| 6q26 | Solute carrier family 22 (organic cation transporter), member 2 | *SLC22A2 (OCT2)* | 808G>T (Ala270Ser) | rs316019 |
| 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* | 388A>G (Asn130Asp) | rs2306283 |
| 12p12 | Solute carrier organic anion transporter family, member 1B1 | *SLCO1B1* | 521T>C (Val174Ala) | rs4149056 |

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|---|---|---|---|---|
| 12p12 | Solute carrier organic anion transporter family, member 1B1 | *SLCO1B1* | 571T>C | rs4149057 |
| 12p12 | Solute carrier organic anion transporter family, member 1B1 | *SLCO1B1* | 597 C>T | rs2291075 |
| 12p12 | Solute carrier organic anion transporter family, member 1B1 | *SLCO1B1* | -11187 G>A | rs4149015 |
| 12p12 | Solute carrier organic anion transporter family, member 1B3 | *SLCO1B3* | 344T>G | rs4149117 |
| 12p12 | Solute carrier organic anion transporter family, member 1B3 | *SLCO1B3* | 699G>A | rs7311358 |
| 11q13 | Solute carrier organic anion transporter family, member 2B1 | *SLCO2B1* | 1457C>T (Ser486Phe) | rs2306168 |
| 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A1 | *UGT1A1* | *60, -3279T>G | rs4124874 |
| 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A1 | *UGT1A1* | *6,211G>A (Gly71Arg) | rs4148323 |
| 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A1 | *UGT1A1* | (TA)6>7, *28 | |
| 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A6 | *UGT1A6* | 541A>G (Thr181Ala) | rs2070959 |
| 2q37 | UDP glucuronosyltransferase 1 family, polypeptide A6 | *UGT1A6* | 552A>C (Arg184Ser) | rs1105879 |
| 4q13 | UDP glucuronosyltransferase 2 family, polypeptide B7 | *UGT2B7* | *2,802C>T (His268Tyr) | rs7439366 † |
| 4q13 | UDP glucuronosyltransferase 2 family, polypeptide B7 | *UGT2B7* | -327A>G | rs7662029 |
| 4q13 | UDP glucuronosyltransferase 2 family, polypeptide B7 | *UGT2B7* | 211G>T (Ala71Ser) | rs12233719 |
| 4q13 | UDP glucuronosyltransferase 2 family, polypeptide B7 | *UGT2B7* | −161T>C | rs7668258 |
| ***Genes involved in statin pharmacodynamics*** | | | | |
| 9q22-q31 | ATP-binding cassette, subfamily A, member 1 | *ABCA1* | T>C | rs2472384 |
| 9q22-q31 | ATP-binding cassette, subfamily A, member 1 | *ABCA1* | 1051G>A (Arg219Lys) | rs2230806 |
| 9q22-q31 | ATP-binding cassette, subfamily A, member 1 | *ABCA1* | 5155G>A (Arg1587Lys) | rs2230808 |

| | | | | |
|---|---|---|---|---|
| 9q22-q31 | ATP-binding cassette, subfamily A, member 1 | *ABCA1* | 69C>T | rs1800977 |
| 9q22-q31 | ATP-binding cassette, subfamily A, member 1 | *ABCA1* | 378G>C | rs1800978 |
| 2p21 | ATP-binding cassette, subfamily G, member 5 | *ABCG5* | Q604E | rs6720173 |
| 2p21 | ATP-binding cassette, subfamily G, member 8 | *ABCG8* | Y54C | rs4148211 |
| 2p21 | ATP-binding cassette, subfamily G, member 8 | *ABCG8* | T400K | rs4148217 |
| 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 ‡ |
| 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 |
| 16p13.11 | Acyl-CoA synthetase medium-chain family member 3 | *ACSM3(SAH)* | 1077G>C (Lys359Asn) | rs5716 ‡ |
| 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)* | -962 ins/del | |
| 3q27 | Adiponectin | *ADIPOQ* | –11377 C>G | rs266729 |
| 3q27 | Adiponectin | *ADIPOQ* | 45T>G | rs2241766 |
| 3q27 | Adiponectin | *ADIPOQ* | 276G>T | rs1501299 |
| 1p31.1-p22.3 | Angiopoietin-like 3 | *ANGPTL3* | C>G | rs1748195 |
| 1q21-q23 | apolipoprotein A-II | *APOA2* | -265T>C | rs5082 |
| 11q23 | Apolipoprotein A-V | *APOA5* | -1131T>C | rs662799 |
| 2p24-p23 | apolipoprotein B | *APOB* | Xba I, 7673C>T | rs693 |
| 2p24-p23 | apolipoprotein B | *APOB* | Ins/Del | |
| 19q13.2 | Apolipoprotein E | *APOE* | 472C>T (Cys158Arg) | rs7412 |
| 19q13.2 | Apolipoprotein E | *APOE* | 334T>C (Cys112Arg) | rs429358 |
| 19q13 | Apolipoprotein E/C-I/C-IV/C-II | *APOE/C1 /C4/C2* | A>G | rs4420638 |

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|---|---|---|---|---|
| 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>A | rs3764261 |
| 16q21 | Cholesteryl ester transfer protein, plasma | *CETP* | | rs12149545 |
| 16q21 | Cholesteryl ester transfer protein, plasma | *CETP* | -971G>A | rs4783961 |
| 16q21 | Cholesteryl ester transfer protein, plasma | *CETP* | 16G>A (Ile405Val) | rs5882 |
| 16q21 | Cholesteryl ester transfer protein, plasma | *CETP* | TaqIB | rs708272 |
| 16q21 | Cholesteryl ester transfer protein, plasma | *CETP* | -629C>A | rs1800775 |
| 1q21-q23 | C-reactive protein, pentraxin-related | *CRP* | 3872G>A | rs1205 |
| 1q21-q24 | C-reactive protein, pentraxin-related | *CRP* | 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 |
| 1q41-q42 | 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 |
| 16q12.2-q13 | Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1 | *HERPUD1* | G>A | rs9989419 |
| 5q13.3-q14 | 3-hydroxy-3-methylglutaryl-Coenzyme A reductase | *HMGCR* | C>T | rs3846662 |
| 5q13.3-q15 | 3-hydroxy-4-methylglutaryl-Coenzyme A reductase | *HMGCR* | A>T | rs12654264 |
| 12q24.2 | Hepatocyte nuclear factor 1 alpha | *HNF1a* | Ile27Leu | rs1169288 |

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|---|---|---|---|---|
| 12q24.2 | Hepatocyte nuclear factor 1 alpha | *HNF1a* | Ala98Val | rs1800574 * |
| 6p21.2 | Kinesin family member 6 | *KIF6* | 2155T>C (Trp719Arg ) | rs20455 |
| 16q22.1 | Lecithin-cholesterol acyltransferase | *LCAT* | A>G | rs255052 |
| 19p13.3 | Low density lipoprotein receptor | *LDLR* | 2052T>C (Val653Val) | rs5925 |
| 19p13.3 | Low density lipoprotein receptor | *LDLR* | 1866C>T (Asn591Asn) | rs688 |
| 19p13.3 | Low density lipoprotein receptor | *LDLR* | T>C | rs1529729 |
| 19p13.3 | Low density lipoprotein receptor | *LDLR* | 44857C>T | rs1433099 |
| 19p13.3 | Low density lipoprotein receptor | *LDLR* | 44964A>G | rs2738466 |
| 1p31 | Leptin receptor | *LEPR* | Gln223Arg | rs1137101 |
| 1p31 | Leptin receptor | *LEPR* | Lys109Arg | rs1137100 |
| 15q21-q23 | Lipase, hepatic | *LIPC* | C>T | rs1532085 |
| 15q21-q23 | Lipase, hepatic | *LIPC* | -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 |
| 7q11.23 | MLX interacting protein-like | *MLXIPL* | 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* | 1679C>G (Leu272Leu) | rs2072183 |
| 7p13 | NPC1 (Niemann-Pick disease, type C1, gene)-like 1 | *NPC1L1* | 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 |

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|---|---|---|---|---|
| 1p32.3 | Proprotein convertase subtilisin/kexin type 9 | *PCSK9* | c.799 + 3A>G | rs2495477 |
| 1p32.3 | Proprotein convertase subtilisin/kexin type 9 | *PCSK9* | 1420G>A (Val474Ile) | rs562556 |
| 1p32.3 | Proprotein convertase subtilisin/kexin type 9 | *PCSK9* | 2009G>A (Glu670Gly) | rs505151 |
| 7q21.3 | Paraoxonase 1 | *PON1* | Gln192Arg | rs662 |
| 22q12-q13.1; 22q13.31 | Peroxisome proliferator-activated receptor alpha | *PPARA* | Leu162Val | rs1800206 * |
| 22q12-q13.1; 22q13.31 | Peroxisome proliferator-activated receptor alpha | *PPARA* | A>G | rs4253776 * |
| 22q12-q13.1; 22q13.31 | Peroxisome proliferator-activated receptor alpha | *PPARA* | C>G | rs4253778 * |
| 3p25 | Peroxisome proliferator-activated receptor gamma | *PPARG* | 167C>G (Pro12Ala) | rs1801282 |
| 4p15.1 | Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha | *PPARGC1A* | 1546G>A | rs8192678 |
| 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 |
| 11q13 | Uncoupling protein 3 (mitochondrial, proton carrier) | *UCP3* | -55C>T | rs1800849 |

* MAF <1%; † Depart from Hardy-Weinberg equilibrium; ‡ Failed for genotyping.

**Table 3-2. List of additional 5 SNPs potentially related to uric acid transport**

| Locus | Candidate Genes / Nearby Genes | Symbol | Polymorphism (Amino acid changes) | dbSNP |
|---|---|---|---|---|
| 17q11.2-q12 | Chemokine (C-C motif) ligand 2 | CCL2 (MCP-1) | -3813C>T | rs1860188 |
| 4p16-p15.3 | Solute carrier family 2 (facilitated glucose transporter), member 9 | SLC2A9 (GLUT9) | T>C | rs1014290 |
| 4p16-p15.3 | Solute carrier family 2 (facilitated glucose transporter), member 9 | SLC2A9 (GLUT9) | T>C | rs12510549 |
| 11q13.1 | Solute carrier family 22 (organic anion/urate transporter), member 12 | SLC22A12 (URAT1) | G>T (His142His) | rs893006 |
| 11q13.1 | Solute carrier family 22 (organic anion/urate transporter), member 12 | SLC22A12 (URAT1) | 426C>T | rs11231825 |

### 3.4.2 DNA extraction

The DNA samples were extracted from the blood specimens by the traditional phenol chloroform method as below.

1. 10 ml peripheral venous blood was collected, centrifuged at 3000 rpm, 4°C for 10 minutes and the plasma was aspirated off.
2. 12 ml of lysis buffer (10 mM KHCO3, 155 mM NH4Cl, 0.1 mM EDTA) was added to the blood and placed on ice for 15 minutes. After centrifuging at 3000rpm, 4°C for 20 minutes, the supernatant was then decanted.
3. The cell pellet was re-suspended in 12 ml lysis buffer and step 2 was repeated.
4. The cell pellet was resuspended in 3ml of TE buffer, then 600ul of 10% SDS and 50ul proteinase K (20mg/ml) was added and incubated at 65°C in a water bath overnight.
5. 1.2 ml of 6M NaCl was added to the overnight digested sample with vigorous

shaking for a few seconds. Then, the sample was centrifuged at 3000rpm, 4°C for 10 minutes.

6. The supernatant was decanted to a clean test tube and 1 ml of TE equilibrated phenol was added and mixed well.
7. One milliliter of chloroform was then added and mixed well. The mixture was centrifuged at 3000 rpm at 4°C for 10 minutes and the upper aqueous layer was transferred to a new test tube.
8. Two milliliters of chloroform was added and mixed well. The mixture was centrifuged at 3000rpm, 4°C for 10 minutes. The upper aqueous layer was transferred to a new test tube for DNA precipitation.
9. After two volumes of ice cold absolute alcohol was added and well-mixed, DNA started to precipitate as a loose mass.
10. The precipitated DNA was transferred to a 1.5 ml Eppendorf tube and washed with 1 ml 70% alcohol. After being centrifuged for 10 minutes at 3,000 rpm, the DNA was pelleted to the bottom of the Eppendorf tube and the alcohol was drained off.
11. The DNA was air-dried for about 10 minutes and then thoroughly dissolved in 200 – 400 µl TE buffer. The optical density (OD) at 260 nm and 280 nm were measured to determine the quantity and quality of the DNA. A ratio of 1.8 was regarded as good quality.

### 3.4.3 Genotyping

The genotyping of the polymorphisms in this study was mainly performed in the Genome Research Centre at The University of Hong Kong using the SEQUENOM MassARRAY system in 2007 and 2008 except for some SNPs that the primers of which could not be compatible with the optimized assay designs.

### 3.4.3.1 Genotyping by the SEQUENOM MassARRAY

With the completion of the Human Genome Project and International HapMap Project, a great number of rapid, and cost effective technologies for SNP analysis have been developed, particularly those high-throughput systems e.g. the mass spectroscopy-based MassARRAY system (Sequenom), the fluorescent microarray-based systems (Affymetrix) and the fluorescent bead-based technologies (Luminex, Illumina, Q-dot), etc.

The iPLEX Gold assay on the MassARRAY® Platform developed by Sequenom Inc. combines the benefits of robust single-base primer extension biochemistry with the sensitivity and accuracy of MassARRAY MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry detection, which can analyse maximally up to 40 SNPs simultaneously within a single well of a 384 well plate (www.sequenom.com).The iPLEX® Gold assay is based on multiplex PCR followed by a single base primer extension reaction. After the PCR (polymerase chain reaction), remaining nucleotides are deactivated by Shrimp Alkaline Phosphatase (SAP) treatment. The single base primer extension step is performed, and the primer extension products analyzed using MALDI TOF MS. Finally, the SpectroCHIP is placed into the MALDI-TOF, and the mass and their correlating genotypes are determined in real time with MassARRAY RT software (www.sequenom.com). This new technology is available in the Genome Research Centre at The University of Hong Kong (www.genome.hku.hk), where we had the majority of the SNPs genotyped.

### 3.4.3.2 TaqMan polymerase chain reaction allelic discrimination

The TaqMan 5' nuclease assay is an established method widely used for real-time PCR quantification. The *ABCG2* 421C>A polymorphism was genotyped in some patients at one stage before all samples were sent to the Genome Research Centre at The University of Hong Kong due to its importance. For some other SNPs that were not compatible with the assay designs of the MassARRAY system and some newly-selected supplemental SNPs, Taqman assays (Applied Biosystems, Foster City, CA, US) were applied. A total of 16 SNPs were genotyped using the Taqman assays (Table **3-3**).

**Table 3-3. SNPs genotyped using Taqman assays in the study**

| Gene | SNP | Drug metabolism genotyping assay | Genotyping assay | Assays-by-design service |
|---|---|---|---|---|
| ABCG2 | 421C>A | C_15854163_70 | | |
| CYP2C9 | 1075A>C (*3) | C_27104892_10 | | |
| CYP2D6* | 188C>T | C_11484460_40 | | |
| | 1934G>A | C_27102431_D0 | | |
| | 1846G>A | C_30634117D_30 | | |
| | 1846G>T | C_30634117C_20 | | |
| | 2938C>T | C_27102425_10 | | |
| | 4268G>C | C_27102414_10 | | |
| SLCO1B1 | -11187G>A | C_32325356_10 | | |
| SLCO2B1 | 1457C>T | C_16193013_20 | | |
| KIF6 | 2155T>C | | C_3054799_10 | |
| PCSK9 | 158C>T | | C_2018187_10 | |
| | 658-7C>T | | C_2018176_10 | |
| | 799+3A>G | | C_2018175_10 | |
| | 1420G>A | | C_998751_10 | |
| | 2009G>A | | C_998744_10 | |
| SAH | | | | -962 I/D |

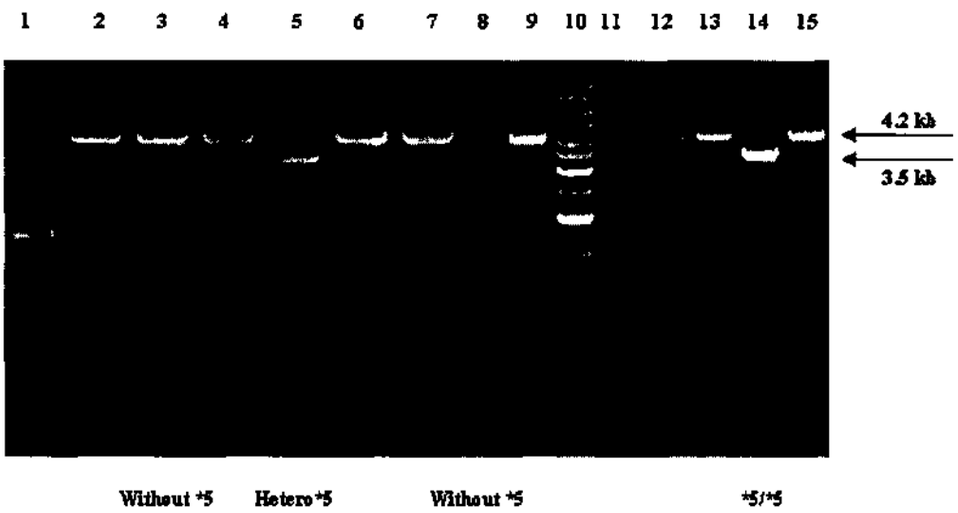
* The 5kb of the cyp2D6 gene was amplified first & then diluted 100 fold with water before performing the Taqman assay.

### 3.4.3.3 Polymerase chain reaction

Polymerase chain reaction was performed to detect the deletion of the *CYP2D6* gene (*5) as described previously (Stamer UM et al., 2002). The primers 5' -F (ACC GGG CAC CTG TAC TCC TCA) and 5' -R (GCA TGA GCT AAG GCA CCC AGA CCG TCT AGT GGG AGA CAA AC) were used to amplify a 3.5-kb PCR product in the presence of allele *5 in contrast to a 4.2-kb PCR product without *5 (Figure 3-1).

All the reagents for PCR reaction were from Roche Diagnostics Mannheim Germany. The PCR reaction was performed with PCR machine 9700.

**Figure 3-1. Polymerase chain reaction for detection of *CYP2D6* *5**

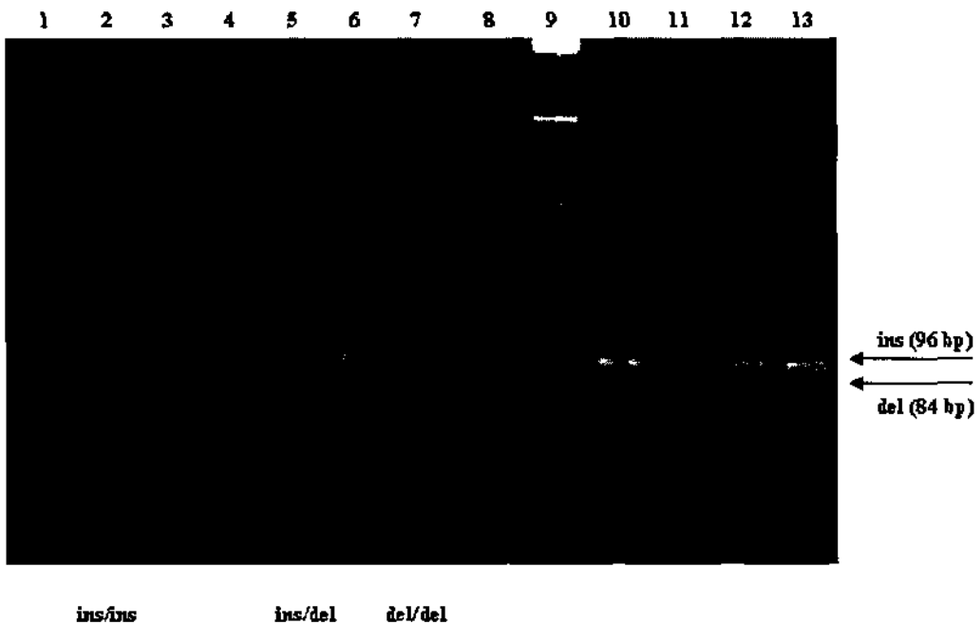



Lane 5 shows heterozygous *5; lane 14 shows homozygous *5, other lanes show absence of *5 apart from lanes 1 and 10 showing controls. Presence of *5 yielded 3.5 kb band (lower arrow) whereas without *5 allele yielded a 4.2 kb band (upper arrow).

For the *APOB* ins/del polymorphism, the primers 5' -F (CAG CTG GCG ATG GAC

CCG CCG A) and 5' -R (ACC GGC CCT GGC GCC CGC CAG CA) were used to amplify a 96 bp PCR product in the presence of allele ins in contrast to a 84 bp PCR product with del allele (Figure **3-2**) (Choong ML et al., 1999).

**Figure 3-2. Polymerase chain reaction for detection of *APOB* ins/del polymorphism**




Lanes 5, 8 and 11 show heterozygous ins/del; lane 7 shows homozygous del, other lanes show homozygous ins apart from lane 9 showing controls. Presence of del yielded 84 bp band (lower arrow) whereas presence of ins allele yielded a 96 bp band (upper arrow).

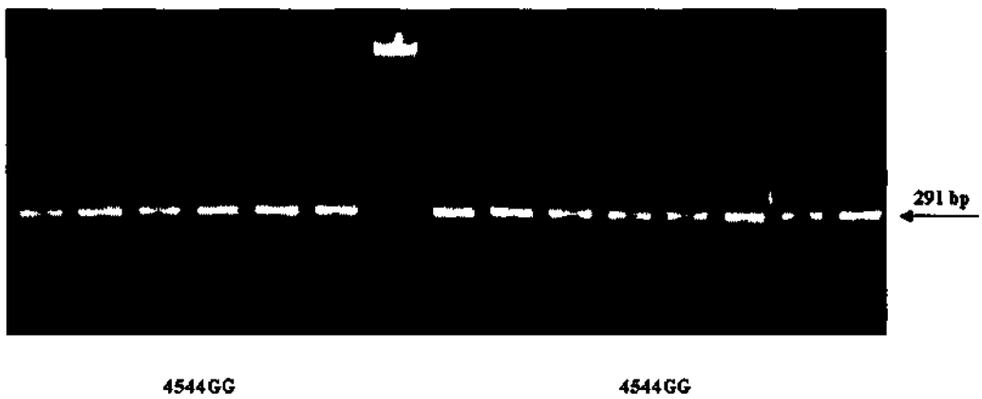
### 3.4.3.4 Polymerase chain reaction - restriction fragment length polymorphism

Traditional polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) tests were performed for determining polymorphisms of *ABCC2* 4544G>A and *APOE* 334T>C. For each polymorphism, about 100 ng DNA template was amplified with specific forward and reverse primers as described previously

(Izzedine H et al., 2006).

7 µl of the amplified 449-bp PCR product for *ABCC2* 4544G>A were digested with 2 U restriction enzyme Rsa I at 37°C overnight in a total volume of 20 µl. The digested mixture was run with 3% agarose gel. The mutated allele of A should have the restriction site giving bands of 95 and 196 bp while the wild-type allele of G gave a band of 291 bp. No variant allele was found in our samples (Figure **3-3**).

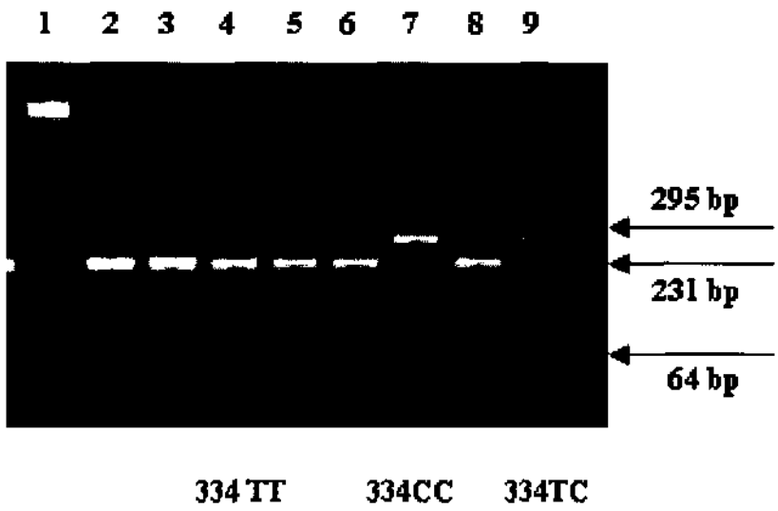
**Figure 3-3. PCR-RFLP for detection of *ABCC2* 4544G>A polymorphism**




The wild-type G allele yielded a 291 bp band. All lanes show homozygous G allele except for control samples.

7 µl of the amplified 318 bp PCR product for *APOE* 334T>C were digested with 2.5 U restriction enzyme Afl III at 37°C overnight in a total volume of 20 µl. The digested mixture was run with 3% agarose gel. The mutated allele of C had the restriction site giving bands of 295 bp while the wild-type allele of T gave bands of 64 and 231 bp (Figure **3-4**).

**Figure 3-4. PCR-RFLP for detection of *APOE* 334T>C polymorphism**



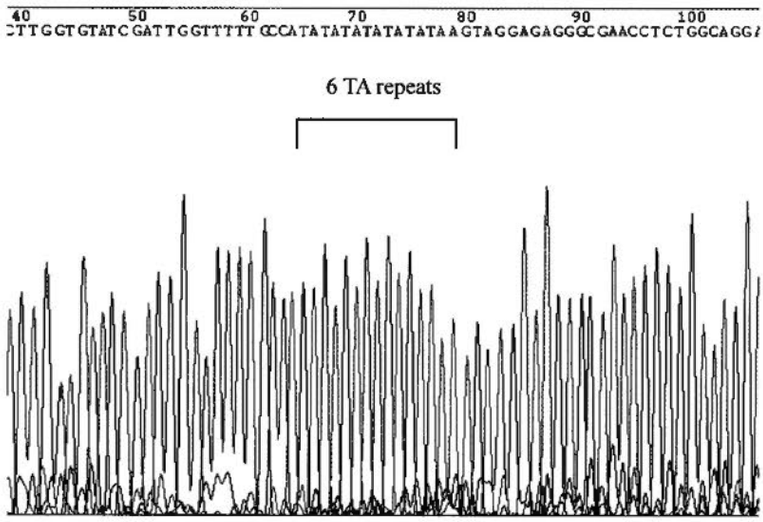

Lane 9 shows heterozygous 334TC; lane 7 shows homozygous 334CC, other lanes show homozygous 334TT apart from lane 1 showing controls. Presence of C varioant allele yielded 295-kb band (upper arrow), whereas presence of T allele yielded 64- and 231-bp bands (middle and lower arrows).

### 3.4.3.5 Sequencing

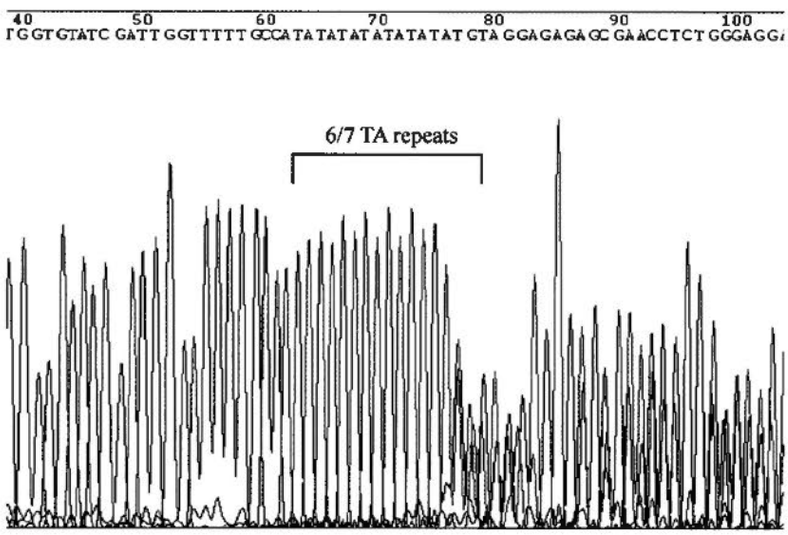
(TA)n repeats in the *UGT1A1* promoter were examined by sequencing in Tech Dragon Limited (www.techdragon.com.hk) using the high throughput Applied Biosystems 3730*xl* DNA Analyzer. The chromatogram for the sequence was viewed by the Chromas Lite 2.01 (Technelysium Pty Ltd).

**Figure 3-5. Sequencing of $(TA)_{6>7}$ repeats in the *UGT1A1* promoter**

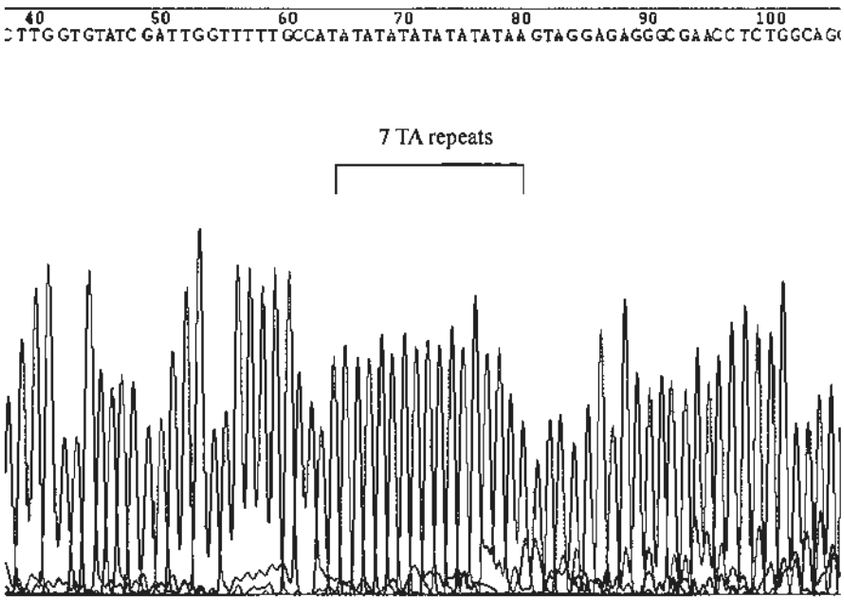
**A. UGT1A1 $(TA)_{6/6}$ (*1/*1)**




**B. UGT1A1 $(TA)_{6/7}$ (*1/*28)**



**C. UGT1A1 $(TA)_{7/7}$ (*28/*28)**




A shows the sample of homozygous 6 TA repeats; B shows the sample of heterozygous 7 TA repeats; C shows the sample of homozygous of 7 TA repeats.

### 3.5 Statistical analysis

Most analyses in this study were performed using the statistical analytical software package Statistical Package for the Social Sciences (SPSS) for windows, version 17.0, SPSS Inc, Chicago, USA. The Haploview program (Daly Lab) was used to estimate pairwise linkage disequilibrium (LD) and construct haplotypes. Study power was calculated using the Genetic Power Calculator (Purcell S et al., 2003).

Skewed data were logarithmically (base 10) transformed before analysis. Continuous variables were expressed as means ± standard deviation or median (Interquartile range). General comparisons between groups were performed using student's t-test or analysis of variance (ANOVA) for normally distributed parameters. Mann-Whitney test or Kruskal-Wallis H test was used for the comparison of continuous variables

that could not be successfully transformed into normally distributed data. The Chi-square ($\chi^2$) test was used for comparison of categorical data and HWE.

Analysis of variance (ANOVA) was utilized to evaluate genetic effects on the lipid responses to rosuvastatin and other CHD risk factors evaluated in the study. In normally distributed data, one-way ANOVA was used. When the data was skewed, the Kruskal-Wallis H test was utilized. Analysis of covariance (ANCOVA) was used to assess the relationship between parameters with adjusting for the confounding factors. Pearson's bivariate correlation or Spearman's bivariate correlation were used to estimate the degree of association between two normally or non-normally distributed continuous variables. Partial correlations examined the linear relationship after adjustment for confounding factors. Univariate and multivariate linear regression analysis were performed to determine contributions of genetic and environment factors to the variations of the lipid responses to rosuvastatin and other CHD risk factors evaluated in the study. Binary logistic regression was used to determine the odds ratio (OR) with 95% confidence interval (CI) for estimating risk associated with genotypes of various genetic parameters.

A two-tailed P value less than 0.05 was considered to be statistically significant. To adjust for multiple testing, we calculated an experiment wide significance level using the conservative method of Bonferroni (ie, 0.05/n) and therefore only those genetic factors with a probability value below this level were considered to be statistical significant.

# Chapter 4 Pharmacogenetic analysis of lipid responses to rosuvastatin in Chinese patients

## 4.1 Introduction

The lipid response to the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins is usually assessed by the percentage change in low-density lipoprotein cholesterol (LDL-C) and this varies widely between individuals, partly for genetic reasons (Chasman DI et al., 2004, Hu M et al., 2009, Kajinami K et al., 2004e, Schmitz G et al., 2007). More intensive reduction of LDL-C with statins is associated with greater reductions in cardiovascular events in a wide range of subject groups (Baigent C et al., 2005, Howard BV et al., 2008, Lewington S et al., 2007), but may increase the risk of toxicity so that optimizing the choice of statin and the dose for an individual may have beneficial effects.

Rosuvastatin in high doses (40 mg) has shown benefits on surrogate endpoints in the ASTEROID (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden) trial (Ballantyne CM et al., 2008, Nissen SE et al., 2004) and the METEOR (Measuring Effects on Intima-Media Thickness: an Evaluation of Rosuvastatin) study (Crouse JR, 3rd et al., 2007). In the recent CORONA (COntrolled ROsuvastatin MultiNAtional Trial in Heart Failure) study, there was no significant reduction in the primary composite outcome with 10 mg rosuvastatin in patients with moderate to severe heart failure, but in a prespecified secondary analysis, there were fewer hospitalizations for cardiovascular causes in the rosuvastatin group than in the placebo group ($P<0.001$) (Kjekshus J et al., 2007).

Rosuvastatin is one of the few drugs for which regulatory authorities including the FDA have recommended starting with lower doses (5 mg) in 'Asian' patients (Food and Drug Administration), but the term 'Asian' was not clearly defined (Po AL, 2007). The dose recommendation was based on single dose pharmacokinetic studies showing that systemic exposure to rosuvastatin was approximately twice as high in Asian Americans than in non-Asians (Grundy SM, 2005) and similar findings reported from Japanese subjects living in Japan compared with Caucasians (Tzeng T et al., 2004). A study from Singapore showed that Chinese subjects had, on average, higher systemic exposure than White subjects living in the same environment, with Malays and Asian Indians having intermediate values (Lee E et al., 2005). These differences in pharmacokinetics are unlikely to be due to ethnic variations in the drug metabolizing enzymes as rosuvastatin undergoes relatively little enzymic modification, but it is a substrate for a number of drug transporters which influence its disposition (Ho RH et al., 2006, Tirona RG, 2005). The uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1, gene *SLCO1B1*) influences hepatic uptake of rosuvastatin, but the common polymorphisms and haplotypes in *SLCO1B1* did not explain the ethnic differences in Singapore (Lee E et al., 2005).

The efflux transporter breast cancer resistance protein (BCRP) or ATP-binding cassette G2 (ABCG2) transporter, plays a significant role in the disposition of rosuvastatin *in vitro* (Huang L et al., 2006). One functional single nucleotide polymorphism (SNP), 421C>A (rs2231142, Gln141Lys) in *ABCG2* reduces the transport function of ABCG2 (Robey RW et al., 2009). The 421C>A polymorphism has been shown to have an important effect on the pharmacokinetics of rosuvastatin in Chinese (Zhang W et al., 2006a) and Caucasians subjects (Keskitalo JE et al., 2009c) with the A variant allele associated with increased systemic drug exposure to

rosuvastatin. The 421A allele is more common in Chinese and Japanese (allele frequency about 35%) than in Caucasians (14%) (Robey RW et al., 2009, Zamber CP et al., 2003), which may contribute to the ethnic difference in pharmacokinetics of rosuvstatin. However, no studies have been published on the effects of *ABCG2* polymorphism on the efficacy of statins.

On the basis of mechanism of action and pharmacokinetics, a number of candidate genes and SNPs have been reported to be associated with lipid responses to some statins as described in the Chapter **1**, although few results can be replicated on subsequent testing which may be due to small sample size in most of studies, low allele frequency of SNPs, small effect size or chance finding and non-genetic confounding factors e.g. drug compliance may also play a role. No studies so far have reported the genetic predictors of lipid response to rosuvastatin, the most potent and recent statin in the market.

The present study was intended to explore whether the 421C>A polymorphism in *ABCG2* and other common polymorphisms in genes related to statin pharmacokinetics, pharmacodynamics, cholesterol synthesis and lipoprotein metabolism pathways including some of those newly identified lipid-related loci and metabolic disorders influence the lipid response to rosuvastatin in Chinese patients. The effects of phenotypic factors on the lipid response were also assessed in the study.

## 4.2 Subjects and Methods

### 4.2.1 Subjects and study design

Please refer to Chapter 3 for the details of study design and methods.

### 4.2.2 SNP selection and genotyping

A total of 135 polymorphisms in 62 candidate genes/loci were selected based on their potential roles in the pharmacokinetics and pharmacodynamics of statin, lipid metabolism or metabolic disorders (Table **3-1**). DNA was extracted and genotyping was performed as described in Chapter 3. Two SNPs failed for genotyping as shown in Table **3-1.** All other polymorphisms were in Hardy-Weinberg equilibrium (HWE) (P>0.05) apart from *UGT2B7* 802C>T which was excluded from the analysis. Seven SNPs with a minor allele frequency (MAF) less than 1% were also excluded (Table **3-1**).

### 4.2.3 Statistical analysis

The percentage reduction in LDL-C was prospectively defined as the primary endpoint, which could largely reduce the influence of the baseline level of LDL-C. The percentage changes in high-density lipoprotein cholesterol (HDL-C), triglycerides, total cholesterol and non-HDL-C were also assessed. The assessment of the lipid responses to rosuvastatin was performed in patients with good adherence with rosuvastatin. The baseline characteristics between gender and other subgroups e.g. familial hypercholesterolaemia (FH) vs. non-FH or rheumatoid arthritis (RA) vs. non-RA were compared using Student's t-test for normally distributed parameters or Mann-Whitney test for continuous variables that could not be successfully transformed into normally distributed data and chi-square tests for categorical

variables. Effects of phenotypic or genotypic factors on the percentage changes in LDL-C, HDL-C, triglycerides, total cholesterol, and non-HDL-C were initially assessed singly using Student's t-test or analysis of variance (ANOVA) for normally distributed variables or Mann-Whitney test or Kruskal-Wallis H test for skewed variables. Assessed phenotypic variables included age, gender, body mass index (BMI), waist circumference (WC), percentage of total body fat, baseline values of lipid parameters, smoking status (current smoker/nonsmoker), FH, type 2 diabetes status, hypertension, RA, as well as history of cardiovascular disease (CVD). The phenotypic factors associated with the outcome measures at $P<0.2$ were considered for inclusion in the multivariate stepwise regression analysis. The genetic effects of 125 polymorphisms on percent changes in LDL-C, HDL-C, triglycerides, total cholesterol and non-HDL-C were evaluated separately using analysis of covariance (ANCOVA) with phenotypic predictors of each outcome as covariates when appropriate. Polymorphisms with P values $< 0.05$ without correction for multiple testing were reported, but statistical significance at this level should be interpreted with caution. To adjust for multiple testing, we calculated an experiment wide significance level using the conservative method of Bonferroni (i.e., $0.05/125=0.0004$), and therefore only those polymorphisms with a probability value below this level were considered to be statistical significant. Multivariate stepwise regression analysis was performed to determine the contribution of phenotypic and genetic factors with nominally significant P values ($P<0.05$) to the variation of the lipid responses. All statistical analyses were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

In post hoc power analysis, we had > 80% power to detect genetic effects that explain 2.5% of the variance in percentage changes in lipid parameters for a variant

with a minor allele frequency of >1% with a type I error rate of 0.05.

## 4.3 Results

### 4.3.1 Baseline characteristics of participants

Baseline characteristics in 386 eligible patients with good adherence to rosuvastatin treatment, including 36 patients with RA are shown in Table **4-1**. The mean (± SD) age was 55.7 ± 11.4 years and about 45% were male. In patients over 60 years old, 58% were female (Figure **4-1**). Two of the subjects were Caucasian and the remainder were Chinese. The mean values for BMI were 25.7 ± 4.0 kg/m$^2$ in males and 24.9 ± 4.3 kg/m$^2$ in females corresponding to WC of 90.7 ± 11.0 cm and % body fat of 25.2 ± 5.9 % in men and WC of 83.3 ± 10.8 cm and % body fat of 34.7 ± 8.1 % in women respectively, suggesting that at least half of the subjects were generally and centrally obese according to the criteria for Asian subjects. Out of the 386 subjects, 166 had a clinical diagnosis of FH, 191 had hypertension, 109 had type 2 diabetes and 55 were known to have a history of CVD. About 10% of subjects were current smokers and the majority of smokers were male.

The baseline LDL-C and total cholesterol were 5.14 ± 1.71 mmol/L and 7.49 ± 1.80 mmol/L, respectively. Male subjects had lower baseline HDL-C concentrations but higher triglycerides than females (Table **4-1**).

**Table 4-1. Baseline characteristics for participants with good adherence with rosuvastatin**

| Characteristics | All<br>n=386 | Males<br>n=173 | Females<br>n=213 | P |
|---|---|---|---|---|
| Age, years | 55.7 ± 11.4 | 54.7 ± 11.6 | 56.6 ± 11.2 | 0.109 |
| Weight, kg | 64.6 ± 13.4 | 71.8 ± 13.0 | 58.9 ± 10.8 | <0.001† |
| Height, m | 1.59 ± 0.09 | 1.67 ± 0.06 | 1.54 ± 0.06 | <0.001 |
| Body mass index, kg/m$^2$ | 25.2 ± 4.2 | 25.7 ± 4.0 | 24.9 ± 4.3 | 0.061† |
| Waist circumference, cm | 86.6 ± 11.5 | 90.7 ± 11.0 | 83.3 ± 10.8 | <0.001† |
| Hip circumference, cm | 97.4 ± 7.5 | 98.4 ± 7.1 | 96.7 ± 7.8 | 0.004† |
| Waist to hip ratio | 0.89 ± 0.08 | 0.92 ± 0.07 | 0.86 ± 0.08 | <0.001 |
| Body fat, % | 29.9 ± 8.5 | 25.2 ± 5.9 | 34.7 ± 8.1 | <0.001† |
| FH, n | 166 (43.0) | 69 (39.9) | 97 (45.5) | 0.264 |
| Hypertension, n | 191 (49.5) | 91 (52.6) | 100 (46.9) | 0.269 |
| Type 2 diabetes, n | 109 (28.2) | 58 (33.5) | 51 (23.9) | 0.038 |
| History of CVD, n | 55 (14.2) | 24 (11.3) | 31 (17.9) | 0.063 |
| RA, n | 36 (9.3) | 9 (5.2) | 27 (12.7) | 0.012 |
| Current smoker, n | 37 (9.6) | 33 (19.1) | 4 (1.9) | <0.001 |
| Total cholesterol, mmol/L | 7.49 ± 1.80 | 7.33 ± 1.66 | 7.62 ± 1.89 | 0.080† |
| LDL-C, mmol/L | 5.14 ± 1.71 | 5.08 ± 1.49 | 5.18 ± 1.86 | 0.800† |
| HDL-C, mmol/L | 1.51 ± 0.40 | 1.34 ± 0.29 | 1.64 ± 0.43 | <0.001† |
| Triglycerides, mmol/L | 2.00 ± 1.46 | 2.15 ± 1.48 | 1.88 ± 1.44 | 0.009† |
| non-HDL-C, mmol/L | 5.98 ± 1.77 | 5.99 ± 1.63 | 5.98 ± 1.88 | 0.162* |
| FPG, mmol/L | 6.2 ± 2.2 | 6.3 ± 2.3 | 6.1 ± 2.2 | 0.225† |
| CK, U/L | 128 ± 117 | 162 ± 158 | 101 ± 57 | <0.001* |
| Creatinine, μmol/L | 79 ± 21 | 91 ± 19 | 70 ± 15 | <0.001† |
| ALT, IU/L | 28.8 ± 23.1 | 32.8 ± 22.9 | 25.6 ± 22.8 | <0.001† |
| ALP, U/L | 75 ± 28 | 73 ± 23 | 76 ± 23 | 0.022† |

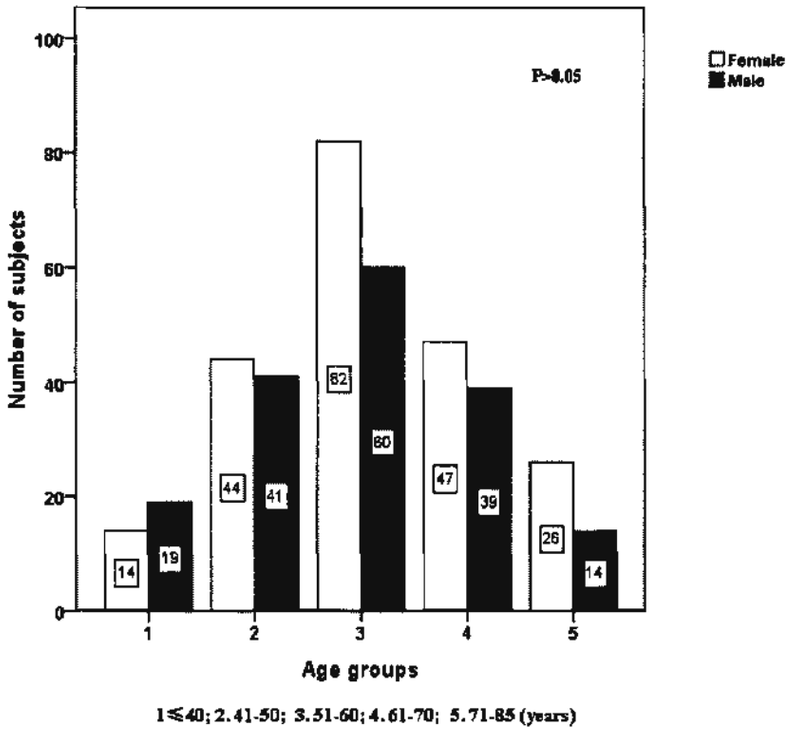
Data were expressed as mean ± SD or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

† Data were compared by Mann-Whitney U test.

Abbreviations: ALP = alkaline phosphatase; ALT = alanine transaminase; CK = creatine kinase; CVD = cardiovascular disease; FH = familial hypercholesterolaemia; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RA = rheumatoid arthritis.

**Figure 4-1. Distribution of ages in male and female subjects**




The mean (± SD) age of 36 patients with RA was 53.6 ± 5.9 years and three-quarters of them were female. Patients with RA tended to have lower body weight, BMI, and WC than patients with hypercholesterolaemia, which may be partly due to the different gender distributions between groups (Table **4-2**). Among 36 RA patients, 6 of them also had hypertension but none of these RA patients had diabetes or CVD. The baseline LDL-C, total cholesterol and triglycerides were significantly lower in patients with RA compared to those in patients with hypercholesterolaemia (Table **4-2**). RA patients had a relatively higher HDL-C level than patients with hypercholesterolaemia (1.64 ± 0.45 mmol/L vs. 1.50 ± 0.39 mmol/L, P = 0.048) but again this may also be due to the high proportion of females in the RA group.

**Table 4-2. Comparison of baseline characteristics for patients with rheumatoid arthritis and patients with hypercholesterolaemia**

| Characteristics | RA n=36 | Non-RA n=350 | P |
|---|---|---|---|
| Age, years | 53.6 ± 5.9 | 55.9 ± 11.8 | 0.051 |
| Male, n | 9 (25.0) | 164 (46.9) | 0.012 |
| Weight, kg | 58.7 ± 9.9 | 65.3 ± 13.6 | 0.004* |
| Height, m | 1.57 ± 0.07 | 1.60 ± 0.09 | 0.092 |
| Body mass index, kg/m$^2$ | 23.8 ± 3.7 | 25.4 ± 4.2 | 0.019† |
| Waist circumference, cm | 82.8 ± 9.4 | 87.0 ± 11.6 | 0.035 |
| Hip circumference, cm | 94.3 ± 6.7 | 97.8 ± 7.6 | 0.001† |
| Waist to hip ratio | 0.88 ± 0.07 | 0.89 ± 0.08 | 0.461 |
| Hypertension, n | 6 (16.7) | 185 (52.9) | <0.001 |
| Type 2 diabetes, n | 0 (0) | 109 (31.1) | <0.001 |
| Total cholesterol, mmol/L | 5.03 ± 0.99 | 7.74 ± 1.67 | <0.001* |
| LDL-C, mmol/L | 2.89 ± 0.84 | 5.37 ± 1.60 | <0.001† |
| HDL-C, mmol/L | 1.64 ± 0.45 | 1.50 ± 0.39 | 0.048* |
| Triglycerides, mmol/L | 1.14 ± 0.53 | 2.09 ± 1.50 | <0.001† |
| non-HDL-C, mmol/L | 3.40 ± 0.98 | 6.25 ± 1.61 | <0.001† |
| Fasting plasma glucose, mmol/L | 4.9 ± 0.5 | 6.3 ± 2.3 | <0.001† |
| Creatine kinase, U/L | 99 ± 80 | 132 ± 121 | 0.020* |
| Creatinine, μmol/L | 67 ± 15 | 81 ± 20 | <0.001* |
| Alanine transaminase, IU/L | 21.9 ± 8.9 | 29.6 ± 24.1 | 0.022† |
| Alkaline phosphatase, U/L | 92 ± 59 | 73 ± 21 | 0.006† |

Data were expressed as mean ± SD or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable, respectively, unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

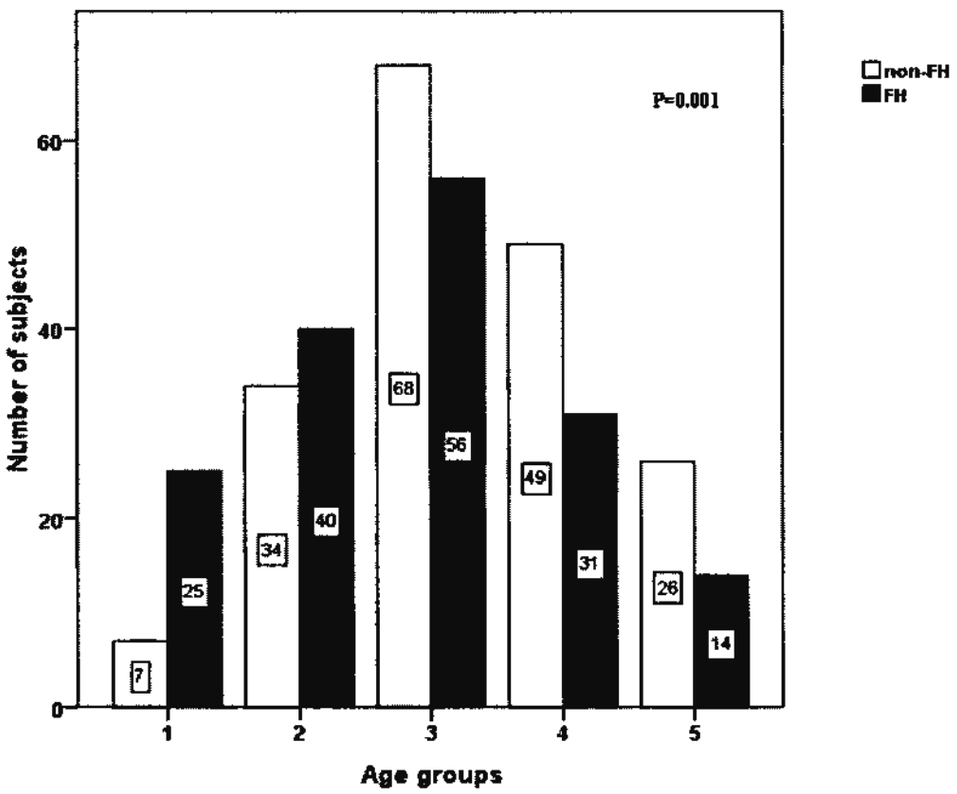
† Data were compared by Mann-Whitney U test.

Abbreviations: HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RA = rheumatoid arthritis.

In 350 patients with hypercholesterolaemia, patients with FH were younger and less obese than non-FH patients (Table **4-3**). There was significant difference in age distributions in FH and non-FH patients (P=0.001) and in 105 patients with age ≤ 50 years, 65 (61.9%) had FH (Figure **4-2**). FH patients had higher baseline levels of

total cholesterol, LDL-C, HDL-C and non-HDL-C, but a lower level of triglycerides, than those of non-FH patients. In contrast, non-FH patients had higher levels of blood pressure and fasting glucose as there were higher proportions of patients with diabetes or hypertension in the non-FH group than in those with FH. The creatinine and ALP were lower in patients with FH compared to non-FH patients (Table 4-3).

**Figure 4-2. Distribution of ages in FH and non-FH patients**




1≤40; 2.41-50; 3.51-60; 4.61-70; 5.71-85 (years)

**Table 4-3. Baseline characteristics for patients with hypercholesterolaemia**

| Characteristics | Combined n=350 | FH n=166 | Non-FH n=184 | P |
|---|---|---|---|---|
| Age, years | 55.9 ± 11.8 | 52.8 ± 12.5 | 58.8 ± 10.4 | <0.001 |
| Male, n | 164 (46.9) | 69 (41.6) | 95 (51.6) | 0.060 |
| Weight, kg | 65.3 ± 13.6 | 62.3 ± 10.8 | 68.1 ± 15.4 | 0.001† |
| Height, m | 1.60 ± 0.09 | 1.60 ± 0.09 | 1.59 ± 0.09 | 0.390 |
| Body mass index, kg/m$^2$ | 25.4 ± 4.2 | 24.2 ± 3.5 | 26.6 ± 4.5 | <0.001* |
| Waist circumference, cm | 87.0 ± 11.6 | 83.5 ± 9.9 | 91.1 ± 12.2 | <0.001* |
| Hip circumference, cm | 97.8 ± 7.6 | 96.8 ± 6.1 | 99.0 ± 8.8 | 0.036† |
| Waist to hip ratio | 0.89 ± 0.08 | 0.86 ± 0.07 | 0.92 ± 0.08 | <0.001 |
| Body fat, % | 29.9 ± 8.5 | 29.1 ± 8.2 | 30.9 ± 8.8 | 0.110 |
| Hypertension, n | 185 (52.9) | 45 (27.1) | 140 (76.1) | <0.001 |
| Type 2 diabetes, n | 109 (31.1) | 19 (11.4) | 90 (48.9) | <0.001 |
| History of CVD, n | 55 (15.7) | 10 (6.0) | 45 (24.5) | <0.001 |
| Current smoker, n | 36 (10.6) | 15 (9.1) | 21 (12.0) | 0.384 |
| Total cholesterol, mmol/L | 7.74 ± 1.67 | 8.80± 1.68 | 6.79 ± 0.92 | <0.001† |
| LDL-C, mmol/L | 5.37 ± 1.60 | 6.44 ± 1.57 | 4.40 ± 0.82 | <0.001† |
| HDL-C, mmol/L | 1.50 ± 0.39 | 1.57 ± 0.37 | 1.43 ± 0.40 | <0.001† |
| Triglycerides, mmol/L | 2.09 ± 1.50 | 1.83 ± 1.29 | 2.32 ± 1.63 | <0.001† |
| non-HDL-C, mmol/L | 6.25 ± 1.61 | 7.22 ± 1.67 | 5.37 ± 0.88 | <0.001* |
| FPG, mmol/L | 6.3 ± 2.3 | 5.5 ± 1.0 | 7.1 ± 2.8 | <0.001† |
| CK, U/L | 132 ± 121 | 143 ± 152 | 119 ± 72 | 0.326 |
| Creatinine, μmol/L | 81 ± 20 | 78 ± 16 | 84 ± 23 | 0.008* |
| ALT, IU/L | 29.6 ± 24.1 | 29.4 ± 23.2 | 29.8 ± 25.0 | 0.737† |
| ALP, U/L | 73 ± 21 | 70 ± 21 | 75 ± 20 | 0.008* |

Data were expressed as mean ± SD or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

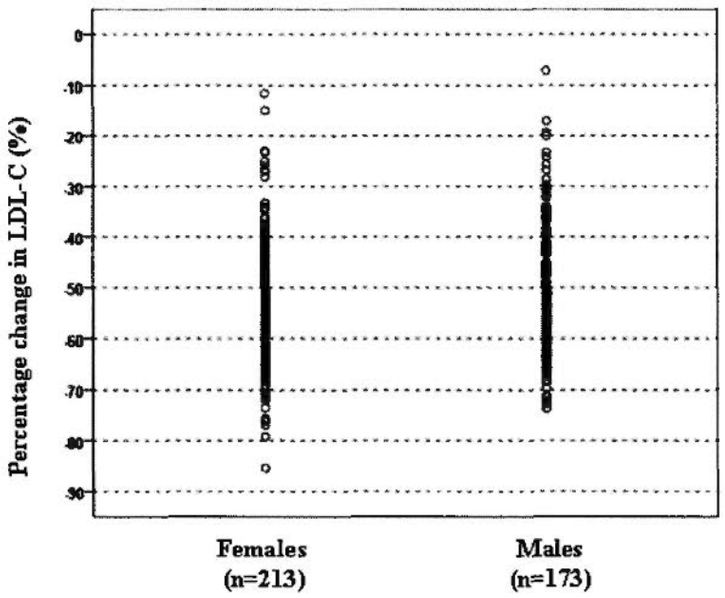
† Data were compared by Mann-Whitney U test.

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; CK = creatine kinase; CVD = cardiovascular disease; FH = familial hypercholesterolaemia; FPG = fasting plasma glucose; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RA = rheumatoid arthritis.

### 4.3.2 Lipid response and safety

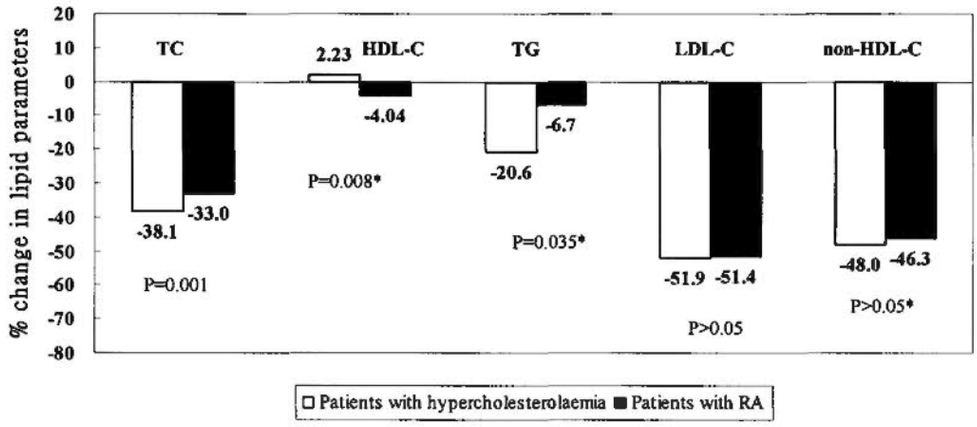
In 386 patients with good adherence to rosuvastatin, there were significant reductions ($P<0.001$) in LDL-C, triglycerides, total cholesterol and non-HDL-C (-51.8 ± 12.2%, -19.3 ± 33.6%, -37.7 ± 9.1% and -47.8 ± 10.9%, respectively), but a nonsignificant change in HDL-C (1.6 ± 13.3%, $P>0.05$). The LDL-C response to rosuvastatin varied considerably among individuals from -7.1% to -85.4% (Figure **4-3**).

**Figure 4-3. The LDL-C response to rosuvastatin in study participants**




Patients with RA had a similar percentage reduction in LDL-C to patients with hypercholesterolaemia (Figure **4-4**), although RA patients had lower baseline LDL-C levels (2.89 ± 0.84 vs. 5.37 ± 1.60, $P<0.001$). However, the changes in HDL-C, triglycerides and total cholesterol were different between the two groups (Figure **4-4**).

**Figure 4-4. Mean lipid responses to rosuvastatin in patients with RA (n=36) and hypercholesterolaemia (n=350)**

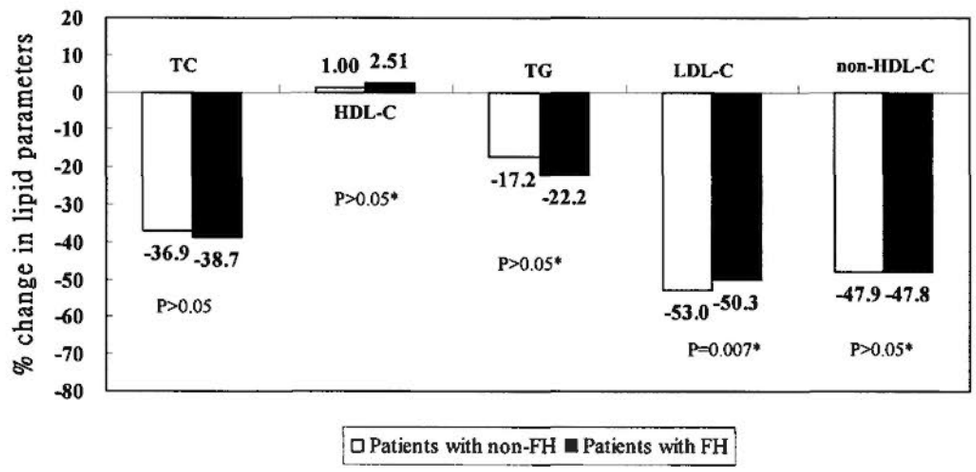



Data were compared by t-test or Mann-Whitney U test (*).

Abbreviations: HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglyceride; RA = rheumatoid arthritis.

FH patients had a significantly smaller percentage LDL-C response to rosuvastatin compared to non-FH but the changes in other lipid parameters in FH were of a similar magnitude to non-FH patients (Figure **4-5**).

**Figure 4-5. Mean lipid responses to rosuvastatin in patients with FH (n=166) and non-FH (n=220)**




Data were compared by t-test or Mann-Whitney U test (*).

Abbreviations: FH = familial hypercholesterolaemia; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TG = triglyceride.

#### 4.3.2.1 Effects of phenotypic factors on the LDL-C response

The analysis of the effects of different phenotypic parameters on the changes in LDL-C levels in response to rosuvastatin 10 mg daily are shown in Table **4-4**. There was no significant effect of baseline LDL-C levels, BMI, WC, % body fat, smoking status, having hypertension, RA or CVD on the percentage change in LDL-C. Post hoc analysis showed that patients with age 51-70 years had a greater LDL-C reduction than that of the younger patients (18-50 years). Female subjects had greater reductions in LDL-C than male subjects. The associations between age and gender and LDL-C response to rosuvastatin were still significant after adjusting for other variables. Patients with FH or without diabetes had a smaller reduction in LDL-C compared to the remainder but this was no longer significant after adjustment for age, gender, having diabetes, FH, or hypertension and baseline LDL-C levels (Table **4-4**).

**Table 4-4. Effects of phenotypic factors on percentage changes in LDL-C in response to rosuvastatin in patients with good adherence**

| Variable | Group, (n) | Baseline LDL-C (mmol/L) | On-treatment LDL-C (mmol/L) | % Change in LDL-C | P | P † |
|---|---|---|---|---|---|---|
| Age, year | ≤40, (33) | 5.83 ± 1.39 | 3.07 ± 0.85 | -46.9 ± 10.6 | | |
| | 41-50, (85) | 5.14 ± 1.69 | 2.66 ± 1.06 | -47.8 ± 12.5 | | |
| | 51-60, (142) | 5.07 ± 1.87 | 2.35 ± 1.05 | -53.7 ± 11.5 | <0.001 | 0.014 |
| | 61-70, (86) | 4.97 ± 1.66 | 2.2 ± 0.84 | -55.0 ± 12.1 | | |
| | >70, (40) | 5.13 ± 1.37 | 2.53 ± 1.01 | -51.1 ± 12.5 | | |
| Gender | Female, (213) | 5.18 ± 1.86 | 2.42 ± 1.04 | -53.1 ± 12.0 | 0.025 | 0.039 |
| | Male, (173) | 5.08 ± 1.49 | 2.51 ± 0.98 | -50.3 ± 12.3 | | |
| BMI, kg/m$^2$ | <22.58, (91) | 5.21 ± 1.93 | 2.50 ± 1.05 | -51.8 ± 11.7 | | |
| (Quartiles) | 22.58-24.84, (93) | 5.33 ± 1.79 | 2.61 ± 1.06 | -50.7 ± 13.1 | 0.617 | |
| | 24.85-27.07, (92) | 4.99 ± 1.43 | 2.32 ± 0.85 | -52.9 ± 11.8 | | |
| | >27.07, (91) | 5.05 ± 1.76 | 2.40 ± 1.12 | -52.6 ± 12.1 | | |
| WC, (cm) | <78.2, (83) | 5.64 ± 2.10 | 2.73 ± 1.16 | -51.3 ± 12.7 | | |
| (Quartiles) | 78.2-85.9, (84) | 5.12 ± 1.70 | 2.42 ± 0.98 | -52.8 ± 10.8 | 0.782* | |
| | 86.0-93.0, (83) | 5.09 ± 1.68 | 2.40 ± 0.93 | -51.9 ± 12.3 | | |
| | >93.0 (84) | 5.06 ± 1.57 | 2.48 ± 1.05 | -50.7 ± 12.7 | | |
| Body fat, % | <23.8, (60) | 5.49 ± 1.63 | 2.61 ± 0.94 | -51.2 ± 12.9 | | |
| (Quartiles) | 23.8-28.7, (59) | 5.54 ± 1.58 | 2.71 ± 0.97 | -50.9 ± 11.9 | 0.691 | |
| | 28.8-35.3, (61) | 5.76 ± 1.81 | 2.78 ± 1.09 | -51.5 ± 11.4 | | |
| | >35.3, (59) | 5.33 ± 1.70 | 2.47 ± 0.96 | -53.3 ± 10.8 | | |
| Baseline | <4.0, (95) | 3.27 ± 0.65 | 1.57 ± 0.45 | -51.4 ± 12.4 | | |
| LDL-C, | 4.0-4.88, (97) | 4.41 ± 0.25 | 2.10 ± 0.65 | -52.5 ± 14.5 | 0.138* | 0.667 |
| mmol/L | 4.89-6.0, (96) | 5.39 ± 0.34 | 2.52 ± 0.62 | -53.2 ± 11.3 | | |
| (Quartiles) | >6.0, (96) | 7.46 ± 1.27 | 3.67 ± 0.85 | -50.5 ± 11.1 | | |
| FH | No, (220) | 4.15 ± 0.99 | 1.93 ± 0.66 | -53.0± 13.1 | 0.007* | 0.078 |
| | Yes, (166) | 6.44 ± 1.57 | 3.17 ± 0.97 | -50.4 ± 10.8 | | |
| Diabetes | No, (277) | 5.34 ± 1.85 | 2.59 ± 1.03 | -51.1 ± 11.2 | 0.007* | 0.060 |
| | Yes, (109) | 4.60 ± 1.09 | 2.14 ± 0.90 | -53.7 ± 14.3 | | |
| Hypertension | No, (200) | 5.49 ± 1.94 | 2.66 ± 1.09 | -51.1 ± 11.6 | 0.157* | 0.593 |
| | Yes, (186) | 4.76 ± 1.32 | 2.25 ± 0.88 | -52.6 ± 12.8 | | |
| History of | No, (331) | 5.22 ± 1.74 | 2.50 ± 1.03 | -52.0 ± 11.8 | 0.966* | |
| CVD | Yes, (55) | 4.61 ± 1.38 | 2.25 ± 0.92 | -50.9 ± 14.4 | | |

| | | | | | |
|---|---|---|---|---|---|
| RA | No, (350) | 5.37 ± 1.60 | 2.57 ± 0.99 | -51.9 ± 12.2 | 0.826 |
| | Yes, (36) | 2.89 ± 0.84 | 1.38 ± 0.48 | -51.4 ± 12.4 | |
| Current smoker | No, (349) | 5.11 ± 1.71 | 2.44 ± 1.00 | -52.1 ± 12.1 | 0.217 |
| | Yes, (37) | 5.39 ± 1.65 | 2.69 ± 1.10 | -49.4 ± 12.9 | |

Data were expressed as mean ± SD and compared by t-test or ANOVA unless otherwise indicated.

* Data were compared by non-parametric test (Mann-Whitney U test or Kruskal-Wallis H test) as data were skewed.

† P value were adjusted for multiple variables with P<0.2 in the univariant analysis including age, gender, FH, diabetes, hypertension and baseline LDL-C levels.

#### 4.3.2.2 Effects of phenotypic factors on the HDL-C response

The analysis of the effects of different phenotypic parameters on the changes in HDL-C levels in response to rosuvastatin 10 mg daily are shown in Table **4-5**. High baseline HDL-C level and having RA or diabetes were associated with an unfavorable effect on HDL-C change even after adjustment for other variables although patients with diabetes had a lower baseline level of HDL-C than that of non-diabetic patients. There was no significant effect of age, gender, BMI, WC, % body fat, smoking status, having hypertension, FH or CVD on the percentage change in LDL-C.

**Table 4-5. Effects of phenotypic factors on percentage changes in HDL-C in response to rosuvastatin in patients with good adherence**

| Variable | Group, (n) | Baseline HDL-C (mmol/L) | On-treatment HDL-C (mmol/L) | % Change in HDL-C | P | P † |
|---|---|---|---|---|---|---|
| Age, year | ≤40, (33) | 1.49 ± 0.35 | 1.46 ± 0.36 | -1.68 ± 11.4 | | |
| | 41-50, (85) | 1.44 ± 0.37 | 1.45 ± 0.39 | 0.87 ± 12.8 | | |
| | 51-60, (142) | 1.54 ± 0.42 | 1.57 ± 0.42 | 2.25 ± 13.4 | 0.316 | |
| | 61-70, (86) | 1.50 ± 0.37 | 1.52 ± 0.37 | 2.70 ± 14.1 | | |
| | >70, (40) | 1.59 ± 0.49 | 1.59 ± 0.44 | 1.66 ± 14.1 | | |
| Gender | Female, (213) | 1.64 ± 0.43 | 1.65 ± 0.42 | 1.09 ± 13.8 | 0.329 | |
| | Male, (173) | 1.34 ± 0.29 | 1.37 ± 0.32 | 2.34 ± 12.6 | | |
| BMI, kg/m$^2$ | <22.58, (91) | 1.66 ± 0.43 | 1.68 ± 0.41 | 2.03 ± 13.0 | | |
| (Quartiles) | 22.58-24.84, (93) | 1.46 ± 0.35 | 1.51 ± 0.41 | 4.04 ± 15.5 | 0.319 | |
| | 24.85-27.07, (92) | 1.54 ± 0.43 | 1.51 ± 0.39 | -0.35 ± 12.0 | | |
| | >27.07, (91) | 1.39 ± 0.34 | 1.39 ± 0.35 | 1.19 ± 12.7 | | |
| WC, cm | <78.2, (83) | 1.68 ± 0.41 | 1.71 ± 0.42 | 2.87 ± 13.7 | | |
| (Quartiles) | 78.2-85.9, (84) | 1.54 ± 0.42 | 1.56 ± 0.40 | 2.62 ± 14.3 | 0.777 | |
| | 86.0-93.0, (83) | 1.48 ± 0.42 | 1.47 ± 0.40 | 0.78 ± 13.7 | | |
| | >93.0 (84) | 1.37 ± 0.31 | 1.39 ± 0.32 | 2.27 ± 13.4 | | |
| Body fat, % | <23.8, (60) | 1.53 ± 0.37 | 1.58 ± 0.42 | 3.68 ± 12.9 | | |
| (Quartiles) | 23.8-28.7, (59) | 1.40 ± 0.30 | 1.42 ± 0.32 | 1.55 ± 12.8 | 0.821 | |
| | 28.8-35.3, (61) | 1.53 ± 0.50 | 1.55 ± 0.49 | 2.55 ± 15.9 | | |
| | >35.3, (59) | 1.53 ± 0.37 | 1.56 ± 0.39 | 2.69 ± 12.0 | | |
| Baseline | <1.23, (98) | 1.08 ± 0.12 | 1.14 ± 0.18 | 4.92 ± 14.6 | | |
| HDL-C, | 1.23-1.43, (95) | 1.32 ± 0.06 | 1.35 ± 0.16 | 2.46 ± 12.6 | 0.017 | $1.2\times10^{-6}$ |
| mmol/L | 1.44-1.72, (95) | 1.57 ± 0.08 | 1.59 ± 0.21 | 1.12 ± 12.8 | | |
| (Quartiles) | >1.72, (98) | 2.05 ± 0.31 | 2.01 ± 0.35 | -1.89 ± 12.3 | | |
| FH | No, (220) | 1.46 ± 0.42 | 1.46 ± 0.39 | 1.00 ± 13.4 | 0.191 | 0.851 |
| | Yes, (166) | 1.57 ± 0.37 | 1.60 ± 0.40 | 2.51 ± 13.2 | | |
| Diabetes | No, (277) | 1.56 ± 0.41 | 1.58 ± 0.40 | 2.46 ± 13.4 | 0.073 | 0.001 |
| | Yes, (109) | 1.38 ± 0.36 | 1.37 ± 0.37 | -0.41 ± 13.0 | | |
| Hypertension | No, (200) | 1.59 ± 0.40 | 1.60 ± 0.41 | 1.41 ± 12.9 | 0.881 | |
| | Yes, (186) | 1.43 ± 0.38 | 1.44 ± 0.38 | 1.90 ± 13.7 | | |
| History of CVD | No, (331) | 1.54 ± 0.41 | 1.54 ± 0.40 | 1.38 ± 13.1 | 0.307 | |
| | Yes, (55) | 1.35 ± 0.32 | 1.39 ± 0.38 | 3.26 ± 14.3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| RA | No, (350) | 1.50 ± 0.39 | 1.52 ± 0.41 | 2.23 ± 13.1 | 0.008 | 0.003 |
| | Yes, (36) | 1.64 ± 0.45 | 1.55 ± 0.38 | -4.04 ± 14.1 | | |
| Current smoker | No, (349) | 1.53 ± 0.41 | 1.54 ± 0.41 | 1.62 ± 13.3 | 0.801 | |
| | Yes, (37) | 1.31 ± 0.28 | 1.33 ± 0.30 | 1.96 ± 13.3 | | |

Data were expressed as mean ± SD and compared by non-parametric test (Mann-Whitney U test or Kruskal-Wallis H test) as data were skewed.

† P value were adjusted for multiple variables with P<0.2 in the univariant analysis including FH, diabetes, RA and baseline HDL-C levels.

#### 4.3.2.3 Effects of phenotypic factors on the triglycerides response

The analysis of the effects of different phenotypic parameters on the changes in triglyceride levels in response to rosuvastatin 10 mg daily are shown in Table 3-6. The baseline triglyceride levels were strongly associated with the percentage changes in triglycerides before and after adjustment for other potential confounding factors ($P<1\times10^{-14}$). Age, and having RA or diabetes was associated with the changes in triglycerides but these were not significant after adjustment for baseline triglycerides. There was no significant effect of gender, BMI, WC, % body fat, smoking status, having hypertension, FH or CVD on the percentage change in triglycerides.

**Table 4-6. Effects of phenotypic factors on percentage changes in triglycerides in response to rosuvastatin in patients with good adherence**

| Variable | Group, (n) | Baseline TG (mmol/L) | On-treatment TG (mmol/L) | % Change in TG | P | P † |
|---|---|---|---|---|---|---|
| Age, year | ≤40, (33) | 1.50 ± 1.25 | 1.18 ± 0.79 | -12.9 ± 35.2 | | |
| | 41-50, (85) | 1.91 ± 1.37 | 1.47 ± 1.11 | -15.5 ± 39.3 | | |
| | 51-60, (142) | 2.04 ± 1.78 | 1.40 ± 0.66 | -18.0 ± 35.9 | 0.045 | 0.457 |
| | 61-70, (86) | 2.20 ± 1.21 | 1.45 ± 0.71 | -29.3 ± 21.5 | | |
| | >70, (40) | 2.02 ± 0.93 | 1.59 ± 0.68 | -15.8 ± 28.8 | | |
| Gender | Female, (213) | 1.88 ± 1.44 | 1.35 ± 0.78 | -19.4 ± 30.4 | 0.459 | |
| | Male, (173) | 2.15 ± 1.48 | 1.53 ± 0.83 | -19.2 ± 37.2 | | |
| BMI, kg/m$^2$ | <22.58, (91) | 1.43 ± 0.88 | 0.98 ± 0.45 | -23.2 ± 29.3 | | |
| (Quartiles) | 22.58-24.84, (93) | 2.00 ± 1.39 | 1.43 ± 0.93 | -19.5 ± 34.7 | 0.609 | |
| | 24.85-27.07, (92) | 2.05 ± 1.29 | 1.52 ± 0.83 | -16.1 ± 35.4 | | |
| | >27.07, (91) | 2.47 ± 1.99 | 1.67 ± 0.71 | -19.9 ± 35.6 | | |
| WC, cm | <78.2, (83) | 1.44 ± 0.98 | 1.04 ± 0.60 | -20.6 ± 25.2 | | |
| (Quartiles) | 78.2-85.9, (84) | 1.76 ± 1.22 | 1.26 ± 0.61 | -19.0 ± 31.7 | 0.869 | |
| | 86.0-93.0, (83) | 2.23 ± 1.37 | 1.66 ± 1.06 | -15.2 ± 45.7 | | |
| | >93.0 (84) | 2.63 ± 2.09 | 1.71 ± 0.73 | -22.1 ± 32.0 | | |
| Body fat, % | <23.8, (60) | 1.74 ± 1.41 | 1.20 ± 0.91 | -24.6 ± 26.4 | | |
| (Quartiles) | 23.8-28.7, (59) | 1.93 ± 0.98 | 1.43 ± 0.69 | -19.3 ± 38.8 | 0.706 | |
| | 28.8-35.3, (61) | 2.08 ± 1.61 | 1.48 ± 0.91 | -14.2 ± 41.5 | | |
| | >35.3, (59) | 2.30 ± 0.99 | 1.61 ± 0.65 | -23.7 ± 28.0 | | |
| Baseline TG, | <1.1, (93) | 0.84 ± 0.16 | 0.82 ± 0.32 | 0.20 ± 40.3 | | |
| mmol/L | 1.1-1.68, (100) | 1.34 ± 0.19 | 1.11 ± 0.34 | -15.8 ± 26.4 | $2.9\times10^{-17}$ | $7.1\times10^{-15}$ |
| (Quartiles) | 1.69-2.44, (97) | 2.03 ± 0.22 | 1.53 ± 0.54 | -24.7 ± 25.1 | | |
| | >2.44, (96) | 3.79 ± 1.88 | 2.23 ± 0.98 | -36.5 ± 30.2 | | |
| FH | No, (220) | 2.13 ± 1.57 | 1.55 ± 0.85 | -17.2 ± 37.1 | 0.279 | |
| | Yes, (166) | 1.83 ± 1.29 | 1.26 ± 0.71 | -22.2 ± 28.1 | | |
| Diabetes | No, (277) | 1.84 ± 1.45 | 1.34 ± 0.76 | -18.1 ± 31.7 | 0.046* | 0.878 |
| | Yes, (109) | 2.40 ± 1.43 | 1.65 ± 0.87 | -22.4 ± 37.9 | | |
| Hypertension | No, (200) | 1.70 ± 1.53 | 1.20 ± 0.73 | -18.6 ± 32.5 | 0.368 | |
| | Yes, (186) | 2.32 ± 1.31 | 1.67 ± 0.81 | -20.1 ± 34.8 | | |
| History of | No, (331) | 1.94 ± 1.49 | 1.39 ± 0.82 | -19.3 ± 33.1 | 0.833 | |
| CVD | Yes, (55) | 2.33 ± 1.23 | 1.66 ± 0.67 | -19.5 ± 36.5 | | |
| RA | No, (350) | 2.09 ± 1.50 | 1.47 ± 0.81 | -20.6 ± 32.0 | 0.035 | 0.300 |
| | Yes, (36) | 1.14 ± 0.53 | 0.98 ± 0.49 | -6.7 ± 44.5 | | |

| | | | | | |
|---|---|---|---|---|---|
| Current | No, (349) | 1.95 ± 1.41 | 1.40 ± 0.80 | -19.5 ± 33.4 | 0.698 |
| smoker | Yes, (37) | 2.45 ± 1.85 | 1.67 ± 0.75 | -17.7 ± 35.3 | |

Data were expressed as mean ± SD and compared by non-parametric test * (Mann-Whitney U test or Kruskal-Wallis H test) as data were skewed.

† P values were adjusted for multiple variables with P<0.2 in the univariant analysis including age, diabetes, RA and baseline triglyceride levels.

#### 4.3.2.4 Effects of phenotypic factors on the total cholesterol response

The analysis of the effects of different phenotypic parameters on the changes in total cholesterol levels in response to rosuvastatin 10 mg daily are shown in Table **4-7**. The baseline total cholesterol level was a strong predictor of total cholesterol response to rosuvastatin before and after adjustment for other potential confounding factors. Age, having diabetes or RA was associated with the changes in total cholesterol but these were not significant after adjustment for baseline total cholesterol levels. Other phenotypic factors examined did not show a significant effect on the changes in total cholesterol in response to rosuvastatin.

**Table 4-7. Effects of phenotypic factors on percentage changes in total cholesterol in response to rosuvastatin in patients with good adherence**

| Variable | Group, (n) | Baseline TC (mmol/L) | On-treatment TC (mmol/L) | % Change in TC | P | P † |
|---|---|---|---|---|---|---|
| Age, year | ≤40, (33) | 7.99 ± 1.35 | 5.05 ± 0.96 | -36.4 ± 9.2 | | |
| | 41-50, (85) | 7.45 ± 1.92 | 4.77 ± 1.37 | -35.7 ± 8.7 | | |
| | 51-60, (142) | 7.42 ± 1.92 | 4.54 ± 1.15 | -38.1 ± 9.1 | 0.011* | 0.430 |
| | 61-70, (86) | 7.40 ± 1.80 | 4.38 ± 1.00 | -40.1 ± 9.0 | | |
| | >70, (40) | 7.64 ± 1.33 | 4.85 ± 1.00 | -36.3 ± 9.1 | | |
| Gender | Female, (213) | 7.62 ± 1.89 | 4.67 ± 1.11 | -38.0 ± 8.8 | 0.432 | |
| | Male, (173) | 7.33 ± 1.66 | 4.58 ± 1.22 | -37.3 ± 9.5 | | |
| BMI, kg/m$^2$ | <22.58, (91) | 7.49 ± 1.98 | 4.62 ± 1.14 | -37.5 ± 9.2 | | |
| (Quartiles) | 22.58-24.84, (93) | 7.60 ± 1.85 | 4.74 ± 1.16 | -37.0 ± 9.2 | 0.575* | |
| | 24.85-27.07, (92) | 7.42 ± 1.62 | 4.56 ± 1.16 | -38.2 ± 9.3 | | |
| | >27.07, (91) | 7.46 ± 1.85 | 4.54 ± 1.22 | -38.8 ± 8.3 | | |
| WC, cm | <78.2, (83) | 7.93 ± 2.11 | 4.91 ± 1.21 | -37.2 ± 9.1 | | |
| (Quartiles) | 78.2-85.9, (84) | 7.37 ± 1.82 | 4.53 ± 1.10 | -37.7 ± 9.3 | 0.974 | |
| | 86.0-93.0, (83) | 7.54 ± 1.86 | 4.65 ± 1.21 | -37.8 ± 8.8 | | |
| | >93.0 (84) | 7.50 ± 1.67 | 4.65 ± 1.16 | -37.7 ± 8.8 | | |
| Body fat, % | <23.8, (60) | 7.72 ± 1.82 | 4.80 ± 1.29 | -37.4 ± 10.1 | | |
| (Quartiles) | 23.8-28.7, (59) | 7.79 ± 1.72 | 4.77 ± 1.08 | -38.3 ± 9.7 | 0.795 | |
| | 28.8-35.3, (61) | 8.09 ± 1.85 | 4.96 ± 1.15 | -38.5 ± 6.7 | | |
| | >35.3, (59) | 7.87 ± 1.74 | 4.76 ± 1.05 | -38.9 ± 8.2 | | |
| Baseline TC, | <6.3, (95) | 5.47 ± 0.74 | 3.54 ± 0.60 | -34.8 ± 9.9 | | |
| mmol/L | 6.3-7.3, (100) | 6.82 ± 0.32 | 4.27 ± 0.71 | -37.4 ± 9.9 | 0.001 | $1.4\times10^{-7}$ |
| (Quartiles) | 7.31-8.38, (95) | 7.82 ± 0.30 | 4.79 ± 0.64 | -38.7 ± 8.4 | | |
| | >8.38, (96) | 9.86 ± 1.42 | 5.93 ± 1.04 | -39.7 ± 7.2 | | |
| FH | No, (220) | 6.51 ± 1.14 | 4.08 ± 0.83 | -36.9 ± 9.4 | 0.052 | 0.541 |
| | Yes, (166) | 8.80 ± 1.68 | 5.36 ± 1.12 | -38.7 ± 8.6 | | |
| Diabetes | No, (277) | 7.69 ± 1.93 | 4.78 ± 1.16 | -37.1 ± 8.7 | 0.008* | 0.003 |
| | Yes, (109) | 6.98 ± 1.26 | 4.25 ± 1.06 | -39.2 ± 9.9 | | |
| Hypertension | No, (200) | 7.80 ± 2.06 | 4.81 ± 1.27 | -37.5 ± 9.3 | 0.76 | |
| | Yes, (186) | 7.15 ± 1.39 | 4.43 ± 0.98 | -37.8 ± 8.9 | | |
| History of | No, (331) | 7.58 ± 1.83 | 4.67 ± 1.17 | -37.9 ± 9.1 | 0.324 | |
| CVD | Yes, (55) | 6.96 ± 1.51 | 4.39 ± 1.07 | -36.5 ± 9.2 | | |
| RA | No, (350) | 7.74 ± 1.67 | 4.76 ± 1.12 | -38.1 ± 8.9 | 0.001 | 0.676 |
| | Yes, (36) | 5.03 ± 0.99 | 3.33 ± 0.59 | -33.0 ± 9.4 | | |

| | | | | | |
|---|---|---|---|---|---|
| Current | No, (349) | 7.48 ± 1.79 | 4.62 ± 1.15 | -37.7 ± 9.1 | 0.795 |
| smoker | Yes, (37) | 7.63 ± 1.85 | 4.76 ± 1.24 | -37.3 ± 9.2 | |

Data were expressed as mean ± SD and compared by t-test or ANOVA unless otherwise indicated.

* Data were compared by non-parametric test (Mann-Whitney U test or Kruskal-Wallis H test) as data were skewed.

† P values were adjusted for multiple variables with P<0.2 in the univariant analysis including age, FH, diabetes, RA and baseline total cholesterol levels.

#### 4.3.2.5 Effects of phenotypic factors on the non-HDL-C response

The analysis of the effects of different phenotypic parameters on the changes in non-HDL-C levels in response to rosuvastatin 10 mg daily are shown in Table **4-8**. There was no significant effect of baseline non-HDL-C levels, BMI, WC, % body fat, smoking status, having hypertension, RA or CVD on the percentage change in LDL-C. Female subjects had greater reductions in non-HLDL-C than male subjects. Age tended to be related to the non-HDL-C response to rosuvastatin but this relationship was not significant after adjusting for gender and having diabetes. The baseline level of non-HDL-C, BMI, WC, % body fat, having diabetes, FH, hypertension, RA or CVD and smoking status did not significantly affect the non-HDL-C response to rosuvastatin (Table **4-8**).

**Table 4-8. Effects of phenotypic factors on percentage changes in non-HDL-C in response to rosuvastatin in patients with good adherence**

| Variable | Group, (n) | Baseline non-HDL-C (mmol/L) | On-treatment non-HDL-C (mmol/L) | % Change in non-HDL-C | P | P † |
|---|---|---|---|---|---|---|
| Age, year | ≤40, (33) | 6.50 ± 1.21 | 3.59 ± 0.86 | -44.4 ± 10.6 | | |
| | 41-50, (85) | 6.01 ± 1.93 | 3.32 ± 1.36 | -44.9 ± 10.7 | | |
| | 51-60, (142) | 5.87 ± 1.89 | 2.98 ± 1.07 | -49.0 ± 10.5 | 0.0004* | 0.142 |
| | 61-70, (86) | 5.90 ± 1.72 | 2.86 ± 0.88 | -50.9 ± 10.3 | | |
| | >70, (40) | 6.05 ± 1.38 | 3.25 ± 1.07 | -46.4 ± 11.9 | | |
| Gender | Female, (213) | 5.98 ± 1.88 | 3.02 ± 1.05 | -49.0 ± 10.5 | 0.028* | 0.019 |
| | Male, (173) | 5.99 ± 1.63 | 3.21 ± 1.17 | -46.4 ± 11.2 | | |
| BMI, kg/m$^2$ | <22.58, (91) | 5.83 ± 1.95 | 2.94 ± 1.09 | -49.1 ± 10.9 | | |
| (Quartiles) | 22.58-24.84, (93) | 6.14 ± 1.78 | 3.22 ± 1.10 | -47.3 ± 11.2 | 0.712 | |
| | 24.85-27.07, (92) | 5.89 ± 1.65 | 3.05 ± 1.13 | -47.9 ± 11.4 | | |
| | >27.07, (91) | 6.07 ± 1.80 | 3.15 ± 1.15 | -48.2 ± 10.1 | | |
| WC, cm | <78.2, (83) | 6.25 ± 2.10 | 3.19 ± 1.19 | -48.5 ± 10.6 | | |
| (Quartiles) | 78.2-85.9, (84) | 5.84 ± 1.76 | 2.97 ± 1.05 | -48.7 ± 11.1 | 0.585 | |
| | 86.0-93.0, (83) | 6.07 ± 1.89 | 3.18 ± 1.20 | -47.2 ± 10.7 | | |
| | >93.0 (84) | 6.13 ± 1.59 | 3.26 ± 1.09 | -46.9 ± 10.5 | | |
| Body fat, % | <23.8, (60) | 6.19 ± 1.77 | 3.22 ± 1.28 | -48.1 ± 12.4 | | |
| (Quartiles) | 23.8-28.7, (59) | 6.39 ± 1.62 | 3.35 ± 0.98 | -47.1 ± 11.2 | 0.794 | |
| | 28.8-35.3, (61) | 6.56 ± 1.80 | 3.41 ± 1.11 | -48.0 ± 8.6 | | |
| | >35.3, (59) | 6.34 ± 1.66 | 3.20 ± 0.96 | -49.1 ± 10.1 | | |
| Baseline | <4.87, (95) | 3.99 ± 0.80 | 2.08 ± 0.57 | -47.2 ± 12.1 | | |
| non-HDL-C, | 4.87-5.79, (94) | 5.30 ± 0.26 | 2.76 ± 0.58 | -47.8 ± 10.8 | 0.910* | |
| mmol/L | 5.8-6.86, (100) | 6.28 ± 0.32 | 3.27 ± 0.75 | -48.0 ± 11.7 | | |
| (Quartiles) | >6.86, (97) | 8.28 ± 1.38 | 4.28 ± 1.07 | -48.4 ± 8.8 | | |
| FH | No, (220) | 5.04 ± 1.15 | 2.62 ± 0.82 | -47.9 ± 11.6 | 0.621* | |
| | Yes, (166) | 7.22 ± 1.67 | 3.76 ± 1.10 | -47.8 ± 9.9 | | |
| Diabetes | No, (277) | 6.13 ± 1.92 | 3.20 ± 1.14 | -47.5 ± 10.3 | 0.079* | 0.174 |
| | Yes, (109) | 5.59 ± 1.22 | 2.88 ± 1.00 | -48.8 ± 12.3 | | |
| Hypertension | No, (200) | 6.21 ± 2.05 | 3.21 ± 1.23 | -47.8 ± 10.9 | 0.985 | |
| | Yes, (186) | 5.73 ± 1.35 | 2.99 ± 0.96 | -47.8 ± 10.9 | | |
| History of | No, (331) | 6.04 ± 1.81 | 3.12 ± 1.12 | -48.1 ± 10.8 | 0.384* | |
| CVD | Yes, (55) | 5.61 ± 1.45 | 3.00 ± 1.05 | -46.5 ± 11.7 | | |

| | | | | | |
|---|---|---|---|---|---|
| RA | No, (350) | 6.25 ± 1.61 | 3.24 ± 1.06 | -48.0 ± 10.8 | 0.559* |
| | Yes, (36) | 3.40 ± 0.98 | 1.78 ± 0.53 | -46.3 ± 12.3 | |
| Current smoker | No, (349) | 5.94 ± 1.77 | 3.07 ± 1.10 | -48.1 ± 10.9 | 0.271* |
| | Yes, (37) | 6.32 ± 1.72 | 3.43 ± 1.18 | -45.7 ± 11.1 | |

Data were expressed as mean ± SD and compared by t-test or ANOVA unless otherwise indicated.

* Data were compared by non-parametric test (Mann-Whitney U test or Kruskal-Wallis H test) as data were skewed.

† P values were adjusted for multiple variables with P<0.2 in the univariant analysis including age, gender, and diabetes.

#### 4.3.2.6 Side effects and laboratory safety profiles

Rosuvastatin was well tolerated in all study participants, including those with poor (<80%) adherence to therapy who were excluded from the analysis. No patients had clinically relevant elevations in creatine kinase (CK), alanine aminotransferase (ALT) or creatinine, and no muscle problems were observed during the study. The main cause for poor adherence reported in those patients with <80% adherence was being forgetful, and none of them complained of significant side effects.

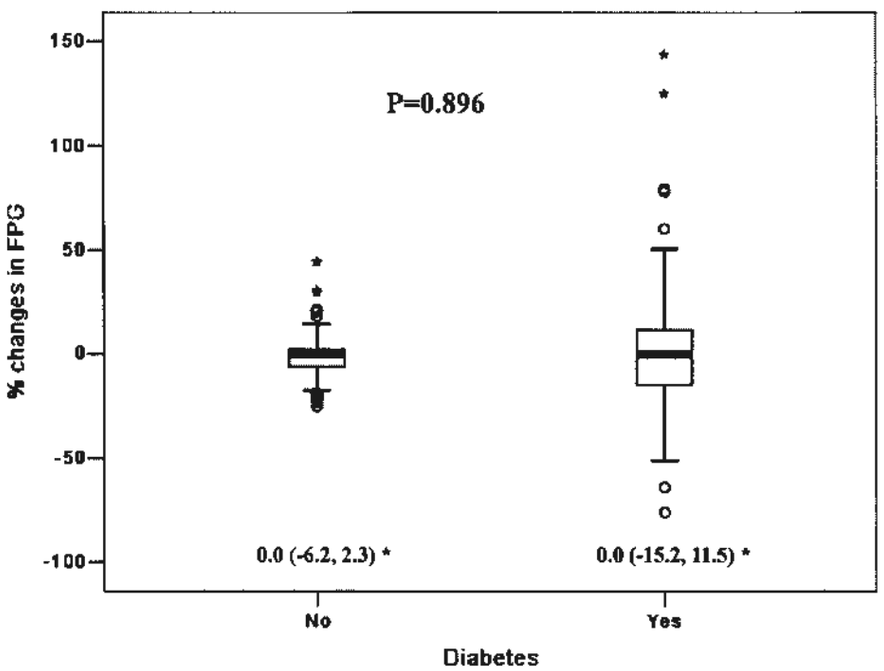
##### 4.3.2.6.1 Side effects

In all patients involved in the study, rosuvastatin 10 mg daily was well tolerated and no patient was withdrawn from the study due to any significant side effects of rosuvastatin. There was no rhabdomyolysis or muscle toxicity observed in the study. One patient, who had a history of muscle pain with some other statins previously, was given rosvuastatin 10 mg together with Coenzyme Q10 (10 mg, three times daily) while joining in the study, and she did not experience any muscle problem with this regimen.

#### 4.3.2.6.2 Glucose

After rosuvastatin treatment, there was a very small but significant change in FPG (-0.26 ± 18.1 %, P=0.009) from baseline mean (±SD) value of 6.2 ± 2.2 mmol/L to 6.1 ± 2.1 mmol/L in this group of patients. Patients with diabetes had no significant change in FPG (median 7.6 mmol/L before and after rosuvastatin treatment) whereas patients with no diabetes had a reduced FPG levels from baseline median value of 5.2 mmol/L to 5.1 mmol/L (P=0.001). However, there was no significant difference in percentage change in FPG between patients with or without diabetes (Figure **4-6**).

**Figure 4-6. Percentage changes in fasting plasma glucose in diabetic and non-diabetic patients**




* Data are median (interquartile range) and values were compared by Mann-Whitney test. Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

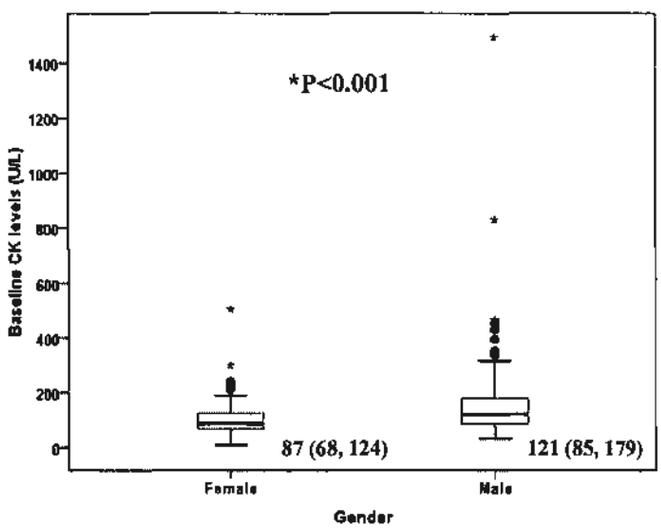
#### 4.3.2.6.3 Creatine kinase

Rosuvastatin treatment was associated with a significant increase (8.8 ± 85.2 U/L; 25.1 ± 95.2 %, P<0.001) in plasma CK levels from baseline mean (±SD) value of 128 ± 117 U/L to 136 ± 96 U/L. Female subjects had lower baseline and on-treatment CK levels than males (Figure 4-7) but there was no gender difference in percentage changes in CK.

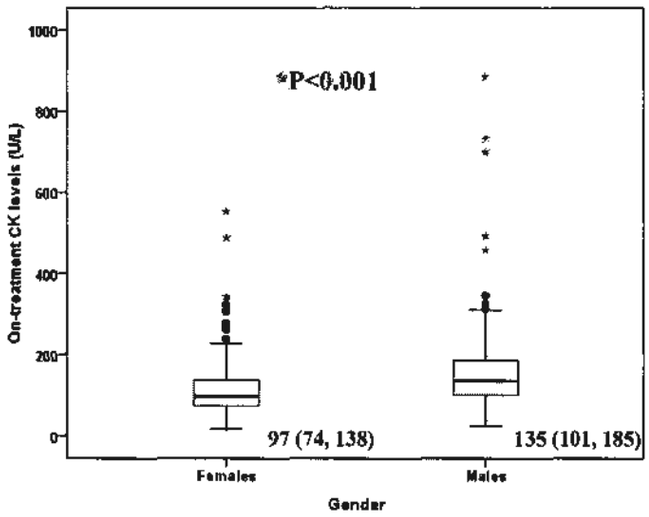
A 40-year-old man had a high baseline CK level of 1496 U/L and he had a history of persistent elevated CK for several years and muscle biopsy in 2001 revealed non-specific myopathy, which was considered to be related to his heavy workload. The CK level was 886 U/L while receiving rosuvastatin 10 mg daily and he had no myalgia. Some other patients also had high baseline and on-treatment CK levels due to their vigorous physical activities but they did not have muscle symptoms and they were monitored carefully during the treatment with statin.

**Figure 4-7. Baseline (A) and on-treatment CPK levels (B) in men and women**

**A. Baseline CK levels**



**B. On-treatment CK levels**




* Data are median (interquartile range) and values were compared by Mann-Whitney test. Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

#### 4.3.2.6.4 Liver and renal function tests

Male subjects had higher baseline and on-treatment levels of creatinine, ALT and total bilirubin but a lower level of ALP than females (Table **4-9**). Rosuvastatin therapy did not affect the plasma creatinine concentrations. After rosuvastatin treatment, the ALT levels were increased in both males and females, whereas the ALP concentrations were slightly decreased. There was a small but significant increase in total bilirubin levels in male subjects. No patients in the study had clinically important elevations in these parameters and there were no reports of liver or renal adverse events.

**Table 4-9. Liver and renal function tests before and after rosuvastatin treatment**

| | Baseline levels | On-treatment levels | % changes from baseline |
|---|---|---|---|
| ***All*** | | | |
| Creatinine (μmol/L) | 77 (65, 89) | 75 (66, 90) | 0.0 (-6.3, 5.7) |
| ALT (IU/L) | 23 (17, 33) | 27 (19, 37) *** | 11.8 (-6.0, 40.3) |
| ALP (U/L) | 70 (58, 85) | 67 (58, 82) *** | -2.38 (-9.46, 4.88) |
| Total bilirubin (μmol/L) | 11 (8, 14) | 11 (9, 14) ** | 0.0 (-14.3, 27.3) |
| ***Males*** | | | |
| Creatinine (μmol/L) | 89 (80, 98) | 90 (79, 100) | 0.0 (-6.2, 5.9) |
| ALT (IU/L) | 28 (20, 38) | 33 (23, 43) *** | 14.3 (-4.0, 40.9) |
| ALP (U/L) | 66 (57, 81) | 65 (57,80) * | -1.59 (-9.46, 5.42) |
| Total bilirubin (μmol/L) | 12 (9, 15) | 12 (10, 16) * | 5.88 (-13.6, 25.0) |
| ***Females*** | | | |
| Creatinine (μmol/L) | 67 (61, 76) | 67 (61, 75) | -1.3 (-6.6, 5.0) |
| ALT (IU/L) | 21 (16, 27) | 24 (19, 32) *** | 9.3 (-9.5, 38.7) |
| ALP (U/L) | 73 (58, 86) | 72 (58, 85) *** | -2.99 (-9.46, 4.17) |
| Total bilirubin (μmol/L) | 10 (8, 13) | 10 (8, 13) | 0.0 (-14.3, 28.6) |

Data are median (interquartile ranges) and values of baseline and on-treatment levels were compared by Wilcoxon Signed Ranks Test.

*P<0.05; ** P<0.01; ***P<0.001.

Abbreviations: ALT= alanine transaminase; ALP= alkaline phosphatase.

### 4.3.3 Genetic determinants of LDL-C response to rosuvastatin

Of 126 polymorphisms evaluated in the present study, 8 SNPs (rs255052 in *LCAT* [lecithin-cholesterol acyltransferase], rs1433099 in *LDLR* [LDL receptor], 421C>A and 34G>A in *ABCG2*, V257M in *FMO3* [flavin containing monooxygenase 3], rs16996148 in *NCAN/CILP2/PBX4* [neurocan / cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4], rs4420638 in *APOE/C1/C4/C2* cluster, and 1421C>G in *LPL* [lipoprotein lipase]) were associated with the percentage changes in LDL-C in response to rosuvastatin before and after adjustment for age, gender and having FH (P<0.05) (Table **4-10**). The *7 variant in *NAT2* (N-acetyltransferase 2) tended to be related to the LDL-C response to rosuvastatin in univariate analysis but

there was no gene-dose effect and the relationship was no longer significant after adjustment for age, gender and having FH.

**Table 4-10. Associations between genetic polymorphisms and LDL-C response to rosuvastatin**

| *Gene or nearby loci* / SNPs | Genotype | N | % change in LDL-C | P | P* | P† | P‡ |
|---|---|---|---|---|---|---|---|
| *LCAT* | GG | 314 | -52.4 ± 12.2 | 0.038 | 0.033 | 0.037 | 0.04 |
| rs255052 | GA/AA | 63/2 | -48.9 ± 11.8 | | | | |
| *LDLR* | CC | 197 | -52.6 ± 12.3 | | | | |
| rs1433099 | CT | 150 | -51.8 ± 11.7 | 0.017 | 0.024 | 0.045 | *0.111* |
| | TT | 28 | -45.6 ± 13.9 | | | | |
| *NAT2* | GG | 251 | -50.7 ± 12.4 | | | | |
| *7, 857G>A | GA | 116 | -54.2 ± 11.7 | 0.035 | *0.062* | *0.111* | --- |
| | AA | 11 | -52.5 ± 11.7 | | | | |
| *ABCG2* | CC | 191 | -48.9 ± 12.2 | | | | |
| 421C>A | CA | 136 | -53.8 ± 11.7 | $3.8\times10^{-6}$ | $5.9\times10^{-6}$ | — | 0.0001 |
| | AA | 55 | -57.0 ± 10.8 | | | | |
| *ABCG2* | GG | 175 | -54.3 ± 11.9 | | | | |
| 34G>A | GA | 162 | -49.9 ± 11.9 | 0.002 | 0.001 | *0.258* | --- |
| | AA | 38 | -49.8 ± 12.6 | | | | |
| *FMO3* | GG | 226 | -53.8 ± 11.3 | | | | |
| Val257Met | GA | 131 | -49.2 ± 12.7 | 0.0005 | 0.0005 | 0.003 | 0.001 |
| | AA | 23 | -47.3 ± 14.2 | | | | |
| *NCAN/CILP2/PBX4* | GG | 308 | -52.5 ± 11.9 | 0.011 | 0.01 | 0.038 | 0.043 |
| rs16996148 | GT | 70 | -48.4 ± 12.9 | | | | |
| *APOE/C1/C4/C2* | AA | 287 | -52.4 ± 12.1 | | | | |
| rs4420638 | AG | 77 | -50.5 ± 12.3 | 0.045 | 0.031 | 0.017 | 0.047 |
| | GG | 8 | -42.5 ± 14.6 | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *LPL* | CC | 290 | -50.8 ± 12.9 | 0.005 | 0.009 | 0.004 | 0.001 |
| 1421C>G | CG/GG | 84/4 | -55.0 ± 9.0 | | | | |

Data were expressed as mean ± SD and values of %LDL-C response among genotype groups were compared by ANOVA unless otherwise indicated.

* P value was calculated after adjustment for age, gender and having familial hypercholesterolaemia.

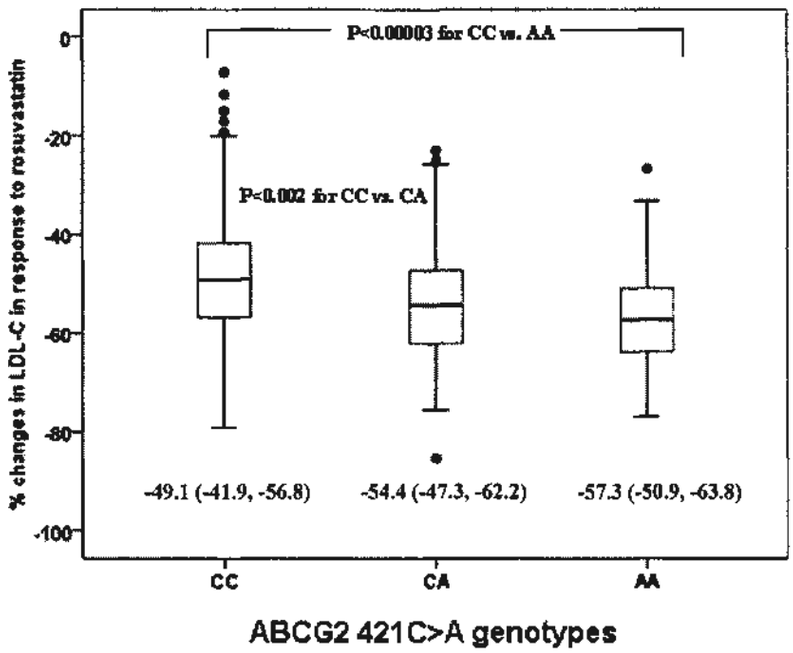
† P value was calculated after adjustment for age, gender, having familial hypercholesterolaemia and *ABCG2* 421C>A polymorphism.

‡ P value was calculated after adjustment for age, gender, having familial hypercholesterolaemia and all other genetic polymorphisms with $P^{\dagger}<0.05$ in the table.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; APOE/C1/C4/C2 = apolipoprotein E/C-I/C-IV/C-II; FMO3 = flavin containing monooxygenase 3; LCAT = lecithin-cholesterol acyltransferase; LDLR = low density lipoprotein receptor; LPL = lipoprotein lipase; NAT2 = N-acetyltransferase 2; NCAN/CILP2/PBX4 = neurocan / cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4.

Of these 8 SNPs, the *ABCG2* 421C>A (MAF = 0.322) that was associated with percentage reduction in LDL-C in the response to rosuvastatin before and after adjustment for age, gender and having FH in a gene-dose dependent manner was the only SNP reaching the experiment-wide significance level ($P<1\times10^{-5}$). Patients with the 421AA genotype (n=55) had a greater reduction in LDL-C (least square mean ± SE) than those with 421CC genotype (n=191) (57.1 ± 1.6 % vs. 49.1 ± 0.8 %) with 421CA heterozygote subjects having intermediate values (-53.6 ± 1.0 % n=136) after adjustment for age, gender and having FH (Figure **4-8**).

**Figure 4-8. Percentage changes in LDL-C by *ABCG2* 421C>A genotypes**

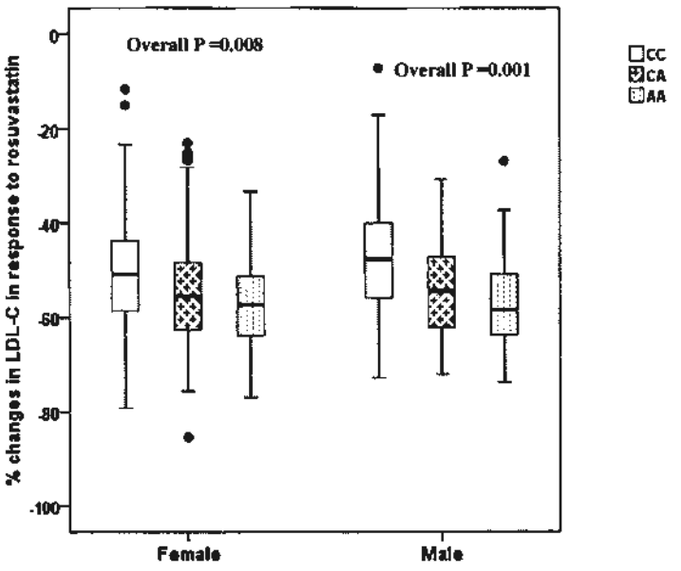



Median (interquartile range) of LDL-C response in each genotype groups was given. Values of LDL-C response among genotype groups were compared by ANCOVA with age, gender, having familial hypercholesterolaemia as covariates.

Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval and circles indicating outliers. CC: n=191; CA: n=136; AA: n=55

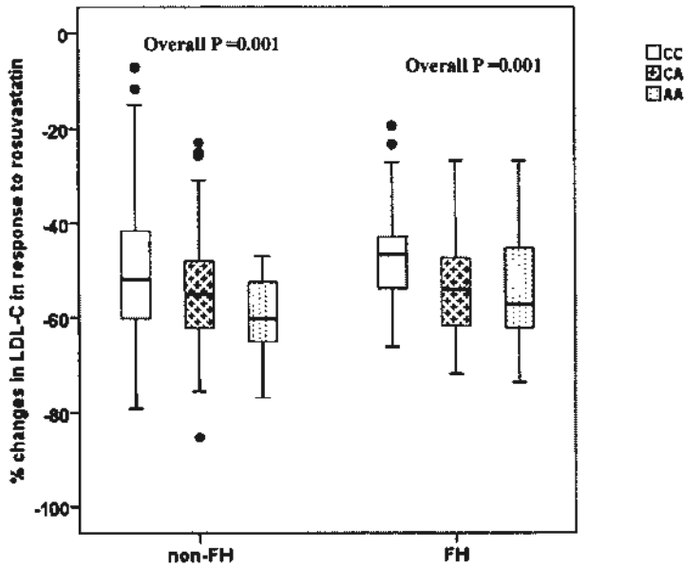
The association between the *ABCG2* 421C>A polymorphism and the percentage changes in LDL-C in response to rosuvastatin remained for both sexes (Figure **4-9**), and for both subgroups of FH and non-FH (Figure **4-10**). This SNP was not related to the baseline LDL-C levels but the A variant allele of this SNP was significantly (P<0.0001) associated with lower on-treatment LDL-C levels (Figure **4-11**).

**Figure 4-9. Associations of percentage changes in LDL-C with *ABCG2* 421C>A genotypes in males and females**



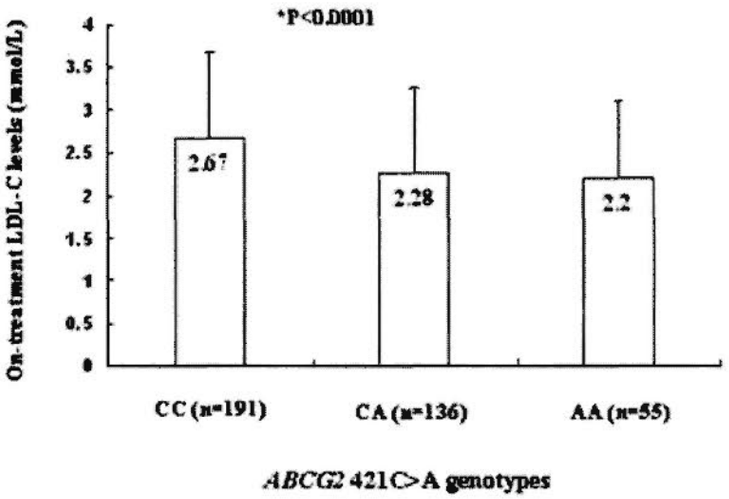

Female: CC: n=98; CA: n=81; AA: n=33. Male: CC: n=93; CA: n=55; AA: n=22.

**Figure 4-10. Associations of percentage changes in LDL-C with *ABCG2* 421C>A genotypes in patients with and without FH**




Non-FH: CC: n=107; CA: n=80; AA: n=31. FH: CC: n=84; CA: n=56; AA: n=24.

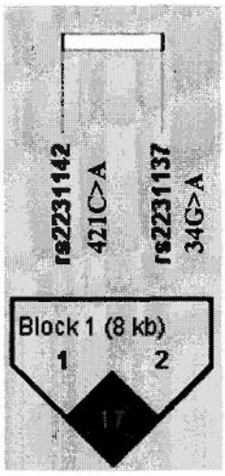
**Figure 4-11. Association of *ABCG2* 421C>A polymorphism and mean LDL-C on-treatment levels**




* P value was calculated by Kruskal-Wallis H test.

Another SNP in *ABCG2* (34G>A, MAF = 0.214) examined in this study was shown to be related to the LDL-C response but there was no gene-dose effect and this association was no longer present after adjustment for the *ABCG2* 421C>A polymorphism (P>0.05), suggesting its effect on the LDL-C response observed was due to its linkage with the 421C>A polymorphism (Figure **4-12**). Indeed, diplotype analysis showed that the 34G>A polymorphism had no effect on LDL-C response to rosuvastatin within each of the 421C>A genotype groups, whereas the 421C>A polymorphism was significantly associated with % change in LDL-C within 34GG and 34GA genotype groups (Table **4-11**).

**Figure 4-12. Degree of linkage disequilibrium of 421C>A and 34G>A polymorphisms in the *ABCG2***




Value in the diamond represent the pairwise LD coefficients ($r^2$) calculated using Haploview.

**Table 4-11. The LDL-C response among diplotypes of 421C>A and 34G>A polymorphisms in the *ABCG2***

| Diplotypes 421C>A / 34G>A | n | % change in LDL-C | Overall P |
|---|---|---|---|
| CC / GG | 48 | -49.8 ± 11.7 * | |
| CC / GA | 100 | -48.5 ± 12.1 †‡ | |
| CC / AA | 38 | -49.8 ± 12.6 | |
| CA / GG | 75 | -55.2 ± 12.1 | 0.0002 |
| CA / GA | 59 | -52.1 ± 11.1 | |
| AA / GG | 52 | -57.2 ± 10.7 | |
| AA / GA | 3 | -54.0 ± 15.4 | |

Data were expressed as mean ± SD and values of LDL-C response among diplotype groups were compared by ANOVA.

* P=0.039 for CC/GG vs. AA/GG;

† P=0.005 for CC/GA vs. CA/GA;

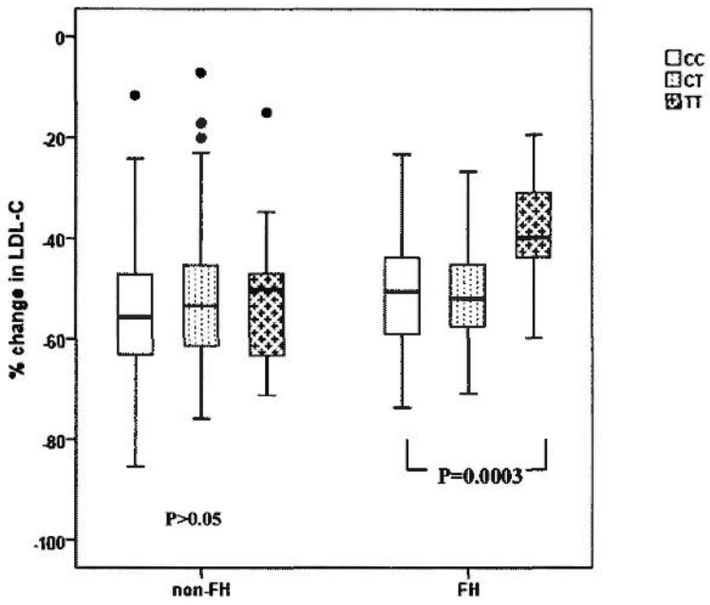
‡ P=0.005 for CC/GA vs. AA/GG..

In addition to the *ABCG2* polymorphisms described above, the other 6 SNPs

(rs255052 in *LCAT* [MAF = 0.088]; rs1433099 in *LDLR* [MAF = 0.275]; V257M in *FMO3* [MAF = 0.233]; rs16996148 in *NCAN/CILP2/PBX4* [MAF = 0.093]; rs4420638 in *APOE/C1/C4/C2* cluster [MAF = 0.125] and 1421C>G in *LPL* [MAF = 0.122]) also tended to be associated with the LDL-C response before and after adjustment for age, gender and having FH but these associations failed to reach the prior defined experiment-wide significance level after multiple correction (Table **4-10**).

In addition, the association between rs1433099 in *LDLR* and LDL-C response was not present after adjustment for age, gender, having FH and other genetic factors. However, further analysis revealed that this polymorphism was significantly associated with LDL-C response to rosuvastatin in patients with FH, but not in non-FH patients (Figure **4-13**). In patients with FH, subjects with two copies of the variant allele (TT: 38.5±10.7, n=12) had significantly (P<0.001) smaller LDL-C reduction compared to those with one or two copies of wild-type alleles (CT: 51.6±9.2%, n=67; CC: 50.7±11.1%, n=82).

**Figure 4-13. Effect of *LDLR* rs1433099 on LDL-C response to rosuvastatin in patients with or without FH**




Non-FH: CC: n=115; CT: n=83; TT: n=16. FH: CC: n=82; CT: n=67; TT: n=12.

Neither the polymorphisms in *HMGCR*, *APOE*, *APOB* or other newly identified lipid-related loci, nor variants in drug metabolizing enzymes or drug transporters showed significant effects on LDL-C reduction (Table **4-12**).

**Table 4-12. Negative associations (P>0.05) between certain genetic factors and LDL-C response to rosuvastatin 10mg daily**

| Gene | SNP | Genotype | n | Baseline LDL-C (mmol/L) | On-treatment LDL-C (mmol/L) | Percentage reduction in LDL-C |
|---|---|---|---|---|---|---|
| ***Polymorphisms in genes related to pharmacokinetics of statins*** | | | | | | |
| *CYP2C9* | *3 (1075A>C) | AA | 260 | 5.13 (1.68) | 2.46 (1.00) | -51.7 ± 12.2 |
| | | AC | 23 | 5.41 (1.91) | 2.56 (1.17) | -53.2 ± 12.3 |
| *CYP2C19* | *2 (681G>A) | GG | 155 | 5.09 (1.67) | 2.45 (0.97) | -51.4 ± 12.0 |
| | | GA | 182 | 5.13 (1.71) | 2.48 (1.05) | -51.6 ± 12.4 |
| | | AA | 26 | 5.30 (2.07) | 2.34 (1.16) | -56.5 ± 10.6 |
| *CYP2C19* | *3 (636G>A) | GG | 353 | 5.12 (1.74) | 2.46 (1.03) | -51.8 ± 12.2 |
| | | GA | 28 | 5.32 (1.32) | 2.54 (0.85) | -51.9 ± 12.4 |
| *CYP2D6* | *10, *5 | *1*1,*2*2 | 38 | 5.39 (1.57) | 2.57 (1.05) | -51.7 ± 13.6 |
| | | *1/*10 | 138 | 5.33 (1.65) | 2.57 (0.99) | -51.8 ± 11.8 |
| | | *10/*10 | 114 | 5.21 (1.60) | 2.51 (0.99) | -51.7 ± 12.2 |
| | | *5 carriers | 32 | 5.19 (1.56) | 2.40 (0.80) | -53.1 ± 11.6 |
| *CYP3A5* | *3 | *1*1 | 28 | 4.69 (1.63) | 2.11 (0.92) | -54.2 ± 14.6 |
| | | *1*3 | 159 | 5.21 (1.66) | 2.50 (1.00) | -51.9 ± 11.8 |
| | | *3*3 | 197 | 5.13 (1.74) | 2.48 (1.04) | -51.4 ± 12.2 |
| *UGT1A1* | *28 | *1*1 | 292 | 5.22 (1.69) | 2.54 (1.05) | -51.4 ± 12.4 |
| | | *1*28 | 82 | 5.01 (1.08) | 2.28 (0.85) | -53.2 ± 11.0 |
| | | *28*28 | 6 | 4.57 (0.87) | 1.94 (0.77) | -58.6 ± 9.0 |
| *ABCB1* | 1236C>T | CC | 152 | 5.12 (1.50) | 2.46 (0.95) | -51.7 ± 12.3 |
| | | CT | 171 | 5.02 (1.70) | 2.40 (0.96) | -51.7 ± 11.9 |
| | | TT | 59 | 5.57 (2.06) | 2.69 (1.26) | -51.8 ± 12.8 |
| | 2677G>T/A | GG | 97 | 5.24 (1.73) | 2.52 (1.08) | -52.0 ± 13.2 |
| | | GT/GA | 204 | 5.08 (1.68) | 2.46 (0.95) | -51.0 ± 12.0 |
| | | TT/AA/TA | 61 | 5.32 (1.75) | 2.51 (1.14) | -53.1 ± 12.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 3435C>T | CC | 140 | 5.15 (1.77) | 2.44 (1.04) | -52.4 ± 12.1 |
| | | CT | 195 | 5.07 (1.67) | 2.44 (0.95) | -51.5 ± 12.0 |
| | | TT | 48 | 5.36 (1.64) | 2.63 (1.21) | -51.2 ± 13.8 |
| | Haplotype | CGC/CGC | 36 | 5.64 (2.05) | 2.72 (1.30) | -52.2 ± 14.9 |
| | | CGC/TTT | 58 | 4.91 (1.75) | 2.37 (1.01) | -51.5 ± 11.6 |
| | | TTT/TTT | 35 | 5.32 (1.69) | 2.61 (1.22) | -51.1 ± 14.4 |
| | | Others | 250 | 5.09 (1.62) | 2.43 (0.93) | -51.9 ± 11.7 |
| *SLCO1B1* | 388A>G | AA | 24 | 4.76 (1.39) | 2.40 (0.89) | -49.7 ± 13.1 |
| | | AG | 121 | 5.22 (1.84) | 2.52 (1.03) | -51.5 ± 11.3 |
| | | GG | 207 | 5.13 (1.66) | 2.48 (1.02) | -51.5 ± 12.6 |
| | 521T>C | TT | 286 | 5.17 (1.68) | 2.48 (0.99) | -51.6 ± 12.5 |
| | | TC | 83 | 5.09 (1.78) | 2.46 (1.09) | -51.9 ± 11.7 |
| | | CC | 10 | 4.79 (0.98) | 2.19 (0.76) | -54.7 ± 9.1 |
| *SLC10A1* | *2 (800C>T) | CC | 313 | 5.10 (1.66) | 2.47 (1.00) | -51.5 ± 12.3 |
| | | CT/TT | 64/5 | 5.27 (1.80) | 2.44 (1.04) | -53.0 ± 11.7 |
| ***Polymorphisms in genes related to pharmacodynamics of statins*** | | | | | | |
| *APOB* | ID | II | 221 | 4.90 (1.63) | 2.39 (0.97) | -51.1 ± 12.8 |
| | | ID | 142 | 5.41 (1.72) | 2.55 (1.05) | -52.8 ± 11.1 |
| | | DD | 19 | 5.68 (2.14) | 2.62 (1.28) | -52.4 ± 13.6 |
| *APOE* | e2/e3/e4 | e2 carrier | 32 | 5.60 (2.31) | 2.66 (1.33) | -52.3 ± 14.0 |
| | | e3e3 | 259 | 5.19 (1.72) | 2.46 (1.04) | -52.4 ± 12.0 |
| | | e4 carrier | 84 | 4.88 (1.28) | 2.44 (0.80) | -49.4 ± 12.4 |
| *HMGCR* | rs3846662 C>T | CC | 124 | 5.10 (1.59) | 2.41 (0.98) | -52.7 ± 11.2 |
| | | CT | 197 | 5.24 (1.83) | 2.52 (1.07) | -51.5 ± 12.6 |
| | | TT | 61 | 4.85 (1.44) | 2.37 (0.90) | -51.0 ± 12.9 |
| | rs12654264 A>T | AA | 121 | 5.12 (1.60) | 2.43 (0.98) | -52.6 ± 11.2 |
| | | AT | 194 | 5.24 (1.84) | 2.52 (1.08) | -51.5 ± 12.8 |
| | | TT | 62 | 4.89 (1.49) | 2.37 (0.89) | -51.1 ± 12.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *PCSK9* | 158C>T | CC | 290 | 5.10 (1.75) | 2.42 (1.02) | -52.2 ± 12.4 |
| | | CT | 86 | 5.32 (1.57) | 2.65 (0.98) | -50.1 ± 11.2 |
| | | TT | 7 | 4.84 (1.01) | 1.90 (0.50) | -60.2 ± 9.7 |
| | 2009G>A | GG | 345 | 5.16 (1.72) | 2.46 (1.02) | -52.0 ± 12.3 |
| | | GA | 39 | 4.95 (1.57) | 2.46 (0.94) | -50.0 ± 11.1 |

Data were expressed as mean ± SD.

Abbreviations: ABCB1 = ATP-binding cassette, subfamily B, member 1; APOB = apolipoprotein B; APOE = apolipoprotein E; CYP2C9 = cytochrome P450 family 2, subfamily C, polypeptide 9; CYP2C19 = cytochrome P450 family 2, subfamily C, polypeptide 19; CYP3A5 = cytochrome P450 family 3, subfamily A, polypeptide 5; CYP2D6 = cytochrome P450 family 2, subfamily D, polypeptide 6; HMGCR = 3-hydroxy-4-methylglutaryl-Coenzyme A reductase; PCSK9 = proprotein convertase subtilisin/kexin type 9; SLCO1B1 = solute carrier organic anion transporter family, member 1B1; SLC10A1 = solute carrier family 10, member 1; UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1.

Multivariate stepwise regression analysis showed that *ABCG2* 421C>A ($P=9.2\times10^{-7}$), *FMO3* V257M (P = 0.0002), *LPL* 1421C>G (P=0.002), *APOE/C1/C4/C2* rs4420638 (P=0.004), and having FH (P=0.009) were predictors of LDL-C response, which totally explained 13.6% of the variance in percentage change in LDL-C in response to rosuvastatin. Age, gender, baseline LDL-C level and other genetic factors were not determinants of LDL-C response to rosuvastatin treatment in the multivariate analysis (P>0.05). The *ABCG2* 421C>A polymorphism explained 6.0% of the variance in percentage reduction in LDL-C in response to rosuvastatin, whereas having FH only contributed to 1.4% of the variance. *FMO3* V257M, *LPL* 1421C>G and *APOE/C1/C4/C2* rs4420638 explained 2.9%, 1.7% and 1.6% of variance in percentage reductions in LDL-C, respectively (Table **4-13**).

**Table 4-13. Multivariate model of predictors of % change in LDL-C in response to rosuvastatin**

| Variables | Standardized Coefficients | P | Adjusted $R^2$ |
|---|---|---|---|
| *ABCG2* 421C>A* | -0.243 | $9.2\times10^{-7}$ | 0.060 |
| *FMO3* V257M* | 0.182 | 0.0002 | 0.029 |
| *LPL* 1421C>G * | -0.153 | 0.002 | 0.017 |
| *APOE/C1/C4/C2* rs4420638 * | 0.141 | 0.004 | 0.016 |
| Having FH | 0.127 | 0.009 | 0.014 |

* Homozygous wild-type allele = 1; heterozygous wild-type allele = 2; homozygous variant allele = 3.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; APOE/C1/C4/C2 = apolipoprotein E/C-I/C-IV/C-II; FH = Familial hypercholesterolaemia; FMO3 = flavin containing monooxygenase 3; LPL = lipoprotein lipase.

### 4.3.4 Genetic determinants of HDL-C response to rosuvastatin

Three genetic polymorphisms (rs1260326 in *GCKR* [glucokinase (hexokinase 4) regulator] [MAF = 0.483] -1131T>C in *APOA5* [apolipoprotein A-V] [MAF = 0.303] and 521T>C in *SLCO1B1* [MAF = 0.136]) tended to be associated with percentage changes in HDL-C ($P<0.05$) as shown in Table **4-14**, but none of these genetic factors reach the prior defined significance level.

**Table 4-14. Associations between genetic polymorphisms and HDL-C response to rosuvastatin**

| Gene/SNPs | Genotype | N | Baseline HDL-C (mmol/L) | % change in HDL-C | P |
|---|---|---|---|---|---|
| *GCKR* | CC | 107 | 1.52 ± 0.45 | -1.49 ± 11.89 | |
| rs1260326 | CT | 178 | 1.51 ± 0.40 | 2.73 ± 13.61 | 0.026 |
| | TT | 94 | 1.49 ± 0.35 | 2.71 ± 13.97 | |
| *APOA5* | TT | 182 | 1.58 ± 0.42 | 0.69 ± 12.82 | |
| -1131 T>C | TC | 131 | 1.50 ± 0.40 | 0.97 ± 11.96 | 0.026 |
| | CC | 42 | 1.35 ± 0.32 * | 7.05 ± 14.82 | |
| *SLCO1B1* | TT | 287 | 1.51 ± 0.41 | 2.39 ± 13.59 | |
| 521 T>C | TC | 83 | 1.52 ± 0.39 | -1.27 ± 12.36 | 0.05 |
| | CC | 10 | 1.59 ± 0.50 | -1.12 ± 10.31 | |
| *SLCO1B1* | TT | 287 | 1.51 ± 0.41 | 2.39 ± 13.59 | 0.023 |
| 521 T>C | TC/CC | 83/10 | 1.52 ± 0.40 | -1.25 ± 12.10 | |

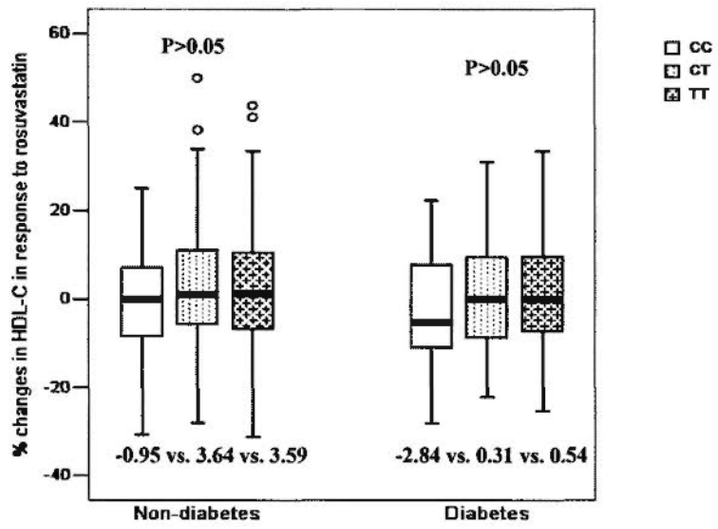
Data were expressed as mean ± SD and values of HDL-C response among genotype groups were compared by Kruskal-Wallis H test.

*P=0.002 for baseline level of HDL-C among 3 genotype groups.

Abbreviations: APOA5 = apolipoprotein A-V; GCKR = glucokinase (hexokinase 4) regulator; SLCO1B1 = Solute carrier organic anion transporter family, member 1B1.

Subjects with one or two copies of the T variant allele of rs1260326 in *GCKR* had a more favorable response in HDL-C levels (Table **4-14**). The effect of this polymorphism remained in patients with or without diabetes although the associations between rs1260326 and HDL-C response in both subgroups were not significant (P>0.05) due to relatively small numbers (Figure **4-14**).

**Figure 4-14. Effect of rs1260326 in *GCKR* on % changes in HDL-C in patients with and without diabetes**




Mean values of % change in HDL-C in each genotype groups were presented in the figure and values among genotype groups in patients with and without diabetes were compared by Kruskal-Wallis H test.

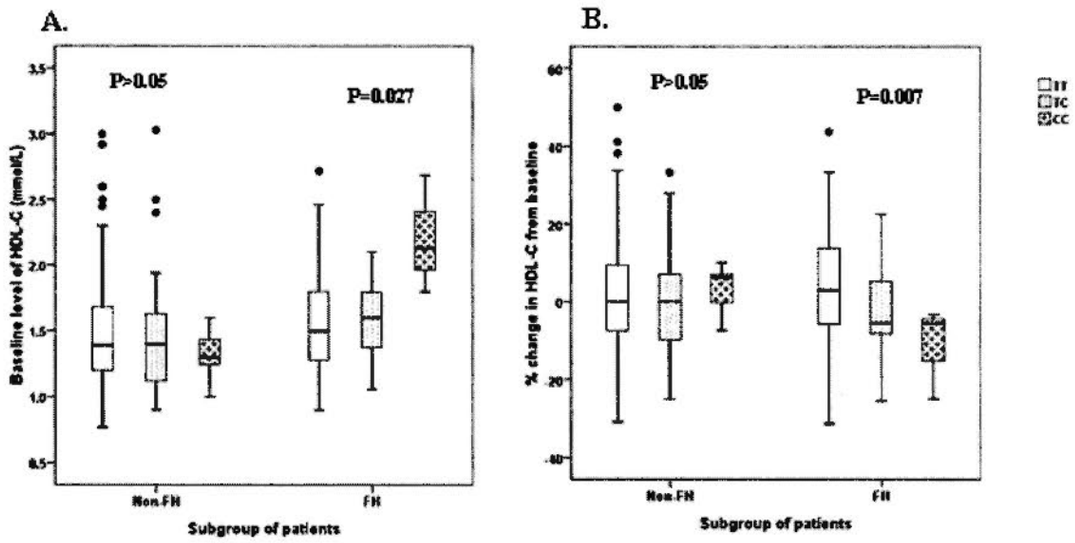
Non-diabetes: CC: n=76; CT: n=129; TT: n=67. Diabetes: CC: n=31; CT: n=49; TT: n=27.

The homozygous variant allele of -1131 T>C in *APOA5* was associated with a low baseline level of HDL-C and a greater HDL-C increase in response to rosuvastatin (Table **4-14**). The relationship between this polymorphism and HDL-C response was no longer present after adjustment for baseline HDL-C levels (P>0.05), suggesting that the *APOA5* -1131 T>C polymorphism affected the baseline HDL-C and thereby HDL-C response to rosuvastatin.

The *SLCO1B1* 521 T>C was associated with percentage change in HDL-C with subjects carrying one or two copies of the variant allele having less increase in HDL-C with rosuvastatin treatment compared to those with homozygous wild-type alleles (Table **4-14**). Further analysis in subgroups of patients with and without FH

showed that the *SLCO1B1* 521T>C polymorphism was associated with baseline HDL-C level and HDL-C response in patients with FH, but not for non-FH patients (Figure **4-15**), suggesting the effect on HDL-C response may be partly due to its impact on baseline HDL-C levels.

**Figure 4-15. Effect of *SLCO1B1* 521T>C polymorphism on baseline HDL-C levels and HDL-C response to rosuvastatin in patients with and without FH**




A. Effect of *SLCO1B1* 521T>C polymorphism on baseline HDL-C levels; B. Effect of *SLCO1B1* 521T>C polymorphism on HDL-C response to rosuvastatin. Values among genotype groups in patients with and without FH were compared by Kruskal-Wallis H test. Non-FH: TT: n=160; TC: n=48; CC: n=7. FH: TT: n=127; TC: n=35; CC: n=3.

In multivariate stepwise regression analysis, baseline HDL-C, having diabetes or RA and the *SLCO1B1* 521T>C polymorphism were determinants of percentage changes in HDL-C in response to rosuvastatin, which total explained 9.9% of the variance in percentage change in HDL-C in response to rosuvastatin whereas genetic factors only contributed to a very small proportion of the variance (0.8%) (Table **4-15**). The

*GCKR* rs1260326 and *APOA5* -1131T>C polymorphisms were not associated with HDL-C response after adjustment for confounding factors (P>0.05).

**Table 4-15. Multivariate model of predictors of % change in HDL-C in response to rosuvastatin**

| Variables | Standardized Coefficients | P | Adjusted $R^2$ |
|---|---|---|---|
| Baseline HDL-C levels | -0.243 | $1.6\times10^{-6}$ | 0.049 |
| Having diabetes | -0.180 | 0.0004 | 0.019 |
| Having RA | -0.159 | 0.002 | 0.023 |
| *SLCO1B1* 521T>C* | -0.105 | 0.031 | 0.008 |

* Homozygous wild-type allele = 1; heterozygous wild-type allele = 2; homozygous variant allele = 3.

Abbreviations: RA = rheumatoid arthritis; SLCO1B1 = Solute carrier organic anion transporter family, member 1B1.

### 4.3.5 Genetic determinants of triglyceride response to rosuvastatin

Among all polymorphisms examined, three SNPs in *CYP3A* tended to be related to triglyceride response but none of them showed a gene-dose effect or reached the experiment-wide significance levels (Table **4-16**). The *DGAT2* (diacylglycerol O-acyltransferase homolog 2) rs10899113 C>T polymorphism also appeared to be associated with triglyceride response in a gene-dose dependent manner (Table **4-16**).

**Table 4-16. Associations between genetic polymorphisms and of triglyceride response to rosuvastatin**

| Gene/SNPs | Genotype | N | Baseline TG (mmol/L) | On-treatment TG (mmol/L) | % change in TG | P |
|---|---|---|---|---|---|---|
| *CYP3A4* | CC | 200 | 2.03 ± 1.61 | 1.44 ± 0.80 | -19.3 ± 29.7 | |
| *1G | CT | 160 | 2.01 ± 1.31 | 1.41 ± 0.82 | -21.7 ± 35.0 | **0.018** |
| | TT | 21 | 1.69 ± 1.11 | 1.52 ± 0.75 | 2.7 ± 50.4 | |
| *CYP3AP1* | AA | 193 | 2.04 ± 1.52 | 1.51 ± 0.88 | -16.2 ± 36.2 | |
| -44G>A | AG | 151 | 1.98 ± 1.46 | 1.31 ± 0.67 | -24.8 ± 27.6 | **0.019** |
| | GG | 30 | 1.95 ± 1.25 | 1.58 ± 0.86 | -8.5 ± 41.2 | |
| *CYP3A5* | AA | 28 | 1.90 ± 1.28 | 1.56 ± 0.88 | -7.3 ± 42.4 | |
| *3 | AG | 159 | 1.98 ± 1.44 | 1.32 ± 0.67 | -24.2 ± 28.8 | **0.028** |
| | GG | 197 | 2.04 ± 1.52 | 1.50 ± 0.88 | -16.9 ± 35.4 | |
| *DGAT2* | CC | 219 | 2.08 ± 1.54 | 1.44 ± 0.87 | -22.0 ± 32.9 | |
| rs10899113 | CT | 152 | 1.91 ± 1.35 | 1.40 ± 0.69 | -16.2 ± 34.8 | **0.047** |
| | TT | 15 | 1.81 ± 1.43 | 1.48 ± 0.92 | -11.8 ± 28.4 | |

Data were expressed as mean ± SD and values of triglyceride response among genotype groups were compared by Kruskal-Wallis H test.

Abbreviations: CYP3A4 = cytochrome P450 family 3, subfamily A, polypeptide 4; CYP3A5 = cytochrome P450 family 3, subfamily A, polypeptide 5; CYP3AP1 = cytochrome P450, family 3, subfamily A, polypeptide 5, pseudogene 1; DGAT2 = diacylglycerol O-acyltransferase homolog 2; TG = triglycerides.

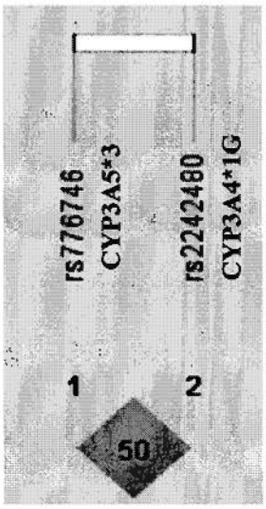
Those 3 SNPs in *CYP3A* are in strong LD, particularly for the -44G>A in *CYP3AP1* (cytochrome P450, family 3, subfamily A, polypeptide 5, pseudogene 1) and *CYP3A5* *3. We found that the *CYP3A5* *3 variant allele was closely linked to the the *CYP3AP1* -44G allele (Table **4-17**). The *CYP3A5* *3 (or *CYP3AP1* -44G>A) were also in strong LD with *CYP3A4* *1G ($r^2$=0.5, Figure **4-16**).

**Table 4-17. Genotype distributions of *CYP3A5**3 and *CYP3AP1* -44G>A in study subjects**

| | | CYP3A5 *3 | | |
|---|---|---|---|---|
| | | *1*1 | *1*3 | *3*3 |
| CYP3AP1 -44G>A | GG | **28** | 2 | 0 |
| | GA | 0 | **150** | 1 |
| | AA | 0 | 3 | **189** |

Values in the table represent subject number in each genotype groups.

**Figure 4-16. Degree of linkage disequilibrium of *CYP3A4* *1G and *CYP3A5* *3**



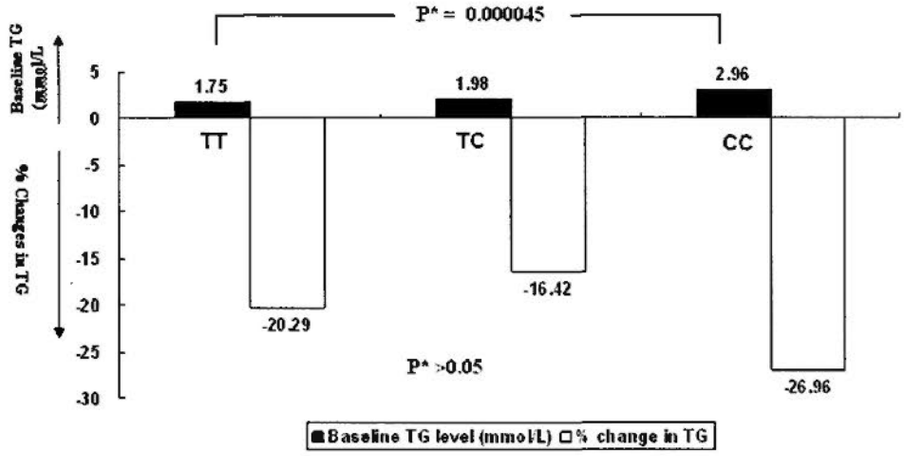

Value in the diamond represent the pairwise LD coefficients ($r^2$) calculated using Haploview.

In multivariate stepwise regression analysis, baseline triglyceride level was the only factor (beta = -0.383, $P = 6.8\times10^{-15}$) that strongly related to the percentage change in triglycerides in response to rosuvastatin, which explained 14.5% of variance in triglyceride response. None of the other genetic or phenotypic factors showed a

significant effect on triglyceride response after adjustment for baseline level of triglyceride (P>0.05).

The *APOA5* -1131 T>C polymorphism was strongly ($P = 4.5\times10^{-5}$) associated with baseline triglyceride levels in a gene-dose dependent manner, but this polymorphism did not affect triglyceride response to rosuvastatin in this study (Figure **4-17**).

**Figure 4-17. Effect of the *APOA5* -1131 T>C polymorphism on baseline triglyceride levels and percentage changes in triglycerides in response to rosuvatatin**




*Mean values of baseline triglyceride and % change in triglycerides in each genotype group were presented in the figure and values among genotype groups were compared by Kruskal-Wallis H test.

TT: n=182; TC: n=131; CC: n=42.

### 4.3.6 Genetic determinants of total cholesterol response to rosuvastatin

Two polymorphisms in *ABCG2* (421C>A, 34G>A), V257M in *FMO3*, *APOE* e2/e3/e4, 1421C>G in *LPL* and 2009A>G in *PSCK9* (proprotein convertase

subtilisin/kexin type 9) tended to be related to percentage changes in total cholesterol in response to rosuvastatin ($P<0.05$), but only the *ABCG2* 421C>A polymorphism was significantly associated with total cholesterol response before and after adjustment for baseline levels of total cholesterol and having diabetes (Table **4-18**). The relationship between total cholesterol response and 1421C>G in *LPL* or 2009A>G in *PSCK9* was no longer present after adjusting for baseline total cholesterol ($P>0.05$) (Table **4-18**). The *APOE* e2/e3/e4 polymorphisms were significant associated with baseline total cholesterol levels with e2 carriers having the highest vales whereas e4 carries had the lowest values, but the total cholesterol response was greater in subjects with homozygous e3 alleles compared to those with e2 or e4 alleles (Table **4-18**).

The multivariate stepwise regression analysis revealed that baseline total cholesterol, having diabetes, *ABCG2* 421C>A and *FMO3* V257M polymorphisms were predictors of the percentage change in total cholesterol in response to rosuvastatin (Table **4-19**), whereas other genetic factors were excluded from the model. Baseline total cholesterol ($P=3.9\times10^{-8}$) and *ABCG2* 421C>A polymorphism ($P=7.7\times10^{-7}$) were strong predictors of total cholesterol response which contributed to 5.2% and 5.5% of variance in total cholesterol response to rosuvastatin, respectively.

**Table 4-18. Associations between genetic polymorphisms and total cholesterol response to rosuvastatin**

| Gene/SNPs | Genotype | N | Baseline TC (mmol/L) | % change in TC | P | P* |
|---|---|---|---|---|---|---|
| *ABCG2* | CC | 194 | 7.58 ± 1.77 | -35.6 ± 9.2 | | |
| 421C>A | CA | 137 | 7.38 ± 1.92 | -39.4 ± 8.6 | $1.8\times10^{-5}$ | $5.0\times10^{-7}$ |
| | AA | 55 | 7.48 ± 1.58 | -40.7 ± 8.0 | | |
| *ABCG2* | GG | 176 | 7.49 ± 1.67 | -39.4 ± 8.8 | | |
| 34G>A | GA | 164 | 7.61 ± 1.91 | -36.7 ± 8.8 | 0.001 | 0.002 |
| | AA | 39 | 6.92 ± 1.74 | -34.4 ± 10.7 | | |
| *FMO3* | GG | 230 | 7.53 ± 1.85 | -38.8 ± 9.0 | | |
| Val257Met | GA | 131 | 7.35 ± 1.62 | -35.9 ± 9.0 | 0.007 | 0.044 |
| | AA | 23 | 7.78 ± 2.23 | -35.6 ± 8.5 | | |
| *LPL* | CC | 294 | 7.41 ± 1.73 | -37.0 ± 9.5 | 0.025 | 0.083 |
| 1421C>G | CG/GG | 84/4 | 7.76 ± 1.98 | -39.5 ± 7.2 | | |
| *APOE* | e2e2/e2e3 | 2/26 | 8.64 ± 2.37 | -36.2 ± 10.1 | | |
| e2/e3/e4 | e3e3 | 233 | 7.79 ± 1.64 | -39.0 ± 8.8 | 0.038 | 0.033 |
| | e3e4/e4e4 | 78/4 | 7.36 ± 1.31[†] | -36.4 ± 8.6 | | |
| *PCSK9* | AA | 347 | 7.51 ± 1.81 | -38.0 ± 9.1 | 0.046 | 0.055 |
| 2009A>G | GA | 39 | 7.34 ± 1.72 | -34.9 ± 8.2 | | |

Data were expressed as mean ± SD and values of total cholesterol response among genotype groups were compared by ANOVA.

* P value was calculated after adjustment for baseline total cholesterol levels and having diabetes.

[†] P = 0.01 for baseline level of total cholesterol among 3 genotype groups by Kruskal-Wallis H test.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; APOE = apolipoprotein E; FMO3 = flavin containing monooxygenase 3; LPL = lipoprotein lipase; PCSK9 = proprotein convertase subtilisin/kexin type 9; TC = total cholesterol.

**Table 4-19. Multivariate model of predictors of % change in total cholesterol in response to rosuvastatin**

| Variables | Standardized Coefficients | P | Adjusted $R^2$ |
|---|---|---|---|
| Baseline TC levels | -0.27 | $3.9\times10^{-8}$ | 0.052 |
| *ABCG2* 421C>A* | -0.24 | $7.7\times10^{-7}$ | 0.055 |
| Having diabetes | -0.169 | 0.0005 | 0.027 |
| *FMO3* V257M* | 0.116 | 0.015 | 0.011 |

* Homozygous wild-type allele = 1; heterozygous wild-type allele = 2; homozygous variant allele = 3.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; FMO3 = flavin containing monooxygenase 3; TC = total cholesterol.

### 4.3.7 Genetic determinants of non-HDL-C response to rosuvastatin

Six SNPs appeared to be related to non-HDL-C response to rosuvastatin (**Table 4-20**). Apart from the rs506696 in *LIPG/ACAA2* (lipase, endothelial/ Acetyl-Coenzyme A acyltransferase 2) (MAF = 0.488), the other 5 SNPs were also associated with the LDL-C response including 421C>A and 34A>G in *ABCG2*, V257M in *FMO3*, rs16996148 in *NCAN/CILP2/PBX4*, and 1421C>G in *LPL*. Among these SNPs, only the 421C>A and 34A>G in *ABCG2* were significantly associated with percentage change in non-HDL-C in response to rosuvastatin after correction for multiple testing and in a gene-dose dependent manner. There was no gene-dose effect of rs506696 in *LIPG/ACAA2* on % change in non-HDL-C response to rosuvastatin.

**Table 4-20. Associations between genetic polymorphisms and non-HDL-C response to rosuvastatin**

| Gene/SNPs | Genotype | N | Baseline non-HDL-C (mmol/L) | % change in non-HDL-C | P |
|---|---|---|---|---|---|
| *LIPG/ACAA2* | AA | 89 | 6.39 ± 1.99 | -45.6 ± 9.3 | |
| rs506696 | CA | 196 | 5.85 ± 1.68 | -48.8 ± 10.9 | 0.037 |
| | CC | 98 | 5.87 ± 1.66 | -47.8 ± 12.0 | |
| *ABCG2* | CC | 194 | 6.11 ± 1.76 | -45.0 ± 11.1 | |
| 421C>A | CA | 137 | 5.83 ± 1.86 | -50.1 ± 9.9 | $5.0\times10^{-7}$ |
| | AA | 55 | 5.92 ± 1.51 | -52.1 ± 9.9 | |
| *ABCG2* | GG | 176 | 5.94 ± 1.62 | -50.2 ± 10.6 | |
| 34G>A | GA | 164 | 6.14 ± 1.87 | -46.3 ± 10.0 | 0.0002 |
| | AA | 39 | 5.41 ± 1.72 | -44.2 ± 13.5 | |
| *FMO3* | GG | 230 | 6.04 ± 1.79 | -49.2 ± 10.5 | |
| Val257Met | GA | 131 | 5.84 ± 1.63 | -45.7 ± 11.0 | 0.007 |
| | AA | 23 | 5.23 ± 2.18 | -45.8 ± 12.4 | |
| *NCAN/CILP2/PBX4* | GG | 312 | 5.91 ± 1.71 | -48.3 ± 10.9 | 0.044 |
| rs16996148 | GT | 70 | 6.33 ± 1.91 | -45.4 ± 10.5 | |
| *LPL* | CC | 294 | 5.90 ± 1.72 | -46.9 ± 11.4 | 0.021 |
| 1421C>G | CG/GG | 84/4 | 6.22 ± 1.88 | -50.5 ± 8.6 | |

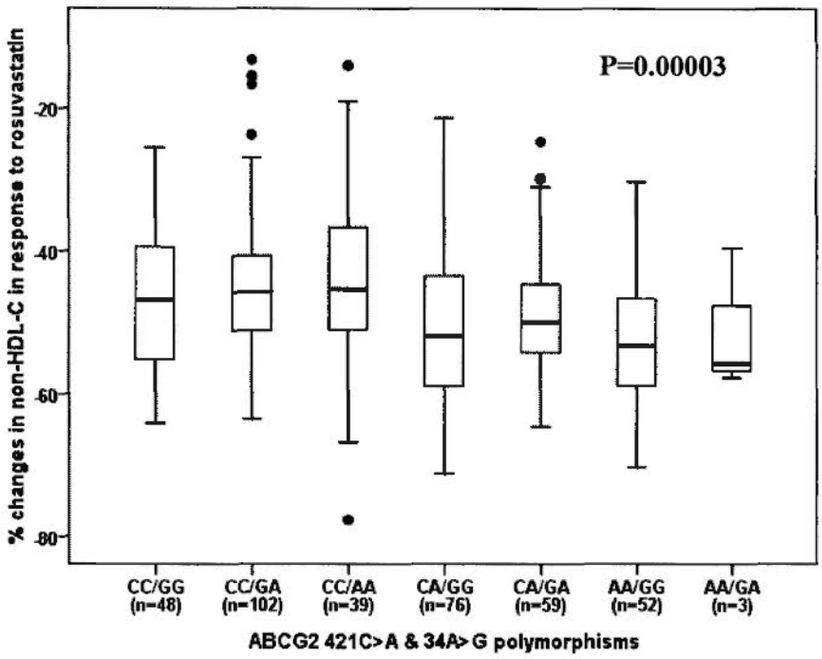
Data were expressed as mean ± SD and values of total cholesterol response among genotype groups were compared by Kruskal-Wallis H test.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; FMO3 = flavin containing monooxygenase 3; LIPG/ACAA2 = Lipase, endothelial / Acetyl-Coenzyme A acyltransferase 2; LPL = lipoprotein lipase; NCAN/CILP2/PBX4 = neurocan / cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4.

Subjects with the 421A variant allele had greater non-HDL-C reduction compared to those with homozygous C alleles in a gene-dose dependent manner, whereas, having the 34A variant allele was associated with a reduced non-HDL-C response to

rosuvastatin (Figure **4-18**).

**Figure 4-18. The non-HDL-C response among diplotypes of 421C>A and 34G>A polymorphisms in the *ABCG2***




* The percentage changes in non-HDL-C in response to rosuvastatin among diplotype groups were compared by Kruskal-Wallis H test.
Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval and circles indicating outliers.

In multivariate stepwise regression analysis, *ABCG2* 421C>A, *LPL* 1421C>G, *FMO3* V257M and *ABCG2* 34G>A polymorphisms were predictors of the percentage change in non-HDL-C in response to rosuvastatin, whereas no phenotypic factors (e.g. age, gender, baseline non-HDL-C or having FH) were found to be determinants of non-HDL-C response to rosuvastatin (Table **4-21**). These 4 genetic factors totally contributed to 9.8% of the variance in non-HDL-C response to rosuvastatin with *ABCG2* 421C>A explaining the highest proportion of variance (6.3%) whereas

34G>A only contributed to 0.9% of the variance in the non-HDL-C response to rosuvastatin.

**Table 4-21. Multivariate model of predictors of % change in non-HDL-C in response to rosuvastatin**

| Variables | Standardized Coefficients | P | Adjusted $R^2$ |
|---|---|---|---|
| *ABCG2* 421C>A* | -0.183 | 0.002 | 0.063 |
| *LPL* 1421C>G* | -0.140 | 0.005 | 0.014 |
| *FMO3* V257M* | 0.127 | 0.01 | 0.012 |
| *ABCG2* 34G>A* | 0.122 | 0.033 | 0.009 |

* Homozygous wild-type allele = 1; heterozygous wild-type allele = 2; homozygous variant allele = 3.

Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; FMO3 = flavin containing monooxygenase 3; LPL = lipoprotein lipase.

### 4.4 Discussion

Multiple large prospective, randomized, placebo-controlled clinical trials have shown that statin therapy could significantly reduce the risk of cardiovascular events by 20-30%, an effect strongly related to the magnitude of LDL-C reduction (Baigent C et al., 2005). However, the lipid response to statins is highly variable and the cause for this is largely unknown, therefore we performed a pharmacogenetic study in Chinese patients to examine genetic determinants of lipid response to rosuvastatin, the most recently introduced statin in Hong Kong public hospitals. As non-genetic factors such as age, gender, race, and smoking status have been shown to have some impact on statin response in some previous studies (Kannel WB et al., 1990, Shear CL et al., 1992, Simon JA et al., 2006), the potential confounding phenotypic factors

were also evaluated in this study.

### 4.4.1 Associations of environmental factors with lipid response to rosuvastatin

#### 4.4.1.1 Age and gender

In this study, age and gender were found to be associated with percentage change in LDL-C from baseline (Table **4-4**). Older participants (>50 years) tended to have a greater response than younger participants (≤50 years), which is in agreement with findings in some previous studies with various statins e.g. lovastatin (Shear CL et al., 1992) and pravastatin (Simon JA et al., 2006), although in the CARE (Cholesterol and recurrent Events) trial the effect of pravastatin on LDL-C reduction was similar among subjects <65 and ≥65 years but the older subjects in the trial did have a nonsignificant 2% greater reduction in LDL-C (Lewis SJ et al., 1998). The finding of an enhanced rosuvastatin-induced effect on LDL-C with advanced age is noteworthy as older patients have an increased CVD risk, which may be related to the different distributions of FH among age groups (Figure **4-2**) as discussed below.

Female patients had greater reductions in LDL-C and non-HDL-C in response to rosuvastatin than males before and after adjustment for other phenotypic factors. The gender difference in LDL-C response to cerivastatin treatment has been reported with a markedly larger LDL-C reduction in females than in males observed (44.4 ± 8.9% vs. 37.0 ± 0.9%, P< 0.05) (Ose L et al., 1999). We did not find a gender difference in HDL-C or triglyceride response to statin in the present study like that of AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study) (Downs JR et al., 1998), although some previous studies with lovastatin and simvastatin reported a greater HDL-C increase in women than in men after statin treatment (Kannel WB et al., 1990, Shear CL et al., 1992, Simon JA et al., 2006).

It is unclear whether the effect of age and gender on lipid-lowering effects of rosuvastatin results from age- and gender-related differences in the pharmacokinetics of rosuvastatin or other age- and gender-associated factors that influence the efficacy of rosuvastatin although there were only small differences in rosuvastatin pharmacokinetics between different age and gender groups and these differences were not considered clinically relevant (Martin PD et al., 2002).

#### 4.4.1.2 Familial hypercholesterolaemia and baseline level of lipids

This study included 166 patients with a clinical diagnosis of FH and identification of mutations in the *LDLR* or other relevant genes was not performed. Data from previous different studies suggested that patients with heterozygous FH respond to rosuvastatin in a similar way to those without FH in both Caucasians (Jones PH et al., 2003c, Stein EA et al., 2003) and Asians (Mabuchi H et al., 2004, Zhu JR et al., 2007) (Table **4-22**), but there was no single study to directly compare the lipid response to rosuvastatin in patients with or without FH. In this study, patients with FH had a 2.6% smaller percentage reduction in LDL-C than patients without FH, suggesting that patients with impaired LDLR function due to genetic defects may have a reduced LDL-C response to statin treatment compared to those with normal LDLR function.

It has previously been suggested that subjects with heterozygous FH might show differing responses to statins depending upon the type of mutation in the *LDLR* as described in Chapter **1** (Table **1-10**). Miltiadous et al reported that apart from *LDLR* mutations, baseline levels of LDL-C and plasma lipoprotein(a) levels appear to be independent factors that affect the LDL-C-lowering effect of atorvastatin in patients

with heterozygous FH (Miltiadous G et al., 2006), but the subject number in that study was rather small (n=49). In the present study, the baseline level of LDL-C was not associated with percentage LDL-C response to rosuvastatin in patients with FH (data not shown), but the role of plasma lipoprotein(a) levels and different types of *LDLR* mutations were not assessed in the study and these warrant further investigations.

**Table 4-22. The LDL-C response to rosuvastatin in patients with and without FH in previous studies**

| Patients | N | Mean age (y) | Populations | Dose (mg) | Baseline LDL-C (mmol/L) | % change in LDL-C | References |
|---|---|---|---|---|---|---|---|
| HomoFH | 41 | 28 | Caucasians (80%) | 20<br>40<br>80 | 13.3 | 18.8<br>22.5<br>21.4 | (Marais AD et al., 2008) |
| HeFH | 436 | 48 | Caucasians (96%) | 20<br>40<br>80 | 7.56 | 47.1<br>53.9<br>57.9 | (Stein EA et al., 2003) |
| | 37 | 51.8 | Japanese | 10<br>20<br>40 | 7.87 | 49.2<br>53.9<br>56.7 | (Mabuchi H et al., 2004) |
| Non-FH | 156<br>160<br>157 | 58 | White (86%) | 10<br>20<br>40 | 4.04<br>4.14<br>4.07 | 45.8<br>52.4<br>55.0 | (Jones PH et al., 2003b) |
| | 515 | 60.3 | Asians (97.8%) | 10 | 4.32 | 47.5 | (Zhu JR et al., 2007) |

Abbreviations: FH = familial hypercholesterolaemia; HeFH = heterozygous FH; HomoFH = homozygous FH;

We defined the percentage changes from baseline in lipid parameters as outcomes, which can largely remove the influence of some baseline lipid levels. Indeed, the percentage reductions in LDL-C and non-HDL-C were not related to their baseline levels. However, the baseline levels of HDL-C, triglyceride and total cholesterol

were still strong predictors of percentage changes in HDL-C, triglyceride and total cholesterol respectively (Table **4-5, 4-6, 4-7**). As has been reported elsewhere, subjects with high total cholesterol and triglyceride or low HDL-C levels had more favorable responses compared to those with relatively "normal" lipid parameters. Since a low baseline level of HDL-C predicts a greater increase in HDL-C (absolute change) with statin treatment, the effect of the baseline level of HDL-C on HDL-C response to statins would be even greater with percentage change in HDL-C from baseline. However, further analysis showed that the absolute change in HDL-C was only slightly less dependent on the baseline level of HDL-C ($r = -0.190$, $P = 0.0002$) compared to the relationship between percentage change and baseline level of HDL-C ($r = -0.192$, $P = 0.0001$).

#### 4.4.1.3 Diabetes, hypertension, rheumatoid arthritis and preexisting cardiovascular disease

Few studies reported in the literature examined the effect of diabetes on the lipid response to statins. Simon et al reported there was no difference in lipid response to simvastatin in patients with and without diabetes in the CAP (Cholesterol and Pharmacogenetics) trial, although patients with diabetes did have a nonsignificant 3% greater LDL-C reduction than those non-diabetic patients (Simon JA et al., 2006). In this study, subjects having diabetes showed greater percentage reductions in LDL-C than non-diabetic patients from the unadjusted data. There may be some confounding factors which contribute to this finding and one of the most obvious to consider is the high proportion of FH in non-diabetic patients (Table **4-3**). Indeed, when the analysis was adjusted for FH and other factors, the difference in LDL-C response to rosuvastatin between patients with and without diabetes was no longer significant. Patients with diabetes also had a greater triglyceride response to

rosuvastatin, which is likely to be related to their high baseline triglyceride levels.

However, although patients with diabetes had a lower level of HDL-C than non-diabetics, they still had a smaller HDL-C response that remained statistically significant after adjusting for a number of potentially confounding variables, including baseline levels of HDL-C and triglyceride. This finding is consistent with previous observations of a smaller increase in HDL-C in diabetics treated with simvastatin in the Heart Protection Study (Collins R et al., 2003) and the finding from a very recent meta-analysis of 32,258 dyslipidaemic patients included in 37 randomized studies using rosuvastatin, atorvastatin, and simvastatin (Barter PJ et al., 2009). The explanation and the clinical implications of a reduced HDL-C response to therapy in people with diabetes warrant further investigation.

Having hypertension, RA or a history of CVD did not appear to be related to LDL-C or other lipid response to rosuvastatin in this study, which agree with findings of some previous studies showing no interactions between blood pressure and statin-induced lipid changes (Downs JR et al., 1998, Kannel WB et al., 1990). RA patients had a higher baseline level of HDL-C than patients without this condition but having hypercholesterolaemia (Table **4-5**), which probably led to a reduced increase in HDL-C levels after statin treatment in the RA group of patients.

#### 4.4.1.4. Obesity, and smoking status

BMI and WC, measures of general and central adiposity, respectively, or percentage total body fat did not seem to affect LDL-C or any other lipid responses to rosuvastatin in this study. We are not aware of any other reports showing a relationship between obesity and LDL-C response to statins but the CAP trial did

reveal that subjects with increased WC had a diminished triglyceride lowering response to simvastatin (Simon JA et al., 2006). In the present study, smoking status was not associated with lipid responses to rosuvastatin, which is consistent with findings from the AFCAPS/TexCAPS and the EXCEL (Expanded Clinical Evaluation of Lovastatin) studies with lovastatin treatment (Downs JR et al., 1998, Shear CL et al., 1992), but not the CAP trial with simvastatin, which showed that smokers had less LDL-C, apolipoprotein-B and triglyceride reductions than nonsmokers (Simon JA et al., 2006).

#### 4.4.1.5 Adherence to rosuvastatin therapy

We assessed adherence to rosuvastatin from the patient's own subjective reporting and tablet counting, the two most common methods used to measure adherence in the clinical settings, which may not be accurate, but no currently available method for measuring adherence is considered as the gold standard (Osterberg L and Blaschke T, 2005). It has been reported from some studies that self-reported adherence to drug therapy may be consistent with the data from electronic monitoring (Walsh JC et al., 2002) and other more objective methods for measurement of adherence (Hugen PW et al., 2002), but this has not always been the case (Garber MC et al., 2004). Furthermore, many aspects of lifestyle e.g. diet and exercise, have been known to influence plasma lipoprotein profiles (Lewis B, 1990). In this study, patients were asked to maintain their lifestyles during the study, but it is possible that some patients who claimed no changes in lifestyle or good drug compliance may not have reported this correctly, which may introduce some bias to the data.

### 4.4.2 Genetic determinants of lipid response to rosuvastatin

#### 4.4.2.1 *ABCG2* polymorphisms

The study extensively examined the effects of polymorphisms in genes potentially related to statin pharmacokinetics, pharmacodynamics, and lipid metabolism on the lipid responses to rosuvastatin in Chinese patients with good adherence to treatment. The most significant finding of this study is that the *ABCG2* 421C>A polymorphism was significantly associated with LDL-C response to rosuvastatin and the difference in percentage reduction in LDL-C between the two homozygote groups is equivalent to more than doubling the dose of rosuvastatin.

Rosuvastatin undergoes little metabolism, but it is a substrate for a number of drug transporters which influence its disposition, including the efflux transporter ABCG2 (Ho RH et al., 2006, Tirona RG, 2005). The findings of greater reduction in LDL-C in subjects with the *ABCG2* 421AA genotype are consistent with the pharmacokinetic studies in Chinese and Caucasian subjects showing about twice the level of systemic exposure to rosuvastatin in subjects with at least one 421A allele compared to those with the 421CC genotype (Keskitalo JE et al., 2009c, Zhang W et al., 2006a). The nonsynonymous 421 C>A SNP is located in exon 5 of the *ABCG2* gene in which a 421C>A transversion results in an amino acid change of glutamine to lysine at codon 141 (Cusatis G and Sparreboom A, 2008). The 421A variant allele results in lower expression levels of the ABCG2 efflux transporter protein in hepatocytes, enterocytes and other tissues and a reduced ability to export substrate, which would increase drug accumulation in hepatocytes and the systemic circulation (Cusatis G and Sparreboom A, 2008, Kondo C et al., 2004, Robey RW et al., 2009). The pharmacokinetic study in Caucasians showing that the AUCs of rosuvastatin and atorvastatin and the Cmax of rosuvastatin are affected by the *ABCG2* genotype, with

no effect on their elimination half-life ($t_{1/2}$) suggests that the increases in plasma concentration of rosuvastatin and atorvastatin in subjects with the 421A variant allele are most likely due to enhanced absorption of these drugs, increasing their bioavailability after oral administration, as a consequence of decreased intestinal efflux of these statins by ABCG2 in association with the 421A variant allele (Keskitalo JE et al., 2009c).

Rosuvastatin shows dose-linear pharmacokinetics (Rosenson RS, 2003), so that having a single 421A variant allele is associated with almost double the plasma concentration or equivalent to doubling the dose compared to 421CC homozygotes, and plasma concentrations will be increased more in those with the 421AA genotype. It has been suggested that the $AUC_{0-\infty}$ after a single dose is equal to the dose-interval AUC at steady state, therefore, the AUCs of rosuvastatin during continuous treatment should be similar to those after a single dose (Keskitalo JE et al., 2009c). Doubling the dose of a statin typically only reduces the LDL-C by a further 6% from the original baseline (Roberts WC, 1997). In the STELLAR (Statin Therapies for Elevated Lipid Levels compared Across doses to Rosuvastatin) trial, a study comparing the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses, doses of 10, 20 and 40 mg rosuvastatin for 6 weeks produced LDL-C reductions of 45.8%, 52.4%, and 55.1%, respectively (Jones PH et al., 2003c). These figures could be compared to the reductions of 48.9 % and 57.0% with 10 mg rosuvastatin in the *ABCG2* 421CC and 421AA genotypes in our study.

The 421A variant is more prevalent in Chinese (35%) or Japanese (35%) than in African-American (2-5%), European (11-14%), Hispanic (10%) or Middle Eastern (13%) subjects (Cusatis G and Sparreboom A, 2008, Robey RW et al., 2009). The

high allele frequency of the 421A variant allele in Chinese and Japanese compared to Caucasians may contribute to the ethnic difference in rosuvastatin pharmacokinetics and potentially the treatment outcomes of rosuvastatin. To our knowledge, the present study was the first to demonstrate the association between the *ABCG2* 421C>A polymorphism and the lipid response to rosuvastatin in Chinese patients. A recent study in Caucasian patients with acute coronary syndrome (ACS), published in an abstract form, has also replicated these findings, showing that the *ABCG2* 421C>A polymorphism was related to the plasma LDL-C concentration after 3 months treatment with rosuvastatin 10 mg (Romaine SP et al., 2008). Patients with one or two A variant alleles (n = 75) achieved significantly lower mean LDL-C levels than the 421CC individuals (1.78 vs. 1.98 mmol/L; P=0.012). These results are similar to the findings in our patients (Figure **4-11**) although the patients involved in this study had a wide range of baseline level of LDL-C. This independent study in Caucasians, in whom the A allele frequency is only 12%, provides strong evidence to support our findings and emphasized that the *ABCG2* 421C>A polymorphism plays an important role in the LDL-C response to rosuvastatin and the importance of this variant on the lipid response to rosuvastatin in different ethnic groups with a variable frequency of the polymorphism.

The other nonsynonymous 34G>A SNP located in exon 2 of the *ABCG2* gene leading to an amino acid change of valine to methionine at codon 12, apparently did not confer an alteration in protein expression or function (Cusatis G and Sparreboom A, 2008, Kondo C et al., 2004, Robey RW et al., 2009). In this study, the 34G>A polymorphism appeared to be related to the LDL-C response to rosuvastatin but this is most likely due to its LD with the 421C>A SNP. However, this polymorpihsm was also associated with the non-HDL-C response before and after adjustment for the

421C>A polymorphism although this association did not reach the experiment-wide significance level.

The list of ABCG2 substrates has been expanding rapidly, which highlights the important role of this transporter in drug disposition and treatment outcomes. Experiments in cell lines demonstrated that some hydrophilic statins and/or their metabolites like rosuvastatin and pitavastatin acid (Fujino H et al., 2005), were substrates of ABCG2, and more recently the lipophilic statin, atorvastatin was identified to be a substrate of this efflux transporter (Keskitalo JE et al., 2009c). The *ABCG2* 421C>A polymorphism was shown to have different impact on the disposition of these statins. The effect was greater in the case of rosuvastatin than atorvastatin (the $AUC_{0-\infty}$ of rosuvastatin was ~140% greater and that of atorvastatin was ~70% greater in the c.421AA genotype compared to the c.421CC genotype) but there was no significant effect on the pharmacokinetics of pravastatin and pitavastatin (Ho RH et al., 2007, Ieiri I et al., 2007). Some reported substrates have also been identified as ABCG2 inhibitors, such as imatinib and gefitinib (Houghton PJ et al., 2004, Ozvegy-Laczka C et al., 2004). It is still unclear whether these substrate statins themselves could influence the activity of ABCG2. However, membrane lipids, especially cholesterol, have been involved in the regulation of various membrane proteins, including several ABC transporters e.g. ABCG2 (Dos Santos SM et al., 2007, Hegedus C et al., 2009). ABCG2 was shown to be located in rafts of mammalian cell membranes, and it has been shown that destroying membrane rafts by cholesterol depletion causes about a 40% decrease in ABCG2 activity (Storch CH et al., 2007). Telbisz et al reported that membrane cholesterol can modulate the activity of ABCG2 in a selective and reversible manner (Telbisz A et al., 2007). Whether cholesterol is an additional substrate for ABCG2 or rather a co-factor

or allosteric modulator promoting its proper transport function requires further evaluation. However, a recent study has shown that the baseline mRNA expression levels of ABC and SLCO transporters (ABCB1, ABCC2, ABCG2, and SLCO2B1) were almost ten-fold higher in subjects with hypercholesterolaemia than those with normal lipid levels and atorvatatin treatment significantly downregulated the gene expression of these influx and efflux transporters (Rodrigues AC et al., 2009). Whether rosuvastatin could affect the function of ABCG2 by altering the concentration of cholesterol in serum and membranes that in turn might affect the efficacy and toxicity of substrate drugs is an important topic that needs to be addressed in future research.

The hepatic expression of ABCG2 was shown to be higher in men compared with women, which may potentially affect the sex-specific variability in the pharmacokinetics and pharmacodynamics of ABCG2 substrates (Merino G et al., 2005). However, gender did not seem to influence the pharmacokinetics of rosuvasatin in healthy subjects although a nonsignificant higher drug exposure to rosuvastain in females than that in males was observed (Martin PD et al., 2002). In this study the lipid responses to rosuvastatin in males and females were not statistically different after adjustment for other genotypic and phenotypic factors. Further studies are needed to investigate environmental and physiological factors that may affect expression of the transporter.

#### 4.4.2.2 *FMO3* polymorphisms

FMO3 catalyzes the oxygenation of various heteroatom-containing compounds (e.g., amine-, sulfide-, phosphorus-, and other nucleophilic heteroatom–containing compounds) in the liver (Cashman JR and Zhang J, 2006). The *FMO3* gene is highly

polymorphic, and some common polymorphic variants of *FMO3* affect the amount or activity of the enzyme and, thereby, alter N-oxygenation of certain amines, including drugs, dietary agents, and other xenobiotics (Cashman JR and Zhang J, 2006, Phillips IR and Shephard EA, 2008). Deficiency in FMO3 activity due to rare inherited defects in the *FMO3* gene causes primary trimethylaminuria (TMAU), the fish-odour syndrome (Phillips IR and Shephard EA, 2008). The frequency of polymorphisms in *FMO3* varies considerably between different ethnic groups (Hao D et al., 2007), which may contribute to interethnic variability in the phenotype of this enzyme. The variant alleles for the two commonly linked SNPs, E158K and E308G ($r^2$=0.86 in Chinese, data from HapMap) can result in a reduced enzyme activity in a substrate-dependent manner. Homozygotes for 158K/308G can exhibit symptoms of mild or transient TMAU. However, some haplotypes inferred from high-activity promoter variants of *FMO3* that were also linked to the variants of E158K/E308G were found to increase transcription of *FMO3* in Hispanic, non-Latino whites, and African Americans with different prevalence among populations (Koukouritaki SB et al., 2007), which may lead to an increase in the amount of protein produced, via promoter SNPs, compensating for the low activity of the enzyme due to the two coding SNPs, E158K and E308G (Phillips IR and Shephard EA, 2008). The presence of LD between these SNPs might contribute to some of the discrepancies between the results of analysis of mutant enzyme activity *in vitro* and drug metabolism *in vivo*.

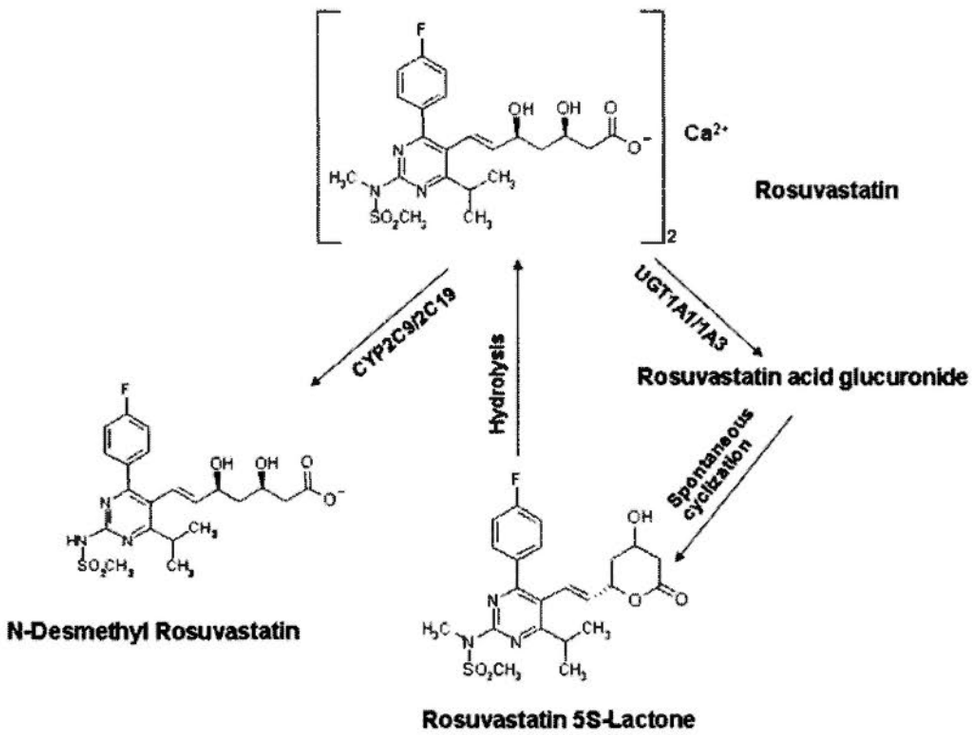
In this study the E158K and E308G polymorphisms were not associated with the LDL-C response to rosuvastatin, but we found *FMO3* V257M was significantly related to the LDL-C response after adjusting for other genotypic and phenotypic factors. The V257M polymorphism was only mildly linked with the E158K and

E308G polymorphisms ($r^2$<0.1 in Chinese, data from HapMap) and the functional effect of this polymorphism on *FMO3* activity has not been fully addressed, but the *in vitro* study showed that the methionine variant at codon 257 of human FMO3 had decreased *N*-oxygenation for the substrate tyramine (Cashman JR et al., 2000) and some previous studies have also shown that this polymorphism linked with another polymorphism causing stop codon ($Trp^{388}Stop$) was responsible for TMAU (Shimizu M et al., 2007). However, in this study subjects with the variant allele of V257M polymorphism had reduced LDL-C response to rosuvastatin suggesting an increased activity of the enzyme associated with this variant allele. This study did not examine those promoter SNPs in the 5'-flanking region which may increase the transcription of *FMO3*, although haplotypes containing these high-activity promoter variants are uncommon. However, a recent study suggested that the expression levels of *trans*-acting factors, i.e. hepatocyte nuclear factor-4 (HNF-4) and nuclear factor Y box-binding protein (NF-Y) might regulate the expression level of the *FMO3* gene (Shimizu M et al., 2008). Further analysis in human liver microsomes from Japanese samples has shown that microsomal FMO3 protein content was significantly correlated with FMO3 mRNA levels, whereas the FMO3 mRNA levels were significantly correlated with HNF-4 mRNA and NF-Y mRNA levels, but not the *FMO3* haplotypes suggesting FMO3 activity was independent of genetic variation in the *FMO3* gene (Nagashima S et al., 2009).

Whether rosuvastatin is a substrate of FMO3 is still unclear, but considering only a small proportion of rosuvastatin (about 10%) is recovered as metabolite, mainly N-desmethyl rosuvastatin formed principally by CYP2C9 (Figure **4-19**) (White CM, 2002), FMO3 activity is unlikely to have a marked effect on the pharmacokinetics of rosuvastatin. The significant association between the *FMO3* V257M polymorphism

and the LDL-C response to rosuvastatin might be due to its linkage to other genes or variants related to the pharmacokinetics or pharmacodynamics of rosuvastatin. A very recent genome-wide association (GWA) analysis in 17,296 women from the Women's Genome Health Study (WGHS) has identified an association between *HNF-4A* polymorphism and lipoprotein fractions, which may link lipoprotein metabolism, FMO3 and lipid response to rosvuastatin, (Chasman DI et al., 2009) that requires further evaluations.

**Figure 4-19. Chemical structure and metabolic pathways of rosuvastatin**




Rosuvastatin undergoes a small degree of enzymic modification, principally by CYP2C9 and CYP2C19, to N-desmethyl rosuvastatin with mean $C_{max}$ values of <10% and approximately one-sixth to one-half the HMG-CoA reductase inhibitory activity of rosuvastatin (Martin PD et al., 2003). Studies *in vitro* suggest rosuvastatin undergoes glucuronidation via *UGT1A1* and *UGT1A3* to form an acyl glucuronide conjugate with subsequent lactonization to form rosuvastatin-5S-lactone which can undergo hydrolysis back to the acid form (Prueksaritanont

T et al., 2002b).

#### 4.4.2.3 Other polymorphisms potentially related to LDL-C response to rosuvastatin

The other SNPs in the genes or loci related to lipoprotein metabolism have also shown some effects on LDL-C response (Table **4-10**), but these associations did not reach the significant level after correction for multiple testing, which may be attributed to under-powering or lack of a true effect.

LPL is a key regulator of lipid metabolism, which governs the delivery of triglyceride-derived free fatty acids to adipose tissue and muscle. The *LPL* rs328 variant involving a C-terminal truncation of two amino acids (Ser447X) has been shown to be associated with increased LPL activity, reduced triglyceride levels and raised HDL-C levels in various studies (Gagne SE et al., 1999, Wung SF et al., 2006). Furthermore, a longitudinal association analyses in 2864 Busselton Health Survey participants has shown that the association of the variant allele of rs328 with raised HDL-C levels significantly increased with age (Webster RJ et al., 2009). The GWA analysis in WGHS participants has identified 36 loci for roles in lipoprotein metabolism defined by 22 traits related to lipoprotein size, concentration and cholesterol content (Chasman DI et al., 2009), and the LPL gene was found to be significantly related to multiple parameters including HDL-C, triglycerides etc. and particularly with the size of lipoprotein particles that may impact on disease risk (Stampfer MJ et al., 1996). Polymorphisms in *LPL* have been shown to affect HDL-C and triglyceride responses to statin therapy and progression of atherosclerosis in 829 subjects from the Post-Coronary Artery Bypass Graft trial

(Goodarzi MO et al., 2007), but we did not find such an association. In this study, the variant allele of rs328 polymorphism was associated with a greater reduction in LDL-C response, which contributes to 2.3% of variance in percentage change in LDL-C in response to rosuvastatin.

Multiple GWA studies showed that the minor allele of SNP rs4420638 in the *APOE-APOC* cluster was strongly associated with increased LDL-C concentrations, decreased CRP levels and increased risk of CHD and Alzheimer disease respectively (Coon KD et al., 2007, Elliott P et al., 2009, Willer CJ et al., 2008b). This polymorphism is situated about 14 kb distal to the APOE locus and was in strong LD with SNPs that characterize the *APOE* e2/e3/e4 locus, rs429358 and rs7412 (Coon KD et al., 2007). However, the *APOE* e2/e3/e4 polymorphism did not seem to affect the LDL-C response in this study ($P$=0.1), although a strong linkage between rs4420638 and APOE e2/e3/e4 was presented ($r^2$= ~0.8 for both).

The rs16996148 SNP located on chromosome 19p13 in an intergenic region between *CILP2* and *PBX4* showed strong association with LDL-C and triglycerides levels in a GWA study, although these loci had no established connections to cholesterol metabolism. LCAT is the major enzyme responsible for the esterification of free cholesterol present in circulating plasma lipoproteins, and catalyzes the transfer of an unsaturated fatty acid from the sn-2 position of lecithin to free cholesterol, generating cholesteryl ester and lysolecithin (Santamarina-Fojo S et al., 2000). A recent GWAS reported that the rs255052 SNP, located 49 kb downstream of *LCAT* was associated with HDL-C levels with the variant allele having an effect size of 0.019 mmol/l on HDL-C (Willer CJ et al., 2008b). Although these 2 polymorphisms tended to be associated with LDL-C response to rosuvastatin, these associations did not reach the

experiment-wide significance level and after adjustment for other genotypic and phenotypic factors the associations were no longer present in the multivariant analysis. Whether these polymorphisms could influence statin efficacy is still an open question and needs to be explored in future studies with a larger sample size.

It has previously been suggested that subjects with heterozygous FH might show differing responses to statins depending upon the type of mutation in the *LDLR*, although this finding has not been consistent (Kajinami K et al., 2004e, Miltiadous G et al., 2005). The present study did not examine the genetic cause of FH, but we found that the *LDLR* rs1433099 polymorphism was associated with the LDL-C response to rosuvastatin in patients with FH with the homozygous variant allele being associated with less response to rosuvastatin (Figure **4-13**) suggesting that the impaired LDLR function may influence the lipid response to statins, particularly for patients with FH.

HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis and the target enzyme for statins and variants (SNP12 and SNP29) in *HMGCR* influenced the LDL-C responses to pravastatin (Chasman DI et al., 2004) and simvastatin (Krauss RM et al., 2008). These polymorphisms are more common in African Americans but are almost absent in Asian populations so we did not examine their effects. However, two other common SNPs in *HMGCR* (rs12654264 and rs3846662), which were identified to be associated with altered LDL-C or HDL-C levels in a GWAS (Kathiresan S et al., 2008b) did not seem to affect lipid responses to rosuvastatin in the present study. Similarly, in another pharmacogenetic study in 707 renal transplant recipients (97% Caucasian) treated with fluvastatin there was no association between *HMGCR* polymorphisms and the LDL-C response to fluvastatin,

which may reflect differences due to drug or ethnicity or choice of SNPs (Singer JB et al., 2007).

In this study, subjects with the variant allele of *CYP2C9* or homozygous variants of *CYP2C19*, *CYP3A5* and *UGT1A1* tended to have greater LDL-C responses to rosuvastatin, which were not statistically significant, possibly due to small numbers in the variant groups (Table **4-12**). These relationships with the less active variants of the enzymes thought to be involved in the metabolism of rosuvastatin are biologically plausible (Figure **4-19**) and should be further explored in future studies with a larger sample size, preferably including pharmacokinetic data.

#### 4.4.2.4 *SLCO1B1* polymorphisms

Although the 521T>C polymorphism in the *SLCO1B1* hepatic uptake transporter was associated with increased systemic exposure to rosuvastatin in Korean and Caucasian subjects (Choi JH et al., 2008, Pasanen MK et al., 2007), this SNP or associated haplotypes did not explain the differences in pharmacokinetics between ethnic groups in the study in Singapore (Lee E et al., 2005). Subjects with a haplotype including 521C showed reduced acute effects on cholesterol synthesis with pravastatin, another SLCO1B1 substrate (Niemi M et al., 2005). However, another study showed no effect of the 521T>C SNP on the lipid responses to 3 weeks dosing with pravastatin, but that study may have been too small or too brief to show a significant effect (Igel M et al., 2006). In the present study, we examined 8 SNPs in *SLCO1B1* including 521T>C, but none of these SNPs or haplotypes showed a significant effect on the LDL-C response to rosuvastatin. In the Heart Protection Study, subjects with the 521C variant had an increased risk of developing myopathy and less reduction in LDL-C with simvastatin (-1.28% per C allele, $P<0.0001$) (Link

E et al., 2008), but in the present study there was no effect of the 521T>C heterozygote on LDL-C reduction and there were only 10 subjects homozygous for the 521C variant, who tended to show a greater effect (Table **4-12**).

However, we found the 521T>C polymorphism tended to be associated with HDL-C response in patients with FH, partly related to its effect on the baseline level of HDL-C (Figure **4-15**) and the association was still significant after adjustment for baseline level of HDL-C. The 521T>C polymorphism has been reported to be associated with increased cholesterol synthesis rate (Pasanen MK et al., 2008a) probably through its impact on bile acid homeostasis (Xiang X et al., 2009) as intracellular bile acid concentrations regulate cholesterol catabolism, which may influence the effect of this polymorphism on lipid response to statins. Animal studies indicate that the liver may be a major source of the cholesterol circulating in HDL particles (Brewer HB, Jr. et al., 2004), and therefore the effect of the 521T>C polymorphism on hepatic cholesterol synthesis may alter the level of circulating HDL-C and consequently the HDL-C response to statin treatment.

#### 4.4.2.5 Genetic determinants of changes in other lipid traits

In this study, changes in HDL-C and triglycerides were examined although these secondary analyses were not expected to yield significant results due to the minimal response of HDL-C and the high variability of triglyceride levels. Indeed, none of the SNPs examined in the study was found to significantly affect these two secondary traits after correction for multiple testing, although the *SLCO1B1* 521T>C tended to affect the HDL-C response in patients with FH.

The mechanism by which statins increase the concentration of HDL-C remains

unclear and is apparently unrelated to the mechanism by which this class of agents lowers the LDL-C levels. It has been recognized that statins could up-regulate hepatic ABCA1 gene expression, which at least partly explains the statin-induced increase in HDL-C (Tamehiro N et al., 2007). A second possible mechanism by which statins increase HDL-C involves their effects on the cholesteryl ester transfer protein (CETP) inhibition due to reduction in the number of triglyceride-rich lipoproteins available to accept cholesterol ester from HDL (Barter PJ et al., 2009, Chapman MJ et al., 2010, McTaggart F and Jones P, 2008). However, statin-induced HDL-raising was much less than expected values (>20%) speculated from the 20 to 25% inhibition of CETP associated with statin treatment, which suggests statins may have other effects that oppose the HDL-raising resulting from CETP inhibition (Barter PJ et al., 2009, Chapman MJ et al., 2010, McTaggart F and Jones P, 2008). Furthermore, statin-induced increase in HDL-C may partly be due to enhancing PPARalpha activity, which may stimulate hepatic apoA-I synthesis and HDL formation (Chapman MJ et al., McTaggart F and Jones P, 2008). Variations in the balance between such opposing effects may be the explanation for the markedly differing patterns of the HDL-C response to treatment with different statins. With the complicated mechanisms involved in the statin-induced HDL-C raising, it is unlikely to be able to identify genetic cause for HDL-C response to rosuvastatin with the design of the present study. Further studies with larger numbers of homogeneous patients are required to identify the genetic predictors of HDL-C response to statins.

The triglyceride response to rosuvastatin was highly variable and was strongly related to baseline levels, as shown in many previous studies. DGAT2 plays a key role in triglyceride biosynthesis (Yen CL et al., 2008) and may be involved in the mechanism of action of niacin (Kamanna VS and Kashyap ML, 2008). The

rs10899113 SNP in *DGAT2* appeared to affect triglyceride response to rosuvstatin suggesting that DGAT2 might also be related to the pharmacological effect of statins, which warrants further evaluation. Those 3 linked SNPs in *CYP3A* appeared to be related to triglyceride response to rosuvastatin but there was no gene-dose effect suggesting the effect may be due to LD with other genetic variants or a chance finding. Although CYP3A has not been considered to play a role in the metabolism of rosuvastatin, polymorphism in *CYP3A5* (*3) was found to be associated with the plasma LDL-C concentration after 3 months treatment with rosuvastatin 10 mg in Caucasian patients with ACS (Romaine SP et al., 2008), but not in patients in the present study. Previous studies have consistently demonstrated that APOA5 is a key determinant of plasma triglyceride concentrations and polymorphisms in this gene have been shown to be related to familial combined hyperlipidaemia in Chinese (Liu ZK et al., 2010) and lipid responses to fenofibrate (Liu Y et al., 2009). In the present study, the *APOA5* -1131T>C polymorphism was strongly associated with baseline triglyceride levels but not the response to rosuvastatin.

The determinants of total cholesterol and non-HDL-C response to rosuvastatin were very similar to those of LDL-C response which are not surprising as the levels of these two parameters are largely determined by the LDL-C concentrations.

### 4.4.3 Limitations of the study

This study had several potential limitations which require consideration. Firstly, this study was conducted in Chinese patients, and the main findings of the present study need to be replicated in an independent cohort in other ethnic groups although the study in Caucasian patients also showed a significant association between *ABCG2* 421C>A and LDL-C on-treatment level in response to rosuvastatin. Secondly, we

selected some common and representative SNPs in those candidate gene/loci based on published data, but it is likely that some rare or unexamined variants also play a role in LDL-C response but we cannot identify those. Thirdly, we have not examined the effect of polymorphisms on the response to other statins or to different doses of statins, which may be important (Voora D et al., 2008). Fourthly, only a small proportion of the variation in LDL-C response could be explained by these SNPs, suggesting that other genetic and lifestyle factors may also be important in determining the response to statins. We attempted to reduce the variation due to poor adherence to therapy by only including the efficacy data from subjects reporting good adherence to treatment. Lastly, changes in LDL-C may be predictive of clinical outcomes, but it would also be important to examine the impact of these polymorphisms on cardiovascular events. Furthermore, increased systemic exporesure to rosuvastatin in patients with ABCG2 421AA genotype might be associated with increased risk of side effects of rosuvastatin e.g. rhabdomyolysis and hepatitis, but we were not able to examine this in the present study as no patients experienced rhabdomyolysis or hepatitis, which is probably due to the small dose used in the study. However, there was no significant difference in changes in liver enzymes and creatine kinase in response to rosuvastatin treatment among *ABCG2* 421C>A genotype groups.

### 4.5 Conclusion

In conclusion, this study is the first to identify the *ABCG2* 421C>A polymorphism to be associated with an increased LDL-C response to rosuvastatin in Chinese subjects. The greater percent reduction in LDL-C in patients with the 421AA genotype compared to those with the 421CC genotype was equivalent to at least doubling the dose of rosuvastatin. This finding confirms the previous observations in the

pharmacokinetic studies and provides importance evidence for genetic factors influencing statin response. Several other polymorphisms, in particular *FMO3* V257M, *LPL* 1421C>G and *APOE/C1/C4/C2* rs4420638, also tended to influence the LDL-C response to rosuvatatin, which need to be replicated in other populations. In this study, patients with FH had a smaller percentage reduction in LDL-C than patients without FH suggesting patients with impaired LDLR function due to genetic defects may have a reduced LDL-C response to statin treatment compared to those with normal LDLR function, but this effect was much smaller than the impact of the *ABCG2* 421C>A polymorphism or other genetic factors on the LDL-C response. Other phenotypic factors did not appear to significantly affect LDL-C response to rosuvastatin after adjustment for genetic confounding factors.

# Chapter 5 Abdominal obesity and *CRP* polymorphisms predict high-sensitivity C-reactive protein levels in Chinese patients on treatment with rosuvastatin

## 5.1 Introduction

Low-grade inflammation plays a pivotal role in the formation and activation of atherosclerotic plaques (Libby P and Ridker PM, 2004, Ross R, 1999). C-reactive protein (CRP), the classic acute-phase response protein, is the most extensively studied systemic marker of inflammation which is produced primarily by hepatocytes and its synthesis is regulated at the transcription level by various cytokines, including interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α (Reiner AP et al., 2008) (Casas JP et al., 2008). The cytokine IL-6, derived in part from adipose tissue as well as vascular tissue, is a potent regulator of a number of acute phase genes in the liver and is the key cytokine responsible for the stimulus of synthesis and secretion of CRP (Schuett H et al., 2009).

Multiple population-based prospective studies have reported associations of slightly elevated circulating CRP levels from 2 to 3 mg/L and above (<10mg/L) with various cardiovascular endpoints as well as with high-risk vascular phenotypes such as high blood pressure, higher body mass index (BMI) and levels of abdominal obesity, smoking, diabetes and other metabolic risk factors (Danesh J et al., 2007, Danesh J et al., 2004, Lakoski SG et al., 2005b, Sakkinen P et al., 2002, Ye X et al., 2007). Although numerous effects have been demonstrated in mechanistic studies, neither the normal functions of human CRP nor its role in disease states have been fully determined (Casas JP et al., 2008, Nordestgaard BG, 2009). It has been questioned

whether CRP is a causal risk factor of coronary heart disease (CHD) or just a marker of established cardiovascular risk factors with which it is associated or whether atherosclerosis causes the increases in CRP. However, recent Mendelian randomization studies have shown that the systemic CRP level is unlikely to be a causal factor for development of ischaemic vascular disease (Elliott P et al., 2009, Zacho J et al., 2008). In contrast to traditional less sensitive methods for the detection of CRP, high-sensitivity CRP (hsCRP) assays can measure the typically low concentrations of CRP that circulate in the absence of acute disease.

Baseline hsCRP concentrations are widely distributed and genetic factors could explain about as much as 50% of the variance of baseline hsCRP concentrations, which is largely attributable to noncoding polymorphisms in the *CRP* gene (Casas JP et al., 2008, Shen J and Ordovas JM, 2009). More recently, genome-wide association studies (GWASs) have identified several other loci associated with circulating hsCRP levels, in particular genes related to the metabolic syndrome, such as the leptin receptor (*LEPR*) and hepatic nuclear factor 1-alpha (*HNF1A*) (Table **5-1**) (Elliott P et al., 2009, Reiner AP et al., 2008, Ridker PM et al., 2008b). Moreover, multiple environmental factors including age, gender, obesity, ethnicity, socioeconomic status and lifestyle factors are also associated with hsCRP levels (Greenfield JR et al., 2004, Kelley-Hedgepeth A et al., 2008, Khera A et al., 2005, Ridker PM et al., 2003, Ye X et al., 2007) and a previous twins study has demonstrated that baseline CRP levels were strongly related to obesity, blood pressure and lipid levels independent of genetic influences (Greenfield JR et al., 2004).

**Table 5-1. Loci associated with plasma hsCRP levels in genome-wide association studies**

| Participants | Stage I (GWAS) (n) | Stage II (Replication) (n) | Loci identified associated with CRP | Reference |
|---|---|---|---|---|
| WGHS | 4418 | 1927 | *APOE*, *CRP*, *GCKR*, *LEPR*, *HNF1A*, *IL6R* | (Ridker PM et al., 2008b) |
| LOLIPOP, NFBC, CoLaus, GEMS, DESIR | 17967 | 13615 | *APOE-APOC cluster*, *CRP*, *LEPR*, *HNF1A*, *IL6R* | (Elliott P et al., 2009) |
| CAP and PRINCE | 1980 | 2930 | *APOE*, *CRP*, *HNF1A* | (Reiner AP et al., 2008) |

**Genes:** *APOE* = Apolipoprotein E; *APOE-APOC* = Apolipoprotein E-Apolipoprotein C; *CRP* = C-reactive protein; *HNF1A* = hepatic nuclear factor 1-alpha; *IL6R* = Interleukin 6 receptor; *LEPR* = leptin receptor.

**Studies:** CAP = the Cholesterol and Atherosclerosis Pharmacogenetics; CoLaus = the Lausanne Cohort; DESIR = the Data from an Epidemiological Study on the Insulin Resistance syndrome study; GEMS = the Genetic Epidemiology of Metabolic Syndrome study; LOLIPOP = the London Life Sciences Population study; NFBC = the 1966 Northern Finnish Birth Cohort; PRINCE = the Pravastatin Inflammation/CRP Evaluation; WGHS = Women's Genome Health Study.

Statin therapy reduces hsCRP levels in a wide range of populations and the recent JUPITER (Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin) study in apparently healthy persons without hyperlipidaemia but with elevated hsCRP levels has demonstrated that rosuvastatin 20 mg daily reduced the median LDL-C by 50% and hsCRP by 37% and was accompanied by a 44% relative risk reduction in combined major cardiovascular events although the overall incidence of the composite primary end point was only 2.8% in the placebo arm with an absolute risk reduction of 1.2%, and the incidence of hard cardiovascular end points was only 1.8% with an absolute risk reduction of 0.9% (Table **5-2**) (Ridker PM et al., 2008a, Ridker PM et al., 2009). This study has

also shown that the LDL-C and hsCRP reductions were only weakly correlated in individual patients, suggesting that a reduction in hsCRP levels after statin treatment is unlikely to be secondary to the reduced LDL-C levels.

So far, a very few studies have examined the determinants of hsCRP on-treatment levels with statins (Liem AH et al., 2008, Ray KK et al., 2005). The Pravastatin or Atorvastatin Evaluation and Infection Therapy – Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) study has shown that hsCRP on-treatment levels varied considerably and were associated with multiple cardiovascular risk factors even among patients receiving intensive statin therapy (Ray KK et al., 2005). Another recent study has also suggested that in patients with longstanding intensive statin therapy with known cardiovascular disease, CRP levels are still associated with the components of metabolic syndrome, with waist circumference (WC), fasting glucose level and smoking being the most important determinants of CRP levels (Liem AH et al., 2008). The effects of genetic factors on hsCRP on-treatment levels with statin have not been reported, but polymorphisms in *CRP* have been shown to modulate the hsCRP response to fenofibrate therapy in patients with metabolic syndrome with alleles associated with higher baseline hsCRP levels being associated with a smaller reduction in hsCRP with fenofibrate (Shen J et al., 2008).

We examined whether rosuvastatin treatment might alter the previously reported relationships between genetic and environmental factors and hsCRP in study patients.

**Table 5-2. Baseline clinical characteristics and hazard ratios for incident cardiovascular events in JUPITER study participants**

| Baseline | Placebo | Rosuvastatin | |
|---|---|---|---|
| | (N=7832) | hsCRP ≥2 mg/L (N=4305) | hsCRP <2 mg/L (N=3411) |
| Age (years) | 66 (60-71) | 66 (61-71) | 66 (60-71) |
| Women | 2957 (37.8) | 1798 (41.8) | 1149 (33.7) |
| Current smoking | 1231 (15.7) | 740 (17.2) | 452 (13.3) |
| BMI (kg/m$^2$) | 28.4 (25.3-32.0) | 29.0 (25.7-33.0) | 27.7 (24.9-30.9) |
| SBP (mm Hg) | 134 (124-145) | 135 (125-146) | 134 (123-145) |
| DBP (mm Hg) | 80 (75-87) | 80 (75-88) | 80 (75-87) |
| Family history of CD | 936 (12.0) | 473 (11.0) | 421 (12.4) |
| Metabolic syndrome | 3274 (42.1) | 1859 (43.5) | 1279 (37.8) |
| hsCRP (mg/L) | 4.3 (2.8-7.1) | 5.4 (3.6-8.6) | 3.2 (2.4-4.7) |
| LDL-C (mmol/L) | 2.8 (2.4-3.1) | 2.8 (2.4-3.1) | 2.8 (2.5-3.1) |
| HDL-C (mmol/L) | 1.3 (1.0-1.6) | 1.3 (1.0-1.5) | 1.3 (1.1-1.6) |
| TG (mmol/L) | 1.33 (0.97-1.91) | 1.36 (0.99-1.92) | 1.30 (0.93-1.88) |
| Glucose (mmol/L) | 5.22 (4.88-5.66) | 5.22 (4.83-5.66) | 5.22 (4.88-5.66) |

| Outcomes | Placebo | Rosuvastatin | |
|---|---|---|---|
| | (N=7832) | hsCRP ≥2 mg/L (N=4305) | hsCRP <2 mg/L (N=3411) |
| Events | 189 | 72 | 31 |
| Event rate | 1.11 | 0.77 | 0.42 |
| HR* (95%CI) | 1 | 0.68 (0.51-0.89) | 0.36 (0.24-0.54) |

| Outcomes | Placebo | hsCRP ≥2 mg/L | | hsCRP <2 mg/L | |
|---|---|---|---|---|---|
| | (N=7832) | LDL-C≥1.8 (N=1384) | LDL-C<1.8 (N=2921) | LDL-C≥1.8 (N=726) | LDL-C<1.8 (N=2685) |
| Events | 189 | 31 | 41 | 8 | 23 |
| Event rate | 1.11 | 1.11 | 0.62 | 0.54 | 0.38 |
| HR* | 1 | 1.06 | 0.53 | 0.42 | 0.35 |
| (95%CI) | | (0.72-1.55) | (0.38-0.74) | (0.18-0.94) | (0.23-0.54) |

| Outcomes | Placebo | hsCRP ≥1 mg/L | | hsCRP <1 mg/L | |
|---|---|---|---|---|---|
| | (N=7832) | LDL-C≥1.8 (N=1874) | LDL-C<1.8 (N=4662) | LDL-C≥1.8 (N=236) | LDL-C<1.8 (N=944) |
| Events | 189 | 36 | 59 | 3 | 5 |
| Event rate | 1.11 | 0.95 | 0.56 | 0.64 | 0.24 |
| HR* | 1 | 0.89 | 0.49 | 0.46 | 0.21 |
| (95%CI) | | (0.62-1.28) | (0.37-0.66) | (0.11-1.85) | (0.09-0.51) |

Baseline characteristics are expressed as median (IQR) or number (%).

*HR controlled for age, baseline LDL-C, baseline hsCRP, baseline HDL-C, blood pressure, sex, body-mass index, smoking status, and parental history of premature CHD.

BMI = Body-mass index; CD = coronary disease; CI = confidence interval; DBP = Diastolic blood pressure; HDL-C high-density lipoprotein cholesterol; HR = hazard ratios; hsCRP = high-sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; SBP = Systolic blood pressure; TG = triglycerides.

## 5.2 Subjects and methods

### 5.2.1 Subjects

This analysis was performed in patients with hypercholesterolaemia who were treated with rosuvastatin for at least 4 weeks (more than 97% of patients had at least 6 weeks treatment with rosuvastatin) with good drug compliance and with on-treatment plasma samples available for hsCRP measurement. This analysis did not include those subjects with rheumatoid arthritis (RA). Anthropometric measurements and routine blood tests were performed as described in Chapter 3.

### 5.2.2 High-sensitive C-reactive protein measurement

The plasma hsCRP on-treatment 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.

### 5.2.3 Single nucleotide polymorphism selection and genotyping

A total of 135 polymorphisms were genotyped in this study as described in Chapter **3**. For the purposes of this focused analysis, we have selected 9 SNPs in 5 genes/loci thought to be related to CRP from recent GWA studies, including two SNPs in *CRP* (3872G>A [rs1205], 5237A>G [rs2808630]), the Ile27Leu (rs1169288) and Ala98Val (rs1800574) in *HNF1A*, Cys112Arg (rs429358, 334T>C) and Cys158Arg (rs7412, 472C>T) in *APOE*, rs4420638 in *APOE-CI-CII* cluster, Lys109Arg (rs1137100) and Gln223Arg (rs1137101) in *LEPR*. (Elliott P et al., 2009, Ridker PM et al., 2008b). In addition, 6 common polymorphisms in genes potentially related to the pharmacokinetics of rosuvastatin (*CYP2C9* *3 [1075 A>C, rs1057910]; *CYP2C19* *2 [681G>A, rs4244285] and *3 [636G>A, rs4986893]; *ABCG2* 421C>A [rs2231142]; *SLCO1B1* 388A>G [rs2306283] and 521T>C [rs4149056]) were also selected to examine whether these variants might affect the hsCRP levels by altering the pharmacokinetics of rosuvastatin in this group of patients.

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) as described in Chapter **3**. The genotyping for detection of the *CYP2C9* *3 polymorphism was performed with the Taqman Drug

Metabolism Genotyping Assay (C_27104892_10) from Applied Biosystems (Foster City, CA, USA) using the Applied Biosystems PCR System 9700 system. All SNPs genotyped were in Hardy-Weinberg equilibrium ($\chi^2$ test P>0.05). No variant allele was found in the Ala98Val in *HNF1A*, therefore this SNP was excluded from the analysis.

### 5.2.4 Statistical analysis

Subjects with hsCRP levels greater than 10 mg/L were excluded from the analysis (Nordestgaard BG, 2009) and for those with hsCRP levels below the limit of detection, the value of 0.1 mg/L was assigned. hsCRP levels were log-transformed for analysis to fulfill the model assumption of residual normality. Univariate and multivariate regression analysis were performed to identify the clinical covariates associated with log-hsCRP. Clinical covariates examined in the study included age, gender, body mass index (BMI), percentage of total body fat, WC, waist to hip ratio (WHR), smoking status, history of cardiovascular event or CHD, diabetes, hypertension, FH, HDL-C, LDL-C, triglycerides and concomitant medications. Variables with P<0.05 were allowed to be retained in the stepwise-selection models. The effects of 14 SNPs on log-hsCRP were analyzed separately in an exploratory analysis with adjustment for clinical variables, and only those genetic variables with significant effects on hsCRP (P<0.05) were selected in the stepwise-selection models. Multivariate linear regression analysis was used to determine contributions of the environmental factors and genetic factors to the variance of log-hsCPR. Associations between genetic polymorphism and log-hsCRP were assessed by analysis of covariance (ANCOVA) followed by a post-hoc test with the Bonferroni test after correction for the effect of clinical variables and other genetic variables. Finally, multivariate logistic regression analysis was performed to examine covariates

associated with having a high relative risk of hsCRP ≥ 2.0 mg/L. To adjust for multiple testing, we calculated an experiment-wide significance level using the conservative method of Bonferroni (0.05/14 = 0.0036), and only those polymorphisms with a probability value below this level were considered to be statistical significant. Data were analyzed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

## 5.3 Results

### 5.3.1 Clinical characteristics of participants

Clinical characteristics of 281 subjects with good compliance to rosuvastatin and having hsCRP less than 10 mg/L were shown in Table **5-3**. The median age was 57 years and about 50% of the study participants were male. The median hsCRP level was 0.811 mg/L (Interquartile range, 0.46 - 1.86 mg/L) including 13 subjects with hsCRP levels less than the limit of detection. About half of the subjects had familial hypercholesterolaemia (FH) or hypertension, and one-quarter of them had diabetes, and most of these patients were receiving treatment for these comorbidities. Only 12.8% of subjects were current smokers and 17.1% of the participants had a history of cardiovascular disease (CVD).

The demographic data for subjects with hsCRP values below and above the median value of 0.811 mg/L were shown in Table **5-4**. Patients with lower hsCRP levels were less obese and had lower levels of fasting glucose, diastolic blood pressure, triglycerides but higher HDL-C and had a relatively lower prevalence of diabetes and hypertension compared to those with hsCRP values above the median. There were no differences in age or LDL-C levels between the two groups. The proportion of FH patients was lower in subjects with hsCRP values below the median than those above.

In addition, for those subjects with extremely low hsCRP level (< 0.146 mg/L), although the number was small, most of these had FH and they were younger compared to the rest with hsCRP greater than 0.146 mg/L (Table **5-5**).

**Table 5-3. Clinical characteristics in subjects with hsCRP < 10 mg/L**

| Characteristics | Total (n=281) | Male (n=137) | Female (n=144) | P |
|---|---|---|---|---|
| Age, years | 57 (49-64) | 55 (48-64) | 58 (50-65) | 0.077 |
| BMI, kg/m$^2$ | 24.9 (22.7, 27.1) | 25.2 (23.3, 27.0) | 24.5 (22.0, 27.2) | 0.130 † |
| WC, cm | 86.0 (79.0, 93.8) | 89.0 (83.2, 99.0) | 83.5 (76.3, 90.5) | |
| WHR | 0.89 (0.84, 0.94) | 0.91 (0.87, 0.97) | 0.87 (0.81, 0.92) | <0.001 |
| Body fat, % | 28.7 (23.6, 35.3) | 24.5 (21.7, 28.4) | 34.2 (29.2, 39.4) | <0.001 † |
| SBP, mmHg | 123 (113, 135) | 123 (114, 135) | 123 (110, 134) | 0.3 |
| DBP, mmHg | 75 (66, 82) | 78 (72, 86) | 71 (63, 80) | <0.001 |
| Pulse, beats/minute | 69 (63, 77) | 70 (63, 76) | 68 (62, 77) | 0.566 * |
| FH | 144 (51.2) | 64 (46.7) | 80 (55.6) | 0.153 |
| Diabetes | 71 (25.2) | 38 (27.7) | 33 (22.9) | 0.41 |
| Hypertension | 151 (53.7) | 77 (56.2) | 74 (52.1) | 0.473 |
| History of CVD | 48 (17.1) | 30 (21.9) | 18 (12.5) | 0.04 |
| Current smoker | 36 (12.8) | 32 (23.4) | 4 (2.8) | <0.001 |
| hsCRP, mg/L | 0.81 (0.46, 1.86) | 0.81 (0.48, 1.76) | 0.83 (0.40, 1.87) | 0.909 * |
| LDL-C, mmol/L | 2.4 (1.9, 3.3) | 2.4 (1.8, 3.3) | 2.4 (1.9, 3.2) | 0.815 * |
| HDL-C, mmol/L | 1.5 (1.24, 1.76) | 1.33 (1.20, 1.55) | 1.6 (1.38, 1.98) | <0.001 * |
| TG, mmol/L | 1.34 (0.92, 1.88) | 1.40 (0.98, 1.99) | 1.20 (0.90, 1.79) | 0.067 * |
| TC, mmol/L | 4.7 (4.0, 5.45) | 4.6 (3.8, 5.55) | 4.85 (4.2, 5.4) | 0.092 † |
| Glucose, mmol/L | 5.4 (4.9, 6.3) | 5.4 (4.9, 6.5) | 5.4 (4.95, 6.3) | 0.803 † |
| Concomitant medications | | | | |
| Antihypertensive & antiarrhythmic drugs | 151 (53.7) | 80 (58.4) | 71 (49.3) | 0.151 |
| Anti-diabetic drugs | 41 (14.6) | 21 (15.3) | 20 (13.9) | 0.739 |
| Aspirin | 41 (14.6) | 28 (20.4) | 13 (9.0) | 0.011 |
| NSAIDs | 21 (7.5) | 6 (4.4) | 15 (10.4) | 0.069 |

Data were expressed as median (interquartile range) or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

† Data were compared by Mann-Whitney U test.

Abbreviations: BMI = body mass index; CVD = cardiovascular disease; DBP = diastolic blood pressure; FH = familial hypercholesterolaemia; hsCRP = high-sensitivity C-reactive protein; NSAIDs = non-steroidal anti-inflammatory drugs; SBP = systolic blood pressure; TC = total cholesterol; TG = triglycerides; WC = waist circumference; WHR = waist to hip ratio.

**Table 5-4. Clinical characteristics in subjects with hsCRP ≤ 0.811 mg/L and > 0.811 mg/L**

| Characteristics | ≤ 0.811 (n=140) | > 0.811 (n=141) | P |
|---|---|---|---|
| Age, years | 57 (49, 65) | 56 (49,64) | 0.749 |
| Male | 69 (49.3) | 68 (48.2) | 0.859 |
| BMI, kg/m$^2$ | 23.7 (21.7, 25.8) | 26.1 (24.0, 28.9) | <0.001† |
| WC, cm | 83.0 (76.9, 89.4) | 90.0 (83.8, 99.6) | <0.001 |
| WHR | 0.88 (0.83, 0.92) | 0.90 (0.85, 0.97) | <0.001 |
| Body fat, % | 26.2 (22.3, 31.4) | 32.3 (26.3, 37.6) | <0.001 |
| SBP, mmHg | 120 (109, 134) | 124 (114, 135) | 0.152 |
| DBP, mmHg | 73 (66, 81) | 76 (67, 85) | 0.028† |
| Pulse, beats/minute | 68 (62, 76) | 70 (63, 79) | 0.142 |
| FH | 80 (57.1) | 64 (45.4) | 0.049 |
| Diabetes | 20 (14.3) | 51 (36.2) | <0.001 |
| Hypertension | 60 (42.9) | 91 (64.5) | <0.001 |
| History of CVD | 18 (12.9) | 30 (21.3) | 0.061 |
| Current smoker | 14 (10) | 22 (15.6) | 0.160 |
| hsCRP, mg/L | 0.46 (0.28, 0.58) | 1.86 (1.08, 2.78) | <0.001 |
| LDL-C, mmol/L | 2.55 (1.9, 3.3) | 2.3 (1.8, 3.2) | 0.185† |
| HDL-C, mmol/L | 1.58 (1.30, 1.89) | 1.39 (1.15, 1.6) | <0.001* |
| Triglycerides, mmol/L | 1.1 (0.8, 1.53) | 1.6 (1.12, 2.1) | <0.001* |
| TC, mmol/L | 4.8 (4.1, 5.58) | 4.6 (3.9, 5.4) | 0.457 |
| Glucose, mmol/L | 5.2 (4.8, 5.8) | 5.7 (5.1, 6.7) | <0.001† |
| Concomitant medications, | | | |
| Antihypertensive & antiarrhythmic drugs | 65 (46.4) | 86 (61.0) | 0.014 |
| Anti-diabetic drugs | 14 (10) | 27 (19.1) | 0.03 |
| Aspirin | 13 (9.3) | 28 (19.9) | 0.012 |
| NSAIDs | 11 (7.9) | 10 (7.1) | 0.807 |

Data were expressed as median (interquartile range) or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

† Data were compared by Mann-Whitney U test.

Abbreviations: BMI = body mass index; CVD = cardiovascular disease; DBP = diastolic blood pressure; FH = familial hypercholesterolemia; hsCRP = high-sensitivity C-reactive protein; NSAIDs = non-steroidal anti-inflammatory drugs; SBP = systolic blood pressure; TC = total cholesterol; WC = waist circumference; WHR = waist to hip ratio.

**Table 5-5. Clinical characteristics in subjects with hsCRP < 0.146 mg/L and ≥ 0.146 mg/L**

| Characteristics | < 0.146 (n=13) | ≥ 0.146 (n=268) | P |
|---|---|---|---|
| Age, years | 45 (35-53) | 57 (50-65) | 0.001 |
| Male, | 9 (69.2) | 128 (47.8) | 0.130 |
| BMI, $kg/m^2$ | 21.8 (20.7, 23.8) | 25.2 (22.9, 27.2) | <0.001† |
| WC, cm | 79.0 (74.6, 84.6) | 86.5 (80.5, 94.8) | 0.001 |
| WHR | 0.84 (0.81, 0.91) | 0.89 (0.84, 0.95) | 0.052 |
| Body fat, % | 23.0 (20.6, 29.0) | 28.9 (23.8, 35.6) | 0.048 |
| SBP, mmHg | 115 (98, 118) | 123 (113, 135) | 0.009 |
| DBP, mmHg | 74 (65, 81) | 75 (66, 83) | 0.028† |
| Pulse, beats/minute | 67.5 (62.8, 73.5) | 69 (63, 77) | 0.671 |
| FH, | 10 (76.9) | 134 (50.0) | 0.058 |
| Diabetes, | 0 (0) | 71 (26.5) | 0.043 |
| Hypertension, | 1 (7.7) | 150 (56.0) | 0.01 |
| History of CVA/CHD, | 1 (7.7) | 47 (17.5) | 0.704 |
| Current smoker, | 2 (15.4) | 34 (12.7) | 0.676 |
| LDL-C, mmol/L | 2.8 (2.4, 3.6) | 2.4 (1.8, 3.2) | 0.09* |
| HDL-C, mmol/L | 1.80 (1.32, 2.02) | 1.48 (1.23, 1.7) | <0.001† |
| Triglycerides, mmol/L | 0.90 (0.64, 1.3) | 1.37 (0.98, 1.9) | 0.003* |
| TC, mmol/L | 5.20 (4.25, 5.85) | 4.7 (4.0, 5.4) | 0.187* |
| Glucose, mmol/L | 5.0 (4.6, 5.3) | 5.4 (4.9, 6.4) | <0.001† |
| Concomitant medications, | | | |
| Antihypertensive & antiarrhythmic drugs | 1 (7.7) | 150 (56.0) | 0.001 |
| Antidiabetic drugs | 0 (0) | 41 (15.3) | 0.227 |
| Aspirin | 0 (0) | 41 (15.3) | 0.227 |
| NSAIDs | 1 (7.7) | 20 (7.5) | 1.000 |

Data were expressed as median (interquartile range) or n (%) and compared by t-test or $\chi^2$-test for continuous variable or categorical variable unless otherwise indicated;

* Data were logarithmically (base 10) transformed for comparison;

† Data were compared by Mann-Whitney U test.

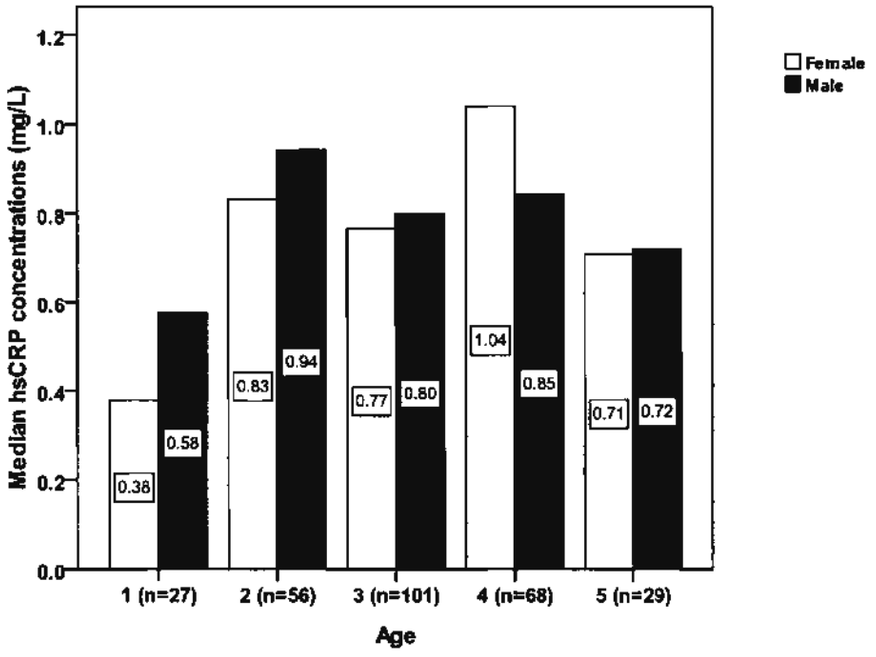
Abbreviations: BMI = body mass index; CVD = cardiovascular disease; DBP = diastolic blood pressure; FH = familial hypercholesterolaemia; hsCRP = high-sensitivity C-reactive protein; NSAIDs = non-steroidal anti-inflammatory drugs; SBP = systolic blood pressure; TC = total cholesterol; WC = waist circumference; WHR = waist-hip ratio.

### 5.3.2 Associations of environmental factors with hsCRP

#### 5.3.2.1 Age and gender

In univariate analysis, age and gender did not seem to be associated with hsCRP levels (Figure 5-1).

**Figure 5-1. hsCRP distribution in different age groups in males and women**

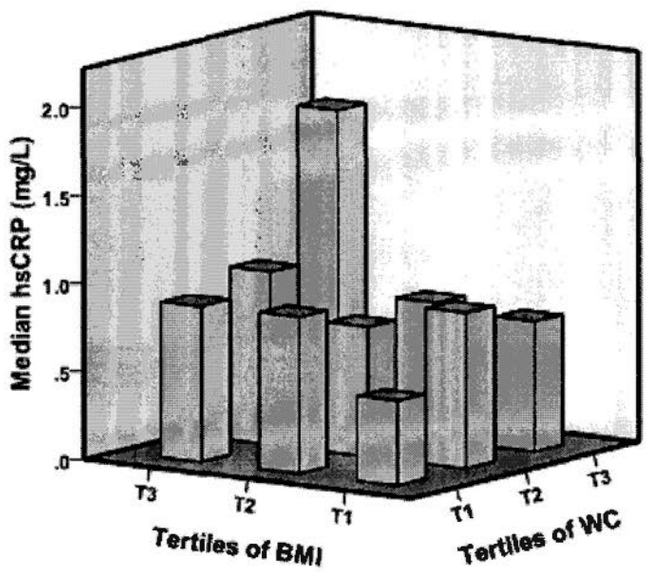



1 = 18-40 years; 2 = 41-50 years; 3 = 51-60 years; 4 = 61-70 years; 5 = 71-85 years

#### 5.3.2.2. Body mass index, waist circumference and percentage body fat

In univariate analysis, WC ($r = 0.437$, $P<5\times10^{-14}$), BMI ($r = 0.422$, $P<4\times10^{-13}$), WHR ($r = 0.328$, $P<4\times10^{-8}$) and percentage body fat ($r = 0.346$, $P<2\times10^{-7}$) were significantly associated with log-hsCRP levels, respectively, but the associations of BMI, WHR and percentage body fat with hsCRP were not significant after adjustment for WC, although patients with both high BMI and WC values had the highest hsCRP concentrations (Figure 5-2).

**Figure 5-2. Median hsCRP by strata of body mass index and waist circumference**




Abbreviations: BMI = body mass index; T = tertile; WC = waist circumference.

The associations of log-hsCRP with BMI, WC and WHR were similar in both males and females (Figure **5-3, 5-4, 5-5**). However, the relationship between log-hsCRP and the percentage body fat was more significant in females than males (Figure **5-6**).

**Figure 5-3. Spearman's correlations between log-hsCRP and body mass index (BMI) in males and females**

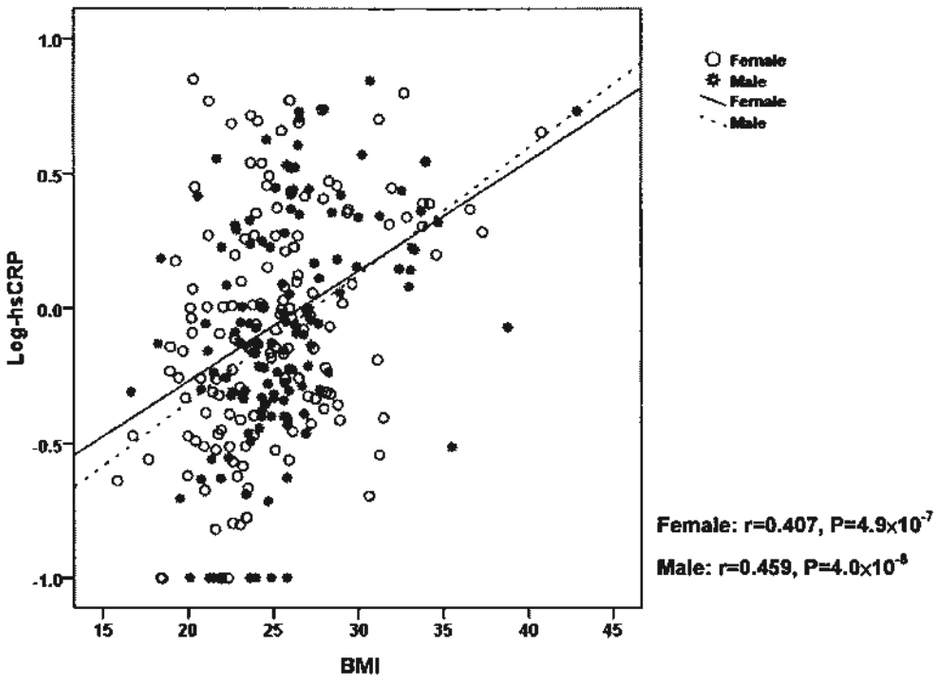



**Figure 5-4. Spearman's correlations between log-hsCRP and waist circumference (WC) in males and females**

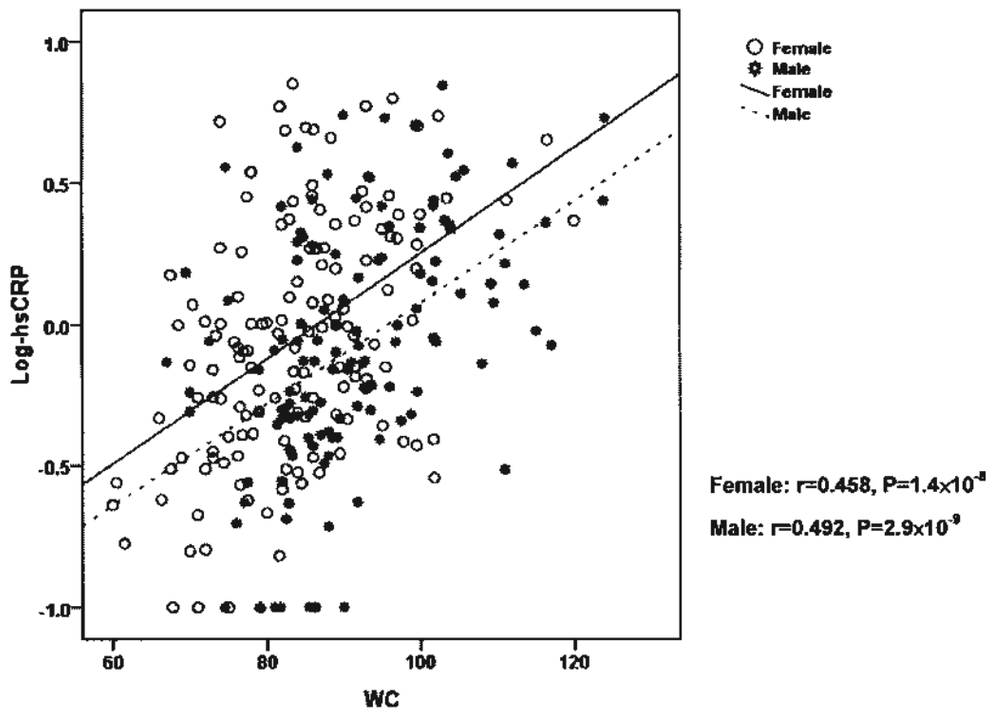


**Figure 5-5. Pearson's correlations between log-hsCRP and waist to hip ratio (WHR) in males and females**

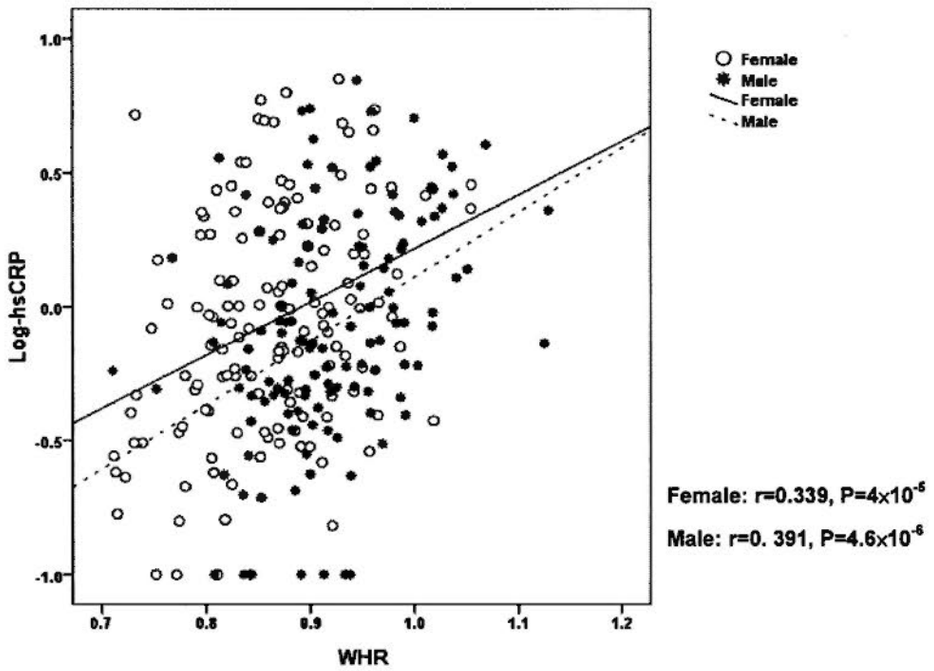
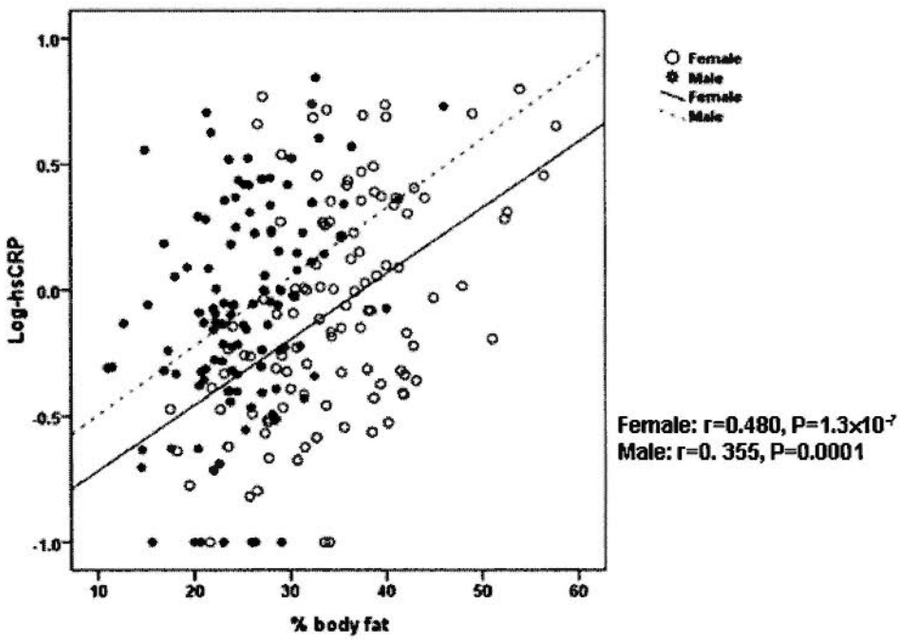



**Figure 5-6. Spearman's correlations between log-hsCRP and percentage body fat in males and females**



#### 5.3.2.3 Lipids and familial hypercholesterolaemia

Low HDL-C and high triglyceride levels were associated with elevated hsCRP concentrations. There was no significant association between LDL-C or total cholesterol with log-hsCRP. Patients with FH tended to have a lower level of hsCRP compared to non-FH, but after adjustment for other confounding factors there was no difference in hsCRP levels between these subgroups.

#### 5.3.2.4 Fasting plasma glucose and diabetes

Fasting plasma glucose was also strongly associated with log-hsCRP levels and patients with diabetes had higher levels of hsCRP than those without.

#### 5.3.2.5 Blood pressure and hypertension

Systolic blood pressure and diastolic blood pressure were weakly associated with log-hsCRP ($r = 0.147$, $P = 0.015$ and $r = 0.126$, $P = 0.037$, respectively) and patients with hypertension had higher hsCRP levels compared to those without.

#### 5.3.2.6 History of cardiovascular disease and smoking

There was no significant difference in hsCRP levels in patients with a history of CVD or smoking compared to those without.

#### 5.3.2.7 Concomitant medications

Apart from rosuvastatin, patients were also treated with various other medications for their comorbidities, particularly antihypertensive and antidiabetic drugs (Table **5-6**).

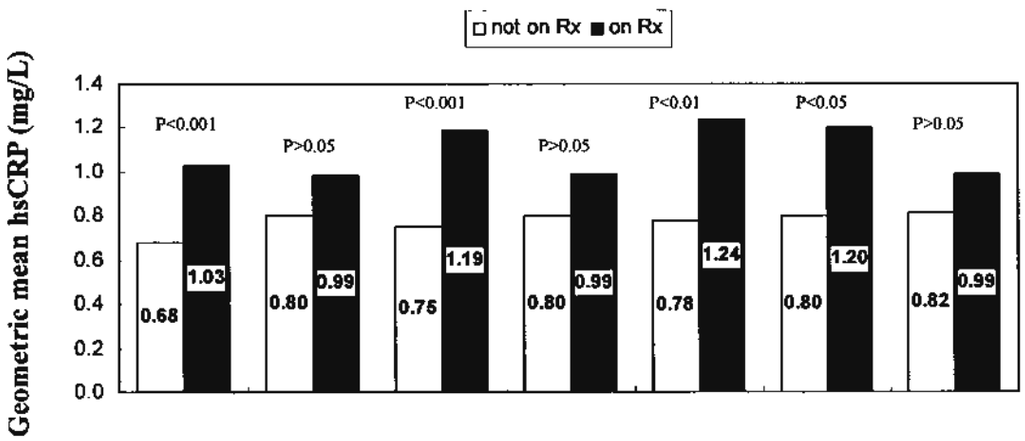
**Table 5-6. Concomitant medications in subjects involved in the analysis**

| **Concomitant medications** | n (%) |
|---|---|
| Antihypertensive & antiarrhythmic drugs | 151 (53.7) |
| ACEI | 64 (22.8) |
| ARB | 16 (5.7) |
| α-blocker, n (%) | 20 (7.1) |
| β-blocker, n (%) | 78 (27.8) |
| CCB, n (%) | 74 (26.3) |
| Diuretics, n (%) | 50 (17.8) |
| HTCZ diuretics, n (%) | 31 (11.1) |
| Nitrate, n (%) | 11 (3.9) |
| Antidiabetic drugs, n (%) | 41 (14.6) |
| Metformin, n (%) | 35 (12.5) |
| Sulfonylureas, n (%) | 27 (9.6) |
| Aspirin, n (%) | 41 (14.6) |
| NSAIDs, n (%) | 21 (7.5) |
| Clopidogrel, n (%) | 5 (1.8) |
| Warfarin, n (%) | 3 (1.1) |

Abbreviations: ACEI = angiotensin converting enzymeinhibitors; ARBs = angiotensin receptor blockers; CCBs = calcium channel blockers; HTCZ = hydrochlorothiazide; NSAIDs = non-steroidal anti-inflammatory drugs.

Patients receiving concomitant medications for diabetes and hypertension tended to have higher hsCRP levels, particularly for β-blockers (Figure **5-7**). Taking aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) did not significantly influence the hsCRP levels.

**Figure 5-7. Geometric mean hsCRP levels in patients with or without concomitant medications**




**Concomitant medications**

1 = antihypertensive drugs; 2 = ACEI or ARB; 3 = β-blocker; 4 = CCB; 5 = Diuretics; 6 = antidiabetic drugs; 7 = Aspirin or NSAIDS

### 5.3.2.8 Multivariate analysis of environmental factors

Multivariate analysis showed that log-hsCRP levels were strongly correlated with WC ($P<1\times10^{-6}$) and moderately correlated with triglycerides and inversely correlated with HDL-C ($P<0.05$). Having diabetes and female gender were also associated with increased hsCRP levels after adjustment for other variables ($P<0.05$) (Table 5-7). Other environmental factors were not associated with hsCRP levels after adjustment for these confounding factors.

### 5.3.3 Associations of genetic factors with hsCRP

Among all the SNPs examined in the analysis, three SNPs were found to be independently associated with log-hsCRP levels, including rs1169288 in *HNF1A* ($P<0.003$); rs1205 ($P<1\times10^{-6}$) and rs2808630 ($P=0.0004$) in *CRP* before and after adjustment for other confounding factors. The association between log-hsCRP and

rs1169288 in *HNF1A* were only observed in females but not in males (Table **5-7**).

**Table 5-7. Multivariate model of predictors of log-hsCRP**

| | ALL | | Male | | Female | |
|---|---|---|---|---|---|---|
| | Beta | P value | Beta | P value | Beta | P value |
| Age | 0.048 | 0.375 | 0.038 | 0.623 | 0.091 | 0.241 |
| Gender | -0.215 | **0.0002** | -- | -- | -- | -- |
| BMI | 0.12 | 0.268 | -0.046 | 0.767 | 0.239 | 0.123 |
| WC | 0.313 | **$8.6\times10^{-7}$** | 0.352 | **$1.2\times10^{-5}$** | 0.365 | **$4\times10^{-6}$** |
| LDL-C | -0.02 | 0.716 | 0.024 | 0.75 | -0.093 | 0.2 |
| HDL-C | -0.197 | **0.001** | -0.218 | **0.0004** | -0.218 | **0.005** |
| TG | 0.122 | **0.048** | 0.155 | 0.065 | 0.106 | 0.233 |
| FH | 0.035 | 0.527 | -0.017 | 0.832 | 0.019 | 0.799 |
| Hypertension | 0.014 | 0.807 | 0.099 | 0.215 | -0.009 | 0.909 |
| Diabetes | 0.109 | **0.045** | 0.081 | 0.293 | 0.122 | 0.114 |
| History of CVD | -0.001 | 0.981 | 0.017 | 0.814 | -0.026 | 0.722 |
| Current Smoker | 0.025 | 0.64 | 0.017 | 0.817 | 0.012 | 0.872 |
| Anti-hypertensive drugs | -0.022 | 0.695 | 0.134 | 0.089 | -0.135 | 0.088 |
| Anti-diabetes drugs | -0.097 | 0.192 | 0.074 | 0.313 | -0.02 | 0.795 |
| Aspirins | 0.043 | 0.411 | 0.072 | 0.322 | -0.005 | 0.947 |
| NSAIDs | 0.007 | 0.886 | 0.035 | 0.636 | -0.011 | 0.885 |
| *CRP* rs1205 | -0.301 | **$6.4\times10^{-7}$** | -0.364 | **$2.6\times10^{-5}$** | -0.227 | **0.008** |
| *CRP* rs2808630 | -0.212 | **0.0004** | -0.169 | **0.043** | -0.273 | **0.002** |
| *HNF1A* rs1169288 | -0.156 | **0.0029** | -0.066 | 0.364 | -0.194 | **0.009** |

Abbreviations: BMI = body mass index; CVD = cardiovascular disease; FH = familial hypercholesterolaemia; HNF1A = Hepatic nuclear factor 1-alpha; hsCRP = high-sensitivity C-reactive protein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; NSAIDs = non-steroidal anti-inflammatory drugs; TG = triglycerides; WC = waist circumference.

Subjects with one or two copies of variant alleles of rs1205, rs2808630 in *CRP* and rs1169288 in *HNF1A* had reduced hsCRP levels compared to those with wild-type alleles (Table **5-8**, Figure **5-8**). hsCRP levels differed across 6 diplotypes of rs1205 and rs2808630 in *CRP* ($P<4\times10^{-5}$). Subjects with wild-type alleles (GG/AA) had

high CRP levels, whereas individuals with 2 mutated alleles had reduced CRP levels with carriers of 1 mutated allele having intermediate levels (Table **5-8**, Figure **5-8**). These associations were statistically significant after correction for multiple testing. None of the polymorphisms in genes potentially related to the pharmacokinetics of rosuvastatin was found to be associated with hsCRP levels.

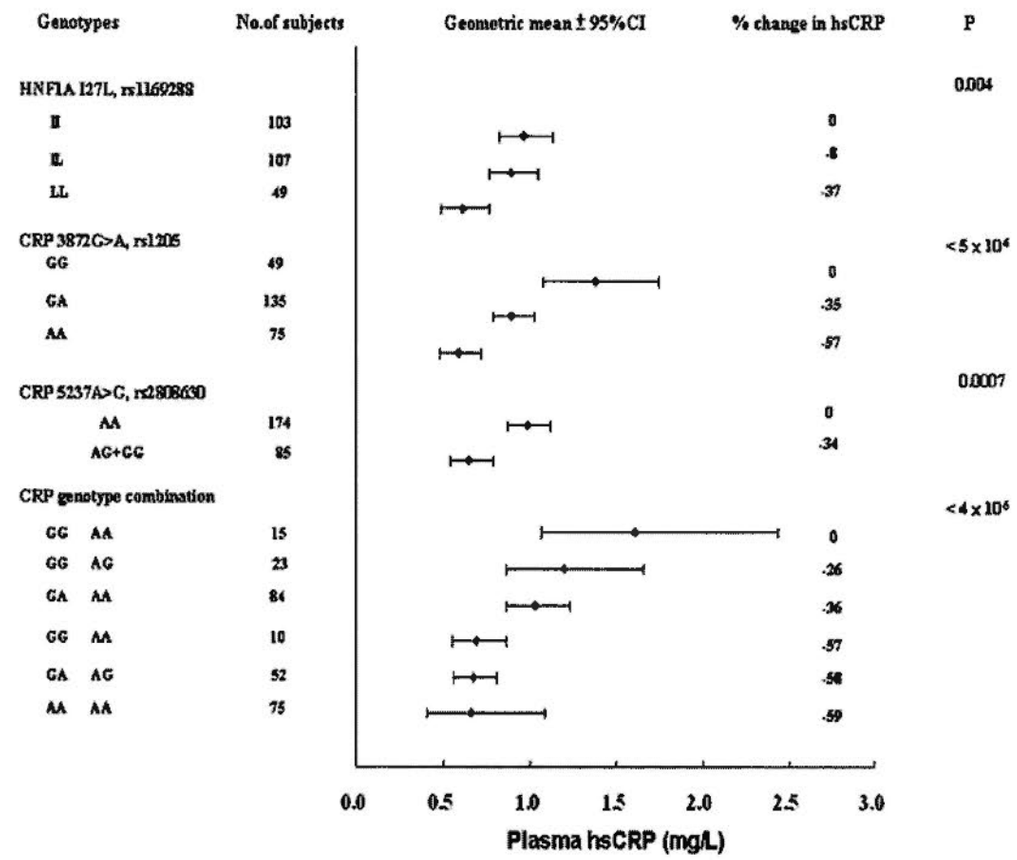
**Table 5-8. Geometric mean hsCRP levels among genotype groups**

| Variant | N | Geometric CRP levels (95%CI) | Overall P value |
|---|---|---|---|
| *CRP* rs1205 | | | |
| GG | 49 | 1.374 (1.079, 1.746) | |
| GA | 135 | 0.893 (0.780, 1.026) | $4.7\times10^{-6}$ |
| AA | 75 | 0.585 (0.480, 0.714) | |
| *CRP* rs2808630 | | | |
| AA | 174 | 0.984 (0.869, 1.117) | 0.0007 |
| AG+GG | 85 | 0.646 (0.533, 0.782) | |
| *CRP* rs1205 + rs2808630 | | | |
| GGAA | 15 | 1.611 (1.064, 2.438) | |
| GGAG | 23 | 1.194 (0.859, 1.660) | |
| GAAA | 84 | 1.028 (0.863, 1.225) | $3.6\times10^{-5}$ |
| GAAG | 52 | 0.689 (0.553, 0.859) | |
| AAAA | 75 | 0.673 (0.561, 0.807) | |
| GGGG | 10 | 0.659 (0.401, 1.084) | |
| *HNF1A* rs1169288 | | | |
| AA | 103 | 0.966 (0.826, 1.130) | |
| AC | 107 | 0.893 (0.767, 1.042) | 0.004 |
| CC | 49 | 0.611 (0.486, 0.766) | |

Data were expressed as geometric mean and 95% CI after adjustment for multiple genetic and environmental variables.

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HNF1α = Hepatocyte nuclear factor 1 alpha.

**Figure 5-8. Association of genetic variants with hsCRP levels in multivariable-adjusted model** *




*Data were expressed as geometric mean and 95% CI after adjustment for multiple genetic and environmental variables.

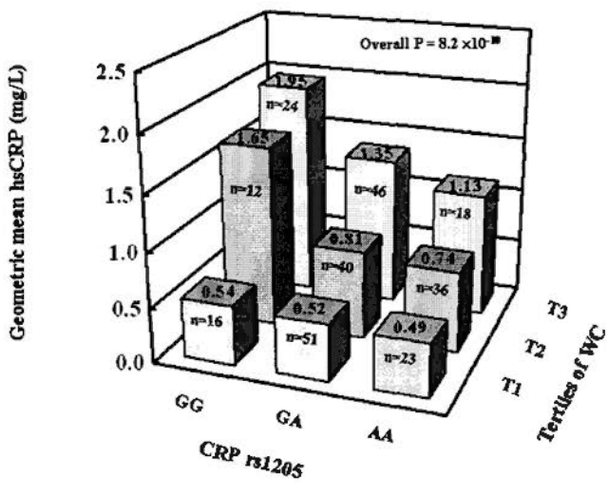
Abbreviations: CI = confidence interval; HNF1α = Hepatocyte nuclear factor 1 alpha; hsCRP = high sensitivity C-reactive protein; SNPs = single-nucleotide polymorphisms.

### 5.3.4 Interactions between waist circumference, rs1205 and plasma hsCRP levels

WC and the rs1205 polymorphism were the most significant factors associated with hsCRP in this study. CRP concentrations increased with increasing WC within each rs1205 genotype group. Similarly, within each WC tertile, the rs1205 variant allele was associated with reduced CRP levels in a gene-dose-dependent manner (overall $P=8.2\times10^{-10}$, Figure **5-9**). Similar interactions were also observed in BMI, rs1205 and

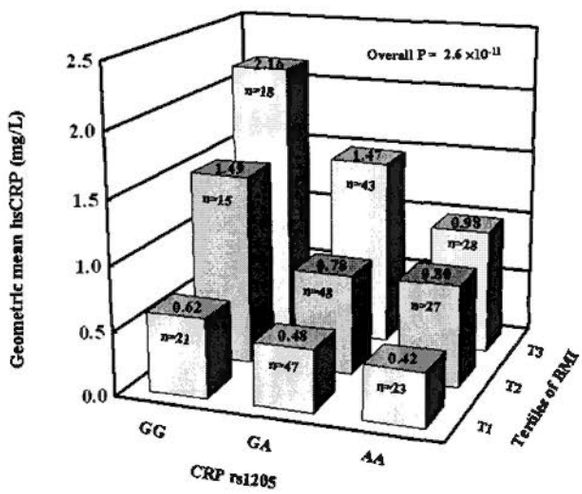
plasma hsCRP levels (overall P=2.6 $\times 10^{-11}$, Figure **5-10**). The relationships remained in men and women separately. In addition, there was no difference in WC among rs1205 genotype groups, suggesting that there was no relationship between abdominal obesity and rs1205.

**Figure 5-9. Plasma hsCRP levels by strata of *CRP* rs1205 genotype and waist circumference**




Abbreviations: CRP = C-reactive protein; T = tertile; WC = waist circumference.

**Figure 5-10. Plasma hsCRP levels by strata of *CRP* rs1205 genotype and BMI**




Abbreviations: BMI = body mass index; CRP = C-reactive protein; T = tertile.

### 5.3.5 Contributions of genetic and environmental factors to the variance of on-treatment hsCRP concentrations

These genetic and environmental factors explained totally 35.5% of the variance in hsCRP levels. WC explained the largest proportion of the variance in hsCRP concentrations (19.1%), followed by HDL-C (3.7%). The 3 genetic variables together explained 8.5% of the variance. Having diabetes and the levels of triglycerides each contributed only 1.0%, and 0.8% of the variance, respectively. Including BMI or percentage body fat in the multivariate model neither explained a higher proportion of variance in hsCRP nor changed the associations between genetic factors and hsCRP.

### 5.3.6 Multivariate odds ratios of predictors of having hsCRP level of ≥ 2 mg/L

Multivariate logistic regression analysis showed that the variants of rs1205 and rs2808630 were inversely associated with the relatively high-risk hsCRP levels. Female gender, higher WC and lower HDL-C levels were also predictors for patients having an hsCRP level of ≥ 2 mg/L. Smoking, having FH and diabetes also tended to increase the risk of having an hsCRP level of ≥ 2 mg/L (Table **5-9**). Similar predictors were observed when using 1mg/L as a cutoff point (data not shown).

**Table 5-9. Multivariable-adjusted odds ratios (ORs)* of predictors of having a high-risk hsCRP concentration (≥2 mg/L)**

| Variables | OR | 95% CI | P |
|---|---|---|---|
| Age, years | 1.006 | 0.970-1.044 | 0.734 |
| Gender | | | |
| Male | 1 | | |
| Female | 2.892 | 1.175-7.120 | 0.021 |
| BMI | | | |
| $< 25$ kg/m$^2$ | 1 | | |
| $\geq 25$ kg/m$^2$ | 1.557 | 0.572-4.237 | 0.387 |
| WC, 10 cm† | 1.875 | 1.150-3.058 | 0.012 |
| FH | | | |
| No | 1 | | |
| Yes | 2.371 | 0.839-6.699 | 0.103 |
| Diabetes | | | |
| No | 1 | | |
| Yes | 2.105 | 0.656-6.757 | 0.211 |
| Hypertension | | | |
| No | 1 | | |
| Yes | 1.047 | 0.220-4.971 | 0.954 |
| Current smoker | | | |
| No | 1 | | |
| Yes | 1.724 | 0.567-5.243 | 0.337 |
| History of CVD | | | |
| No | 1 | | |
| Yes | 1.016 | 0.266-3.884 | 0.982 |
| LDL-C, mmol/L | 0.828 | 0.529-1.298 | 0.411 |
| HDL-C, 0.1 mmol/L† | 0.858 | 0.758-0.971 | 0.016 |
| TG, mmol/L | 1.570 | 0.895-2.756 | 0.116 |
| Antihypertensive drugs | | | |
| No | 1 | | |
| Yes | 1.180 | 0.254-5.480 | 0.832 |
| Antidiabetic drugs | | | |
| No | 1 | | |
| Yes | 0.486 | 0.136-1.734 | 0.266 |
| Aspirin | | | |
| No | 1 | | |
| Yes | 0.699 | 0.164-2.969 | 0.627 |

| | | | |
|---|---|---|---|
| NSAIDs | | | |
| No | 1 | | |
| Yes | 0.479 | 0.105-2.175 | 0.340 |
| *CRP* 3872G>A, rs1205 | | | |
| GG | 1 | | |
| GA | 0.402 | 0.147-1.100 | 0.076 |
| AA | 0.106 | 0.030-0.367 | 0.0004 |
| *CRP* 5237A>G , rs2808630 | | | |
| AA | 1 | | |
| AG+GG | 0.251 | 0.099-0.632 | 0.003 |
| *HNF1A* I27L, rs1169288 | | | |
| AA | 1 | | |
| AC | 1.000 | 0.442-2.261 | 0.999 |
| CC | 0.656 | 0.219-1.960 | 0.450 |

*Multivariate logistic regression for OR and P value.

† The OR estimated for a 10 cm increase in WC or 0.1 mmol/L incresase in HDL-C of having an hsCRP level of ≥2 mg/l when controlling for other variables in the model.

Abbreviations: BMI = body mass index; CI: confidence interval; CVD = cardiovascular disease; FH = familial hypercholesterolaemia; HNF1A = Hepatic nuclear factor 1-alpha; hsCRP = high-sensitivity C-reactive protein; NSAIDs = non-steroidal anti-inflammatory drugs; OR = odds ratio; TG = triglycerides; WC = waist circumference.

### 5.4 Discussion

Plasma hsCRP concentration is a heritable trait and there is substantial evidence for the significant impact of *CRP* polymorphisms on baseline hsCRP levels. Variants and haplotypes in the *CRP* gene have been shown to be associated with circulating CRP levels in childhood and adulthood, with the strongest effects being found for average serum CRP level across the life course in a population-based prospective cohort study (1980-2001) of 1609 young Finns, which supports the assumption that genetic variants define groups with long-term difference in circulating CRP levels (Kivimaki M et al., 2007).

In the present study, the variant alleles of rs1205 and rs2808630 in *CRP* were significantly associated with reduced hsCRP levels, which is consistent with previous findings in different populations (Kathiresan S et al., 2006, Kolz M et al., 2008, Lee CC et al., 2009, Teng MS et al., 2009) although patients in this study were on treatment with a potent statin which is known to reduce hsCRP levels. The mechanism of the associations of rs1205 and rs2808630 with hsCRP might be through their linkage disequilibrium (LD) with the functional triallelic SNP rs3091244 (Kathiresan S et al., 2006) or their own functional effects as these two SNPs are located in the 3' untranslated region (Figure **1-11**) potentially affecting mRNA transcription and stability (Kolz M et al., 2008). The triallelic SNP rs3091244 is located in the promoter sequence 301 bases upstream of the *CRP* gene transcription start site. It has been considered to be functional as it resides within the hexameric core of transcription factor-binding elements and and the mutation alters a transcription factor–binding motif for upstream stimulating factor 1 (USF1), thus affecting the transcriptional activity of the CRP gene (Carlson CS et al., 2005). The variant alleles of T and A of this triallelic SNP rs3091244 were highly correlated with the wild type alleles of rs1205 and rs2808630 in Framingham Heart Study participants (Kathiresan S et al., 2006).

The SNP rs1205 was recently found to capture the strongest effects of 8 SNPs covering the *CRP* gene including 10kb surrounding the gene on circulating CRP levels and the haplotypes did not add to the effect on CRP levels beyond the effect of this SNP that was considered to be due to the extremely strong LD over and upstream of the *CRP* gene (Eiriksdottir G et al., 2009). The frequency of the rs1205 polymorphism varies among ethnic groups corresponding to different population levels of CRP (Table **5-10**). This SNP is highly prevalent in Chinese (the variant

allele frequency of 54% in this study) and Japanese (73%) and is likely to be the major determinant of lower CRP concentrations in these populations (Kelley-Hedgepeth A et al., 2008).

**Table 5-10. Frequencies of rs1205 and median CRP levels by ethnicity**

| Ethnic Groups | Median CRP levels * (mg/L) | Frequency of the variant allele of rs1205† |
|---|---|---|
| **Sub-Saharan African** | 3.2 | 0.15 |
| **Caucasians** | 1.5 | ~ 0.30 |
| **Chinese** | 0.7 | 0.54 |
| **Japanese** | 0.5 | 0.73 |

*Data are from the study of Women's Health Across the Nation (Kelley-Hedgepeth A et al., 2008).

†Data are from the present study and the NCBI SNP website.

The effect of the rs1205 polymorphism in *CRP* on hsCRP on-treatment levels observed in the present study appeared to be greater than its impact on the baseline level of hsCRP with no treatment in Caucasians (12% and 23% reductions in CRP levels in subjects with 1 and 2 copies of variants respectively comparing to subjects with homozygous wild-type alleles) (Zacho J et al., 2008), but a recent study in Taiwanese showed the effect of this polymorphism on baseline hsCRP (35% and 56% reductions in CRP levels in subjects with 1 and 2 copies of variants respectively comparing to subjects with homozygous wild-type alleles) (Teng MS et al., 2009) was similar to the findings of on-treatment hsCRP levels in the present study which suggests that rosuvastatin treatment did not alter the association between this genetic factor and hsCRP levels. The *CRP* polymorphism may affect the binding of the transcriptional complex with c-Fos, STAT3, and HNF-1α caused by cytokine

stimulation that induces synergistic expression of the *CRP* gene and thereby *CRP* expression. Statins were shown to inhibit cytokine-induced STAT3 activation (Arnaud C et al., 2005), which may reduce the formation of the transcriptional complex, but this effect is unlikely to be influenced directly by polymorphisms in *CRP*.

Hepatic nuclear factor 1-α (HNF1-α) is a homeodomain-containing transcription factor which is known to regulate the expression of a number of liver genes, including those of the acute phase proteins such as CRP (Armendariz AD and Krauss RM, 2009). It has been shown that the human *CRP* promoter contained two functional HNF1A-binding sites, which is necessary but not sufficient for induction of CRP (Toniatti C et al., 1990). More recently, Nishikawa et al have demonstrated that the formation of a complex of three proteins including HNF1A is essential for cytokine-driven CRP gene expression (Nishikawa T et al., 2008). Recent GWASs have consistently demonstrated the strong association between polymorphisms in *HNF1A* and CRP phenotypes (Reiner AP et al., 2008, Ridker PM et al., 2008b). In the GWAS in the Cardiovascular Health Study (CHS) participants, the most significant association between *HNF1A* haplotypes and CRP was observed in the haplotype which harbored the minor allele of I27L and S486N in *HNF1A*. In the present study, the common non-synonymous coding SNP I27L was associated with reduced hsCRP levels with a gender difference observed. The mechanisms behind this gender difference in the relationships between this polymorphism and hsCRP are unknown, but *HNF1A* also plays a role in bile acid and cholesterol homeostasis and glucose metabolism and mutations in *HNF1A* are also associated with one type of maturity-onset diabetes of the young (MODY-3) in Chinese (Ng MC et al., 1999) and in other populations (Fajans SS et al., 2001), These observations reveal a complex

interaction between metabolic and inflammatory pathways mediated by this transcription factor (Armendariz AD and Krauss RM, 2009).

Apart from the *CRP* gene itself and *HNF1A*, *APOE* is the other gene that was consistently related to CRP levels in the GWASs (Reiner AP et al., 2008, Ridker PM et al., 2008b). The *APOE* gene has been extensively investigated for its impact on plasma lipid levels and risk of CVD. The two polymorphisms at the amino acids 112 and 158 that define the major e2, e3, and e4 haplotypes (Table **1-11**) were associated with CRP levels in previous candidate-gene studies in Caucasians and Japanese-Americans with lower levels of CRP among *APOE* e4 carriers or higher levels of CRP among e2 carriers compared to the remaining haplotype carriers reported (Austin MA et al., 2004, Chasman DI et al., 2006, Marz W et al., 2004). However, in the present study the associations of e2, e3, and e4 haplotypes with hsCPR were not significant although e4 homozygote subjects (n=3) did have the lowest level of CRP but the number in this group was too small to compare and e4 heterozygote subjects did not seem to have a reduced CRP level. The mechanism of the correlation between *APOE* polymorphisms but not apolipoprotein E protein with CRP was still unclear (Chasman DI et al., 2006). It has been speculated that the association was related to the changes in the metabolism of plasma lipoproteins determined by *APOE* polymorphisms which may in turn influence the half life of lipoprotein-bound CRP (Austin MA et al., 2004) and this effect was independent of inflammation since other inflammation markers like fibrinogen and white cell count were not related to the *APOE* genotype (Marz W et al., 2004). Therefore, it is possible that rosuvastatin treatment affected the association between *APOE* polymorphisms and CRP by reducing plasma LDL-C concentrations.

Most recently, the minor allele of SNP rs4420638 in the *APOE-CI-CII* cluster was shown to be strongly associated with increased LDL-C, triglycerides and total cholesterol, reduced HDL-C and CRP levels, as well as an increased risk of CHD in various studies (Elliott P et al., 2009, Willer CJ et al., 2008b). This polymorphism is situated about 14 kb distal to the *APOE* locus and was in strong LD with the two SNPs that characterize the *APOE* e2/e3/e4 locus. In the present study, the rs4420638 polymorphism was also found to be strongly linked with *APOE* e2/e3/e4 but it did not show a significant impact on hsCRP levels.

Leptin, the adipocyte-derived protein product of the leptin (*LEP*) gene, is involved in appetite regulation and obesity, blood pressure regulation and immune response (Shamsuzzaman AS et al., 2004). Circulating leptin levels are consistently associated with CRP levels, although the exact mechanism is not clear but it is conceivable that leptin may act via induction of IL-6 production, or perhaps via the leptin receptor to upregulate CRP production (Shamsuzzaman AS et al., 2004). A number of studies have shown that *LEPR* polymorphisms were associated with leptin and CRP levels (Ridker PM et al., 2008b, Zhang YY et al., 2007), but such an association was not found in the present study, which may be due to the small number of subjects in this analysis.

In the patients in this study, rosuvastatin 10 mg reduced LDL-C by 52% and a recent study in Chinese patients with hypercholesterolaemia (Qu HY et al., 2009) has reported 40% and 47.5% mean reductions in hsCRP and LDL-C, respectively, after 12 weeks treatment with rosuvatatin 10 mg, which are similar in magnitude to those effects observed in the JUPITER study with 20 mg of rosuvastatin. The systemic exposure to rosuvastatin in Asians has been shown to be 2-fold higher than in

Caucasians (Carlson CS et al., 2005), which may contribute to the greater efficacy in Chinese (Qu HY et al., 2009). The functional SNP 421C>A in *ABCG2* was associated with altered systemic exposure to rosuvastatin in Chinese and Caucasians subjects (Keskitalo JE et al., 2009c, Zhang W et al., 2006a) and the higher allele frequency of *ABCG2* 421A in Chinese and Japanese (35%) compared to Caucasians (14%) may suggest that this polymorphism contributes to the elevated plasma concentrations of rosuvastatin in Asians. The 421C>A polymorphism was found to be the major genetic determinant from a number of candidate polymorphisms of the on-treatment LDL-C level and the LDL-C response to rosuvastatin in these patients as shown in Chapter **4**, but not for the hsCRP levels. It is noteworthy that the ABCG2 was newly identified as a uric acid transporter and the 421C>A polymorphism in *ABCG2* was strongly associated with increased uric acid level and risk of gout in a GWAS (Dehghan A et al., 2008). Increasing evidence suggests that elevated uric acid levels are associated with hypertension, renal disease, and increased risk of CVD and importantly may play a role in the metabolic syndrome. The oxidative changes uric acid induces in adipocytes may be one of the potential mechanisms to explain how hyperuricaemia might induce the metabolic syndrome (Feig DI et al., 2008), which may also be associated with elevated CRP levels. Therefore, the complex interaction between the *ABCG2* 421C>A polymorphism, increased systemic exposure of rosuvastatin, and elevated uric acid levels may have obscured the association of this polymorphism with hsCRP.

In prospective observational studies, baseline hsCRP levels have been consistently linked with the traditional cardiovascular risk factors and other environmental factors (Miller DT et al., 2005). The post hoc analysis of the PROVE IT-TIMI 22 trial has shown that hsCRP levels varied considerably and were strongly associated with

multiple cardiovascular risk factors including age, female gender, smoking, obesity, low HDL-C, high triglycerides etc. in patients receiving standard or intensive statin therapy (Ray KK et al., 2005). The present study also found similar associations and the proportions of the variance in hsCRP concentrations explained by these factors are similar to the other studies in populations on no treatment (Kathiresan S et al., 2006).

Adiposity, especially abdominal obesity is the strongest predictor of hsCRP concentrations across different populations as a result of up-regulation of the cytokines IL-6 and TNF-α by obesity (Kelley-Hedgepeth A et al., 2008, Timpson NJ et al., 2005). A recent study has shown that abdominal adiposity was associated with elevated hsCRP levels independent of BMI, a measure of general adiposity (Lapice E et al., 2009). Large prospective studies have shown that BMI and WC are associated with risk factors of CVD and are both predictors of CVD mortality. A meta-analysis has suggested indices of abdominal obesity were superior over BMI for detecting cardiovascular risk factors in both men and women (Lee CM et al., 2008) but individual studies have demonstrated equally strong associations of BMI and WC with CVD risk (van Dis I et al., 2009) and independent associations of BMI and WC with the risk of death (Pischon T et al., 2008). Changes in visceral adiposity with age may also partly contribute to age-related variations in plasma CRP concentrations (Cartier A et al., 2009). Females had a higher level of hsCRP than males, which may be due to the effect of sex hormones since oestrogen is known to increase CRP levels in women (Lakoski SG et al., 2005a) or different fat distributions, particularly subcutaneous adiposity between males and females (Cartier A et al., 2009). A recent study has shown the interaction of adiposity (BMI and WC) with *CRP* rs1205 genotypes to influence CRP levels where the relationship of increasing CRP with

adiposity is carried by selected genotypes in men (Eiriksdottir G et al., 2009), but this interaction was not observed in our study.

In this study, patients with FH who have high LDL-C levels due to monogenetic defects had a lower average level of CRP than non-FH, which may be largely attributable to this group having fewer other metabolic risk factors. However, further logistic regression analysis showed that FH patients tended to have a 2.4-fold increased risk of having hsCRP ≥2 mg/L after adjustment for multiple confounding factors, suggesting that FH itself may be associated with higher levels of CRP and proinflammatory cytokines compared to non-FH subjects as demonstrated in a previous small study (El Messal M et al., 2006). The lack of association between smoking status or presence of CVD with hsCRP in this study may be due to the small number of smokers and patients having had CVD events.

Various cardiovascular drugs apart from statins have been suggested to lower circulating CRP levels in some but not all studies, including fibrate, nicotinic acid, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), antidiabetic agents, anti-inflammatory and antiplatelet agents ect (Prasad K, 2006). However, a recent randomized trial of open-label insulin and placebo-controlled metformin in 500 patients with recent-onset type 2 diabetes has shown that there was no significant difference in hsCRP reduction among those allocated to insulin (-11.8%; 95% CI, -18.7 - -4.4%) or no insulin (-17.5%; 95% CI, -23.9 - -10.5%) (P=0.25), or among those allocated to metformin (-18.1%; 95% CI, -24.4 - -11.1%) or placebo (-11.2%; 95% CI, -18.1 - -3.7%) (P=0.17) (Pradhan AD et al., 2009). In this study some of these medications appeared to be associated with higher hsCRP levels (Figure **5-5**) suggesting that the hsCRP levels were determined by the

diseases and/or risk factors that these medications treated, although patients treated with antidiabetic drugs, NSAIDs and aspirin tended to have a lower risk of having an hsCRP level of ≥ 2 mg/L (Table **5-9**).

Recent Mendelian randomization studies have shown that the systemic hsCRP level is unlikely to be a causal factor for development of ischaemic vascular disease (Elliott P et al., 2009, Zacho J et al., 2008). Although the JUPITER study has shown that the maximal treatment benefits were obtained in those who achieved the lowest levels of both LDL-C and CRP with adjustment for some traditional risk factors (Ridker PM et al., 2009) these may not account entirely for features of the metabolic syndrome, visceral obesity or insulin resistance. In fact, patients with on-treatment hsCRP levels < 2 mg/L who had the greatest reduction in cardiovascular events were also those who had the lowest initial values of hsCRP in the JUPITER study (Danchin N, 2009, Ridker PM et al., 2009). In addition, an hsCRP concentration of less than 1 mg/L with an LDL-C level of at least 1.8 mmol/L did not significantly reduce the risk of the primary outcome in the JUPITER study (95% CI for the hazard ratios for incident cardiovascular events: 0.11-1.85) whereas the hazard for hsCRP levels of less than 2 mg/L with an LDL-C level of at least 1.8 mmol/L did (95% CI for the hazard ratios for incident cardiovascular events: 0.18-0.94) (Table **5-2**) and this inconsistency also raises concerns about the causal effect of CRP in CVD. The lower CRP levels in those patients in the JUPITER study may be due to the low levels of other risk factors that are associated with hsCRP e.g. adiposity, smoking, blood pressure and other lipid parameters, as observed in the present study and previous studies (Liem AH et al., 2008, Ray KK et al., 2005). Therefore patients with persisting elevation of hsCRP ≥2.0 mg/l despite achieving the LDL-C target <70 mg/dl with the rosuvastatin dosage used in the JUPITER study (or 10 mg dosage in

Chinese patients) may be candidates for interventions targeted to the other risk factors related to the metabolic syndrome rather than intensified lowering of LDL-C.

### 5.5 Study limitations

This is a post hoc analysis and there are several limitations requiring consideration. The most important is the lack of baseline hsCRP levels in this group of patients, so we are unable to assess if the genetic variants or obesity indices affected the CRP-lowering effect of rosuvastatin. The *CRP* polymorphisms and obesity, which is unlikely to change with statin therapy, are the main determinants of on-treatment hsCRP levels suggesting the baseline level of CRP driven by the *CRP* polymorphisms and obesity is the major determinant of on-treatment levels of hsCRP but not the determinant of changes in hsCRP in response to statins, although interactions between genetic factors, obesity, other metabolic risk factors and the change in hsCRP response cannot be excluded. Secondly, we only examined 2 common SNPs in *CRP*, but there are several other *CRP* SNPs which affect hsCRP levels, and may also contribute to the variance in hsCRP, although these two SNPs are highly correlated with the functional triallelic SNP rs3091244 that was the strongest variant associated with CRP levels out of 13 well-selected SNPs capturing the underlying common genetic variation in *CRP* in the Framingham Heart Study. Thirdly, although in the present study WC appeared to be a better predictor of hsCRP than BMI and percentage body fat, these obesity measures and/or other obesity indexes like measures of visceral fat may have additional ability to predict hsCRP levels. Fourthly, our sample consisted of a small number of smokers, and thus, we cannot assess the contribution of this cardiovascular risk factor to the variance of hsCRP levels. Further studies with a large sample size are required to address the effects of genetic and environmental factors on the hsCRP response to statins.

## 5.6 Conclusions

In summary, this study demonstrated close relationships between genetic variants and cardiovascular risk factors and levels of hsCRP in Chinese patients receiving treatment with rosuvastatin, which are similar to the relationships reported in patients not on statin treatment. These findings support the concept that hsCRP is a useful biomarker for CHD risk, partly through its association with traditional CHD risk factors, but it may not be a true risk factor for CHD.

# Chapter 6 Genetic determinants of some cardiovascular risk factors in Chinese patients

## 6.1 Introduction

There have been rapid developments in the genomics of complex traits and recent genome-wide association studies (GWASs) have localized common DNA sequence variants that contribute to many human phenotypes, including those influencing plasma lipids, uric acid and bilirubin levels (Chasman DI et al., 2009, Dehghan A et al., 2008, Johnson AD et al., 2009, Kolz M et al., 2009, Wallace C et al., 2008) and many of these associations have also been reported in candidate gene studies (Saito A et al., 2009, Yamada Y et al., 2007). Furthermore, multiple novel loci have been identified to be related to these phenotypes in the GWASs which may provide a better understanding of the genetic basis of dyslipidaemia, hyperuricaemia and hyperbilirubinaemia and the role of different molecular players in the pathogenesis of these disorders.

It has been well recognized that serum lipids are important determinants of cardiovascular disease (CVD) and are related to morbidity. Blood concentrations of lipoproteins and lipids are highly heritable and in some studies the heritabilities of these plasma lipid concentrations are as high as 50 - 70% (Lusis AJ and Pajukanta P, 2008). Different types of studies have clearly shown that elevated LDL-C causes human atherothrombosis, and that individuals with lifelong exposure to high plasma LDL-C levels due to the genetic disorder familial hypercholesterolaemia (FH) develop early atherothrombotic CVD (Garg A and Simha V, 2007). In addition, epidemiological studies have shown that low levels of HDL-C and high triglyceride concentrations, common features of type 2 diabetes mellitus and the metabolic

syndrome, are associated with increased risk of coronary artery disease (CAD) (Gordon DJ et al., 1989, Nordestgaard BG et al., 2007, Patel JV et al., 2009) although the causality has not been established. The GWAS reported by Willer et al investigated whether the lipid-associated variants were associated with CAD in the Wellcome Trust Case Control Consortium (WTCCC) sample of ~2000 CAD and ~13000 British individuals, which showed that nearly all alleles that were associated with increased LDL-C concentrations were also associated with increased risk of CAD but there was no similar pattern of association for alleles associated with HDL-C and triglyceride levels (Willer CJ et al., 2008b).

Uric acid is the final catabolic, heterocyclic purine derivative resulting from the oxidation of purines in humans. Elevated serum uric acid concentrations may be related to high dietary intake of purine-rich foods, excess alcohol consumption, impaired renal function with decreased uric acid clearance, and impaired renal uric acid excretion in various conditions e.g. obesity, insulin resistance, hypertension, low-sodium diet, diuretic therapy (Feig DI et al., 2008, Strazzullo P and Puig JG, 2007). Elevated serum uric acid concentration causes gout and is related to a wide variety of cardiovascular conditions, e.g. hypertension, metabolic syndrome, renal disease, CVD and stroke etc. (Feig DI et al., 2008, Strazzullo P and Puig JG, 2007). Epidemiological evidence suggests that serum uric acid is an independent predictor of CVD in subjects with hypertension and established vascular disease but not in healthy subjects suggesting that the influence of uric acid on CVD is explained by the secondary association of uric acid with other established risk factors (Strazzullo P and Puig JG, 2007, Wannamethee SG, 2005). However, a large prospective cohort study in over 90,000 Chinese subjects aged >35 years conducted in Taiwan has shown that hyperuricaemia is an independent risk factor for all-cause and CVD

mortality not only in all subjects representing the general public, but also in patients with hypertension and diabetes and potentially low-risk subgroups (Chen JH et al., 2009). It has been reported that uric acid also has a positive role as an antioxidant substance and is associated with longevity in mammals (Cutler RG, 1984), which may provide a possible explanation for the previously reported controversial association between uric acid and CVD (Bo S et al., 2008, Strazzullo P and Puig JG, 2007). Serum uric acid concentration is an inherited trait with heritability estimates of up to about 70% (Whitfield JB and Martin NG, 1983, Yang Q et al., 2005), suggesting genetic variation may determine uric acid level through regulation of uric acid metabolic pathways. More recently, several new loci were identified to be associated with serum uric acid levels and gout in GWASs, including the solute carrier family 2, member 9 gene (*SLC2A9*) and ATP-binding cassette, subfamily G, member 2 gene (*ABCG2*) etc (Kolz M et al., 2009), which expands our understanding of physiological mechanisms of the disease. The strongest association was detected for *SLC2A9* variants with uric acid levels with a gender-specific effect observed in various populations (Dehghan A et al., 2008, Doring A et al., 2008, Vitart V et al., 2008). However, the genetic determinants of uric acid levels in Chinese patients with normal uric acid levels have not been reported.

Bilirubin is excreted as a major component of bile as the breakdown product of haeme catabolism, which has been shown to be inversely related to CVD in both retrospective and prospective studies and supported by meta-analysis suggesting that it could be a protective factor (Novotny L and Vitek L, 2003, Schwertner HA and Vitek L, 2008). It has also been reported that low serum bilirubin concentrations were closely associated with multiple risk factors of CVD in Hong Kong Chinese subjects (Ko GT et al., 1996). Serum bilirubin levels are also heritable and recent

GWASs have identified genetic polymorphisms that contribute to variability in serum bilirubin levels, in particular variants in *UGT1A1* and *SLCO1B1* (Johnson AD et al., 2009). UGT1A1 controls bilirubin conjugation and clearance, and a TA dinucleotide insertion in the TATA box of the *UGT1A1* promoter, *28 ($TA_{6>7}$), resulting in 70% reduction of UGT1A1 gene transcription is strongly associated with hyperbilirubinaemia (Schwertner HA and Vitek L, 2008). The SLCO1B1 transporter is also a logical candidate for effects on bilirubin considering it has transport affinity for bilirubin into hepatocytes (Johnson AD et al., 2009).

Given the growing importance of these risk factors of CVD, we further analyzed whether polymorphisms in candidate genes examined in the study were associated with lipids, uric acid and bilirubin levels in Chinese patients.

## 6.2 Methods

### 6.2.1 Subjects and data collection

The baseline lipid profiles and bilirubin levels were collected in all patients involved in this pharmacogenetic study including those with poor adherence to therapy, whereas, the uric acid level for individuals was retrieved retrospectively with a representative value selected since this test was not routinely examined during the study. For patients who had been taking urate-lowering drugs, the baseline uric acid level prior to the drug treatment was used.

### 6.2.2 Single nucleotide polymorphism selection

#### 6.2.2.1 Polymorphisms in genes related to lipids

A total of 94 polymorphisms in 46 candidate genes potentially related to cholesterol

synthesis and lipoprotein metabolism including some drug transporters were analyzed for association with baseline lipid traits, including LDL-C, HDL-C and triglycerides.

#### 6.2.2.2 Polymorphisms in genes related to uric acid

Eight single nucleotide polymorphisms (SNPs) in 5 candidate genes potentially related to uric acid transport (Kolz M et al., 2009) were selected for analysis of association with uric acid concentrations (Table **6-1**).

**Table 6-1. SNPs in genes potentially related to uric acid transport**

| Genes | rs number | Polymorphism |
|---|---|---|
| *ABCG2* | rs2231142 | 421C>A |
| *ABCG2* | rs2231137 | 34G>A |
| *CCL2* | rs1860188 | -3813C>T |
| *GCKR* | rs1260326 | C>T |
| *SLC2A9* | rs1014290 | T>C |
| *SLC2A9* | rs12510549 | T>C |
| *SLC22A12* | rs893006 | G>T |
| *SLC22A12* | rs11231825 | 426C>T |

ABCG2 = ATP-binding cassette, subfamily G, member 2; CCL2 = Chemokine (C-C motif) ligand 2; GCKR = glucokinase regulatory protein; SLC2A9 = Solute carrier family 2, member 9; SLC22A12 = Solute carrier family 22, member 12.

#### 6.2.2.3 Polymorphisms in genes potentially related to bilirubin

Fifteen polymorphisms in *UGTs* and *SLCO1B1* were selected for analysis of association with bilirubin levels (Table **6-2**).

**Table 6-2. SNPs in genes potentially related to bilirubin disposition**

| Genes | rs number | Polymorphism |
|---|---|---|
| *SLCO1B1* | rs2291073 | T>G |
| | rs4149036 | C>A |
| | rs4149080 | G>C |
| | rs2306283 | 388A>G |
| | rs4149056 | 521T>C |
| | rs4149057 | 571T>C |
| | rs2291075 | 597 C>T |
| | rs4149015 | -11187 G>A |
| *UGT1A1* | rs4124874 | *60, -3279T>G |
| | rs4148323 | *6, 211G>A |
| | | (TA)6>7, *28 |
| *UGT1A6* | rs2070959 | 541A>G |
| | rs1105879 | 552A>C |
| *UGT2B7* | rs7662029 | -327A>G |
| | rs12233719 | 211G>T |
| | rs7668258 | -161T>C |

SLCO1B1 = Solute carrier organic anion transporter family, member 1B1; UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1; UGT1A6 = UDP glucuronosyltransferase 1 family, polypeptide A6; UGT2B7 = UDP glucuronosyltransferase 2 family, polypeptide B7.

### 6.2.3 Statistical analysis

Baseline levels of LDL-C, HDL-C, triglyceride, bilirubin and uric acid concentrations were logarithmically transformed due to non-normal distributions. Univariate analysis was performed to identify the genetic determinants of these traits and only those genetic variants with $P<0.05$ were reported. Multivariate linear regression analysis was used to determine contributions of the environmental factors

and genetic factors to the variance of these selected traits. Data were analyzed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

## 6.3 Results

### 6.3.1 Genetic determinants of lipids

Baseline lipid levels were obtained from 410 subjects including 171 patients with FH. The association between genetic polymorphisms and lipid traits were analyzed in FH and non-FH patients separately due to their different lipid profiles.

#### 6.3.1.1 Low-density lipoprotein cholesterol

In patients without FH, 3 SNPs were found to be related to LDL-C levels including two tightly linked SNP in *ABCA1* (ATP-binding cassette, subfamily A, member 1) and rs266729 in *ADIPOQ* (adiponectin) (Table **6-3**), but the *ADIPOQ* SNP did not show a gene-dose effect with heterozygous subjects having the lowest value of baseline level of LDL-C suggesting lack of true effect of this polymorphism.

In 171 patients with FH, 9 polymorphisms appeared to be related to baseline LDL-C concentrations but none of them was in *ABCA1* or *ADIPOQ* and some SNPs did not show a gene-dose effect (Table **6-3**).

**Table 6-3. Associations between genetic polymorphism and baseline LDL-C levels in patients with and without FH**

| Subjects | Polymorphisms | Genotypes | N | Mean LDL-C levels (mmol/L) | P |
|---|---|---|---|---|---|
| **non-FH** | | | | | |
| | *ABCA1* | TT | 86 | 3.90 ± 0.95 | |
| | rs2472384 T>C | TC | 109 | 4.13 ± 0.96 | 0.045* |
| | | CC | 43 | 4.39 ± 0.99 | |
| | *ABCA1* | GG | 88 | 3.92 ± 0.96 | |
| | R219K | GA | 105 | 4.09 ± 0.95 | 0.018* |
| | | AA | 43 | 4.45 ± 0.96 | |
| | *ADIPOQ* | CC | 129 | 4.18 ± 1.01 | |
| | rs266729 | CG | 88 | 3.92 ± 0.85 | 0.045* |
| | | GG | 22 | 4.29 ± 1.19 | |
| **FH** | | | | | |
| | *ABCB1* | CC | 28 | 7.14 ± 1.86 | |
| | 1236C>T | CT | 71 | 6.41 ± 1.51 | 0.019 |
| | | TT | 71 | 6.18 ± 1.37 | |
| | *ACSM2B* | CC | 104 | 6.63 ± 1.55 | 0.028† |
| | rs1133607 C>T | CT | 64 | 6.13 ± 1.50 | |
| | *APOB* | CC | 135 | 6.30 ± 1.54 | 0.016† |
| | rs693 | CT | 33 | 6.99 ± 1.50 | |
| | *APOB* | II | 87 | 6.23 ± 1.52 | |
| | ID | ID | 72 | 6.59 ± 1.52 | 0.032* |
| | | DD | 8 | 7.54 ± 1.75 | |
| | *APOE* | e2 carrier | 19 | 6.79 ± 1.81 | |
| | e2e3e4 | e3e3 | 112 | 6.56 ± 1.54 | 0.005 |
| | | e4 carrier | 38 | 5.75 ± 1.23 | |
| | *APOE-APOC* cluster | AA | 131 | 6.61 ± 1.59 | 0.004† |
| | rs4420638 | AG/GG | 36 | 5.82 ± 1.28 | |

| | | | | |
|---|---|---|---|---|
| *LDLR* | TT | 84 | 6.81 ± 1.55 | |
| rs1529729 T>C | TC | 70 | 6.08 ± 1.36 | 0.01 |
| | CC | 17 | 6.14 ± 1.92 | |
| *NCAN/CILP2/PBX4* | GG | 135 | 6.32 ± 1.54 | 0.042† |
| rs16996148 G>T | GT | 35 | 6.89 ± 1.50 | |
| *NPC1L1* | AA | 52 | 6.71 ± 1.59 | |
| rs2301935 A>C | AC | 84 | 6.00 ± 1.38 | 0.0007 |
| | CC | 33 | 7.09 ± 1.62 | |
| *PPARG* | CC | 158 | 6.49 ± 1.56 | 0.046† |
| 167C>G | CG | 11 | 5.55 ± 0.96 | |

Data are given as mean ± SD and values of LDL-C levels among genotype groups were compared by ANOVA unless otherwise indicated.

* Data were compared by Kruskal-Wallis test.

† Data were log transformed for comparison.

Abbreviations: ABC = ATP-binding cassette; ACSM2B = Acyl-CoA synthetase medium-chain family member 2B; ADIPOQ = adiponectin; APO = apolipoprotein ; LDLR = LDL receptor; NCAN/CILP2/PBX4 = Neurocan/cartilage intermediate layer protein 2 / Pre-B-cell leukemia homeobox 4; NPC1L1 = NPC1 (Niemann-Pick disease, type C1, gene)-like 1; PPARG = Peroxisome proliferator-activated receptor gamma.

#### 6.3.1.2 High-density lipoprotein cholesterol

In patients without FH, 2 SNPs in *ABCA1* and 2 SNPs in *LIPC* (hepatic lipase) were found to be related to HDL-C levels, but the SNPs in *ABCA1* did not present a gene-dose effect, whereas 8 polymorphisms in genes other than *ABCA1* and *LIPC* were shown to be associated with baseline HDL-C concentrations in FH patients (Table **6-4**).

**Table 6-4. Associations between genetic polymorphism and baseline HDL-C levels in patients with and without FH**

| Subjects | Polymorphisms | Genotypes | N | Mean HDL-C levels (mmol/L) | P |
|---|---|---|---|---|---|
| **Non-FH** | | | | | |
| | *ABCA1* | TT | 86 | 1.44 ± 0.45 | |
| | rs2472384 T>C | TC | 109 | 1.40 ± 0.34 | 0.031† |
| | | CC | 44 | 1.59 ± 0.45 | |
| | *ABCA1* | GG | 88 | 1.47 ± 0.47 | |
| | R219K | GA | 105 | 1.39 ± 0.33 | 0.026† |
| | | AA | 44 | 1.59 ± 0.45 | |
| | *LIPC* | CC | 67 | 1.32 ± 0.35 | |
| | rs1532085 | CT | 124 | 1.51 ± 0.45 | 0.002* |
| | | TT | 46 | 1.53 ± 0.38 | |
| | *LIPC* | CC | 93 | 1.36 ± 0.39 | |
| | -514C>T | CT | 102 | 1.46 ± 0.37 | 0.002* |
| | | TT | 44 | 1.64 ± 0.51 | |
| **FH** | | | | | |
| | *ABCB1* | GG | 38 | 1.70 ± 0.37 | |
| | 2677G>T/A | GT/GA | 94 | 1.55 ± 0.36 | 0.007† |
| | | TT/AA/AT | 31 | 1.43 ± 0.36 | |
| | *ABCB1* | CC | 59 | 1.62 ± 0.35 | |
| | 3435C>T | CT | 85 | 1.61 ± 0.38 | 0.005† |
| | | TT | 27 | 1.37 ± 0.31 | |
| | *APOA2* | TT | 146 | 1.54 ± 0.37 | 0.011* |
| | -265T>C | TC/CC | 26 | 1.72 ± 0.34 | |
| | *APOA5* | TT | 77 | 1.63 ± 0.39 | |
| | -1131T>C | TC | 66 | 1.55 ± 0.35 | 0.024* |
| | | CC | 22 | 1.39 ± 0.34 | |

| | | | | |
|---|---|---|---|---|
| *LDLR* | TT | 109 | 1.52 ± 0.36 | |
| 2052T>C | TC | 56 | 1.66 ± 0.37 | 0.046† |
| | CC | 7 | 1.69 ± 0.28 | |
| *LDLR* | CC | 115 | 1.52 ± 0.37 | |
| 1866C>T | CT | 47 | 1.69 ± 0.38 | 0.019† |
| | TT | 7 | 1.69 ± 0.28 | |
| *NPC1L1* | AA | 52 | 1.69 ± 0.39 | |
| rs2301935 A>C | AC | 84 | 1.54 ± 0.36 | 0.025* |
| | CC | 33 | 1.48 ± 0.33 | |
| *SLCO1B1* | TT | 133 | 1.55 ± 0.38 | |
| 521T>C | TC | 35 | 1.61 ± 0.29 | 0.026* |
| | CC | 3 | 2.21 ± 0.45 | |

Data are given as mean ± SD and values of HDL-C levels among genotype groups were compared by ANOVA unless otherwise indicated.

* Data were compared by Kruskal-Wallis test.

† Data were log transformed for comparison.

Abbreviations: ABC = ATP-binding cassette; APO = apolipoprotein; LDLR = LDL receptor; LIPC = lipase, hepatic; NPC1L1 = NPC1 (Niemann-Pick disease, type C1, gene)-like 1; SLCO1B1 = Solute carrier organic anion transporter family, member 1B1.

#### 6.3.1.3 Triglycerides

Five SNPs were found to be related to baseline triglyceride levels in patients without FH as shown in Table **6-5** including the *APOA5* (apolipoprotein A 5) -1131T>C and the *LDLR* (LDL receptor) 44964A>G. These two polymorphisms along with other 3 SNPs were related to triglyceride levels in patients with FH but interestingly, the *LDLR* 44964A>G polymorphism presented an opposite effect on baseline level of triglyceride in patients with and without FH separately.

**Table 6-5. Associations between genetic polymorphism and baseline triglyceride levels in patients with and without FH**

| Subjects | Polymorphisms | Genotypes | N | Mean triglyceride levels (mmol/L) | P |
|---|---|---|---|---|---|
| **non-FH** | | | | | |
| | *ACSM3* | GG | 52 | 2.39 ± 1.23 | |
| | rs886433G>T | GT | 111 | 1.93 ± 1.31 | 0.03† |
| | | TT | 77 | 2.12 ± 1.93 | |
| | *ACSM3* | II | 53 | 2.38 ± 1.23 | |
| | 962ID | ID | 109 | 1.94 ± 1.32 | 0.009† |
| | | DD | 79 | 2.10 ± 1.91 | |
| | *APOA5* | TT | 114 | 1.91 ± 1.36 | |
| | -1131T>C | TC | 83 | 1.91 ± 0.89 | 0.001* |
| | | CC | 20 | 3.43 ± 3.22 | |
| | *HNF1A* | AA | 87 | 1.98 ± 1.32 | |
| | I27L | AC | 117 | 2.01 ± 1.71 | 0.011† |
| | | CC | 37 | 2.56 ± 1.30 | |
| | *LDLR* | AA | 101 | 2.34 ± 1.45 | |
| | 44964A>G | AG | 101 | 1.99 ± 1.74 | 0.007† |
| | | GG | 36 | 1.72 ± 0.91 | |
| **FH** | | | | | |
| | *ABCB1* | GG | 38 | 1.47 ± 0.83 | |
| | 2677G>T/A | GT/GA | 94 | 1.83 ± 1.19 | 0.026* |
| | | TT/AA/AT | 31 | 2.39 ± 1.85 | |
| | *ABCB1* | CC | 59 | 1.48 ± 0.74 | |
| | 3435 C>T | CT | 85 | 1.75 ± 1.13 | 0.004* |
| | | TT | 27 | 2.75 ± 2.06 | |
| | *APOA5* | TT | 77 | 1.49 ± 0.93 | |
| | -1131T>C | TC | 66 | 1.95 ± 1.18 | 0.005* |
| | | CC | 22 | 2.53 ± 2.12 | |

| | | | | |
|---|---|---|---|---|
| *LDLR* | AA | 60 | 1.70 ± 1.20 | |
| 44964A>G | AG | 88 | 1.75 ± 1.29 | 0.03* |
| | GG | 23 | 2.37 ± 1.39 | |
| *LEPR* | GG | 149 | 1.88 ± 1.29 | 0.015* |
| rs1137101 | GA/AA | 21 | 1.40 ± 1.16 | |

Data are given as mean ± SD and values of triglyceride levels among genotype groups were compared by ANOVA unless otherwise indicated.

* Data were compared by Kruskal-Wallis test.

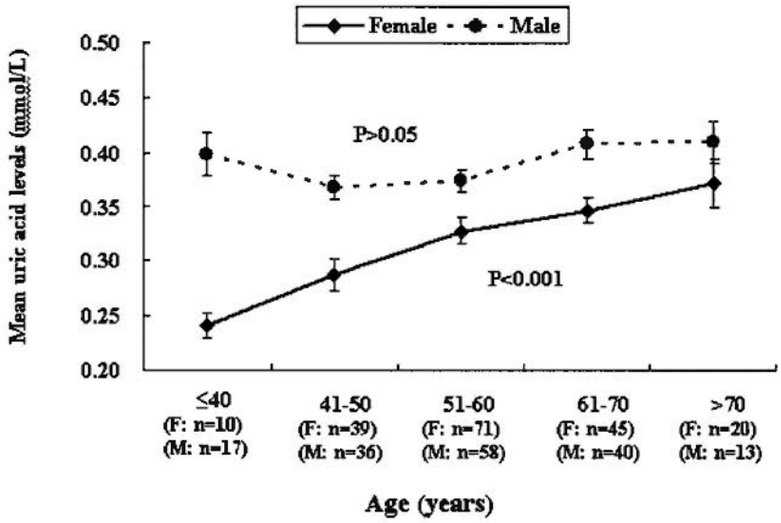
† Data were log transformed for comparison.

Abbreviations: ABC = ATP-binding cassette; ACSM3 = Acyl-CoA synthetase medium-chain family member 3; APO = apolipoprotein; HNF1A = hepatic nuclear factor 1-alpha; LDLR = LDL receptor; LEPR = leptin receptor;

### 6.3.2 Determinants of uric acid

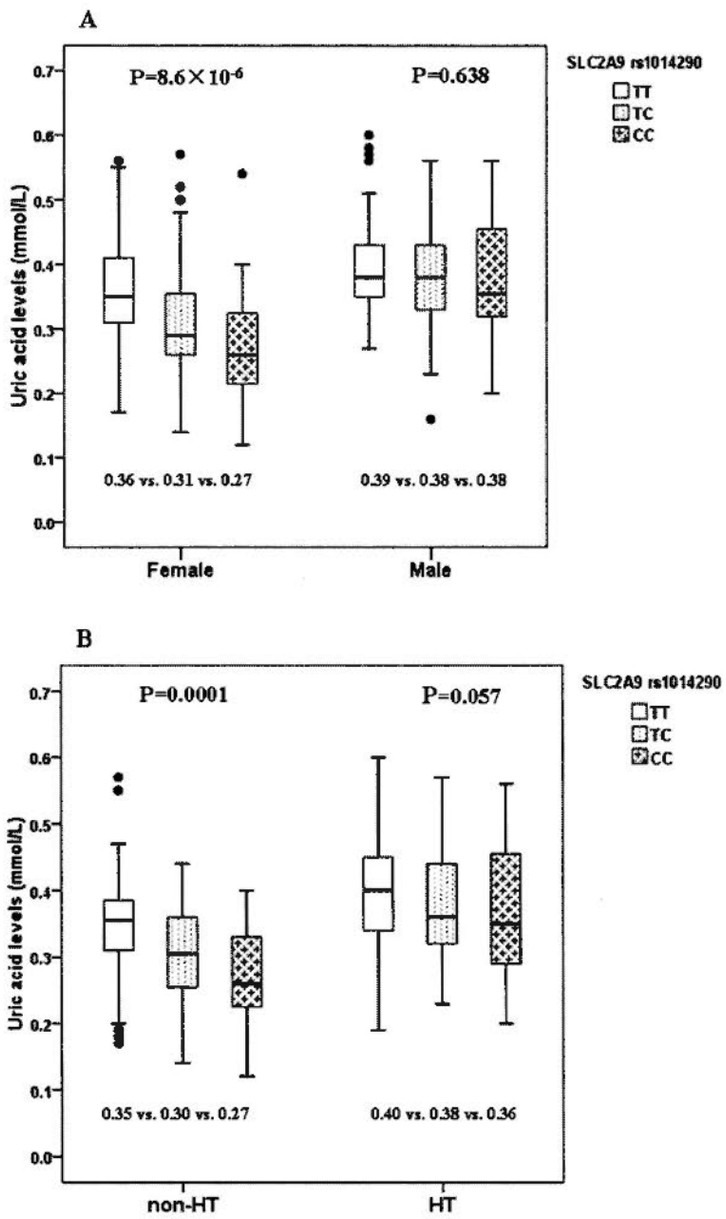
Plasma uric acid levels were collected from 349 patients with a mean (± SD) of 0.35 ± 0.09 mmol/L. The mean age of study participants was 56.1 ± 10.8 years and about 47% of them (n=164) were male. Among these subjects, 137 had FH, 105 had diabetes, and 186 had hypertension. The uric acid levels were strongly related to ages in female subjects whereas there was no difference in uric acid levels among age groups in males although male subjects with age above 60 years tended to have increased uric acid levels (Figure 6-1).

**Figure 6-1. Effect of age on uric acid levels in study participants**




Among the 8 SNPs examined in this analysis, the *SLC2A9* rs1014290 T>C was the only polymorphism that was significantly associated with plasma uric acid levels in a gene-dose dependent manner (TT vs. TC vs. CC = 0.38 ± 0.08 mmol/L: 0.34 ± 0.09 mmol/L: 0.31 ± 0.10 mmol/L, $P=1.0\times10^{-5}$). The association between the *SLC2A9* rs1014290 T>C and uric acid levels was more pronounced in women or in patients without hypertension than in men or patients with hypertension (Figure **6-2**). Male subjects and patients with hypertension had higher uric acid levels than females and patients without hypertension respectively across all genotype groups (Figure **6-2**). The other SNP in *SLC2A9* or SNPs in other candidate genes was not significantly associated with uric acid levels in all subjects combined (Figure **6-3**) or in any subgroup of patients.

**Figure 6-2. Effect of *SLC2A9* rs1014290 polymorphism on uric acid levels**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

* Uric acid levels among genotype groups were compared by Kruskal-Wallis test.

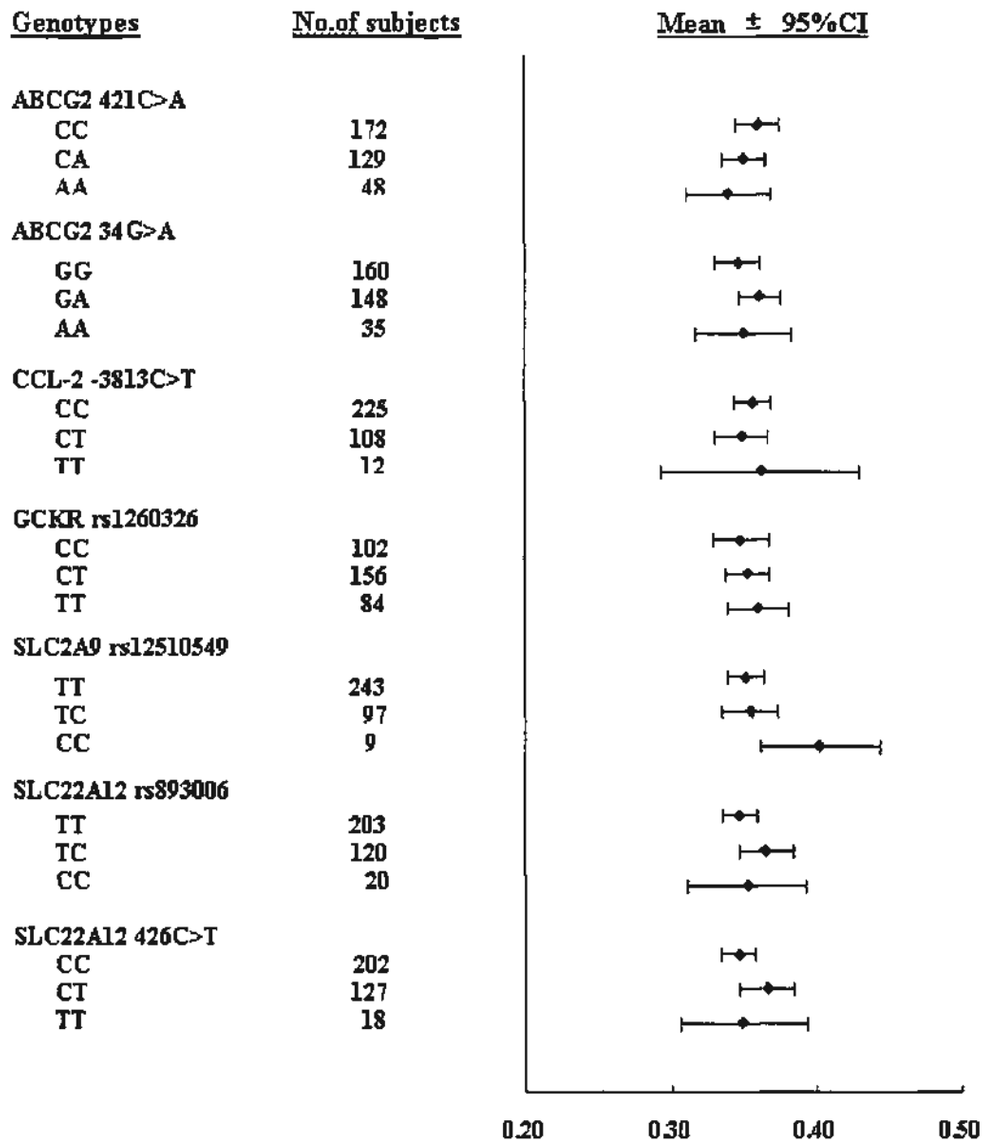
In females: TT, n=70; TC, n=83; CC, n=31.

In males: TT, n=74; TC, n=68; CC, n=20.

In patients without hypertension: TT, n=64; TC, n=72; CC, n=27.

In patients with hypertension: TT, n=80; TC, n=79; CC, n=24.

**Figure 6-3. Non-significant associations (P>0.05) between other SNPs examined and uric acid levels**




Abbreviations: ABCG2 = ATP-binding cassette, subfamily G, member 2; CCL2 = Chemokine (C-C motif) ligand 2; GCKR = glucokinase regulatory protein; SLC2A9 = Solute carrier family 2, member 9; SLC22A12 = Solute carrier family 22, member 12.

In univariate analysis, male gender, advanced age, presence of hypertension and diabetes, absence of FH, high body mass index (BMI), high waist circumference (WC), high creatinine level, low HDL-C, levels and high triglycerides were associated with increased uric acid levels ($P<0.05$). However, the associations between uric acid with age, presence of FH, BMI, and HDL-C levels were no longer significant after adjustment for other confounding factors (Table **6-6**). Multivariate regression analysis revealed that WC, creatinine levels, triglyceride levels, presence of hypertension and the *SLC2A9* rs1014290 polymorphism were predictors of plasma uric acid concentrations in all subjects combined or subgroup of female subjects, which totally explained 34.4% and 44.7% of the variance in uric acid levels, respectively with serum creatinine level and *SLC2A9* rs1014290 polymorphism being strongest predictors (Table **6-6**). In contrast, having hypertension, but not creatinine level was the strongest predictor of uric acid levels in male subjects. Having diabetes and SLC2A9 rs1014290 polymorphism tended to have some ability to predict uric acid levels in males.

**Table 6-6. Multivariate analysis of predictors of serum uric acid concentrations**

| | All | | Male | | Female | |
|---|---|---|---|---|---|---|
| **Variables** | **B** | **P** | **B** | **P** | **B** | **P** |
| Age (year) | 0.043 | 0.443 | 0.047 | 0.636 | 0.069 | 0.321 |
| Gender* | 0.096 | 0.115 | -- | -- | -- | -- |
| BMI ($kg/m^2$) | 0.003 | 0.977 | 0.086 | 0.346 | 0.175 | 0.165 |
| WC (cm) | 0.197 | 0.0007 | 0.138 | 0.153 | 0.204 | 0.004 |
| Creatinine (μmol/L) | 0.273 | $6.0\times10^{-7}$ | 0.004 | 0.965 | 0.39 | $3.8\times10^{-8}$ |
| LDL-C (mmol/L) | -0.032 | 0.536 | -0.041 | 0.658 | -0.071 | 0.263 |
| HDL-C (mmol/L) | -0.056 | 0.318 | -0.138 | 0.121 | -0.021 | 0.76 |
| TG (mmol/L) | 0.112 | 0.037 | 0.096 | 0.276 | 0.142 | 0.036 |
| FH† | -0.062 | 0.243 | -0.077 | 0.424 | -0.102 | 0.113 |
| Hypertension† | 0.208 | 0.0002 | 0.422 | $1.9\times10^{-5}$ | 0.159 | 0.024 |
| Diabetes† | -0.056 | 0.308 | -0.19 | 0.046 | 0.06 | 0.37 |
| SLC2A9, rs1014290‡ | -0.226 | $1.3\times10^{-5}$ | -0.193 | 0.028 | -0.235 | 0.0003 |

*0 = Female; 1 = male. †0 = absence of the disease; 1= presence of the disease; ‡ 1 = homozygous wild-type allele; 2 = heterozygous variant allele; 3= homozygous variant allele. Abbreviations: BMI = body mass index; FH = familial hypercholesterolaemia; TG = triglycerides; SLC2A9 = Solute carrier family 2, member 9; WC = waist circumference.

### 6.3.3 Genetic determinants of bilirubin

Baseline bilirubin levels were obtained from 403 subjects with a median (interquartile range [IQR]) value of 10 (8, 13) μmol/L for women and 12 (9, 15) μmol/L for men. Male subjects had higher mean (±SD) baseline bilirubin levels than that of females (12.6 ± 4.5 μmol/L vs. 10.5 ± 3.8 μmol/L, $P = 6.6\times10^{-7}$). There was no difference in bilirubin levels in patients with hypertension, diabetes, or FH comparing to those without these conditions. Triglyceride level was found to be inversely associated with bilirubin levels ($r = -0.155$, $P = 0.002$). Other phenotypic factors including age, general or central obesity etc were not associated with bilirubin levels.

Among 17 polymorphisms in 4 candidate genes, we found variants in the *UGT1A1* (*6, *28 and *60) and *1A6* (541A>G and 552A>C) examined in this study were significantly associated with bilirubin levels (Table **6-7**), whereas none of the SNPs in *SLCO1B1* and *UGT2B7* was related to bilirubin concentrations. The *UGT1A1* *28 appeared to have a stronger effect on bilirubin level than other genetic variants (Table **6-7**).

**Table 6-7. Associations between polymorphisms in *UGT1A1* and *UGT1A6* and bilirubin levels**

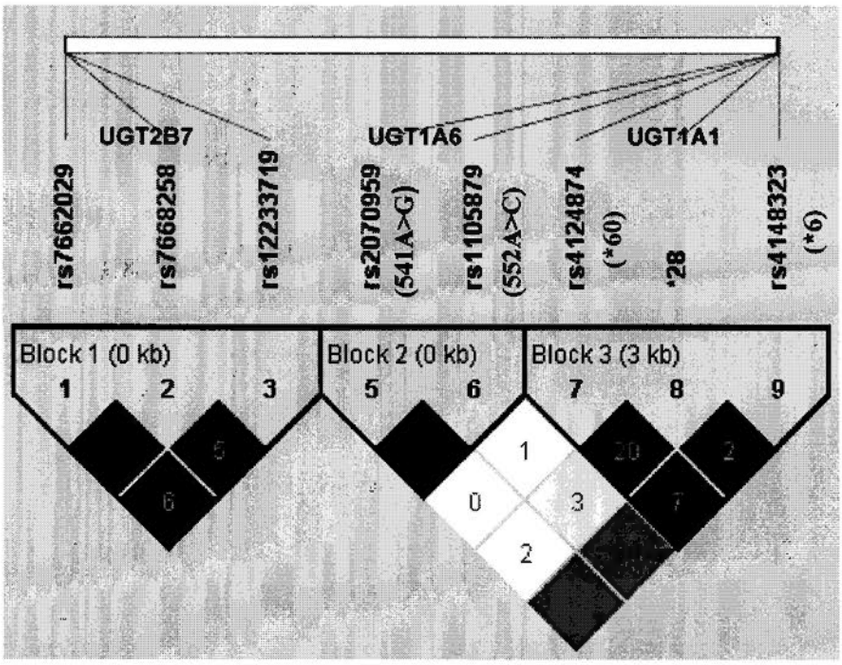
| Polymorphisms | Genotype | N | Baseline bilirubin levels (μmol/L) | P |
|---|---|---|---|---|
| *UGT1A1* | | | | |
| -3279T>G (*60) | TT | 159 | 10 (8, 13) | |
| | TG | 191 | 11 (8, 14) | 0.001 |
| | GG | 50 | 13 (9.8, 16) | |
| 211G>A | GG | 293 | 10 (8, 13) | 0.00007 |
| | GA/AA | 102/5 | 13 (9, 15) | |
| $(TA)_{6>7}$ (*28) | $(TA)_{6/6}$ | 308 | 10 (8, 13) | |
| | $(TA)_{6/7}$ | 83 | 12 (10, 15) | $7.8\times10^{-8}$ |
| | $(TA)_{7/7}$ | 7 | 21 (19, 28) | |
| *UGT1A6* | | | | |
| 541A>G | AA | 275 | 10 (8, 13) | |
| | AG | 116 | 13 (10, 15) | 0.00002 |
| | GG | 9 | 13 (9.5, 19) | |
| 552 A>C | AA | 248 | 10 (8, 13) | |
| | AC | 135 | 12 (9, 15) | $6.8\times10^{-6}$ |
| | CC | 16 | 13.5 (11.2, 21) | |

Data are given as median (IQR) and were compared by Mann-Whitney U or Kruskal-Wallis test.

Abbreviations: UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1; UGT1A6 = UDP glucuronosyltransferase 1 family, polypeptide A6.

To determine the extent of linkage disequilibrium (LD) of the polymorphisms in the UGTs cluster in this group of subjects, standardised LD coefficients were calculated (Figure **6-4**). The 2 SNPs in *UGT1A6* were highly linked ($r^2 = 0.75$) and were also in LD with *UGT1A1* *6 ($r^2 = 0.36$ and 0.48, respectively). The *UGT1A1* $(TA)_{6>7}$ (*28) polymorphism in the promoter region of UGT1A1 was moderately linked to the other SNP in the same region, the -3279T>G (*60).

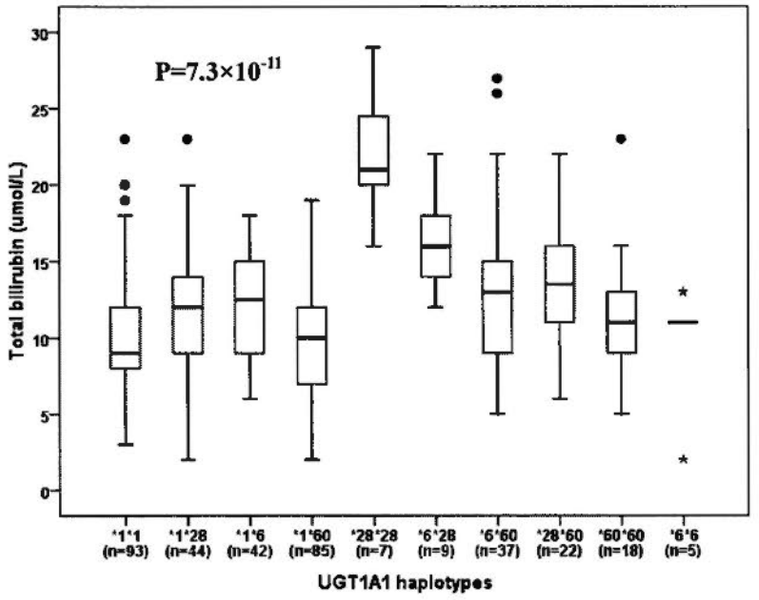
**Figure 6-4. Degree of linkage disequilibrium present within UGTs cluster**




Values in each diamond represent the pairwise linkage disequilibrium coefficients ($r^2$) calculated using Haploview 4.0.

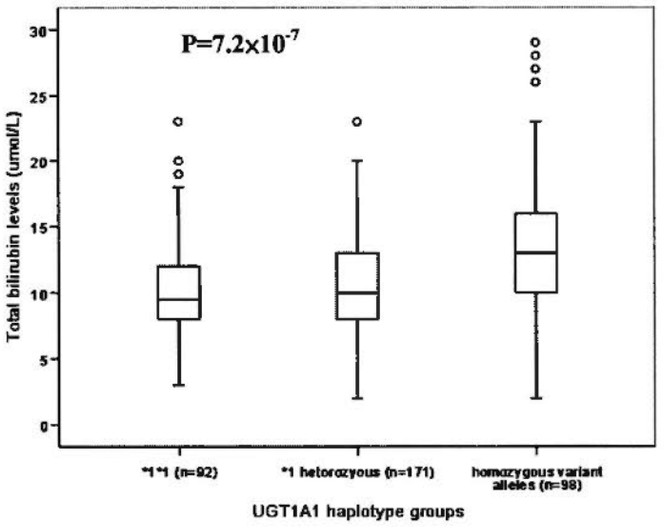
Further analysis showed that the *UGT1A1* *28 and *6 polymorphisms had a greater effect on bilirubin levels than *60 (Figure **6-5**). Subjects with two variant alleles had higher values of bilirubin than those with homozygous wild-type alleles with heterozygous having intermediate values (Figure **6-6**).

**Figure 6-5. Association of the*UGT1A1* haplotypes and bilirubin levels**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

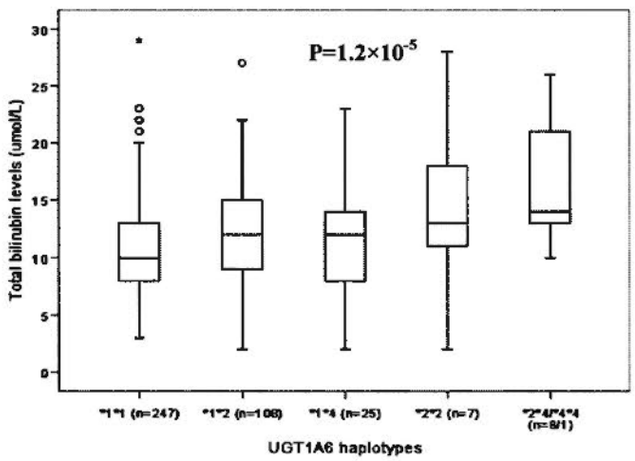
**Figure 6-6. Effect of *UGT1A1* haplotypes on bilirubin levels**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

The haplotype analysis of the closely linked SNPs 541A>G and 552A>C in *UGT1A6* showed that subjects with *2 (G-C) and *4 (A-C) had higher bilirubin levels than those with wild type alleles (A-A) in a gene-dose dependent manner (Figure **6-7**) and the effect of *UGT1A6* haplotypes on bilirubin levels was mainly determined by the 552A>C SNP as the 541G variant in *2 had no additional effect compared to *4.

**Figure 6-7. Effect of *UGT1A6* haplotypes on bilirubin levels**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

Multivariate regression analysis showed that triglyceride level ($P=2.6\times10^{-15}$), gender ($P=2.8\times10^{-8}$), *UGT1A1* *28 ($P=1.5\times10^{-9}$) and *6 ($P=2.2\times10^{-7}$) were independently associated with log-bilirubin levels which total explained 29.2% of variance of log-bilirubin levels (Table **6-8**). The 2 SNPs in *UGT1A6* were not associated with bilirubin level after adjustment for variants in *UGT1A1* suggesting their effects on bilirubin level may be due to the LD with *UGT1A1* *6 (Figure **6-4**).

**Table 6-8. Multivariate model of predictors of bilirubin levels**

| Variables | Standardized Coefficients | P | Adjusted $R^2$ |
|---|---|---|---|
| Triglyceride levels | -0.367 | $2.6\times10^{-15}$ | 0.122 |
| Gender * | 0.252 | $2.8\times10^{-8}$ | 0.065 |
| *UGT1A1* *28 † | 0.280 | $1.5\times10^{-9}$ | 0.053 |
| *UGT1A1* *6 † | 0.238 | $2.2\times10^{-7}$ | 0.052 |

*0 = Female; 1 = male. † 1 = Homozygous wild-type allele; 2 = heterozygous variant allele; 3 = homozygous variant allele.

Abbreviations: UGT1A1 = UDP glucuronosyltransferase 1 family, polypeptide A1.

## 6.4 Discussion

### 6.4.1 Associations between genetic polymorphisms and baseline levels of lipids

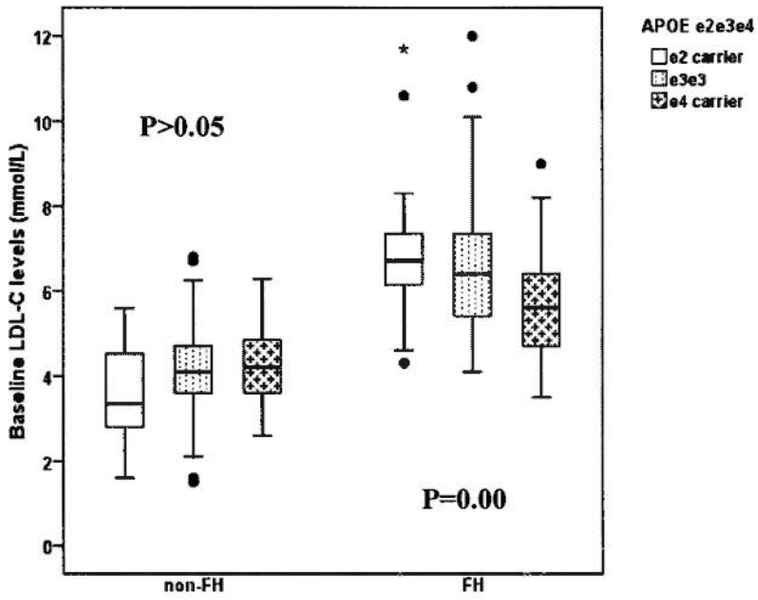
The analysis of associations between lipid concentration traits and polymorphisms in genes related to cholesterol synthesis and lipoprotein metabolism has identified several different genetic predictors of LDL-C or HDL-C levels in patients with and without FH. Furthermore, more genetic factors appear to affect the baseline lipid levels in patients with FH compared to non-FH patients. These findings suggest the complex interactions between genetic and environmental factors and plasma cholesterol levels in patients with and without FH.

In contrast to the high LDL-C in FH resulting from a monogenic defect in one of several genes that affect receptor-mediated uptake of LDL, including *LDLR*, *APOB*, and proprotein convertase subtilisin-like kexin type 9 (*PCSK9*), the pathogenesis of common dyslipidaemia is more complicated and largely unclear. It has been known that dyslipidaemia is more often secondary to other causes than a primary genetic defect. Even in patients with known genetic disorders, secondary factors may affect

lipid levels considerably (Pimstone SN et al., 1998). These include obesity; lifestyle influences such as diet, exercise, smoking, and alcohol use; endocrine disorders such as diabetes mellitus and hypothyroidism; liver and renal diseases; and the use of pharmacological agents (Garg A and Simha V, 2007, Watanabe H et al., 2003), so it is not surprising that the variants identified in those large GWASs explained only a small fraction (~5%) of interindividual variability in lipoprotein levels (Lusis AJ and Pajukanta P, 2008).

APOE is a multifunctional protein that plays a key role in the metabolism of cholesterol and triglycerides and 2 common polymorphisms in this gene lead to 3 *APOE* isoforms (e2, e3, and e4) with different functions in lipid metabolism (Chapter **1**, Table **1-11**). Multiple epidemiologic studies have shown that individuals carrying the e4 allele have higher and those carrying the e2 allele have lower LDL-C levels than people with the commonest e3/e3 genotype. In the present study, the APOE polymorphism also tended to present a similar association with LDL-C levels in patients without FH but this was not significant (e2 carriers vs. e3e3 vs. e4 carriers = 3.57 ± 1.20 mmol/L: 4.11 ± 0.95 mmol/L : 4.25 ± 0.91 mmol/L, P>0.05). However, a reverse association between *APOE* polymorphisms and LDL-C levels was observed in patients with FH (Figure **6-8**). It has been suggested that the effect of *APOE* polymorphisms on LDL-C levels is based on the differences in the LDLR affinity and the different regulatory effects on LDLR among *APOE* variants (Kolovou G et al., 2009, Mahley RW et al., 2009), therefore, the impaired function of LDLR in patients FH may alter the association between LDL-C and *APOE* polymorphism which requires further evaluations.

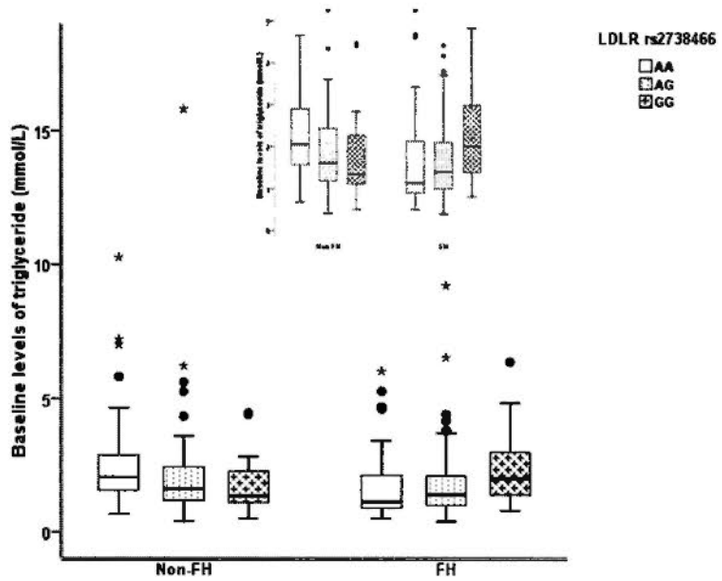
**Figure 6-8. Different associations between *APOE* polymorphisms and baseline levels of LDL-C in patients with and without FH**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.
In non-FH: e2 carriers, n= 14; e3e3, n= 167; e4 carriers, n=51.
In FH: e2 carriers, n= 19; e3e3, n= 112; e4 carriers, n=38.

It is also worthy to note that the *LDLR* 44964A>G polymorphism showed an opposite relationship with baseline triglyceride levels in FH and non-FH patients separately (Figure **6-9**). This SNP has been found to be associated with increased baseline triglyceride levels but a decreased LDL-C level and a greater incidence of CHD or CVD in PROSPER (Prospective Study of Pravastatin in the Elderly at Risk) participants (Polisecki E et al., 2008). Again, the reduced function of LDLR in patients with FH may play a role in the reverse relationship between the *LDLR* 44964A>G polymorphism and baseline triglyceride levels in patients with and without FH.

**Figure 6-9. Different associations between the *LDLR* rs2738466 polymorphism and baseline levels of triglyceride in patients with and without FH**




Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, circles indicating outliers and stars indicating values of greater than 3×IQR.

Previous studies have consistently demonstrated that APOA5 is a key determinant of plasma triglyceride concentrations and polymorphisms in this gene have been shown to be related to familial combined hyperlipidaemia in Chinese (Liu ZK et al., 2010). In the present study, the *APOA5* -1131T>C polymorphism was strongly associated with baseline triglyceride levels in both FH and non-FH patients suggesting the significant role of this polymorphism on plasma triglyceride levels (Table **6-3**).

Most of these associations did not reach a significant level after correction for multiple testing, which may be due to the small sample size in the subgroups of FH and non-FH. In addition, we may not have adequate power to detect some potential

associations with the obtained sample size, but the results from this analysis can be used as a basis for further evaluation with expanded number of subjects.

### 6.4.2 Determinants of uric acid

This analysis shows that the *SLC2A9* rs1014290 polymorphism was significantly associated with reduced uric acid levels in Chinese patients particularly in female subjects, which is consistent with the findings from GWASs in Caucasian and Black subjects (Dehghan A et al., 2008, Doring A et al., 2008, Vitart V et al., 2008). In previous genome-wide studies, the strongest effect on serum uric acid concentrations was detected for the *SLC2A9* with several linked noncoding genetic variants of SLC2A9 including rs1014290 in intron 3 and rs12510549 in intergenic region examined in the study strongly associated with decreased serum uric acid concentrations and increased fractional excretion of uric acid suggesting it as a key player in the renal excretion of uric acid (Dehghan A et al., 2008, Doring A et al., 2008, Vitart V et al., 2008) (Kolz M et al., 2009). Nonsynonymous SNPs in SLC2A9 showed less significant associations with serum uric acid levels than intronic SNPs (Vitart V et al., 2008), although the missense SNP rs16890979 in *SLC2A9* leading to a valine to isoleucine aminoacid substitution (V253I) showed strong associations with uric acid level and gout in the Framingham cohort and the Atherosclerosis Risk in Communities (ARIC) participants (Dehghan A et al., 2008, Doring A et al., 2008, Vitart V et al., 2008). This nonsynonymous SNP rs16890979 is in high LD ($r^2$=0.88) with an intronic SNP rs7345553, the SNP showing the strongest association ($P=5.2\times10^{-201}$) at the *SLC2A9* locus with uric acid levels in meta-analysis of the GWA data from 28,141 individuals of European ancestry (Kolz M et al., 2009). It has been proposed that SLC2A9 may play an important role in urate reabsorption and polymorphisms in the gene associated with reduced activity of the transporter impair

the reabsorption of urate leading to reduced levels of serum uric acid (Le MT et al., 2008).

Before being identified as a modulator of uric acid levels in recent GWASs, SLC2A9 coding for glucose transporter 9 (GLUT9), was newly identified as a member of the SLC2A gene family of hexose facilitative transporters functioning in the transport of both glucose and fructose (Manolescu AR et al., 2007). Although the exact mechanism of *SLC2A9* affecting urate excretion is unclear, functional assay has suggested that SLC2A9 has a direct role in urate transport (Matsuo H et al., 2008, Vitart V et al., 2008) and this function has been shown to be facilitated by glucose and fructose in one study (Caulfield MJ et al., 2008). It is worthy noting that fructose itself promotes uric acid formation (Hallfrisch J, 1990), so it would be of interest to examine the interactions between fructose intake, *SLC2A9* polymorphisms and uric acid levels. A very recent study in 1,822 Croatians has suggested that there is a certain extent of interaction between *SLC2A9* and dietary patterns in serum uric acid determination and the metabolic effect of soft drinks seems to be determined by the underlying genotype of *SLC2A9* rs1014290 (Jeroncic I et al., 2010).

An elevated uric acid level has been consistently associated with an increased relative risk for development of hypertension (Feig DI et al., 2008). In this study, further analysis in subgroups of patients with and without hypertension revealed that the *SLC2A9* rs1014290 polymorphism had a more predominant effect on uric acid levels in patients without hypertension than those with hypertension, which may be due to the influence of disease itself or diuretic use in patients with hypertension altering this association. The *SLC2A9* polymorphism was not associated with hypertension in this study and previous GWASs suggesting uric acid is not a direct

causal factor for hypertension (Vitart V et al., 2008).

The ABCG2 is highly expressed in the placenta, kidney, liver and small intestine, and has also been newly identified to be a urate transporter. The functional SNP 421C>A polymorphism was shown to be significantly associated with increased uric acid levels and risk of gout in different populations (Dehghan A et al., 2008, Wang B et al., 2010). However, the present study only replicated the association between the *SLC2A9* rs1014290 polymorphism and uric acid levels, the strongest relationship identified in previous GWASs, but not *ABCG2* 421C>A or other SNPs in candidate genes, which may be largely due to the influence of other confounding factors such as concomitant medications, lifestyles e.g. dietary intake and physical activities or lack of power to detect the variance in serum uric acid levels related to the genotypes investigated in this study. It is worth noting that patients in this study were treated with rosuvastatin, which is the substrate of ABCG2 and the variant allele of 421C>A polymorphism was associated with increased systemic exposure to rosuvastatin (Keskitalo JE et al., 2009c) and the lipid response as described in Chapter **4** (Tomlinson B et al., 2010). Statins have been shown to reduce uric acid levels in patients with CHD in the GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study (Athyros VG et al., 2004), therefore, lack of association between *ABCG2* 421C>A and uric acid level in the present study may be due to the complicated interaction between genetic effects and drug effects on uric acid levels.

In the present study, male subjects had higher uric acid levels than female subjects as shown in many previous studies, but this gender difference in uric acid levels is unlikely to be due to the different effects of the *SLC2A9* rs1014290 polymorphism on uric acid concentrations in males and females as male patients had higher uric acid

levels than females within each *SLC2A9* rs1014290 genotype group. In this study, we found a gender-specific association between age and uric acid level. Age did not seem to affect uric acid levels in males but in females the uric acid level increased with increasing age. It has been reported that postmenopausal women had higher uric acid levels than in premenopausal women (Hak AE and Choi HK, 2008), and therefore, it is possible that menopause status or oestrogen hormone affects the observed association between age and uric acid in women.

It has been well known that multiple phenotypic factors are associated with uric acid levels, including age, gender, obesity, blood pressure and other metabolic disorders. In the present study, serum creatinine level, waist circumference, triglyceride levels and having hypertension were independent phenotypic predictors of uric acid level in female subjects, whereas serum creatinine level was not related to uric acid levels in male subjects after adjustment for other phenotypic factors. Similarly, having hypertension is the strongest predictor of uric acid levels in males but was only moderately associated with uric acid level in females. Patients with hypertension had higher creatinine levels than those non-hypertensive patients in females (76.2 ± 19.1 μmol/L vs. 64.3 ± 19.1 μmol/L, $P<0.001$), but there was no significant difference in creatinine levels in male subjects with and without hypertension (93.3 ± 24.2 μmol/L vs. 88.0 ± 12.4 μmol/L, $P>0.05$). In multivariate analysis, waist circumference was associated with uric acid levels in females but not in males, which was similar to the finding from a previous study in Korean subjects (Jang WC et al., 2008). The underlying mechanism of gender-specific relationships between hypertension, creatinine level, waist circumference and uric acid level observed in the study is unclear and may be related to gender-related differences in uric acid metabolism and other clinical features.

This analysis has several limitations which require consideration. Patients in the present study had increased risk of CVD and were treated with multiple pharmacotherapy for these risk factors, which may influence uric acid level and/or association between genetic polymorphism and uric acid level, therefore, the results of this study should be interpreted with caution. In addition, lifestyle factors, in particular diet, also play an important role in uric acid concentrations and these were not evaluated in this study.

### 6.4.3 Determinants of bilirubin

SLCO1B1 and UGT1A1 appear to play an important role in the disposition of bilirubin. It has been shown that homozygous or compound heterozygous mutations of the *UGT1A1* gene can result in inheritable unconjugated hyperbilirubinaemia (Kadakol A et al., 2000). The *UGT1A1* *28 and *6 variants are known to reduce enzymatic activity of *UGT1A1* and the *28 polymorphism in the *UGT1A1* has been proven to be associated with hyperbilirubinaemia (Schwertner HA and Vitek L, 2008). Several other studies have also shown that the 211G>A SNP (*6) in *UGT1A1* is associated with increased bilirubin concentrations in different populations (Lin R et al., 2009, Saito A et al., 2009). The *28 allele is more common in White and Black subjects (0.25-0.56) than in Asians (~0.1) (Hall D et al., 1999), whereas the *6 variant is prevalent in Asian populations with a frequency of 0.13-0.25 but almost absent in other populations (Kaniwa N et al., 2005).

In the present study, we have confirmed the well-known associations between *28 and *6 polymorphisms and increased bilirubin level in Chinese patients with increased risk of CVD. Polymorphisms in *SLCO1B1* did not appear to affect

bilirubin levels in this study nor in previous studies in Chinese and Japanese subjects (Lin R et al., 2009, Saito A et al., 2009). We also examined whether common SNPs in other UGT genes e.g. *UGT1A6* and *UGT2B7* influence bilirubin levels although UGT1A1 has been considered to be the only enzyme that contributes substantially to bilirubin glucuronidation, but none of the others showed a significant effect after adjustment for *UGT1A1* polymorphisms.

Several prospective studies have shown that high normal and moderately elevated serum bilirubin levels protect against CVD, which suggests that bilirubin or some other factors associated with bilirubin prevents future CVD (Schwertner HA and Vitek L, 2008). The protective effect of bilirubin against CVD is probably related to its antioxidant and anti-inflammatory properties (Nakagami T et al., 1993, Stocker R and Keaney JF, Jr., 2004). In 1,780 individuals participating in the Framingham Offspring Study who had been followed up for 24 years, homozygote *UGT1A1* *28 allele carriers with higher serum bilirubin concentrations had approximately one third the risk of CVD and CHD of those with wild and heterozygous genotypes (Lin JP et al., 2006). Future prospective studies are needed to determine if elevated serum bilirubin levels due to these genetic factors are associated with decreased CVD mortality.

Serum bilirubin has been shown to be independently related to many of the existing risk factors for CVD (Schwertner HA and Vitek L, 2008). A previous retrospective study on the association of cardiovascular risk factors and bilirubin levels in Hong Kong Chinese (n=1,508) showed an inverse relationship between serum bilirubin levels and triglycerides, insulin, glycated hemoglobin and VLDL (very-low-density lipoprotein) (Ko GT et al., 1996). A strong inverse correlation of plasma bilirubin

concentrations with triglyceride levels ($P<5\times10^{-15}$) was also observed in this study. It is still unclear whether bilirubin is responsible for the protection of CVD or some other factors associated with bilirubin prevents future CVD.

*In vitro* and animals studies have suggested that statins upregulate the antioxidant defense protein haeme oxygenase-1 (HO-1), the enzyme that plays an important role in haeme metabolism and production of biliverdin, bilirubin and carbon monoxide (Grosser N et al., 2004, Muchova L et al., 2007). In this study, rosuvastatin appeared to increase the bilirubin levels by 5.9% from baseline levels in male subjects, but had no effect in female subjects (Chapter **4**, Table **4-9**). The gender-dependent effect of statins on bilirubin level or HO-1 induction has not been reported, however, the association between bilirubin levels and cardiovascular event has been shown to be gender-dependent in Framingham offspring study (Djousse L et al., 2001) and some other large prospective studies (Kimm H et al., 2009). In 78,724 Korean subjects (41,054 men, aged 30-89 years) with stroke cases of 1137 in men and 827 in women, participants with a higher level of bilirubin (>15.3 μmol/L) showed lower hazard ratios in men with ischaemic stroke after adjustment for multiple confounding factors compared to the lowest level of bilirubin (≤10.2 μmol/L), but these associations were not found in haemorrhagic stroke or in studies of women (Kimm H et al., 2009). The mechanism of gender difference in bilirubin response to rosvuastatin in the present study is unclear, but this may be related to the gender-specific associations between bilirubin levels and CVD risk, which needs to be further investigated. The polymorphisms in *UGT1A1* or other candidate genes did not affect bilirubin response to rosuvastatin.

## 6.5 Conclusion

In summary, our findings from the large-scale SNP database of drug-related and lipid-related genes confirms some previous associations between genetic polymorphisms and lipid parameters, uric acid and bilirubin levels, the commonly measured quantitative traits associated with risk of CVD. This analysis also provides some novel findings regarding different genetic determinants of lipoprotein levels in patients with and without FH, which warrant further evaluations.

# Chapter 7 Summary of the study

## 7.1 Summary of the study

This study has shown that there is a wide variation in LDL-C response to rosuvastatin (Figure **4-3**) in Chinese patients with increased risk of cardiovascular disease (CVD). The *ABCG2* 421C>A polymorphism was strongly associated with an increased LDL-C response to rosuvastatin, which explained about 5% of the variance in LDL-C response to rosuvastatin. Several other polymorphisms, in particular *FMO3* V257M, *LPL* 1421C>G and *APOE/C1/C4/C2* rs4420638, also tended to influence the LDL-C response to rosuvatatin. In this study, patients with familial hypercholesterolaemia (FH) had a 2.6% smaller percentage reduction in LDL-C than patients without and age and gender also tended to affect LDL-C response but these effects were much smaller than the impact of the *ABCG2* 421C>A polymorphism or other genetic factors on the LDL-C response.

The HDL-C response to treatment with rosuvastatin 10 mg daily in study participants was minimal and was strongly related to baseline HDL-C levels. The *SLCO1B1* 521T>C polymorphism may also contribute to the difference in baseline HDL-C and HDL-C response to rosuvastatin in patients with FH. The triglyceride response to rosuvastatin was highly variable and was strongly related to baseline levels. A common SNP in *DGAT2* (rs10899113) appeared to have some impact on the triglyceride response to rosuvstatin.

This study also demonstrated close relationships between genetic variants and

cardiovascular risk factors (e.g. obesity, low HDL-C, and high triglycerides) and levels of hsCRP in Chinese patients receiving treatment with rosuvastatin, which are similar to the relationships reported in patients not on statin treatment.

Analysis of associations between genetic factors and baseline lipid levels in FH and non-FH patients showed that multiple polymorphisms in candidate genes/loci tended to be associated with baseline lipoprotein levels with different genetic polymorphisms contributing to the variance in lipid levels in subgroup of FH and non-FH.

The present study also confirmed prior reported associations between *UGT1A1* polymorphisms and bilirubin levels, and gender-specific effects of SLC2A9 polymorphisms on uric acid levels.

### 7.2 Clinical implications

It is well recognized that lowering elevated levels of LDL-C reduces cardiovascular risk. Statins are indicated for the prevention of cardiovascular events in a very large number of the population in most developed countries throughout the world. However, there is a considerable variation between individuals in the response to these drugs in terms of the main primary lipid parameter, which is the reduction in plasma LDL-C concentration and this is thought to be the major determinant of the cardiovascular outcome. Pharmacogenetic studies have shown that genetic variation may determine statin response and side effects.

This study was performed to examine whether genetic variants predicted lipid

responses to rosuvastatin in Hong Kong Chinese patients, which might help to optimize statin therapy by identifying individuals with increased risk or benefit. One of the major novel findings was that a genetic polymorphism in *ABCG2*, a protein in the cell membrane that helps to pump drugs out of liver cells into the bile, was associated with a greater reduction in plasma LDL-C with rosuvastatin. The greater reduction in LDL-C in patients with the AA genotype compared to those with the CC genotype was equivalent to at least doubling the dose of rosuvastatin. This corresponds with previously published pharmacokinetic data indicating that the *ABCG2* 421C>A polymorphism plays an important role in the LDL-C response to rosuvastatin through a pharmacokinetic effect. This genetic variant or polymorphism is relatively common in Chinese people and about 50% of the population carries at least one copy of the variant allele and they may respond to smaller doses of rosuvastatin. The finding of this study helped to optimize the lipid response in the cohort of patients studied by choosing the most appropriate statin at the most appropriate dose and this is likely to have greater benefits in reducing cardiovascular events in the long term.

Today, there are about 10% of labels for FDA-approved drugs which contain pharmacogeneomic information (Frueh FW et al., 2008). There are several examples where pharmacogenetic tools already impact therapeutics with the most promising examples in the field of oncology. For example, testing for HER2/neu and epidermal growth factor receptor expression is required before starting therapy with specific oncology agents (Frueh FW et al., 2008). Pharmacogenetics has also influenced the clinical practise in Hong Kong, for example, testing of *HLA-B**1502 allele should be performed in patients prior to starting carbamazepine treatment as recommended by the local regulatory authority due to the strong association between certain serious

skin reactions to carbamazepine and *HLA-B**1502 in Asian ancestry (Ferrell PB, Jr. and McLeod HL, 2008). The application of pharmacogenetics to cardiovascular disease is less clear at present. The best example of the clinical implications of utilizing pharmacogenetic-based theray is the impact of the vitamin K epoxide reductase complex, subunit 1 (*VKORC1*) and *CYP2C9* variants on warfarin response which totally explain about 50% of the variability in response to warfarin (Manolopoulos VG et al., 2010). The present study identified certain genetic polymorphisms that determine the lipid response to rosuvastatin in Chinese patients which provides important pharmacogenetic information on rosuvastatin and a better understatanding of the mechamisms of action of the drug. With the rapid development of technology and more clinical outcome data available, the pharmacogenetic information on statins could be used as a basis for reaching a therapeutic decision in the future.

The importance of C-reactive protein (CRP) as a biomarker of inflammation and an indicator of risk for cardiovascular events has received considerable attention in recent years. Achieving lower levels of CRP during statin treatment is associated with improved cardiovascular outcome but baseline plasma CRP levels vary considerably among individuals and are driven by multiple genetic factors and cardiovascular correlates. Whether CRP represents a risk marker or risk factor is an area of hot debate. Based on results from the JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluation Rosuvastatin) trial, FDA has now approved rosuvastatin for reducing the risk of stroke, myocardial infarction, and revascularization procedures in individuals who have normal LDL-C levels and no clinically evident coronary heart disease but who do have an increased risk based on age ($\geq$ 50 years in men; $\geq$ 60 years in women), elevated hsCRP, and the presence of

at least one additional CVD risk factor, which include high blood pressure, low HDL-C, smoking, or a family history of premature coronary heart disease (FDA, 2010). In JUPITER, age was the only cardiovascular risk factor, in addition to elevated CRP, in 25% of patients, while nearly 50% of patients had at least one other risk factor. In a post hoc subgroup analysis, the benefit of treatment was observed only in those who met the age and elevated-CRP criteria with at least one additional risk factor. Among patients who strictly qualified for treatment based on age and CRP only, the reduction in the primary end point was not statistically significant. The levels of CRP during statin therapy have been shown to be still related to traditional cardiovascular risk factors, but we believe the present study is the first to examine the effects of traditional risk factors together with relevant genotypes on CRP during statin treatment and the results of the study provide additional insights into the interpretation of CRP levels.

### 7.3 Potential for further development of the research

This study has identified genetic predictors of LDL-C response to rosuvastatin, the most recently introduced statin in Hong Kong public hospitals. It would be interesting to see if these genetic polymorphisms also influence the lipid response to other commonly used statins, e.g. simvastatin. Furthermore, it will be useful to measure the concentrations in the plasma samples obtained and perform the population pharmacokinetic analysis to examine the impact of the ABCG2 421C>A polymorphism and other variants in drug metabolizing enzymes and drug transporters on the pharmacokinetics of rosuvastatin and to link this to the pharmacodynamic result described in this thesis. It is also worth examining whether certain ABCG2 inducers/inhibitors or other environmental and physiological factors

could alter expression of the transporter and thereby the pharmacokinetics and pharmacodynamics of rosuvastatin.

In addition, the exact mechanism of effect of *FMO3* V257M, the other common genetic polymorphism associated with the LDL-C response to rosuvatatin with an effect size similar to the *ABCG2* 421C>A polymorphism in the study is still unclear, and needs to be further evaluated and replicated in an independent cohort.

Changes in LDL-C may be predictive of clinical outcomes, but it would also be important to examine the impact of polymorphisms identified in this study on cardiovascular events. Future analyses of clinical outcome studies are warranted to identify whether genetic variations influencing the lipid response to statins can be translated to differences in the actual clinical outcome response to this class of medications.

In this study, patients with FH had a smaller LDL-C response to rosuvastatin than non-FH patients suggesting impaired LDLR function in FH may influence the statin response. However, the diagnosis of FH was made on the basis of phenotypic criteria which may be not accurate. Further genetic tests could be performed to verify the diagnosis and most importantly to examine whether different genetic causes of FH influence the lipid response to statins in Chinese patients with FH.

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## Original Publications arising from the thesis

# Pharmacogenetics of HMG-CoA Reductase Inhibitors: Optimizing the Prevention of Coronary Heart Disease

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**Abstract:** The statins are the most important group of drugs for lipid-lowering therapy in the prevention of coronary heart disease. Greater reductions in LDL-cholesterol appear to be associated with greater benefits but the clinical efficacy and safety of statin treatment varies considerably from person to person because of a combination of phenotypic and genotypic factors. Pharmacogenetic studies have investigated the relationship between common genetic variants and the lipid responses to statin therapy and adverse events, and some candidate genes related to the pharmacodynamics and pharmacokinetics of different statins have been identified. Some of these genetic variants show a different frequency in different ethnic groups. This field of pharmacogenetic research is receiving considerable attention and many new findings have been reported recently. Pharmacogenetic and pharmacogenomic studies of statin therapy are likely to provide a better understanding of the effects of these drugs and to help with prediction of the most appropriate drug and dosage for each individual and whether the addition or substitution of other lipid modifying drugs may be necessary to achieve the most safe and effective prevention of coronary heart disease.

**Key Words:** Coronary heart disease, ethnic differences, HMG-CoA reductase inhibitors, LDL-cholesterol, pharmacogenetics.

## INTRODUCTION

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins comprise the most important group of drugs for lipid-lowering therapy in the prevention of coronary heart disease (CHD). Statins achieve their pharmacological effects by competitive inhibition of the rate-limiting step in hepatic cholesterol synthesis and this in turn results in up-regulation of the low-density lipoprotein (LDL) receptor expression, and finally leading to lowering the plasma LDL-cholesterol concentration. Greater reductions in LDL-cholesterol appear to be associated with greater benefits [Baigent, C. *et al.* 2005], but the clinical efficacy and safety of statin treatment varies considerably from person to person because of a combination of phenotypic and genotypic factors [Kajinami, K. *et al.* 2004e; Schmitz, G. *et al.* 2007].

Pharmacogenetic studies have investigated the relationship between common genetic variants and the lipid responses to statin therapy or adverse events, which could help to optimize statin therapy by identifying individuals with increased risk or benefit. Some candidate genes related to the pharmacodynamics and pharmacokinetics of different statins have been identified. Although statins are generally well tolerated, there are some adverse effects which occur in a few percentages of patients, such as gastrointestinal disturbances or muscle discomfort, but these are relatively mild and often transient in nature [Tomlinson, B. *et al.* 2001]. Potentially more serious adverse effects, which are usually dose-dependent, include elevations in hepatic transaminase levels and myopathy and these occur in about 0.2% and ≤ 0.03% respectively of patients on standard doses of most statins [Brewer, H.B., Jr. 2003]. The genetic basis of statin-related muscle disorders is largely unknown, but some studies have identified genetic variants that are associated with statin-induced myopathy [Link, E. *et al.* 2008]. A number of these genetic variants show a different frequency in different ethnic groups, which may be responsible for the interethnic variations in pharmacokinetics or pharmacodynamics of some statins. This review summarizes the main findings and some of the more recent publications in this field, aiming to highlight the contributions of some common polymorphisms in candidate genes to the variation in response to statin therapy or the potential toxic effects of statins.

## PHARMACOKINETICS OF STATINS

The chemical structures of the statins are shown in Fig. (**1**). They all share the same active pharmacophore which resembles HMG-CoA, the substrate of their target enzyme HMG-CoA reductase. The differences in the other aspects of their chemical structure determine their affinity for binding to the enzyme active site, their lipophilicity and ability to enter hepatocytes. These parameters and other physicochemical and pharmacokinetic properties are summarized in Table **1**. Lovastatin and simvastatin are administered in the lactone forms which are more lipophilic and can pass through cell membranes more easily. They are better absorbed from the gastrointestinal tract. All the other statins are administered as the active acid form. Lovastatin and simvastatin are rapidly hydrolyzed in the body by esterases and paraoxonases. The active hydroxy acids of the statins can be converted to lactones (Fig. (**2**)) by a Co-enzyme A (CoASH)-

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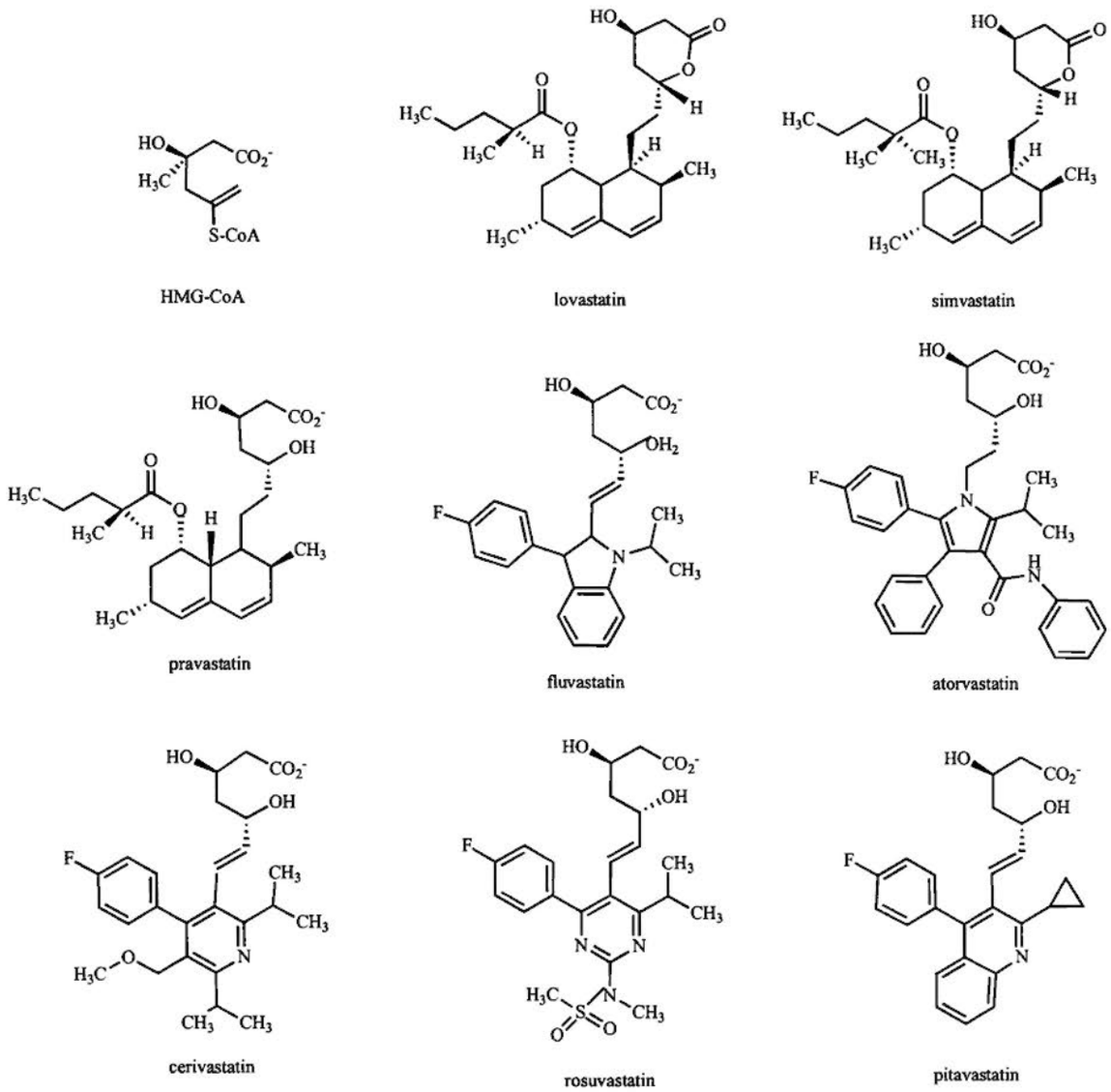


**Fig. (1).** Chemical structures of the statins and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA).

dependent pathway [Prueksaritanont, T. *et al.* 2001] and by a pathway involving uridine diphosphate glucuronosyltransferases (UGTs), mainly UGT1A1 and UGT1A3, forming the acyl glucuronide conjugates, which undergo spontaneous cyclization to the lactone [Prueksaritanont, T. *et al.* 2002b]. Inhibition of the UGT-mediated pathway appears to be one of the mechanisms for the interaction of most statins with gemfibrozil [Prueksaritanont, T. *et al.* 2002b].

The more lipophilic statins are subject to considerable metabolism by cytochrome P-450 (CYP) enzymes, which act predominantly on the statin lactones producing various active and inactive oxidative metabolites within both the enterocytes and hepatocytes. The main CYP enzymes involved are CYP3A4, CYP3A5, CYP2C9, CYP2C19 and CYP2D6. Substrates for CYP3A enzymes are commonly also substrates for the efflux drug transporter p-glycoprotein, also know as multidrug resistance protein (MDR1, *ABCB1*). This is true for some of the statins, and other efflux transporters located in the enterocytes and at the canalicular membrane of the hepatocytes may also be involved. The list includes multidrug resistance-associated protein 2 (MRP2, *ABCC2*), breast cancer resistance protein (BCRP, *ABCG2*) and the bile salt exporting pump (BSEP, *ABCB11*), which are also involved in excretion of statins and their metabolites into the gut lumen or biliary system (Fig. (3)).

Uptake of the more hydrophilic statin acids or lactones into hepatocytes is highly dependent on various drug influx transporters including the solute carrier transporter (SLC) proteins, such as organic anion transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*). All the statins appear to be sub-

**Table 1. Pharmacokinetic Properties of Statins**

| | Lovastatin | Simvastatin | Pravastatin | Fluvastatin | Atorvastatin | Cerivastatin | Rosuvastatin | Pitavastatin |
|---|---|---|---|---|---|---|---|---|
| $IC_{50}$ (nM) | N/A | 11.2 | 44.1 | 27.6 | 8.2 | 10.0 | 5.4 | NA |
| Lipophilicity | 3.91* | 4.4* | -0.75 – -1.00 | 1.0 – 1.25 | 1.0 – 1.25 | 1.50 – 1.75 | -0.25 – - 0.5 | 1.5* |
| Absorption (%) | 30 | 60-85 | 35 | 98 | 30 | >98 | 50 | 80 |
| Bioavailability (%) | 5 | <5 | 18 | 24-30 | 12 | 60 | 20 | 60-80 |
| Hepatic extraction (%) | ≥70 | ≥80 | 45 | ≥70 | 70 | 50-60 | 63 | NA |
| Renal excretion (%) | 10 | 13 | 20 | 6 | <5 | 30 | 10 | NA |
| Protein binding (%) | >98 | >95 | 50 | >98 | >98 | >99 | 90 | 96 |
| Half-life (h) | 2-5 | 2-5 | 1-3 | 1-3 | 7-20 | 1-3 | 20 | 10-13 |
| Metabolism | +++ | +++ | + | +++ | +++ | +++ | + | ++ |
| Active metabolites | 3 | 3 | 2 | No | 2 | 2 | Minor | Minor |
| CYP enzyme metabolism | 3A4/5, 2C8 | 3A4/5, 2C8 | 3A4 | 2C9 | 3A4, 2C8 | 2C8, 3A4 | 2C9, 2C19 | 2C9 |
| UGTs | 1A1, 1A3, 2B7 | 1A1, 1A3, 2B7 | | | 1A1, 1A3, 2B7 | 1A1, 1A3 | | 1A3, 2B7 |
| Uptake transporters | SLCO1B1, MCT4 | SLCO1B1 | SLCO1B1/2B1, OAT3, MCT1 | SLCO1B1 | SLCO1B1 | SLCO1B1 | SLCO1B1/1B3/ 2B1/1A2, SLC10A1 | SLCO1B1/1B3 |
| Efflux transporters | ABCB1 | ABCB1 | ABCB1/B11/C2/G2 | ABCG2 | ABCB1/G2 | ABCB1/C2/G2 | ABCB1/C2/G2 | ABCB1/C2/G2 |

$IC_{50}$ (inhibitor concentration to produce 50% inhibition) in purified human HMG-CoA reductase catalytic domain.
Lipophilicity as log*D* at pH7.4 of administered form, except * at pH7.0.
Adapted from references [Neuvonen, P.J. *et al.* 2006; Schmitz, G. *et al.* 2007; Shitara, Y. *et al.* 2006; Tirona, R.G. 2005].

strates of OATP1B1 to a varying degree and the efficiency of this transport mechanism may be a major determinant for the ability of individual statins in the hydrophilic acid form to enter hepatocytes where their main pharmacological activity is exerted.

Most of the statins appear to have pharmacokinetic parameters that increase in proportion to the dose. However, with each doubling of the dose the increase in effect is on average an additional 7% reduction in LDL-cholesterol and 5% reduction in total cholesterol [Roberts, W.C. 1997]. The dose-response relationships for average changes in lipid parameters with different statins have been established in a number of large studies mainly performed in Caucasians [Jones, P. *et al.* 1998; Jones, P.H. *et al.* 2003]. Thus it may be anticipated that any polymorphism in an enzyme or transporter, or a drug interaction, which increased the drug concentration in plasma or hepatocytes by 100% might only result in an additional 6% reduction in LDL-cholesterol.

Drug interactions with the statins are mostly pharmacokinetic, involving the CYP enzymes or transporters. For example, the interactions between statins and azole antifungal drugs are largely due to the inhibition of CYP3A enzymes [Neuvonen, P.J. *et al.* 2006], whereas those with cyclosporine involve the inhibition of CYP3A enzymes, P-glycoprotein and other drug transporters by this co-administrated inhibitor [Simonson, S.G. *et al.* 2004; Palumbo, G. *et al.* 2005]. Polymorphisms in the genes for these enzymes and transporters may influence the interactions mediated through these pathways. The pharmacogenetic approach has been used to study drug-drug interactions, and we have suggested this could be used more widely to include study of herb-drug interactions [Tomlinson, B. *et al.* 2008]. One study has shown that the herbal compound baicalin reduces systemic exposure of rosuvastatin by induction of hepatic rosuvastatin uptake through OATP1B1 in an OATP1B1 haplotype-dependent manner [Fan, L. *et al.* 2008]. Polymorphisms or mutations in the genes encoding these proteins will also alter the pharmacokinetics and potentially the efficacy of the statins [Neuvonen, P.J. *et al.* 2006]. For some of the polymorphisms there is a moderate difference in the allelic frequencies between different ethnic groups. These have been summarized for the drug metabolizing enzymes in Table 2 and the main drug transporters in Table 3. These have been described in more detail recently [Saito, Y. *et al.* 2007]. Data from Chinese, Japanese and Korean studies have been combined in a group called East Asians. There are insufficient data from some large ethnic groups such as South Asian Indians so these are not shown in the tables. Ethnic differences in the disposition of rosuvastatin have been reported with greater systemic exposure in Japanese, Chinese and other Asian groups compared to Caucasians [Lee, E. *et al.* 2005; Tirona, R.G. 2005]. This resulted in recommendations in the rosuvastatin labeling to use

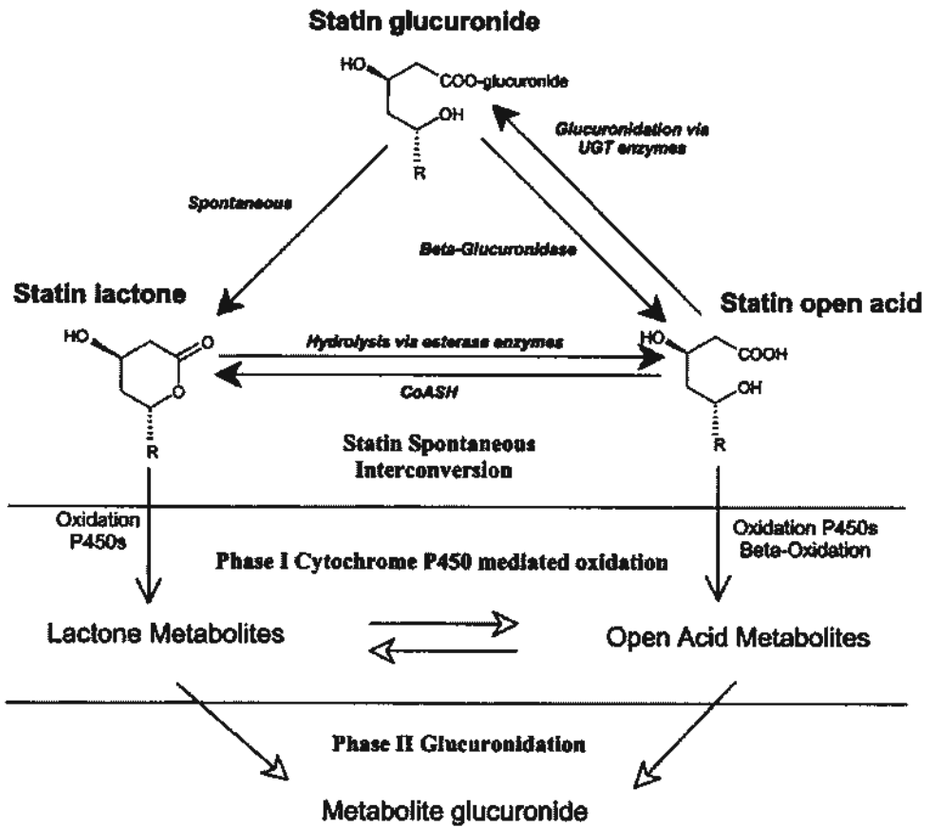


**Fig. (2).** Proposed metabolic pathway of a typical statin (adapted from Prueksaritanont 2002, with permission from American Society for Pharmacology and Experimental Therapeutics) [Prueksaritanont, T. *et al.* 2002a].

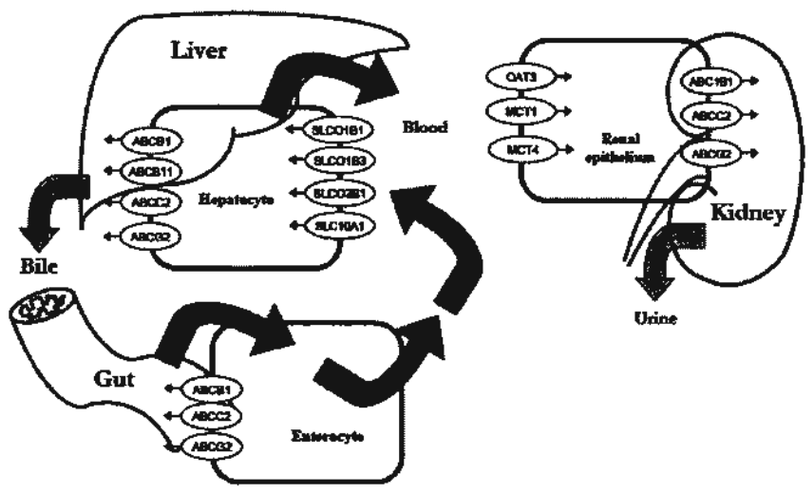


**Fig. (3).** Vectorial pathway for statin distribution from enterocytes to hepatocytes for their pharmacological action and metabolism and elimination *via* the bile and kidneys.

Listed across the compartment interfaces are efflux or uptake transporter proteins that may be involved.

Abbreviations: ABC, ATP-binding cassette; MCT, monocarboxylate transporter; OAT, organic anion transporter; SLC, solute solute carrier family; SLCO, solute carrier organic anion transporter family member.

**Table 2. Allele Frequencies of Common Polymorphisms in Drug Metabolizing Enzymes in Different Ethnic Groups**

| Metabolizing Enzymes | Polymorphisms or Haplotype | Amino Acid Exchange | Enzyme Activity | Allele Frequency (%) | | |
|---|---|---|---|---|---|---|
| | | | | White | Black | East Asian |
| **CYP2C8** | | | | | | |
| | *2 (805A>T) | Ile269Phe | Decreased | 0-4.1 | 1.1-18 | 0 [a] |
| | *3 (416G>A, 1196A>G) | Arg139Lys, Lys399Arg | Decreased | 13 | 2 | 0 [a] |
| | *4 (792C>G) | Ile264Met | Unclear | 0.2 | 14.1 | 0 [a] |
| **CYP2C9** | | | | | | |
| | *2 (430C>T) | Arg144Cys | Decreased | 10-15 | 2-4 | 0 |
| | *3 (1075A>C) | Ile359Leu | Decreased | 5-10 | <2 | 1-4 |
| **CYP2C19** | | | | | | |
| | *2 (681G>A) | Val331Ile | None | 13-19 | 11-25 | 21-45 |
| | *3 (636G>A) | W212end | None | 0-0.3 | 0-1.8 | 5-13 |
| **CYP3A4** | | | | | | |
| | *1B (-392A>G) | | Slightly decreased | 3.9-4.2 | 53-69 | 0-4.7 |
| | *2 (15713T>C) | Ser222Pro | Substrate specific | 2.7 | 0 | 0 |
| | *4 (352A>G, 13871A>G) | Ile118Val | Decreased | | | 3.3 |
| **CYP3A5** | | | | | | |
| | *1 | | Normal | 0-15 | 36-45 | 23-40 |
| | *3 (6986A>G) | Premature stop | Severely decreased | 85-98 | 27-84 | 60-77 |
| | *6 (14690G>A) | Premature stop | Decreased | 0 | 8.7 | 1.6 |
| **CYP2D6** | | | | | | |
| | *1 | Wild-type | Normal | 33.4-83.8 | 27.8-90.4 | 22.7-49 |
| | *2 (-1584C>G, 2850C>T, 4180G>C) | Wild-type | Normal | 32.4-35.3 | 9.9-40 | 8.0-13.4 |
| | *3 (2549A>del) | 260 terminate | Inactive | 0-2.5 | 0-1 | 0 |
| | *4 (100C>T, 974C>A, 984A>G, 1846G>A splice, 4180G>C) | 182 terminate | Inactive | 11.3-28.6 | 0.9-9.3 | 0.2-0.8 |
| | *5 gene deletion | Gene deletion | No activity | 0.6-7.3 | 3.3-9 | 1.2-6.2 |
| | *10 (100C>T, 4180G>C) | Pro34Ser, Ser486Thr | Decreased | 1.4-6.1 | 1-8.6 | 38.1-70 |
| | *17 (1023C>T, 2850C>T, 4180G>C) | Thr107Ile, Arg296Cys, Ser486Thr | Decreased | 0-1.1 | 9-34 | 0 |
| | *41 (-1584C>G, 2850C>T, 2988G>A, 4180G>C) | Arg296Cys, Ser486Thr | Decreased | 10-20 | 0 | 0 |
| | *2 X N (1661G>C, 2850C>T, 4180G>C) (N≥2) | Arg296Cys, Ser486Thr | Increased | 5-10 | 1.9-13.6 | 0.5 |
| **UGT1A1** | | | | | | |
| | *6 (211G>A) | Gly71Arg | Decreased | 0.7 | NA | 5-24.1 |
| | *28 (-53(TA)$_{6>7}$) | | Decreased | 29.5-40 | 34.6-44.6 | 6.8-23 |
| | *60(-3279T>G) | | Decreased | 43.9-55 | 85 | 16.7-32.7 |

[a] In 380 Japanese subjects

Adapted from references: CYP3A4: [Hsieh, K.P. *et al.* 2001; Kim, K.A. *et al.* 2004; Sata, F. *et al.* 2000; Wang, A. *et al.* 2005; Sinues, B. *et al.* 2007; Dally, H. *et al.* 2003], CYP3A5: [Daly, A.K. *et al.* 2006; Hustert, E. *et al.* 2001; Kim, K.A. *et al.* 2007; Kuehl, P. *et al.* 2001; Roy, J.N. *et al.* 2005; Balram, C. *et al.* 2003], CYP2C9: [Kim, K.A. *et al.* 2004; Kirchheiner, J. *et al.* 2003; Schwarz, U.I. 2003], CYP2D6: [Zanger, U.M. *et al.* 2004; Cascorbi, I. 2003; Meyer, U.A. and Zanger, U.M. 1997], UGT1A1: [Beutler, E. *et al.* 1998; Lampe, J.W. *et al.* 1999].

**Table 3. Allele Frequencies of Common Polymorphisms in Drug Transporters in Different Ethnic Groups**

| Drug Transporters | Polymorphisms or Haplotype | Amino Acid Exchange | Effect on Transporter Activity | Allele Frequency (%) | | | References |
|---|---|---|---|---|---|---|---|
| | | | | White | Black | East Asian | |
| **SLCO1B1** | | | | | | | |
| | *1a | | | NA | NA | 35.2 | [Ho, R.H. *et al.* 2007; Hsiang, B. *et al.* 1999; Iida, A. *et al.* 2001; Jada, S.R. *et al.* 2007; Mwinyi, J. *et al.* 2008; Nozawa, T. *et al.* 2002; Pasanen, M.K. *et al.* 2006a; Pasanen, M.K. *et al.* 2008; Tirona, R.G. *et al.* 2001] |
| | 388A>G | Asn130Asp | Uncertain | 30-46 | 70-80 | 60-90 | |
| | 463C>A | Pro155Thr | Unchanged | 1.6-18 | 2-4.8 | 0-2.5 | |
| | 521T>C | Val174Ala | Reduced | 14-20 | 1.0-2.5 | 11-16 | |
| | *14 (388A>G +463C>A) | Asn130Asp + Pro155Thr | Unchanged | 13.0-15.4 | NA | NA | |
| | *15 (388A>G +521T>C) | Asn130Asp + Val174Ala | Reduced | 2.7 | NA | 7-10.3 | |
| | *17 (-11187G>A +388A>G +521T>C) | Asn130Asp + Val174Ala | Reduced | 6.9 | NA | 2.9-13.3 | |
| **ABCB1** | | | | | | | |
| | 3435C>T | Ile1145Ile, wobble | Decreased P-gp expression | 48-54 | 16-26 | 37-47 | [Fromm, M.F. 2002; Ito, S. *et al.* 2001; Lee, C.G. *et al.* 2004; Ozawa, S. *et al.* 2004; Tang, K. *et al.* 2002] |
| | 2677G>T | Ala893Ser | Decreased P-gp expression | 41.6-46 | 6.5-10 | 36-43.7 | |
| | 2677G>A | Ala893Thr | Decreased P-gp expression | 0-3.6 | 0-0.5 | 5.8-21.8 | |
| | 1236C>T | Gly412Gly, wobble | Decreased P-gp expression | 34.4-42 | 15 | 61.5-69.4 | |
| | -129T>C | Non-coding | Decreased P-gp expression | 5.9 | NA | 1.6-8.3 | |
| **ABCC2** | | | | | | | |
| | 1249 G>A | Val417Ile | No significant effect | 15-21 | 14 | ~12 | [Ito, K. *et al.* 2001; Itoda, M. *et al.* 2002; Niemi, M. *et al.* 2006] |
| | 1446 C>G | Thr482Thr | Unknown | 1.3 -3.7 | NA | NA | |
| | 3563 T>A | Val1188Glu | Unknown | 5 | 4 | NA | |
| | 4544 G>A | Cys1515Tyr | Unknown | 5 | 17 | NA | |
| **ABCG2** | | | | | | | |
| | 34G>A | Val-12Met | Uncertain | 2-10.3 | 4 | 15-36 | [Lee, S.S. *et al.* 2007; Mizuarai, S. *et al.* 2004; Zamber, C.P. *et al.* 2003] |
| | 421C>T | Gln-141Lys | Reduced | 9.0-14 | 0 | 28-35 | |
| | 376C>T | Gln-126Term | Lack of function | 0 | NA | 0.4-1.9 | |

small doses in people of Asian origin [Po, A.L. 2007]. For the other statins there have been no systematic studies of ethnic differences, except for cerivastatin for which a retrospective analysis of the pharmacokinetic data found no evidence for any clinically relevant inter-ethnic differences between Caucasians, Black and Japanese subjects [Muck, W. *et al.* 1998].

## CYP3A ENZYMES

### CYP3A4

The CYP3A enzyme group constitutes the largest amount of CYP enzymes in the liver and enterocytes. They play a key role in the metabolism of over 50% of the drugs in common use in clinical practice [Li, A.P. *et al.* 1995; de Wildt, S.N. *et al.* 1999; Dresser, G.K. *et al.* 2000; Tang, W. and Stearns, R.A. 2001]. The importance of both CYP3A4 and CYP3A5 in the metabolism of some of the statins in both the lactone and acid forms has been known for many years [Prueksaritanont, T. *et al.* 1997]. Indeed, simvastatin has been considered as a potential probe drug for studying CYP3A drug interactions, but it was found to be suboptimal compared with midazolam because of its lack of CYP3A specificity [Chung, E. *et al.* 2006]. Many drug interactions have been described between statins and potent inhibitors of CYP3A4 such as itraconazole, ketconazole and even grapefruit juice, which acts only on enterocyte CYP3A4 [Neuvonen, P.J. *et al.* 2006]. These combinations should

generally be avoided because of the increased risk of myopathy and rhabdomyolysis but the combination of a low dose of statin with a moderate inhibitor of CYP3A4 such as diltiazem might potentiate the lipid-lowering effect. This was supported by a retrospective study of patients treated with simvastatin showing that with co-prescription of diltiazem, the reduction in cholesterol appeared approximately equivalent to doubling the dose of simvastatin [Yeo, K.R. *et al.* 1999]. With simvastatin, and potentially other statins, inhibition of CYP-mediated metabolism might reduce the formation of downstream active metabolites so the overall efficacy may not increase [Gruer, P.J. *et al.* 1999], but a recent study from Japan found an approximate doubling of plasma HMG Co-A reductase inhibitory activity when diltiazem was added to simvastatin 5-mg daily in patients with hyperlipidemia and hypertension and there was an additional reduction in LDL-cholesterol from 22.3% to 28.3% (calculated from the mean values of LDL-cholesterol given in the report) which might be predicted as an effect similar to doubling the dose [Watanabe, H. *et al.* 2004].

The enzyme activity of CYP3A4 shows wide inter-individual variability which is not currently explained by polymorphisms in *CYP3A4* [Xie, H.G. *et al.* 2001]. More than 40 SNPs of the *CYP3A4* gene have been identified on the Human Cytochrome P450 Allele Nomenclature Committee homepage (http://www.imm.ki.se/CYPalleles-updated 21st May, 2008), but most of these are not very frequent. The distribution in different ethnic groups of the three common alleles in the *CYP3A4* gene located in chromosome 7q.21.1 is shown in Table **2**. The allele *CYP3A4*1B* is the most frequent variant, particularly among black subjects. Patients who were homozygous for *CYP3A4*1B* had significantly higher post-treatment LDL-cholesterol levels in a study with atorvastatin 10-mg daily, but there was no difference in absolute and percentage changes in LDL-cholesterol [Kajinami, K. *et al.* 2004b]. The *CYP3A4*2* allele showed substrate specific pharmacokinetics [Xie, H.G. *et al.* 2001]. Its effect on statins has yet to be confirmed.

The *CYP3A4*4* allele results in the amino acid sequence change of Ile118Val. In a study among Chinese hyperlipidemic patients, in whom the incidence was quoted at 3.32%, the lipid lowering effects of simvastatin were increased for total cholesterol and triglycerides but not for LDL cholesterol in subjects with this allele compared to those with the wild-type allele [Wang, A. *et al.* 2005]. It is difficult to say whether this difference in changes in lipid fractions is simply due to differences in pharmacokinetics as it may be expected that the main effect would be on LDL-cholesterol if the systemic and hepatic exposure to simvastatin acid is greater in those subjects with the less frequent *CYP3A4*4* allele.

Another recent study in Chinese hyperlipidemic patients identified the frequency of a common variant *CYP3A4*1G* (*G* to *A* substitution at position 82266) as 2.76% and found a gene-dose-dependent effect ($P < 0.01$) with increasing percentage reduction in serum total cholesterol in **1/*1, *1/*1G, *1G/*1G* genotypes with atorvastatin but not with simvastatin [Gao, Y. *et al.* 2008]. These differences in percentage reduction in total cholesterol resulted from the sum of differences in changes in both LDL-cholesterol and HDL-cholesterol, but the changes were not significant for either cholesterol fraction separately so it is difficult to interpret these results.

### CYP3A5

CYP3A5 contributes to simvastatin metabolism and subjects with the *CYP3A5*3* allele, with decreased enzyme activity, have increased systemic exposure to simvastatin [Kim, K.A. *et al.* 2007]. The frequency of this variant is similar in Chinese (76%), Japanese (77%) and Caucasian subjects (85%) [Balram, C. *et al.* 2003]. The high frequency of this polymorphism may contribute a substantial amount to inter-individual variability in CYP3A substrates [Schmitz, G. and Langmann, T. 2006]. However, a study comparing the clinical response to atorvastatin 10-mg daily in 1902 subjects with the **3* or **1* alleles showed no significant effect [Thompson, J.F. *et al.* 2005]. It has been argued that atorvastatin may be less sensitive than simvastatin to pertubations in CYP3A activity through interactions with inhibitors because atorvastatin is given in the open acid form and its hepatic uptake is more dependent on the OATP1B1 transporter than the more lipophilic simvastatin lactone [Shitara, Y. and Sugiyama, Y. 2006]. Another study with simvastatin 20-mg daily in Caucasian patients in Brazil did not show any effect of the *CYP3A5*3* allele on lipid responses [Fiegenbaum, M. *et al.* 2005b]. However, in another study in subjects being treated with various statins (lovastatin, simvastatin and atorvastatin) there were smaller lipid-lowering responses in the *CYP3A5*1* wild-type expressors compared to homozygous *CYP3A5*3* enzyme non-expressors, supporting the theory that CYP3A5-related metabolism of statins may influence the clinical efficacy [Kivisto, K.T. *et al.* 2004].

### CYP2C8

CYP2C8 is known to be involved in the arachidonic acid pathway relating to vasoactivity [Dreisbach, A.W. *et al.* 2005; King, L.M. *et al.* 2005]. Its related gene *CYP2C8* is located on chromosome 10q23.33 and has three major variant alleles **5* (*475delA*), **7* (*4517C>T*) and **8* (*4517C>G*). Cerivastatin and its active metabolites are substrates of CYP2C8 [Schmitz, G. *et al.* 2007]. It has been reported that gemfibrozil greatly increased plasma concentration of cerivastatin, its lactone and metabolites, which is likely to be partly due to inhibition of the CYP2C8 pathway [Shitara, Y. *et al.* 2004]. Inhibitors of CYP3A4 have relatively little effect on cerivastatin pharmacokinetics because CYP2C8 seems to be the dominant pathway [Shitara, Y. *et al.* 2004]. The consequence from the high level of systemic exposure to cerivastatin was a high incidence of myopathy and increased risk of fatal rhabdomyolysis [Backman, J.T. *et al.* 2002; Ishikawa, C. *et al.* 2004] which contributed to the withdrawal of cerivastatin from the market [Furberg, C.D. and Pitt, B. 2001]. Both the *CYP2C8*5* and *CYP2C8*7* alleles result in non-expression of the gene and null activity of the enzyme, so these two alleles are likely to be associated with increased levels of CYP2C8 substrates. Both simvastatin acid and lovastatin acid undergo a minor degree of metabolism by CYP2C8 and it was shown that gemfibrozil increased the AUC values of these two acids but not their lactones, possibly though inhibition of the CYP2C8 pathway [Backman, J.T. *et al.* 2000; Kyrklund, C. *et al.* 2001]. Intui-

tively, the effect of the non-expressor *CYP2C8* alleles may have some influence on metabolism of some statins but no studies on this appear to have been reported.

### CYP2C9 and CYP2C19

CYP2C9 is responsible for the hydroxylation of a wide range of drugs, being particularly important for the narrow therapeutic range agents warfarin and phenytoin [Schwarz, U.I. 2003]. The **3* allele results in significant reduction in catalytic activities for all CYP2C9 substrates [Xie, H.G. *et al.* 2001]. Fluvastatin is the only statin known to undergo a major degree of CYP2C9-mediated metabolism. The *CYP2C9*3* allele is more common in Caucasians and AUC values for fluvastatin were found to be higher in subjects with the **3* allele with reduced enzyme activity [Kirchheiner, J. *et al.* 2003]. However, no correlation was found with the clinical effects in terms of lipid lowering efficacy [Kirchheiner, J. *et al.* 2003]. Other minor alleles, e.g. **4, *5, *6,* are only found in particular ethnic groups with low frequencies. Their effects on statin pharmacokinetics have not been reported. A large study involving 707 renal transplant patients on cyclosporine (ALERT study) found no significant effect of the *CYP2C9*2* and **3* alleles on the LDL-cholesterol reduction with fluvastatin 40-mg [Singer, J.B. *et al.* 2007].

Pitavastatin and to a lesser extent rosuvastatin undergo a minor degree of metabolism by CYP2C9 and the latter also by CYP2C19 [Igel, M. *et al.* 2002], but there are no reports of the effects of variants in these enzymes on the pharmacokinetics or pharmacodynamics of these statins.

### CYP2D6

The involvement of the CYP2D6 enzyme in the metabolism of statins is more controversial than the other enzymes mentioned above. CYP2D6 may not be directly involved in the metabolism of the statin lactone or hydroxy acid, but its action on the downstream oxidative metabolites is not clear. However, there have been previous reports of increased cholesterol-lowering efficacy with simvastatin treatment in subjects with *CYP2D6* genotypes associated with decreased enzyme activities [Mulder, A.B. *et al.* 2001; Nordin, C. *et al.* 1997; Zuccaro, P. *et al.* 2007], but *in vitro* studies found that CYP2D6 was not involved in the metabolism of simvastatin [Prueksaritanont, T. *et al.* 2003]. The *CYP2D6* gene is highly polymorphic with as many as 70 alleles described [Xie, H.G. *et al.* 2001]. Previous studies on the effects of *CYP2D6* polymorphisms on clinical responses were performed in Caucasians with alleles **3, *4* or **5* resulting in poor metabolizer status. A common allele **10* in the Chinese population has a frequency as high as 65% [Garcia-Barcelo, M. *et al.* 2000], but the effect of this allele on simvastatin or other statin efficacy or metabolism has not been identified.

## URIDINE DISPHOSPHATE-GLUCURONOSYL TRANSFERASE (UGT)

The uridine disphosphate-glucuronosyl transferase (UGT) superfamily can be subdivided into four subfamilies, UGT1, UGT2, UGT3, and UGT8 [Mackenzie, P.I. *et al.* 2005]. The *UGT1A* locus is located on chromosome 2q37 and consists of 9 active and 4 inactive first exons and common exons 2 to 5. Nine functional proteins (UGT1A1, UGT1A3-1A10) can be made [Tukey, R.H. and Strassburg, C.P. 2000]. There is tissue-specific expression of the UGT1A proteins with five of the nine functional UGT1A proteins primarily expressed in hepatic tissues (UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9) and three in extrahepatic tissues (UGT1A7, UGT1A8 and UGT1A10) [Tukey, R.H. *et al.* 2000]. At least 113 variant alleles of *UGT1A1* have been recorded (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/).

The UGT enzymes catalyze the conjugation of glucuronic acid to certain target substrates and they are involved in the inter-conversion of statin lactones and acids through a glucuronidation pathway as shown in Fig. (**2**) [Prueksaritanont, T. *et al.* 2002a]. Gemfibrozil was shown in an animal model to inhibit both CYP enzymes and UGT-mediated metabolism of simvastatin, atorvastatin and cerivastatin [Prueksaritanont, T. *et al.* 2002b]. However, the comparably lower rates of UGT-mediated metabolism compared to that *via* CYP P-450 pathways suggested UGT-mediated effects are less likely to be an influential factor in the intrinsic clearance of either the lactone or acid forms. In a study with pitavastatin with an *in-vitro* model Fujino *et al.* reported that the lactone form was an end-product from glucuronidation then lactonization [Fujino, H. *et al.* 2003]. UGT1A3 and UGT2B7 were suggested to be the major enzymes involved in pitavastatin lactonization, but other UGT enzymes, including UGT1A1, UGT1A4 and UGT1A6 are also thought to be involved [Fujino, H. *et al.* 2003]. The number of alternative pathways may reduce the contribution of changes in individual enzyme activities to result in altered pharmacokinetics of the statins.

## DRUG TRANSPORTERS

It is well recognized that drug transporters play an important role in the disposition of numerous drugs, particularly those which are more hydrophilic [Shitara, Y. *et al.* 2006]. Of the statins, pravastatin, rosuvastatin and pitavastatin are hydrophilic and are subject to relatively little metabolism, so drug transporters have a major effect on their disposition. Other statins or their active or inactive metabolites have also been shown to be substrates for some transporters. Furthermore, as the hepatic uptake and efflux determine the drug concentration in the liver, which is the target organ of statins, the drug transporters are likely to influence the pharmacological effects and potentially the adverse effect of some statins. The major drug transporters involved in statin disposition are shown in Table **1** and Fig. (**3**).

Functional polymorphisms in genes encoding these drug transporters are likely to influence the pharmacokinetic profiles and the efficacy of transporter-dependent drugs. As with the CYP and UGT enzymes, the frequency of the polymorphisms in the genes for the SLCO influx transporters and ABC efflux transporters varies between different ethnic groups, and this may account for some of the interethnic variability in the pharmacokinetics and pharmacodynamics of drugs [Konig, J. *et al.* 2006; Cascorbi, I. 2006]. The frequencies of common polymorphisms in some of the well documented transporters, which have a close relationship with statin disposition are shown in Table **3**.

Multiple membrane transporters facilitate the transfer of statins into or out of hepatocytes, and a number of studies have shown that the polymorphisms in these transporters may alter the pharmacokinetic profiles, the safety and the efficacy of the specific statins (Table 4), although results in different studies have not always been consistent.

## SLCO1B1 (OATP1B1)

The organic anion-transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*), previously known as OATP-C or OATP2, is an uptake transporter expressed on the sinusoidal membrane of human hepatocytes. It is highly polymorphic with different allele frequencies in different ethnic groups. A

**Table 4. Effect of Polymorphisms in Drug Transporters on the Pharmacokinetics, Efficacy or Safety of Statins in Recent Studies**

| Drug Transporters | Polymorphisms or Haplotypes | Statins | Effects | References |
|---|---|---|---|---|
| SLCO1B1 | 521T>C | Atorvastatin 20 mg | 144% or 61% greater AUC(0-48 h) of atorvastatin in 521CC than those in 521TT or 521TC, respectively. 100% greater AUC(0-48 h) of 2-hydroxyatorvastatin in 521CC than those in 521TT | [Pasanen, M.K. *et al.* 2007] |
| | *1b, *15 | Pitavastatin 2mg | Higher AUC in *15 carries (*15/*15 and *1b/*15) than non carriers (*1b/*1b) | [Ieiri, I. *et al.* 2007] |
| | *1a *1b, *15 | Pitavastatin 8mg | Higher AUC in *15 carries than non carriers | [Chung, J.Y. *et al.* 2005] |
| | 521T>C | Pravastatin 20 mg | Greater reduction in total cholesterol (22.4% vs. 14.5%) in 521TC than 521TT in Chinese CHD patients | [Zhang, W. *et al.* 2007] |
| | 521T>C | Pravastatin 40mg | Higher AUC in those carrying the 521C allele | [Ho, R.H. *et al.* 2007] |
| | 521T>C | Rosuvastatin 10mg | 65% and 79% higher AUC(0-48 h) and Cmax in 521CC than those in 521TT | [Pasanen, M.K. *et al.* 2007] |
| | *1a, *1b, *15 | Rosuvastatin 10mg | Pharmacokinetic exposure of rosuvastatin higher in OATP1B1*15/*15 subjects than others in Koreans | [Choi, J.H. *et al.* 2008] |
| | 521T>C | Rosuvastatin 40mg | Higher AUC in those carrying the 521C allele (521CC>CT>TT) in Caucasians | [Lee, E. *et al.* 2005] |
| | 521T>C | Simvastatin 40mg | AUC of simvastatin acid 120% and 221% higher in 521CC than in 521TC and 521TT, respectively. Cmax of simvastatin acid 162% and 200% higher in 521CC than in 521TC and 521TT, respectively. | [Pasanen, M.K. *et al.* 2006b] |
| | 521 T>C | Simvastatin 80mg | The odds ratio for myopathy was 4.5 per copy of the C allele, and 16.9 in CC as compared with TT homozygotes. | [Link, E. *et al.* 2008] |
| | 463C>A | Fluvastatin 80mg | Greater reduction in LDL-cholesterol (-41% vs. -31.5%) in 463AA than 463CC in European elderly subjects | [Couvert, P. *et al.* 2008] |
| ABCB1 | 3435C>T | Atorvastatin 10mg | Smaller reduction in LDL-C but larger increase in HDL-C in Female 3435CC than those in variant allele carriers | [Kajinami, K. *et al.* 2004a] |
| | MDR1-h4 MDR1-h10 | Fluvastatin 40mg | MDR1-h4 and MDR1-h10 associated with greater LDL-C reduction and decreased TG reduction in FH patients | [Bercovich, D. *et al.* 2006] |
| | 1236C>T, 2677G>A/T, 3435C>T | Simvastatin 20mg | Greater reductions in TC and LDL-C in 1236T and 2677G>A/T variant allele carriers than those with wild-type allele or homozygous for the 2677G allele, respectively. The 1236T, 2677non-G, and 3435T alleles were less frequent in ADR cases than in the non-ADR group | [Fiegenbaum, M. *et al.* 2005b] |
| ABCC2 | 1446C>G | Pravastatin 40mg | 70% lower AUC(0-12) and Cmax in heterozygous 1446C>G than in non-carriers | [Niemi, M. *et al.* 2006] |
| ABCG2 | 421C>T | Rosuvastatin 20mg | Lower AUC and Cmax but higher CL/F value in 421CC than in 421CA+421AA group in Chinese male subjects | [Zhang, W. *et al.* 2006] |

number of the *SLCO1B1* alleles and haplotypes are associated with altered OATP1B1-mediated transport function. The *388A>G (*1b)* variant is highly prevalent in East Asian ethnic groups, with the highest allelic frequency of approximately 87% reported in the Malay population [Jada, S.R. *et al.* 2007], but it is less frequent in Caucasians. However, the allelic frequency of the *c.521T>C* variant (**5*) in East Asians (11-16%) is similar to that reported in Caucasians (14-20%) [Pasanen, M.K. *et al.* 2006a], but is less common in Blacks (1%) [Ho, R.H. *et al.* 2007]. These two single nucleotide polymorphisms (SNPs) are in linkage disequilibrium and together they define a haplotype termed *SLCO1B1 *15*. It has been reported that this haplotype shows a decreased transporter activity for the SLCO1B1 substrate, pravastatin [Nozawa, T. *et al.* 2005]. The frequency of the **15* variant is relatively higher in East Asians (7-10.3 %) compared with the frequency in Caucasians (2.7%).

It is worth noting that almost all of the statins are substrates of SLCO1B1 to a greater or lesser extent. Indeed, many studies have shown that the SNPs or haplotypes of *SLCO1B1* associated with reduced SLCO1B1 function have an impact on the pharmacokinetics and pharmacodynamics of most statins [Shitara, Y. *et al.* 2006].

The *521T>C (*5)* variant of *SLCO1B1* has recently been reported to influence the pharmacokinetics of certain statins in different ethnic populations. Pasanen, *et al.* [Pasanen, M.K. *et al.* 2007] found that subjects with the *521CC* genotype had a 144% or 61% greater $AUC_{0\text{-}48h}$ values for atorvastatin than those with *521TT* or *521TC* genotypes, respectively, and the $AUC_{0\text{-}48h}$ values of the metabolite 2-hydroxyatorvastatin were also 100% higher in those with *521CC* compared to those with *521TT*. A relatively smaller but statistically significant effect of the *SLCO1B1 521T>C* polymorphism on the $AUC_{0\text{-}48h}$ of rosuvastatin 10-mg (65% higher in those with *521CC* compared to those with *521TT*) was also shown in this study in Finland. However, a similar study comparing single dose pharmacokinetics of rosuvastatin 40-mg daily among Caucasians, Chinese, Malay, and Asian-Indian subjects all living in Singapore concluded that the higher systemic exposure in Asians compared to Caucasians was not related to the *SLCO1B1 521T>C (*5)* polymorphism although there were some effects of the polymorphism within these ethnic groups [Lee, E. *et al.* 2005].

The relationship between the *SLCO1B1 521T>C* polymorphism and the pharmacokinetics of simvastatin and simvastatin acid in healthy volunteers has also been investigated [Pasanen, M.K. *et al.* 2006b]. The *SLCO1B1* polymorphism had a marked influence on the pharmacokinetics of active simvastatin acid with a 120 and 221% higher $AUC_{0\text{-}\infty}$ for simvastatin acid in the *521CC* group compared to those with the *521TC* and *521TT* genotypes. $C_{max}$ for simvastatin acid was 162 and 200% higher in the *521CC* group than in those with the *521TC* and *521TT*, respectively. However, there was no significant effect on the parent simvastatin lactone, which suggests that simvastatin acid but not the lactone is a substrate for the SLCO1B1 influx transporter [Pasanen, M.K. *et al.* 2006b].

A recent study using a genomewide scan for genetic markers of simvastatin-related myopathy in 85 subjects with definite or incipient myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily as part of a trial involving 12,000 participants, identified a single strong association of myopathy with the noncoding rs4363657 SNP in *SLCO1B1* [Link, E. *et al.* 2008]. The rs4363657 SNP was in nearly complete linkage disequilibrium with the nonsynonymous rs4149056 SNP (*521T>C*), and the odds ratio for myopathy was 16.9 (95% CI, 4.7 to 61.1) in CC as compared with TT homozygotes. This finding may suggest that the genotyping of SLCO1B1 polymorphisms may be one of the most useful approaches in the future for tailoring both the statin dose and safety monitoring [Link, E. *et al.* 2008].

The *SLCO1B1*15* variant has also been shown to have a significant effect on the pharmacokinetics of rosuvastatin in Korean subjects. The *SLCO1B1*15* allele (*388A>G, 521C>T*) was associated with increased systemic exposure to rosuvastatin. Homozygous **15* subjects had $AUC_{0\text{-}\infty}$ and $C_{max}$ values 1.7- and 2.2-fold higher respectively, than homozygous **1* subjects [Choi, J.H. *et al.* 2008]. Similar effects of the *SLCO1B1*15* variant on the pharmacokinetics of pitavastatin and pravastatin have also been reported [Chung, J.Y. *et al.* 2005; Ho, R.H. *et al.* 2007; Ieiri, I. *et al.* 2007]. In another study in Korea comparing homozygous wild-type **1* subjects with homozygous *SLCO1B1*15* subjects, the mutant group showed increased values for $C_{max}$ of 3.12- and 2.12-fold, and for $AUC_{0\text{-}\infty}$ of 2.62- and 1.99-fold with pitavastatin and pravastatin, respectively [Deng, J.W. *et al.* 2008]. However, the same study did not find any effect of the *SLCO1B1*15* genotype on fluvastatin pharmacokinetics, highlighting the substrate specificity which may be based on the hydrophilicity of the statins studied.

In addition to these pharmacokinetics findings, Niemi *et al.* [Niemi, M. *et al.* 2005] reported that the three heterozygous carriers of the *SLCO1B1 *17* haplotype (containing the *-11187G>A, 388A>G* and *521T>C* SNPs) had a significantly smaller effect with pravastatin on cholesterol synthesis determined by plasma lathosterol and the lathosterol to cholesterol ratio as compared with non-carriers in 41 healthy Caucasian subjects. Recently, Zhang *et al.* [Zhang, W. *et al.* 2007] reported that the *SLCO1B1 521T>C* polymorphism significantly modulated the total cholesterol-lowering efficacy of pravastatin in Chinese CHD patients with less reduction in total cholesterol (14.5% vs. 22.4%) with *521TC* genotype than those with the *521TT* genotype, which is in accordance with the previous findings in Japanese patients with hypercholesterolemia [Tachibana-Iimori, R. *et al.* 2004]. All these findings support the hypothesis that impaired hepatic uptake activity in those carrying the *521C* allele results in an increased plasma concentration of pravastatin but decreased intracellular concentrations of pravastatin in hepatocytes, the primary site of action. However, in contrast to these supportive findings, a retrospective study involving 462 patients on pravastatin treatment did not find an important effect of the *SLCO1B1 521T>C* polymorphism on the lipid response to pravastatin in Caucasian subjects [Thompson, J.F. *et al.* 2005].

Couvert *et al.* [Couvert, P. *et al.* 2008] have recently reported that the non-synonymous SNP *463C>A* (Pro155Thr) as well as the *SLCO1B1*14* allele, which is distinguished by the presence of the c.*388A>G* and c.*463C>A* polymor-

phisms, were significantly associated with enhanced lipid-lowering efficacy of fluvastatin in 420 European elderly hypercholesterolemic subjects aged 70-85 years. Subejcts homozygous for the *463CC* genotype (n = 294) exhibited significantly less LDL-cholesterol reduction (-31.5%) relative to heterozygous patients (-36.2%, n = 111), and to homozygous *A/A* subjects (-41%, n = 15). This SNP is only prevalent in European populations (~16%) but almost absent in Asians and Blacks, [Mwinyi, J. *et al.* 2008] which might contribute to the interethnic differences in the pharmacokinetics and pharmacodyanamic of statins.

**ABCB1 (MDR1, P-glycoprotein)**

The multidrug resistance protein (MDR1), P-glycoprotein (P-gp) or ABCB1 is an efflux transporter, which is expressed in multiple organs in humans, including the small intestine, liver, kidney and brain. Although the expression of ABCB1 was found to be 7-fold higher in small-intestine enterocyte homogenates than in the liver [von Richter, O. *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.

At least 32 SNPs have been described in the *ABCB1* gene [Marzolini, C. *et al.* 2004; Choudhuri, S. and Klaassen, C.D. 2006]. Two of the synonymous SNPs (*1237C>T* in exon 12 and *3435C>T* in exon 26) and a nonsynonymous SNP (*2677G>T*, Ala893Ser) in exon 21 were found to be in linkage disequilibrium, forming a SNP-haplotype [Choudhuri, S. *et al.* 2006]. It has been suggested that the haplotype could provide more useful information than single SNPs in pharmacogenetic analysis of *ABCB1* [Kim, R.B. *et al.* 2001]. Although results are not all consistent, most studies have shown a synonymous polymorphism, *3435C>T*, associated with lower expression of P-gp in man, which leads to lower transporter activity. The molecular mechanism underlying this clinical association is still unclear [Hoffmeyer, S. *et al.* 2000]. It has been hypothesized that this SNP was linked to a nonsynonymous SNP or SNPs in the regulatory regions of the *ABCB1* gene controlling the expression, such as *2677G>T/A*, leading to an Ala893Ser amino acid exchange; however, results from *in vitro* transfection of *ABCB1* expression on *2677G>T* vectors in cell culture were inconclusive [Kimchi-Sarfaty, C. *et al.* 2002]. Recently, Wang and Sadee used allelic expression imbalance as a quantitative phenotype in the search for functional cis-acting polymorphisms in *ABCB1*. They found that *3435C>T* represented a main functional polymorphism, accounting for 1.5- to 2-fold changes in mRNA levels, the mechanism of which was probably that different folding structures resulted in increased mRNA turnover [Wang, D. and Sadee, W. 2006].

The frequency of the *3435C>T* varies between different ethnic groups. In Caucasians and Asian groups, the frequency of the wild-type *3435C* has been reported as 34-59%, comparatively lower than that of the African population (73-84%) [Ameyaw, M.M. *et al.* 2001]. Similarly, the *2677G>T* variant is more frequent in East Asians and Caucasians (36-43.7%, 41.6-46%, respectively) than Africans (6.5-10%) [Ozawa, S. *et al.* 2004; Kroetz, D.L. *et al.* 2003]. However, another base change in this allele, *2677G>A*, has a relatively higher frequency in East Asians (5.8-21.8%), but is almost absent in Africans (0-0.5%) and has a low frequency in Caucasians (0-3.6%). The *1236C>T* variant is quite common in East Asians with frequencies of 61.5-69.4%, but is less prevalent in Caucasians (34.4-42%) and Africans (15%). The haplotype frequencies with these three alleles (*1236C>T, 2677G>T/A,* and *3435C>T*) have been examined in different ethnic groups, and they are also distributed with an ethnic-dependent manner [Ozawa, S. *et al.* 2004]. Kim *et al.* also reported that the SNP-haplotype of *1236C>T-2677G>T-3435C* has a higher frequency of 62% in European Americans compared to 13% in African Americans [Kim, R.B. *et al.* 2001]. Hence, the association between ethnicity and *ABCB1* polymorphisms may be an important factor.

Kajinami *et al.* analyzed the association between 2 prevalent polymorphisms (*2677G>T/A* and *3435C>T*) in the *ABCB1* gene and the variability in response to atorvastatin in 344 hypercholesterolemic patients [Kajinami, K. *et al.* 2004d]. They found that female *3435C* homozygotes showed significantly smaller reductions in LDL-cholesterol, but larger increases in HDL-cholesterol, relative to those with a variant allele, and haplotype determination with these combined polymorphisms identified a subgroup that showed an even greater response to treatment, which was not defined by a single polymorphism [Kajinami, K. *et al.* 2004d].

Another study examined the interactions between common polymorphisms (*1236C>T, 2677G>A/T,* and *3435C>T*) in the *ABCB1* gene and the lipid-lowering efficacy and safety of simvastatin in Brazil [Fiegenbaum, M. *et al.* 2005b]. In this study, the patients carrying the *ABCB1 1236T* variant allele had a greater reduction in total cholesterol and LDL-cholesterol with simvastatin treatment than those who were homozygotes with the wild-type allele. Similar results were observed for the *2677G>A/T* polymorphism and haplotype data. The *1236T, 2677non-G,* and *3435T* alleles were less frequent in cases that had adverse drug reactions (ADRs) to simvastatin than in the group without ADRs [Fiegenbaum, M. *et al.* 2005b].

In a compliance-monitored clinical study with fluvastatin 40-mg for 20 weeks in 76 patients with familial hypercholesterolemia, 5 tagging SNPs in the MDR1 *(ABCB1)* gene, including *2677G>T* (Ala893Ser), were used to reconstruct six haplotypes which accounted for 90.2% of the observed haplotypes [Bercovich, D. *et al.* 2006]. The *MDR1-h4* haplotype (including 893Ala) was associated with an increase in LDL-cholesterol response by 16.4%, whereas *MDR1-h10* (including 893Ser) was associated with decreased triglyceride response following fluvastatin treatment, although fluvastatin is not thought to be a substrate for ABCB1-mediated transport [Bercovich, D. *et al.* 2006].

However, some statins or their metabolites, such as the acid, methyl ester and lactone of atorvastatin, lovastatin lactone and simvastatin lactone, were found to modulate ABCB1 transporter properties in a concentration-dependent manner in a murine monocytic leukemia cell line, which may introduce another variable when assessing the genetic effects of *ABCB1* polymorphisms [Bogman, K. *et al.* 2001]. It has also been reported that ABCB1 might participate in lipid translocation across membranes, so that ABCB1 could also modulate pharmacodynamic effects independent of its influ-

ence on the pharmacokinetic properties of statins [van Helvoort, A. *et al.* 1996].

### ABCC2 (MRP2)

The ATP-binding cassette transporter ABCC2 or MRP2, encoded by the *ABCC2* gene plays an important role in drug excretion processes. Impaired function of MRP2 may result in increased gastrointestinal absorption and decreased biliary and/or urinary excretion of its substrates. More than 40 SNPs in the *ABCC2* gene have been identified, but little is currently known about the effects of genetic variation in *ABCC2* on the pharmacokinetics of drugs in humans.

Niemi *et al.* reported a significant association between an *ABCC2* polymorphism and the pharmacokinetics of pravastatin [Niemi, M. *et al.* 2006]. In that study, a synonymous SNP, *1446C>G* of the *ABCC2* gene, was associated with increased hepatic expression of *ABCC2* mRNA and reduced plasma concentrations of pravastatin. The $AUC_{0\text{-}12h}$ and $C_{max}$ of pravastatin were 67% and 68% lower in three subjects heterozygous for the *ABCC2 c.1446C>G* than in those not carrying this SNP, which corresponded with a 95% higher mRNA expression in human liver samples with the *1446CG* genotype than in those with the *1446CC* genotype. However, the mechanism by which this synonymous SNP might increase MRP2 expression is still unclear [Niemi, M. *et al.* 2006]. Recently, another study failed to find a relationship between 3 commonly occurring nonsynonymous polymorphisms in *ABCC2* (*1249G>A, 3563T>A* and *4544G>A*) and the pharmacokinetics of pravastatin [Ho, R.H. *et al.* 2007]. The effects of *ABCC2* polymorphisms on the pharmacokinetics of pravastatin and other ABCC2 substrates warrant further study.

### ABCG2 (BCRP)

The ABCG2 transporter, also known as breast cancer resistance protein (BCRP), mitoxantrone resistant protein (MXR) and placenta-specific ATP-binding cassette transporter (ABCP), is highly expressed in the placenta, colon, liver and intestine [Krishnamurthy, P. and Schuetz, J.D. 2006; Staud, F. and Pavek, P. 2005], and contributes to the disposition of many drugs and some statins. Rosuvastatin [Huang, L. *et al.* 2006], and pitavastatin, [Hirano, M. *et al.* 2005b] have been shown to be substrates of ABCG2. A SNP in *ABCG2* (*421C>A*) resulting in a change of glutamine to lysine at codon 141, has been shown to have a significant effect on the single dose pharmacokinetics of rosuvastatin 20-mg in male Chinese subjects after exclusion of the impact of *SLCO1B1* and *CYP2C9* polymorphisms [Zhang, W. *et al.* 2006]. In that pharmacokinetic study, the $AUC_{0\text{-}72}$, $AUC_{0\text{-}\infty}$ and $C_{max}$ values of rosuvastatin were lower by 44%, 44% and 48% respectively in the 7 subjects in the *421CC* genotype group, as compared with the 7 subjects in the *421CA+421AA* combined genotype group. The oral clearance CL/F value was lower in the *421CA+421AA* group than that in the *421CC* group [Zhang, W. *et al.* 2006]. This SNP is more frequent in Asians (34%) than Caucasians (11%) or Africans (1-5%) [de Jong, F.A. *et al.* 2004].

However, although pitavastatin and pravastatin are also thought to be substrates of ABCG2, this polymorphism apparently did not alter the pharmacokinetics of either of these two statins in recently published pharmacokinetic studies [Ho, R.H. *et al.* 2007; Ieiri, I. *et al.* 2007]. This disparity may be attributed to the existence of multiple organic anion transporters in the liver, including ABCB1 and ABCC2, which are involved in the biliary excretion of pitavastatin and pravastatin, and ABCG2 may only play a limited role in the disposition of these statins [Ieiri, I. *et al.* 2007; Kivisto, K.T. and Niemi, M. 2007].

### Other Transporters

Some studies have shown that statins are substrates for some other influx and efflux transporters, including SLCO2B1 (OATP2B1), SLC22A8 (OAT3), ABCB11 (BSEP), and sodium-dependent taurocholate cotransporting polypeptide (NTCP) [Hirano, M. *et al.* 2005a; Ho, R.H. *et al.* 2006; Kivisto, K.T. *et al.* 2007]. The possible effects of polymorphisms in these genes on statin pharmacokinetics have not been reported yet.

Human organic anion transporter 3 (OAT3 gene, *SLC22A8*), a member of the SLC22 superfamily, is mainly expressed in the kidney and localized on the basolateral membrane of the proximal tubules [Cha, S.H. *et al.* 2001]. In rat Oat3-expressing LLCPK1 cells, pravastatin appeared to be a relatively specific substrate of OAT3 [Hasegawa, M. *et al.* 2002]. Nishizato *et al.* have investigated the contribution of polymorphisms of the *OAT3* genes to the pharmacokinetics of pravastatin in 23 healthy volunteers [Nishizato, Y. *et al.* 2003]. Two polymorphisms in the *OAT3* gene, the synonymous *723T>A* (n = 12) and the nonsynonymous Ala389Val (n = 1), did not appear to be associated with changes in renal and tubular secretory clearance of pravastatin [Nishizato, Y. *et al.* 2003].

ABCB11, also known as sister of P-glycoprotein, is the bile acid export pump (BSEP) at the hepatocyte canalicular membrane, which mediates ATP-dependent bile acid secretion [Choudhuri, S. *et al.* 2006; Suchy, F.J. and Ananthanarayanan, M. 2006; Noe, J. *et al.* 2002]. It was found that pravastatin is a substrate of ABCB11 [Hirano, M. *et al.* 2005a]. However, the polymorphisms in *ABCB11* (*1331T>C, 2029A>G*) did not show a significant effect on the pharmacokinetics of pravastatin in a study conducted in European-American and African-American participants [Ho, R.H. *et al.* 2007].

NTCP has been reported to be important in the disposition of rosuvastatin and accounted for 35% of rosuvastatin uptake *in vitro* [Ho, R.H. *et al.* 2006]. The *NTCP*2* allele, which shows almost complete loss of function for bile acid uptake, showed a remarkable gain of transport activity for rosuvastatin and is only seen in Asians with an allele frequency of 7.5% [Ho, R.H. *et al.* 2004; Ho, R.H. *et al.* 2006]. However, the effects of the *NTCP* polymorphism on the pharmacokinetics of rosuvastatin remains unclear and warrant further study.

## GENETIC VARIANTS IN PHARMACODYNAMIC PATHWAYS AND LIPID RESPONSES

In addition to the genes which might be involved in the pharmacokinetics of statins, a number of candidate genes involved in lipid metabolism pathways that may affect the

pharmacodynamic lipid-regulating effect of statins have been studied [Kajinami, K. *et al.* 2005c; Kajinami, K. *et al.* 2004e; Schmitz, G. *et al.* 2006; Siest, G. *et al.* 2005]. The polymorphisms in these genes may contribute to the variation in the lipid response and clinical outcome with statin therapy. These have been examined in several clinical studies, initially on a single gene basis, but more recent studies have examined the effects of multiple SNPs in candidate genes [Chasman, D.I. *et al.* 2004; Singer, J.B. *et al.* 2007; Thompson, J.F. *et al.* 2005].

In addition to the polymorphisms in well-established lipid regulators like apolipoprotein E (Apo E), a number of recent large genome-wide studies, which examined hundreds of thousands of SNPs in thousands of subjects in European Populations, have identified some new loci related to lipoprotein levels, which may be useful to examine their relationship with statin responses [Kathiresan, S. *et al.* 2008; Kooner, J.S. *et al.* 2008; Sandhu, M.S. *et al.* 2008; Willer, C.J. *et al.* 2008]. These studies verified some SNPs in previously implicated loci, but also identified several new loci associated with lipid concentrations and/or the risk of coronary artery disease. The polymorphisms in these newly identified loci may also contribute to the variation in lipid response to statins and may be promising targets for further investigation.

To date, the most extensively examined genes related to lipid metabolism pathways and statin effects are the LDL receptor gene (*LDLR*), *Apo E*, the cholesteryl ester transfer protein gene (*CETP*) and the target enzyme for statins, HMG-CoA reductase (*HMGCR*). Some of the positive findings of the effects of polymorphisms in the genes related to lipid metabolism on the lipid responses to statins and clinical outcomes in previous studies are summarized in Table **5**. The results have not always been consistent.

### LDL Receptor

The LDL receptor in the liver plays a key role in LDL particle uptake and catabolism. The lack of the LDL receptor (LDLR) or dysfunctional receptors reduces the clearance of LDL particles, resulting in an increase in the plasma LDL-cholesterol levels. To date, more than 1,000 mutations in the LDLR have been described causing familial hypercholesterolemia (FH) [Garg, A. and Simha, V. 2007; Soutar, A.K. and Naoumova, R.P. 2007; Varret, M. *et al.* 2008]. Mutations in the *LDLR* gene have been divided into five classes. Class I and II mutations are known as receptor negative mutations, and Class III - V mutations are receptor defective mutations. Different types of *LDLR* mutation have been reported to be associated with different response to statins in FH patients in some of the previous studies. In some studies patients with mutations having a mild effect or with receptor defective mutations showed a better response in terms of LDL-cholesterol reduction than that in patients with severe or receptor negative mutations (Table **5**), but other studies failed to observe this association [Couture, P. *et al.* 1998; Sijbrands, E.J. *et al.* 1998; Sun, X.M. *et al.* 1998]. The differences in sample size, gene-gene interactions and gene-environment interactions may contribute to these discrepancies.

Mutations in the gene encoding the proprotein convertase subtilisin/kexin type 9 (PCSK9), which encodes a proprotein convertase that causes degradation of cell surface LDLRs could result in an increased number of LDLRs and may reduce baseline cholesterol levels and alter the cholesterol-lowering effect of statins [Abifadel, M. *et al.* 2003; Berge, K.E. *et al.* 2006; Dedoussis, G.V. *et al.* 2004; Garg, A. *et al.* 2007]. The reported frequency of *PCSK9* mutations is relatively low at about 2% and reports on the effects of *PCSK9* mutations on the responses to treatment with simvastatin or atorvastatin have been inconclusive [Berge, K.E. *et al.* 2006; Pisciotta, L. *et al.* 2007].

### Apolipoprotein E

Apolipoprotein E is a plasma protein that serves as a ligand for low density lipoprotein receptors and, through its interaction with these receptors, participates in the metabolism of cholesterol and other lipids. [Mahley, R.W. 1988] Apo E is present in three main isoforms (E2, E3, E4), which show increasing affinities to the LDL receptor and do account for some of the interindividual variability of baseline LDL-cholesterol levels. The effect of polymorphisms in the *Apo E* gene on the plasma lipid response to statins has been addressed in many studies. Several reports found that subjects with Apo E2 had greater lipid responses compared to those with E3 and E4 [Maitland-van der Zee, A.H. *et al.* 2006; Ordovas, J.M. *et al.* 1995; Pedro-Botet, J. *et al.* 2001; Zuccaro, P. *et al.* 2007], whereas others failed to show a significant interaction between *Apo E* polymorphisms and the lipid response to statins [Chasman, D.I. *et al.* 2004; Karayan, L. *et al.* 1994; O'Malley, J.P. and Illingworth, D.R. 1990; Pena, R. *et al.* 2002; Sanllehy, C. *et al.* 1998; Vohl, M.C. *et al.* 2002]. Kajinami *et al.* reviewed the results and concluded that carriers of the E4 allele tend to show a greater percentage reduction in LDL-cholesterol than those carrying the wild-type Apo E allele, whereas E2 allele carriers showed a smaller reduction [Kajinami, K. *et al.* 2005c; Kajinami, K. *et al.* 2004e]. However, Schmitz *et al.* reached the opposite conclusion in their review [Schmitz, G. *et al.* 2006; Schmitz, G. *et al.* 2007]. More recently, Tavintharan *et al.* [Tavintharan, S. *et al.* 2007] reported that Apo E4 was associated with a greater reduction in LDL-cholesterol in Chinese patients with type 2 diabetes mellitus, but an earlier study in Japanese patients with type 2 diabetes did not find this but did report that apo B was reduced to a lesser extent in subjects with the E3/E2 phenotype [Watanabe, J. *et al.* 1993].

### Cholesteryl Ester Transfer Protein

The cholesteryl ester transfer protein (CETP) plays a key role in determining plasma HDL-cholesterol concentration and both LDL and HDL particle size, and perhaps the risk of CHD [Boekholdt, S.M. *et al.* 2004]. The *CETP TaqIB* polymorphism has been reported to be associated with altered CETP plasma concentrations and activity, and with plasma HDL-cholesterol levels in different populations [Boekholdt, S.M. *et al.* 2005; Corella, D. *et al.* 2000; Park, K.W. *et al.* 2003]. However, the effect of this polymorphism on lipid responses or clinical outcome with statin treatment in different groups of patients is inconsistent (Table **5**), but a meta-analysis performed on individual patient data from 10 trials

**Table 5. Positive Effects of Polymorphisms in Various Genes Related to the Lipid Response to Statin Therapy**

| Genes | Polymorphisms | Statins | Patients | Effects | References |
|---|---|---|---|---|---|
| LDL receptor | C660X, D147H, 652delGGT | Fluvastatin 40-mg | FH | Smaller reduction in LDL-C in patients with Sephardic and Lithuanian mutations ($p < 0.005$). | [Leitersdorf, E. *et al.* 1993] |
| | D206E, V408M | Simvastatin 40-mg | FH $_{Afrikaner-1}$ (FH1), FH $_{Afrikaner-2}$ (FH2) | Greater reduction in TC and LDL-C in patients with FH2 | [Jeenah, M. *et al.* 1993] |
| | Del 6kb-ex15 P664L | Pravastatin 20-mg + cholestyramine 12-g | FH$_{Tonami-1}$ FH$_{Kanazawa-2}$ | Greater reduction in LDL-C in patients with FH$_{Kanazawa-2}$ | [Kajinami, K. *et al.* 1998] |
| | W66G, Del 15kb-ex1, C646Y | Simvastatin 20-mg | Children & adolescents with heterozygous FH | Smaller reduction in LDL-C in those with W66G mutation than those with the deletion>15 kb and the C646Y mutation | [Couture, P. *et al.* 1998] |
| | Mild Severe | Simvastatin 40-mg | FH | Fewer achieved LDL- C goals with severe LDLR mutation compared to those with 'mild' mutations | [Heath, K.E. *et al.* 1999] |
| | Null mutation Defective mutation | Simvastatin 20-mg | FH | Smaller reduction in TC and LDL-C in patients with null mutations than those with defective mutations | [Chaves, F.J. *et al.* 2001] |
| | Receptor-negative, Receptor-defective | Simvastatin 20-mg | Adolescents with heterozygous FH | Smaller reduction in LDL-C in those with receptor-negative mutation than those with receptor-defective mutation | [Vohl, M.C. *et al.* 2002] |
| | AvaII | Pravastain 20-mg | Hypercholesterolemia | Greater reduction in LDL-C in patients with AvaII mutations | [Lahoz, C. *et al.* 2005b] |
| Apo E | E2, E3, E4 | Lovastatin 40-mg | FH | Smaller reduction in LDL-C, but greater increase in HDL-C in male E4 carries | [Carmena, R. *et al.* 1993] |
| | E2, E3, E4 | Atorvastatin 10-mg | FH | E4 allele more frequent in good responders | [O'Neill, F.H. *et al.* 2001] |
| | E2, E3, E4 | Pravastatin 40-mg | Primary hyperlipidemia | Greater LDL-C response with E2 carries | [Ordovas, J.M. *et al.* 1995] |
| | E2, E3, E4 | Simvastatin 20-mg | Primary hyperlipidemia | Responsiveness was greatest in those with E2 carries | [Nestel, P. *et al.* 1997] |
| | E2, E3, E4 | Fluvastatin 40-mg | Coronary artery disease | Less reduction in LDL-C in those with E4 allele but similar benefit in terms of progression. | [Ballantyne, C.M. *et al.* 2000] |
| | E2, E3, E4 | Atorvastatin 10-mg | Primary hyperlipidemia | Greater LDL-C and TG response in male with E2 carries | [Pedro-Botet, J. *et al.* 2001] |
| | -491A/T | Atorvastatin 10 – 40- mg | Combined hyperlipidemia | Greater LDL-C-lowering effect with -491T allele carriers | [Garcia-Otin, A.L. *et al.* 2002] |
| | E2, E3, E4 | Pravastatin | Males with coronary artery disease | E2 carriers had largest improvement of HDL-C and LDL/HDL ratios, compared with E3 and E4 carriers, but not with regard to angiographic parameters. | [Maitland-van der Zee, A.H. *et al.* 2006] |
| | E2, E3, E4 | Statins | Dyslipidemia | Greater HDL-C response in E2 carriers | [Zuccaro, P. *et al.* 2007] |
| | E2, E3, E4 | Simvastatin 10-mg Lovastatin 20-mg | Diabetes | Greater LDL-C response in E4 carries than those with E2 | [Tavintharan, S. *et al.* 2007] |
| CETP | Taq1B (B1, B2) | Pravastatin 40-mg | Males with coronary artery disease | Pravastatin slowed progression of coronary disease in B1B1 carriers but not in B2B2 carriers | [Kuivenhoven, J.A. *et al.* 1998] |
| | Taq1B (B1, B2) | Atorvastatin 10-mg and 80-mg | Type 2 diabetes | Patients with B1B1 or 629CC genotype had a greater increase in HDL-C and a larger reduction in TG | [van Venrooij, F.V. *et al.* 2003] |
| | Haplotype | Pravastatin, Atorvastatin, Cerivastatin | Dyslipidemias | SNPs1-6 associated with increase in HDL-C and SNPs4-9 with decrease in TG | [Winkelmann, B.R. *et al.* 2003] |

**Table 5. Contd....**

| Genes | Polymorphisms | Statins | Patients | Effects | References |
|---|---|---|---|---|---|
| CETP | TaqIB (B1, B2) | Simvastatin 20-mg | Hypercholesterolemia | Patients with B2B2 genotype having more benefit in HDL-C improvement than carriers of B1 allele | [Fiegenbaum, M. *et al.* 2005a] |
| | TaqIB (B1, B2) | Simvastatin 20-40-mg, pravastatin 40-mg, atorvastatin 20-40-mg | FH | Patients with the B2B2 genotype have a higher CVD risk in comparison with the B1 allele carriers. | [Mohrschladt, M.F. *et al.* 2005] |
| | Haplotype: -2600, -867, -525, TaqIB, Ex 14/I405V | Fluvastatin 40-mg | FH | CETP-H13 associated with greater reduction in LDL-C. CETP-H5 was significantly associated with decreased TG and HDL-C response. | [Bercovich, D. *et al.* 2006] |
| LPL | Haplotype: 12 SNPs | Lovastatin | CABG | Haplotypes related to HDL-C response, TG response and graft progression protection | [Goodarzi, M.O. *et al.* 2007] |
| LIPC | C-514T | Lovastatin (40-mg) + colestipol (30-g), or niacin (4-g) + colestipol (30-g). | Males with CAD | Subjects with the CC genotype had the greatest improvement in LDL density and HDL(2)-C and the greatest angiographic improvement | [Zambon, A. *et al.* 2001] |
| | C-514T | Pravastatin 20-mg | Hypercholesterolemia | Greater increase in HDL-C in those carrying T allele than those with CC genotype | [Lahoz, C. *et al.* 2005a] |
| HMGCR | SNP12, SNP29 | Pravastatin 40-mg | Hypercholesterolemia | 22% smaller reduction in TC and 19% smaller reduction in LDL-C in hyterozygotes | [Chasman, D.I. *et al.* 2004] |
| ApoB | XbaI, ins/del, EcoRI | Fluvastatin | FH, Non-FH | Greater reduction in LDL-C in ins homozygotes | [Guzman, E.C. *et al.* 2000] |
| PON | R192Q M55L | Pravastatin 40-mg | Hypercholesterolemic males | R/Q192 genotype modulates the responses of serum HDL-C and apo AI. | [Malin, R. *et al.* 2001] |
| | -107C>T and 192Q>R | atorvastatin simvastatin | FH | HDL-C increment more pronounced in subgroups of -107CT/TT or 192QR/RR genotype combined with low baseline HDL-C (+13.9%, P<0.001, respectively+15.4%, P<0.001). | [Himbergen, T.M. *et al.* 2005] |
| ApoA I | G-75A | Pravastatin 20-mg | Hypercholesterolemia | Higher increase in HDL-C in non-smoking male GG carriers | [Lahoz, C. *et al.* 2003] |
| | G-75A, +83 | Atorvastatin 10-mg | Hypercholesterolemia | Gender-difference in HDL-C increase in +83 variant carriers | [Kajinami, K. *et al.* 2005a] |
| ABCG5/G8 | Q604E, D19H, Y54C, T400K, and A632V | Atorvastatin 10-mg | Hypercholesterolemia | D19H variant associated with greater reduction in LDL-C | [Kajinami, K. *et al.* 2004c] |
| CYP7A1 | A-204C | Atorvastatin 10-mg | Hypercholesterolemia | A-204C promoter variant associated with poor response of LDL-C reduction | [Kajinami, K. *et al.* 2004c; Kajinami, K. *et al.* 2005b] |
| ABCA1 | C-477T, A-419C, G-320C | Fluvastatin 40-mg | CAD | -477C/T variants associated with the severity of coronary atherosclerosis. | [Lutucuta, S. *et al.* 2001] |
| PPAR | Haplotype | Fluvastatin 40-mg | CAD | PPARδ haplotype 2 determine TG and apoC-III response and coronary lesions, PPARγ haplotypes 7 associated with reduced mean lumen diameter. | [Chen, S. *et al.* 2004] |
| LEPR | Arg223Gln | Simvastatin 5-mg | Hypercholesterolemia | Subjects with Arg/Arg genotype had significantly weaker improvement in TC levels than those with Arg/Gln genotype and Gln/Gln genotype. | [Takahashi-Yasuno, A. *et al.* 2003] |

found that the *CETP TaqIB* variant was associated with HDL-cholesterol plasma levels but did not influence the response to pravastatin therapy [Boekholdt, S.M. *et al.* 2004]. Although genetic deficiency of *CETP* is associated with higher plasma HDL-cholesterol concentrations, the effect of these genetic variants on the cardiovascular disease is still controversial. Some studies showed that *CETP* polymorphisms were associated with a lower risk of cardiovascular

disease which was independent of the effect on HDL-cholesterol [Blankenberg, S. *et al.* 2003], but others failed to find any association [de Grooth, G.J. *et al.* 2004; McCaskie, P.A. *et al.* 2007; Tsai, M.Y. *et al.* 2008].

### ABCG5/G8 and CYP7A1

Cholesterol excretion by ATP binding cassette transporters G5 and G8 (ABCG5/G8) and bile acid biosynthesis by cholesterol 7alpha-hydroxylase (CYP7A1) are major pathways for the removal of cholesterol into bile. Kajinami *et al.* reported a significant association between the *ABCG8* Asp19His variant and the LDL-cholesterol lowering response to atorvastatin therapy [Kajinami, K. *et al.* 2004a; Kajinami, K. *et al.* 2004c; Kajinami, K. *et al.* 2005b]. In the same study, they also found that the *CYP7A1 –204A>C* promoter polymorphism could influence the LDL-cholesterol reduction. The combination of these two polymorphisms could explain more of the variation in LDL-cholesterol reduction in these patients [Kajinami, K. *et al.* 2004a; Kajinami, K. *et al.* 2004c; Kajinami, K. *et al.* 2005b].

### HMG-CoA Reductase

The largest study reported so far to investigate the pharmacogenetics of statins was that from the 1536 patients treated with pravastatin 40-mg daily for 24 weeks in the Pravastatin Inflammation/CRP Evaluation (PRINCE) study, in which 148 SNPs in 10 candidate genes related to drug or lipid metabolism were examined [Chasman, D.I. *et al.* 2004]. The only significant findings were that the carriers of the minor alleles of two closely linked SNPs (SNP12 and SNP29) in the gene coding for HMG-CoA reductase (heterozygote prevalence 6.7% for both) had significantly smaller reductions in total cholesterol and LDL-cholesterol (20.1% vs. 25.2%) compared to subjects homozygous for the major alleles. A more detailed examination of the relationships of the *HMGCR* gene polymorphisms and haplotypes to the lipid responses to simvastatin 40-mg was performed in the CAP (Cholesterol and Pharmacogenetics) study with 326 black and 596 whites subjects[Medina, M.W. *et al.* 2008]. There was an effect in black subjects with the 38 black carriers of haplotype *H7*, which included SNP12 of the PRINCE study, and/or *H2* having a significantly attenuated LDL-cholesterol response compared with black non-carriers as measured by percent reduction in LDL-cholesterol (-36.9% vs. -40.6%, P=.02), but no haplotype effect was observed in whites. The *HMGCR* polymorphisms examined in the study explained <2% of the overall variance in LDL-cholesterol response to simvastatin. Subsequently, Donnelly and colleagues [Donnelly, L.A. *et al.* 2008] replicated the finding that rs17238540 (SNP 29 of the PRINCE study) is markedly associated with lower efficacy of total cholesterol lowering in a large population-based cohort of patients with diabetes. They found that the heterozygotes had a 13% smaller reduction in total cholesterol ( – 32.3 vs. – 37.1%, P =0.0081) and a 27% smaller reduction in triglycerides ( – 27.5 vs. – 37.6%, P = 0.0046) compared to homozygotes for the wild type allele. However, another large clinical trial involving 707 renal transplant patients all receiving cyclosporine (ALERT study) examined the association between 42 polymorphism in 18 candidate genes and the lipid response to fluvastatin 40-mg [Singer, J.B. *et al.* 2007]. In that study, there was no significant effect of the polymorphisms in *HMGCR* on the LDL-cholesterol reduction with fluvastatin treatment, and none of the other selected polymorphisms had any significant effect either [Singer, J.B. *et al.* 2007].

### Other HDL-Related Proteins

Some of the factors related to HDL metabolism have also been investigated, such as apolipoprotein A-I (Apo A1) and paraoxonase (PON). Some studies showed that the effect of statin therapy on HDL-cholesterol concentration was influenced by PON1 (paraoxonase-1) genotype and/or phenotype status in patients with FH [Himbergen, T.M. *et al.* 2005] or non-FH patients [Malin, R. *et al.* 2001] but some other studies failed to find a relationship [Christidis, D.S. *et al.* 2007].

Lahoz, C. *et al.* have reported that the *G/A* polymorphism of the *apo A-I* promoter region (*-75G>A*) could affect not only baseline HDL-cholesterol concentrations but also the response to pravastatin treatment [Lahoz, C. *et al.* 2003]. However, the results from another study suggested that the Apo A1 +83 polymorphism or another unknown causative sequence variant close to this polymorphism played a more important role in HDL-cholesterol response to statin therapy than the *-75G>A* polymorphism and in a gender-dependent manner [Kajinami, K. *et al.* 2005a]. In that study, the associations between the estrogen receptor alpha (ESR1) gene haplotype and the plasma lipid response to atorvastatin were also examined. The *ESR1* haplotype 2 [PvuII(-)XbaI(+)] was significantly and independently associated with greater HDL-cholesterol response in a gender-specific manner, and the gene-gene interaction between *ESR1* and *Apo A1* influenced by gender was also observed [Kajinami, K. *et al.* 2005a].

## PHARMACOGENETICS AND PLEIOTROPIC EFFECTS OF STATINS

In addition to their lipid-lowering properties, statins are also thought to have a number of protective effects on the cardiovascular system, which may be independent of their action on lipids and have been called "pleiotropic" effects. These potentially beneficial effect of statins include effects on endothelial function, oxidative stress, inflammation, thrombogenic factors and other processes which might contribute to atherosclerosis [Lahera, V. *et al.* 2007]. The complicated mechanisms underlying the anti-atherosclerotic effects of statins have been extensively investigated and appear to involve effects on numerous factors modulating endothelial function, inflammatory and atherothrombotic process, such as adhesion molecules, interleukins, C reactive protein, and so on. Polymorphisms in these modulating genes could not only alter the progression of atherosclerosis and the risk of cardiovascular events, but also influence the beneficial effect of statins in clinical outcomes.

### Kinesin-Like Protein 6

Kinesin-like protein 6 (KIF6) is a member of the superfamily of molecular motors that are involved in intracellular transport. A Trp719Arg polymorphism in this gene has been found to be associated with CHD incidence in both males and females in large population based studies [Morrison, A.C. *et al.* 2007; Shiffman, D. *et al.* 2008]. Iakoubova *et al.*

examined the association between 35 genetic polymorphisms previously found to be associated with cardiovascular disease, including the *KIF6* Trp719Arg polymorphism, and recurrent myocardial infarction (MI) in the CARE (Cholesterol and Recurrent Events) trial and CHD in the WOSCOPS (West of Scotland Coronary Prevention Study) trial and whether the risk associated with these polymorphisms could be reduced by pravastatin treatment [Iakoubova, O.A. *et al.* 2008b]. Of the 35 polymorphisms tested, only the *KIF6* Trp719Arg was associated with coronary events. Carriers of the *KIF6* 719Arg allele had an increased risk of MI in the CARE trial with an odds ratio of 1.50 (95% confidence interval [CI] 1.05 to 2.15) and CHD in the WOSCOPS trial with an odds ratio of 1.55 (95% CI 1.14 to 2.09), and pravastatin treatment substantially reduced the absolute risk by 4.98% and 5.49% among carriers, which suggested that carriers of the 719Arg allele had a greater benefit from statin therapy than noncarriers. More functional studies of the KIF6 kinesin are warranted to explain the observed difference in treatment benefit.

A similar finding was also identified from the PROVE-IT TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22) trial by the same group of researchers, which showed a significant difference in benefit from high-dose atorvastatin therapy (80-mg daily) compared with standard-dose pravastatin therapy (40-mg daily) between carriers and non-carriers of *KIF6* 719Arg [Iakoubova, O.A. *et al.* 2008a]. In carriers of the 719Arg allele, high-dose atorvastatin therapy, compared with standard-dose pravastatin therapy, reduced the risk of death or major cardiovascular events by 41%. In contrast, high-dose atorvastatin, compared with standard-dose pravastatin, was of no significant benefit in non-carriers. The absolute risk reduction was 10.0% in carriers versus 0.8% in non-carriers after 2 years of treatment, but carriers and non-carriers did not differ in on-treatment LDL-cholesterol, triglyceride, or C-reactive protein (CRP) levels, which suggested a pleiotropic mechanism distinct from lipid or CRP lowering effects [Iakoubova, O.A. *et al.* 2008a].

**C-reactive Protein and Other Factors**

C-reactive protein (CRP), a member of the pentraxin family, plays an important role in acute and chronic inflammation. Inflammation contributes to all phases of atherosclerosis and thrombosis. Clinical studies suggested that inflammation is the underlying cause of about 80% of all sudden cardiac deaths [Albert, C.M. *et al.* 2002]. Plasma levels of high sensitivity (hs) CRP level have been shown to be a reliable marker of systemic inflammation and a strong predictor of future vascular events [Ridker, P.M. 2007; Ridker, P.M. and Cook, N.R. 2007]. Genetic variations in *CRP* gene have been found to be associated with plasma hsCRP levels and CHD risk [Suk, H.J. *et al.* 2005; Crawford, D.C. *et al.* 2006; Lange, L.A. *et al.* 2006; Danik, J.S. and Ridker, P.M. 2007]. Whether polymorphisms in the *CRP* gene could affect the beneficial effect of statins is still unknown.

However, polymorphisms in some other genes related to the progression and complication of atherosclerosis have been shown to influence the benefit of statins. A functional polymorphism (*-786T>C*) in endothelial nitric oxide synthase (eNOS) gene may modulate the activity of this gene. Nagassaki *et al.* found that this polymorphism could modulate the effects of atorvastatin on NO bioavailability and oxidative stress [Nagassaki, S. *et al.* 2006]. Furthermore, Souza-Costa *et al* [Souza-Costa, D.C. *et al.* 2007] reported that this polymorphism influenced atorvastatin-induced anti-inflammatory effects in healthy volunteers, although in that study, no effects for the *-786T>C* polymorphism on the concentrations of inflammatory markers was observed. Subjects with the *CC* genotype had a significant reduction of inflammatory markers (CD40 ligand, vascular adhesion molecule-1, sP-selectin and modulating matrix metalloproteinase-9) after 14 days treatment with atorvarstatin 10-mg per day, but not for those with *TT* genotype ($P<0.05$). This observation suggested that for the primary prevention of cardiovascular events, subjects with *CC* genotype, who may be at increased cardiovascular risk, could benefit from treatment with statins.

The ADAMTS1 (*a d*isintegrin-like *a*nd *m*etalloproteinase with *t*hrombo*s*pondin motifs) matrix metalloproteinase is a protein that has been shown to cleave versican, a key proteoglycan that regulates vascular smooth muscle cell migration and contributes to the structural integrity of the fibrous cap in atherosclerotic lesions. Recently, the Ala227Pro polymorphism in the *ADAMTS1* gene has been found to be associated with CHD risk and benefit from pravastatin therapy in 2421 male subjects from the CARE study, a randomized trial of pravastatin versus placebo [Sabatine, M.S. *et al.* 2008]. In men not on pravastatin, those homozygous for the 227Pro allele of *ADAMTS1* had a nearly 2-fold increased risk of CHD events compared with non-carriers. In this high-risk group, treatment with pravastatin was highly efficacious, reducing the odds of fatal CHD or nonfatal MI by approximately 75%, as compared with 25% in non-carriers or heterozygotes [Sabatine, M.S. *et al.* 2008]. It was concluded that the greater benefit of pravastatin in individuals homozygous for the 227Pro allele could be due to either enhanced inhibition of ADAMTS-1 *via* statin-mediated pathways or that in such individuals the effect of statins on plaque stabilization is particularly important.

Sterol regulatory element binding factor (SREBF) -1a and SREBF cleavage activating protein (SCAP) regulate lipid homeostasis. Previous studies showed that polymorphisms in these two genes could modulate the lipid response to fluvastatin [Salek, L. *et al.* 2002] and simvastatin [Fiegenbaum, M. *et al.* 2005c], respectively. There was a strong graded genotype-treatment interaction between SREBF-1a *-36del/G* genotypes and change in Apo A-I levels in response to fluvastatin (16.5% increase in *GG*, 10.5% in *del/G*, and 0.4% in *del/del* groups). In another study, the mean percentage decrease in total cholesterol was greater in carriers of the *SCAP 2386G* allele compared with those homozygous for the *2386A* allele (-29.6 ± 13.4% vs. -22.1 ±13.8%, $P=0.007$) after treatment with simvastatin 20-mg per day over 6 months. However, more recently, Arazi *et al* did not find a significant association between these variants and the response to atorvastatin, which suggested that polymorphisms in *SREBF1* and *SCAP* may play a minor role in determining the pharmacological response to statins [Arazi, S.S. *et al.* 2008].

Oxidized-LDL (ox-LDL) is involved in atherothrombosis by induction of endothelial dysfunction and thrombosis. The specific receptor lectin-like oxidized-LDL receptor-1 (LOX-1) is thought to be important in plaque formation, progression and thrombosis due to the expression both in endothelial cells and monocytes. Polymorphisms in this gene (3'UTR/T) have been found to be related with reduced anti-platelet activity associated with statin therapy [Puccetti, L. *et al.* 2005] and cardiovascular event rates during statin treatment [Puccetti, L. *et al.* 2007].

Recently, more and more genetic variants have been identified to be associated with cardiovascular risk and various manifestations of atherosclerosis [Jang, Y. *et al.* 2008; Humphries, S.E. *et al.* 2008; Remmler, C. and Cascorbi, I. 2008; Trompet, S. *et al.* 2007], which may influence the beneficial effects from treatment with statins and may be worthy of more attention in future studies.

## CONCLUDING REMARKS

As the statins are already used on a very wide scale and with increasing evidence to support aggressive lipid lowering with these drugs, this field of pharmacogenetic research is receiving considerable attention and many new findings have been reported recently. Currently the data are not sufficient to support any genetic testing to predict the efficacy or adverse effects from statins, but recent studies have identified areas of potential importance. Polymorphisms in drug metabolizing enzymes and transporters have been shown to influence single dose pharmacokinetics with the statins, but their effect on long term lipid responses is less clear. The polymorphisms in genes related to the lipid metabolism and other pleiotropic effects of statins are likely to influence the beneficial effects of all statins, except perhaps for those in the HMG-CoA reductase enzyme itself which may have variable effects with different statins. Certain haplotypes of *HMGCR* in particular ethnic groups appear to be associated with reduced statin efficacy and it may be expedient to use additional alternative treatments in such cases. Conversely, subjects at high risk with particular *KIF6* or *ADAMTS1* genotypes may benefit more from statin therapy. Future pharmacogenetic studies of statins regarding the pharmacodynanic outcomes will provide more solid evidence for optimizing statin usage in clinical practice.

Although the reason for statin-related myopathy in some patients is largely unknown, various mechanisms have been proposed to explain statin myotoxicity including altered pharmacokinetics due to drug metabolism or drug-drug interactions, physicochemical properties of the drugs, effects on metabolic end products such as coenzyme Q10, and interference with metabolic pathways regulating muscle repair [Ruano, G. *et al.* 2007]. Potential candidate genes related to these mechanisms might be determinants of statin intolerance and genetic variation in candidate genes might be associated with inter-individual variation in statin-induced myotoxicity, which need to be verified in future studies. The recent finding of the common variant in *SLCO1B1* being the only polymorphism in the genomewide scan study associated with increased risk of myopathy with high dose simvastatin suggests this may be an important target for future study to improve statin safety [Link, E. *et al.* 2008].

The rapid developments in this field of pharmacogenetic and pharmacogenomic studies of statin therapy are likely to provide a better understanding of the effects of these drugs in different people and to help with prediction of the most appropriate drug and dosage for each individual and whether the addition or substitution of other lipid modifying drugs may be necessary to achieve the most safe and effective prevention of coronary heart disease.

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## ABBREVIATIONS

| | | |
|---|---|---|
| ABC | = | ATP-binding cassette |
| ABCP | = | Placenta-specific ATP-binding cassette transporter |
| ADAMTS1 | = | A disintegrin-like and metalloproteinase with thrombospondin motifs |
| ADRs | = | Adverse drug reactions |
| Apo A1 | = | Apolipoprotein A-I |
| Apo E | = | Apolipoprotein E |
| BCRP | = | Breast cancer resistance protein |
| BSEP | = | Bile acid export pump |
| CABG | = | Coronary artery bypass graft |
| CAD | = | Coronary artery disease |
| CETP | = | Cholesteryl ester transfer protein gene |
| CHD | = | Coronary heart disease |
| CRP | = | C-reactive protein |
| CYP | = | Cytochrome P-450 |
| CYP7A1 | = | Cholesterol 7alpha-hydroxylase |
| eNOS | = | Endothelial nitric oxide synthase |
| ESR1 | = | Estrogen receptor alpha |
| FH | = | Familial hypercholesterolemia |
| HDL-C | = | High-density lipoprotein-cholesterol |
| HMG-CoA | = | 3-hydroxy-3-methylglutaryl coenzyme A |
| IC50 | = | Inhibitor concentration to produce 50% inhibition |

| | | |
|---|---|---|
| KIF6 | = | Kinesin-like protein 6 |
| LDL-C | = | Low-density lipoprotein-cholesterol |
| LDLR | = | LDL receptor |
| LEPR | = | Leptin receptor |
| LIPC | = | Hepatic lipase |
| LOX-1 | = | Lectin-like oxidized-LDL receptor-1 |
| LPL | = | Lipoprotein lipase |
| MCT | = | Monocarboxylate transporter |
| MDR1 | = | Multidrug resistance protein |
| MI | = | Myocardial infarction |
| MRP2 | = | Multidrug resistance-associated protein 2 |
| MXR | = | Mitoxantrone resistant protein |
| NA | = | Not available |
| NO | = | Nitric oxide |
| NTCP | = | Sodium-dependent taurocholate cotransporting polypeptide |
| OAT | = | Organic anion transporter |
| ox-LDL | = | Oxidized-LDL |
| PCSK9 | = | Proprotein convertase subtilisin/kexin type 9 |
| P-gp | = | P-glycoprotein |
| PON | = | Paraoxonase |
| PPAR | = | Peroxisome proliferator- activated receptor |
| SCAP | = | SREBF cleavage activating protein |
| SLC | = | Solute carrier transporter |
| SLCO | = | Solute carrier organic anion transporter family member |
| SNPs | = | Single nucleotide polymorphisms |
| SREBF-1a | = | Sterol regulatory element binding factor-1a |
| TC | = | Total cholesterol |
| TG | = | Triglycerides |
| UGT | = | Uridine diphosphate glucuronosyltransferases |

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# ABCG2 Polymorphism Is Associated With the Low-Density Lipoprotein Cholesterol Response to Rosuvastatin

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**The ATP-binding cassette G2 (*ABCG2*) c.421C>A (rs2231142) polymorphism influences the pharmacokinetics of rosuvastatin. We examined whether this polymorphism influences the low-density lipoprotein cholesterol (LDL-C)-lowering efficacy of the drug. In 305 Chinese patients with hypercholesterolemia who were treated with rosuvastatin at a dosage of 10 mg daily, the c.421A variant was found to be significantly associated with greater reduction in LDL-C level, in a gene-dose-dependent manner. As compared with subjects with the c.421CC genotype, those with the c.421AA genotype showed a 6.9% greater reduction in LDL-C level, which would be equivalent to the effect obtained by doubling the dose of rosuvastatin.**

The extent of reduction in the level of low-density lipoprotein cholesterol (LDL-C) in response to doses of any of the hydroxymethylglutaryl coenzyme A reductase inhibitors or statins varies widely between individuals, partly because of genetic differences.[1] A more intensive reduction in LDL-C level with the use of statins is associated with greater reduction in cardiovascular events in a wide range of subject groups;[2] however, there is potential for a concomitant increase in the risk of toxicity, and therefore optimizing the choice of statin and dose for an individual patient may have beneficial effects.

Rosuvastatin is one of the few drugs for which regulatory authorities, including the US Food and Drug Administration, have recommended starting with lower doses (5 mg instead of 10 mg) in Asian patients.[3] This recommendation was based on data from single-dose pharmacokinetic studies that showed that systemic exposure to rosuvastatin was approximately twice as high in Asians as in non-Asians.[4] These differences are unlikely to be due to ethnicity-related variations in drug metabolizing enzymes, given that rosuvastatin undergoes relatively little enzymic modification and is a substrate for a number of drug transporters that influence its disposition.[5,6]

The efflux transporter ATP-binding cassette G2 (ABCG2) plays a significant role in the disposition of rosuvastatin *in vitro*.[7] The c.421C>A (rs2231142, Gln141Lys) single-nucleotide polymorphism of *ABCG2* influences the pharmacokinetics of rosuvastatin in Chinese and Caucasian subjects.[8,9] We examined whether this *ABCG2* single-nucleotide polymorphism influences the reduction of LDL-C levels when rosuvastatin is administered to Chinese patients with hypercholesterolemia, including some with familial hypercholesterolemia (FH).

## RESULTS

### Characteristics of participants

The frequency of the c.421A variant allele in 305 Han Chinese subjects with hypercholesterolemia who showed good adherence to the treatment regimen was 30.5%. This is similar to the prevalence previously reported among other Asians.[10] The genotype distribution was consistent with Hardy–Weinberg equilibrium. The baseline characteristics and lipid profiles after rosuvastatin treatment, stratified by *ABCG2* c.421C>A genotypes, are shown in **Table 1**. Rosuvastatin was well tolerated by all the participants; none had clinically relevant elevations in creatine kinase, alanine aminotransferase, or creatinine, and no muscle problems were observed.

### Association between *ABCG2* c.421C>A polymorphism and lipid responses

There were significant differences (overall $P = 0.0006$) in the percentage reductions in LDL-C level between c.421C>A

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**Table 1 Characteristics and lipid profiles of patients stratified by *ABCG2* c.421C>A genotype**

| | *ABCG2* c.421C>A | | | | |
|---|---|---|---|---|---|
| | All genotypes ($n = 305$) | c.421CC ($n = 158$) | c.421CA ($n = 108$) | c.421AA ($n = 39$) | *P* value[a] |
| *Characteristics* | | | | | |
| Age (years) | 56.7 ± 11.3 | 56.8 ± 12.3 | 57.2 ± 9.9 | 55.0 ± 0.9 | 0.91 |
| Male, *n* (%) | 139 (45.6) | 77 (48.7) | 45 (41.7) | 17 (43.6) | 0.51 |
| Body weight (kg) | 65.1 ± 13.7 | 65.1 ± 14.0 | 64.4 ± 14.0 | 67.2 ± 11.5 | 0.32 |
| Body height (m) | 1.59 ± 0.09 | 1.59 ± 0.09 | 1.59 ± 0.08 | 1.60 ± 0.08 | 0.81 |
| Body mass index (kg/m$^2$) | 25.4 ± 4.2 | 25.5 ± 4.1 | 25.1 ± 4.4 | 26.1 ± 3.7 | 0.17 |
| Waist circumference (cm) | 87.1 ± 11.0 | 87.0 ± 11.5 | 86.6 ± 11.6 | 89.1 ± 11.2 | 0.93 |
| Hip circumference (cm) | 97.9 ± 7.6 | 97.7 ± 7.9 | 97.5 ± 7.6 | 99.8 ± 6.7 | 0.07 |
| Waist/hip ratio | 0.89 ± 0.08 | 0.89 ± 0.08 | 0.89 ± 0.07 | 0.89 ± 0.08 | 0.92 |
| Body fat (%) | 30.5 ± 8.6 | 30.0 ± 8.2 | 30.9 ± 8.8 | 31.2 ± 9.4 | 0.59 |
| FH, *n* (%) | 137 (44.9) | 67 (42.4) | 51 (46.3) | 20 (51.3) | 0.57 |
| Diabetes mellitus, *n* (%) | 94 (30.8) | 55 (34.8) | 30 (27.8) | 9 (23.1) | 0.25 |
| Hypertension, *n* (%) | 166 (54.4) | 90 (57.0) | 57 (52.8) | 19 (48.7) | 0.59 |
| Current drinker, *n* (%)[b] | 28 (9.6) | 18 (11.9) | 5 (4.9) | 5 (12.8) | 0.14 |
| Current smoker, *n* (%)[b] | 29 (9.9) | 13 (8.6) | 11 (10.8) | 5 (12.8) | 0.69 |
| *Baseline lipid levels (mmol/l)* | | | | | |
| Total cholesterol | 7.68 ± 1.62 | 7.64 ± 1.54 | 7.69 ± 1.83 | 7.77 ± 1.35 | 0.71 |
| LDL-C | 5.51 ± 3.87 | 5.70 ± 5.14 | 5.30 ± 1.77 | 5.33 ± 1.29 | 0.60 |
| HDL-C | 1.52 ± 0.40 | 1.48 ± 0.37 | 1.56 ± 0.43 | 1.56 ± 0.43 | 0.30 |
| Triglycerides | 1.88 ± 0.89 | 1.88 ± 0.91 | 1.84 ± 0.87 | 1.93 ± 0.87 | 0.86 |
| *Lipid levels on treatment (mmol/l)* | | | | | |
| Total cholesterol | 4.69 ± 1.04 | 4.80 ± 1.05 | 4.57 ± 1.06 | 4.56 ± 0.89 | 0.09 |
| LDL-C | 2.52 ± 0.94 | 2.64 ± 0.94 | 2.42 ± 0.99 | 2.31 ± 0.77 | 0.023 |
| HDL-C | 1.54 ± 0.41 | 1.52 ± 0.40 | 1.56 ± 0.42 | 1.58 ± 0.44 | 0.73 |
| Triglycerides | 1.38 ± 0.63 | 1.41 ± 0.62 | 1.31 ± 0.61 | 1.48 ± 0.74 | 0.38 |

Data are given as mean ± SD or *n* (%).

ANOVA, analysis of variance; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

[a]Significance determined by ANOVA or Kruskal–Wallis test for continuous variables with normal or non-normal distribution, respectively, or by $\chi^2$ test for categorical characteristics. [b]Data missing for 13 patients, with 7 in CC and 6 in CA.

genotype groups, with a gene–dose effect observed (**Figure 1**). Patients with the c.421AA genotype had a 6.9% greater reduction in LDL-C level than did those with the c.421CC genotype; c.421CA heterozygote subjects had intermediate values after adjusting for age, gender, presence of FH, and baseline LDL-C level (**Table 2**). This polymorphism accounted for 5.4% of the interindividual variance in LDL-C response to rosuvastatin. The significant association of the *ABCG2* c.421C>A polymorphism with lipid responses to rosuvastatin remained even when the data from FH and non-FH patient subgroups were analyzed separately. The magnitude of the difference in LDL-C response among the genotype groups was similar for FH and non-FH patient subgroups (**Table 2**).

The percentage reduction in total cholesterol showed a similar pattern, with 4.1 and 3.2% greater reduction in total cholesterol in c.421AA subjects and c.421CA subjects, respectively, than in c.421CC subjects. No significant differences were found among the genotype groups with regard to high-density lipoprotein cholesterol and triglyceride responses (**Table 2**).

### Findings in patients with rheumatoid arthritis treated with rosuvastatin

In the group of 36 patients with rheumatoid arthritis (RA), the mean age was 53.6 ± 5.9 years, and 25 (75%) of them were women. The frequency of the c.421A variant allele in patients with RA was 41.7%, and the genotype distribution was in Hardy–Weinberg equilibrium. The baseline lipids in this group of patients were lower than in the patients with hypercholesterolemia (except for high-density lipoprotein cholesterol), but the percentage changes in lipids after administration of rosuvastatin to this group with RA were similar to the results of the main study, and subjects with the *ABCG2* c.421C>A variant allele showed a higher percentage of reduction in levels of LDL-C, total cholesterol, and triglycerides than did subjects with c.421CC (**Table 2**).

**Table 2 Association of lipid responses to rosuvastatin 10 mg/day treatment and *ABCG2* c.421C>A genotype**

| | % Change | c.421CC ($n = 158$) | c.421CA ($n = 108$) | c.421AA ($n = 39$) | *P* value[a] |
|---|---|---|---|---|---|
| All hypercholesterolemic patients combined | LDL-C | −50.2 (−52.0, −48.4) | −54.3 (−56.5, −52.1) | −57.0 (−60.6, −53.4) | 0.0006 |
| | TC | −36.9 (−38.2, −35.6) | −40.1 (−41.7, −38.5) | −41.0 (−43.7, −38.4) | 0.0016 |
| | HDL-C | 2.8 (0.8, 4.9) | 0.6 (−1.8, 3.0) | 2.2 (−1.9, 6.2) | 0.372 |
| | TG[b] | −26.5 (−39.4, −2.6) | −28.9 (−40.1, −15.2) | −17.6 (−41.7, 6.2) | 0.434 |
| FH patients | | ($n = 67$) | ($n = 50$) | ($n = 20$) | *P* value[a] |
| | LDL-C | −48.8 (−51.2, −46.4) | −53.5 (−56.3, −50.7) | −55.4 (−59.9, −50.9) | 0.009 |
| | TC | −37.4 (−39.3, −35.5) | −41.8 (−44.0, −39.6) | −41.7 (−45.2, −38.3) | 0.0056 |
| | HDL-C | 6.2 (3.1, 9.3) | −2.5 (−6.1, 1.0) | 1.4 (−4.4, 7.1) | 0.002 |
| | TG[b] | −27.3 (−40.0, −10.0) | −29.4 (−37.0, −16.5) | −24.4 (−38.2, 0.0) | 0.767 |
| Non-FH patients | | ($n = 91$) | ($n = 58$) | ($n = 19$) | *P* value[a] |
| | LDL-C | −51.3 (−53. 9, −48.7) | −54.8 (−58.0, −51.5) | −58.7 (−64.4, −53.1) | 0.038 |
| | TC | −36.4 (−38.3, −34.5) | −38.6 (−40.9, −36.2) | −40.8 (−45.0, −36.7) | 0.099 |
| | HDL-C | 0.6 (−1.9, 3.2) | 3.0 (−0.2, 6.2) | 2.8 (−2.8, 8.4) | 0.49 |
| | TG[b] | −24.8 (−38.0, 3.6) | −27.9 (−44.9, −14.9) | −12.5 (−44.2, 10.0) | 0.399 |
| RA patients | | ($n = 14$) | ($n = 14$) | ($n = 8$) | *P* value[c] |
| | LDL-C | −47.8 ± 11.3 | −49.6 ± 13.0 | −60. 9 ± 9.1 | 0.022 |
| | TC | −28.6 ± 8.2 | −33.5 ± 8.9 | −39.7 ± 8.9 | 0.041 |
| | HDL-C[b] | −6.9 (−8.8, 7.7) | −9.9 (−20.0, 6.6) | −2.9 (−10.8, 6.2) | 0.52 |
| | TG[b] | −9.2 (−19.9, −9.2) | −19.6 (−40.6, 0.0) | −31.0 (−38.7, −20.9) | 0.006 |

Data are given as least-square mean (95% confidence interval) or mean ± SD except as noted.

ANCOVA, analysis of covariance; ANOVA, analysis of variance; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RA, rheumatoid arthritis; TC, total cholesterol; TG, triglycerides.

[a]Overall significance determined by ANCOVA, including age, gender, presence of FH (in all participants combined only), and baseline lipid level as covariates for percentage change in TC, LDL-C, and HDL-C from baseline, and by Kruskal–Wallis test for percentage change in TG from baseline, respectively. [b]Median (interquartile range). [c]Significance as determined by ANOVA or Kruskal–Wallis test for continuous variables with normal and non-normal distribution, respectively.

## DISCUSSION

The finding that there is greater reduction in LDL-C level in subjects with the *ABCG2* c.421AA genotype is consistent with the data from pharmacokinetics studies in Chinese and Caucasian subjects that showed approximately twice the level of systemic exposure to rosuvastatin in subjects with at least one c.421A allele as compared with those with the c.421CC genotype.[8,9] The c.421A allele results in lower expression levels of the ABCG2 efflux transporter protein and a reduced ability to export substrate, leading to an increase in drug accumulation both in hepatocytes and in the systemic circulation.[11] In a recent pharmacokinetics study with rosuvastatin, c.421A heterozygote and c.421A homozygote subjects were found to have area under the curve values 22 and 144% higher, respectively, than those for c.421CC homozygote subjects.[8,9] Because rosuvastatin shows dose-linear pharmacokinetics,[12] the plasma concentrations achieved in c.421AA homozygote subjects would be equivalent to having received at least double the dose received by c.421CC homozygote subjects. The high frequency of the c.421A allele in Chinese and Japanese populations (~35%) as compared with Caucasians (14%) suggests that this polymorphism contributes to the variation between populations in the pharmacokinetics of rosuvastatin and potentially to the treatment outcomes of rosuvastatin.[13]

Although the average plasma exposure of rosuvastatin in Asians was twofold greater than that in Caucasians, the efficacy and safety of rosuvastatin—or other statins, for that matter—have not been shown to be different between Asian and Western populations.[14] A study of rosuvastatin at a dosage of 10 mg daily administered to Asian subjects found that the reduction in LDL-C level in 515 evaluable patients was 47.5%, which is similar to data from studies in Caucasians.[15,16] It may be difficult to identify ethnicity-related differences in the lipid response to statins, because doubling the dose of a statin typically only reduces the LDL-C level by a further 6% from the original baseline, and many other variables influence the lipid responses.[17]

The disposition of various statins is influenced to a variable extent by the *ABCG2* c.421C>A polymorphism. The effect was found to be greater with rosuvastatin than with atorvastatin,[8] and there was no significant effect on the pharmacokinetics of pravastatin and pitavastatin.[18,19] Some substrates of ABCG2, such as imatinib and gefitinib, have also been reported to be inhibitors of this transporter.[20] It is still unclear whether these substrate statins could influence the activity of ABCG2. The *ABCG2* c.421C>A polymorphism might also have a direct influence on cardiovascular risk; in a recent genome-wide association study, this was found to be one of three loci associated with increased serum uric acid concentrations.[21]

The c.521T>C polymorphism in the uptake transporter gene *SLCO1B1* is associated with increased systemic exposure to rosuvastatin;[22,23] however, in a study carried out in Singapore,

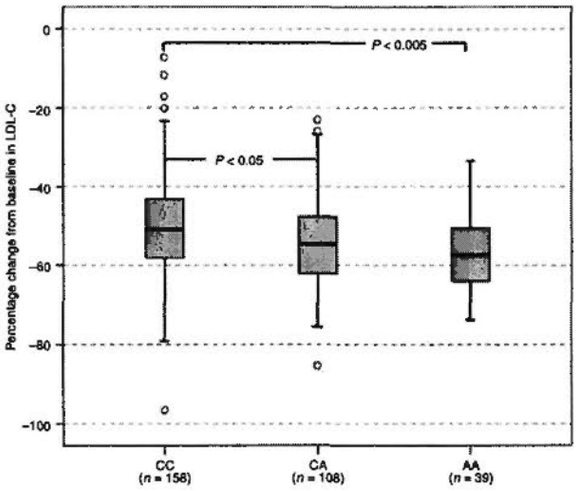


**Figure 1** Percentage change in LDL-C level from baseline after rosuvastatin 10 mg/day treatment, stratified by *ABCG2* c.421C>A genotypes, in 305 patients with good adherence to the treatment regimen. Box and whisker plots with median, interquartile range, bar indicating 95% confidence interval, and circles indicating outliers. *ABCG2*, ATP-binding cassette G2; LDL-C, low-density lipoprotein cholesterol.

this single-nucleotide polymorphism and associated haplotypes (*5 and *15) did not explain the differences in rosuvastatin pharmacokinetics between different ethnic groups.[24] Decreased activity of this transporter may result in reduced statin efficacy because less of the drug enters the hepatocytes, but plasma concentrations would be increased, thereby increasing the risk of muscle toxicity.[6,25] Indeed, a genome-wide scan for genetic markers for increased risk of myopathy associated with high-dose simvastatin yielded a single strong association with a non-coding *SLCO1B1* single-nucleotide polymorphism that was in nearly complete linkage disequilibrium with c.521T>C. The latter was also associated with a decrease in the reduction of LDL-C level, despite the presumed increase in plasma levels of the drug.[26] Further studies are warranted to examine whether variants in this transporter and other candidate genes related to statin pharmacokinetics would affect the lipid response.

We considered it reasonable to combine data from subjects with and without FH. Although it has previously been suggested that subjects with heterozygous FH might show differing responses to statins depending on the type of mutation in the LDL receptor, there have been no consistent data to support this view.[27,28] In the present study, the percentage changes in lipids were not significantly different between patients with and without FH, although the baseline values of LDL-C and high-density lipoprotein cholesterol were higher in the FH patients. Furthermore, the significant effect of this polymorphism on the lipid response to rosuvastatin was observed in both subgroups separately.

To our knowledge, this study is the first to demonstrate the association between the *ABCG2* c.421C>A polymorphism and the lipid response to rosuvastatin in Asians. The association of the *ABCG2* c.421C>A polymorphism and lipid response to rosuvastatin in the small number of RA subjects suggests a robust effect. A recent study in Caucasian patients with acute coronary syndrome, published as an abstract, reported similar findings. It showed that the *ABCG2* c.421C>A polymorphism was related to the plasma LDL-C concentration after 3 months of treatment with rosuvastatin at a dosage of 10 mg daily.[29] Patients with one or two c.421A variant alleles achieved significantly lower mean LDL-C levels than did individuals with c.421CC (1.78 vs. 1.98 mmol/l; $P = 0.012$), which was similar to our findings (**Table 1**). This independent study in Caucasians, in whom the frequency of the c.421A variant allele was only 12%, supports our findings and emphasizes the importance of this variant in the lipid response to rosuvastatin in different ethnic groups with different frequencies of the polymorphism.

In conclusion, the *ABCG2* c.421C>A polymorphism plays an important role in LDL-C response to rosuvastatin in Chinese subjects. The greater reduction in LDL-C level in patients with the c.421AA genotype as compared with those with the c.421CC genotype was equivalent to at least doubling the dose of rosuvastatin. This polymorphism is likely to play a major role in the ethnicity-related differences observed in rosuvastatin pharmacokinetics, given the high frequency of the A allele in Chinese and other East Asian populations. Also, these pharmacokinetic differences are likely to correspond to differences in efficacy of reduction in LDL-C levels. Future analyses of clinical outcome studies are warranted to identify whether genetic variations influencing the lipid response to statins can be translated into differences in the actual clinical outcome response to this class of medications.

## METHODS

**Study design.** The patients recruited were of Han Chinese origin, aged ≥18 years, who were considered to be at increased risk of coronary heart disease, or who had FH and baseline LDL-C level >2.6 mmol/l, and were being treated or were about to be treated with rosuvastatin at a dosage of 10 mg daily. The diagnosis of FH was made on the basis of clinical criteria appropriate for Chinese subjects,[30] and the FH patients in the study belonged to different families. Patients who were already on 10 mg of rosuvastatin were included only if their baseline lipid profile for at least 4 weeks without any lipid-lowering treatment was available within the previous year. Patients who were not yet on lipid-lowering treatment were prescribed rosuvastatin at a dosage of 10 mg once daily. All the subjects were advised to continue with their usual diet and other aspects of lifestyle during the study. The participants were interviewed in order to assess drug compliance and tolerability and efficacy of rosuvastatin at a scheduled follow-up visit after at least 4 weeks on treatment (median 12 weeks). Blood samples were collected in the fasting state for measuring lipid profiles and laboratory safety data. The levels of LDL-C were calculated according to the Friedewald formula. Safety was assessed in all participants by recording laboratory safety data and adverse events. Individuals with uncontrolled medical conditions, triglyceride levels >4.5 mmol/l, or poor compliance (<80%) were excluded from the study. The primary outcome of this study was the difference in the percentage reduction in LDL-C level among the different genotype groups.

The study protocol was approved by the local clinical research ethics committee, and the participants gave written informed consent before any study procedures were undertaken.

**Findings in patients with RA.** We also tested the association with genotype in 36 patients with RA who had participated in a double-blind, placebo-controlled, randomized study to assess the effect of rosuvastatin at a dosage of 10 mg daily on carotid intima-media thickness and pulse-wave velocity.

**Genotyping of ABCG2 c.421C>A polymorphism.** DNA taken from the participants was genotyped for the ABCG2 c.421C>A polymorphism using the Taqman Drug Metabolism Genotyping Assay (C_15854163_70) from Applied Biosystems (Foster City, CA). The polymerase chain reaction (PCR) solution consisted of 2.5 μl of 2× Taqman Universal PCR Master Mix (P/N 4304437, Applied Biosystems), 0.25 μl of the 20× Taqman Drug Metabolism Genotying Assay Mix, and 2.25 μl of ~5 ng/μl genomic DNA, and the PCR was performed using a PCR System 9700 machine (Applied Biosystems, Foster City, CA). The PCR cycle consisted of 2 min at 50 °C and 10 min at 95 °C, followed by 50 cycles of 15 s at 92 °C and 90 s at 60 °C. The end-point reading was recorded using an ABI Prism 7700 sequence detector (Applied Biosystems) in order to discriminate the alleles of the DNA samples.

**Statistical analysis.** Skewed data were logarithmically transformed before analysis. The $\chi^2$ test was used to test the Hardy–Weinberg equilibrium. Associations between *ABCG2* c.421C>A polymorphism and lipid responses were assessed by analysis of covariance followed by a *post hoc* test with the Bonferroni test, taking age, gender, presence of FH, and baseline level of lipids as covariates. The data were analyzed using SPSS, version 13.0 (SPSS, Chicago, IL).


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## CONFLICT OF INTEREST

B.T. has received research funding to perform clinical studies from AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi Sankyo, Kowa, Merck, Merck Sharp and Dohme, Novartis, Otsuka, Pfizer, Roche, Sanofi-Aventis, and Servier and has acted as a consultant or speaker for AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Kowa, Merck, Merck Sharp and Dohme, Novartis, Pfizer, Roche, Sanofi-Aventis, Schering-Plough, and Servier. G.T.C.K. has received research grants to perform clinical studies from Merck Sharp and Dohme. V.W.Y.L. has received research funding to perform clinical studies from AstraZeneca, Bayer, Boehringer Ingelheim, Merck Sharp and Dohme, and Pfizer. L.B. has received research funding from Novartis to perform animal studies. The other authors declared no conflict of interest.




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