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Effects of statin treatments and polymorphisms in *UGT1A1* and *SLCO1B1* on serum bilirubin levels in Chinese patients with hypercholesterolaemia

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ABSTRACT

Objectives: In vitro and animal studies showed that statins could increase bilirubin levels by activation of haem oxygenase-1, whereas the effect of statins on serum bilirubin levels in humans remains controversial. The organic anion transporting polypeptide 1B1 (OATP1B1, gene *SLCO1B1*) and UDP-glucuronosyltransferase 1A1 (*UGT1A1*) play an important role in the disposition of bilirubin. This study investigated 1) whether common polymorphisms in *UGT1A1* and *SLCO1B1* influence bilirubin levels; 2) whether statin treatments affect bilirubin levels; and 3) whether the polymorphisms examined influence the drug effect.

Methods: Associations between common polymorphisms in *UGT1A1* and *SLCO1B1* and the serum bilirubin levels on no lipid-lowering treatment were analyzed in 379 Chinese patients with hypercholesterolaemia. Effects of simvastatin 40 mg daily and rosuvastatin 10 mg daily on the bilirubin levels were compared in 236 subjects with good compliance to both statins.

Results: The *UGT1A1* polymorphisms associated with reduced enzyme activity were significantly associated with increased baseline bilirubin levels. The bilirubin levels were increased from a geometric mean (95% CI) of 10.9 (10.3–11.4) μmol/L at baseline to 11.6 (11.1–12.0) μmol/L with rosuvastatin and 12.5 (11.9–13.0) μmol/L with simvastatin and the increase was greater with simvastatin ($P < 0.001$). There was no relationship between polymorphisms in *UGT1A1* or *SLCO1B1* and changes in bilirubin levels with the two statins.

Conclusions: This study showed that the polymorphisms in *UGT1A1*, but not *SLCO1B1*, were associated with serum bilirubin levels in Chinese patients. Statins increased bilirubin levels and this effect was independent of the polymorphisms in *UGT1A1* and *SLCO1B1*.

1. Introduction

Serum total bilirubin concentrations have been inversely associated with the risk for coronary artery disease (CAD) [1]. Several recent studies have shown that low bilirubin levels are associated with coronary artery calcification in subjects with clinical suspicion of CAD [2] and in-stent restenosis in patients with CAD who underwent coronary stenting during an average follow-up period of 8 months [3]. Although the underlying mechanism for the association between bilirubin and atherosclerosis is not fully understood, there is increasing evidence suggesting the beneficial effects of bilirubin are probably related to its antioxidant and anti-inflammatory properties [4].

Haem oxygenase-1 (HO-1) is a stress-responsive enzyme that catalyzes the pro-inflammatory molecule haem into carbon monoxide, ferrous iron and biliverdin, which is subsequently converted to bilirubin by biliverdin reductase [5]. *In vitro* and animal studies showed that statins could upregulate HO-1 [6,7] and increase formation of carbon monoxide and bilirubin [8] which may contribute to the pleiotropic anti-atherogenic actions of this class of drug. However, this hypothesis has not been well tested in humans and the effect of statins on serum bilirubin levels remains controversial.

Ong et al have reported an unexpected association of statin usage with lower total bilirubin levels in subjects at high cardiovascular risk based on data from the United States National Health and Nutrition Examination Survey (NHANES) 1999–2008 [9]. However, due to the cross-sectional study design the study cannot establish the causality between serum bilirubin levels and statin treatment. More recently, a prospective study performed in 514 Caucasian patients with familial hypercholesterolaemia (FH) showed

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a significant 7% median increase in bilirubin levels after a high dose (80 mg) simvastatin treatment for a period of two years [10].

Serum bilirubin level is a heritable trait and recent genome-wide association studies (GWASs) have identified several genetic polymorphisms that contribute to variability in serum bilirubin levels, in particular variants in the organic anion transporting polypeptide 1B1 (OATP1B1) gene (*SLCO1B1*) and UDP-glucuronosyltransferase 1A1 (*UGT1A1*) [11], which are involved in the hepatic uptake and metabolism of bilirubin, respectively (Fig. 1). Although the hepatic efflux transporters ABCC2 and ABCC3 appear to play a major role in the basolateral excretion of bilirubin-glucuronides (Fig. 1) and loss of function mutations in ABCC2 cause Dubin–Johnson syndrome [12,13], a condition in humans characterized by conjugated hyperbilirubinaemia, these genes were not found to affect serum bilirubin levels in the GWAS reports.

A TA dinucleotide insertion in the TATA box of the *UGT1A1* promoter, *UGT1A1*28* ($TA_{6>7}$) resulting in 70% reduction of UGT1A1 gene transcription, has been strongly associated with unconjugated hyperbilirubinaemia and this polymorphism is more common in Europeans and Africans (~40%) than in that in Asians (16%) [14]. Moreover, the association between the *SLCO1B1* polymorphism and bilirubin levels has been less evident in Asians than that in Caucasians [15,16].

The aim of the present study was to verify 1) whether common polymorphisms in *UGT1A1* and *SLCO1B1* influence the serum bilirubin levels; 2) whether statin treatments increase serum bilirubin levels; and 3) whether these genetic polymorphisms would influence any drug effect in Chinese patients with hypercholesterolaemia.

2. Methods

2.1. Study design and subjects

The present analysis was performed in a cohort of Han Chinese patients who had previously been involved in a study on pharmacogenetics of statins [17], in which after a 4–6 weeks washout period, subjects started monotherapy with rosuvastatin 10 mg daily for at least 4 weeks (more than 97% of patients had at least 6 weeks treatment). These patients were subsequently switched to treatment with simvastatin 40 mg daily if appropriate. If they had already been treated with simvastatin 40 mg daily before starting rosuvastatin 10 mg and had lipid values available after at least 4 weeks treatment with good self-reported adherence, those data were used for the analysis of the response on simvastatin. These study participants were Han Chinese patients aged ≥18 years with established coronary heart disease (CHD) or CHD risk equivalent [18] including some with FH. The characteristics of the study design have been previously reported [17]. Patients were excluded if they had uncontrolled diabetes, hypertension, or thyroid disease; had acute renal/liver disease, or significant renal or hepatic dysfunction; or had experienced a cardiovascular event within the 3 months before recruitment. No other lipid-lowering medication was allowed and all other concomitant medications were unchanged during the study period.

Blood samples were taken after overnight fasting at baseline and after treatment with rosuvastatin and simvastatin, respectively for measurement of lipids and laboratory safety tests including total bilirubin and liver enzymes using standard methods in the local hospital laboratory. The study protocol was approved by the local Clinical Research Ethics Committee and was performed in accordance with the Declaration of Helsinki. All participants gave written informed consent.

2.2. Genotyping and sequencing for polymorphisms

Three common variants in *UGT1A1*: *6 (211G>A, rs4148323), *28 and *60 (-3279T>G, rs4124874) and two nonsynonymous single nucleotide polymorphisms (SNPs) 388A>G (rs2306283) and 521T>C (rs4149056) in *SLCO1B1* were selected due to their potential effect on gene expression or enzyme/transporter activity. Genotyping of *UGT1A1* *6 and *60 and *SLCO1B1* 388A>G and 521T>C was performed in the Genome Research Centre, University of Hong Kong using the mass-spectroscopy based, high-throughput MassARRAY iPLEXTM platform (Sequenom, San Diego, CA).

The (TA)n repeats in the *UGT1A1* promoter were examined for determining *28 genotype by sequencing in Tech Dragon Limited (www.techdragon.com.hk) using the high throughput Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). The chromatogram for the sequence was viewed by the Chromas Lite 2.01 (Technelysium Pty Ltd, Helensvale, Australia). Patients were designated as 6/6 (*1/*1), 6/7 (*1/*28), or 7/7 (*28/*28) depending on the number of TA repeats in the *UGT1A1* promoter region. All SNPs genotyped were in Hardy–Weinberg equilibrium (χ^2 test $P > 0.05$).

2.3. Statistical analysis

Continuous variables with skewed distributions were log-transformed before analysis and are expressed as a geometric

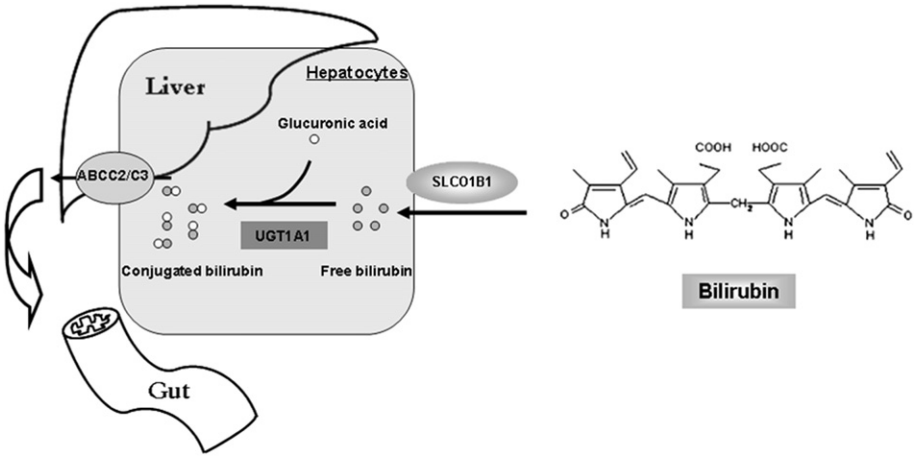

Fig. 1. Roles of *SLCO1B1* and *UGT1A1* in the disposition of bilirubin.