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ScienceDirect
Biochemical and Biophysical Research Communications 363 (2007) 395–398
BBRC
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# Effect of ScrF I polymorphism in the 2nd intron of the HMGCR gene on lipid-lowering response to simvastatin in Chinese diabetic patients

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Received 23 August 2007
Available online 10 September 2007

**Abstract**

*Objective:* To investigate whether ScrF I polymorphism in the 2nd intron of the HMG-COA reductase gene (HMGCR) influences serum lipid levels and whether this polymorphism affects the efficiency of the cholesterol lowering HMG-CoA reductase inhibitor, simvastatin.

*Methods:* One hundred sixty-eight patients with type 2 diabetes mellitus (T2DM) prospectively received simvastatin as a single-agent therapy (20 mg day-1 p.o.) for 12 weeks. Serum lipid levels were determined before and after simvastatin treatment. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP).

*Results:* Subjects with the AA homozygotes had significantly higher serum very low-density lipoprotein cholesterol (VLDL-C) levels than those with the aa homozygotes. In addition, in 168 patients with T2DM who took 20 mg simvastatin, the VLDL-C lowering effect by simvastatin in subjects with the aa homozygotes was significantly lower than in those with the Aa heterozygotes and AA homozygotes.

*Conclusions:* Simvastatin treatment significantly decreased plasma lipids in all patients ($P < 0.01$). Importantly, we demonstrate that ScrF I polymorphism of the HMGCR gene in patients with T2DM groups is associated with significant elevation of serum VLDL-C levels. Subjects with the AA homozygotes had significantly higher serum high VLDL-C levels than those with the Aa heterozygotes and aa homozygotes (AA: $2.18 \pm 0.51$; Aa: $2.04 \pm 0.59$, aa: $1.86 \pm 0.43$, $P < 0.05$ for comparison among three genotypes and $P < 0.01$ for difference between AA and aa). Furthermore, this polymorphism tends to show an enhanced response to an HMG-CoA reductase inhibitor in terms of the cholesterol-lowering effect. In 168 patients with T2DM who took 20 mg simvastatin, the VLDL-C lowering effect by simvastatin in subjects with the AA homozygotes was significantly lower than in those with the Aa heterozygotes and aa homozygotes (the reduction in serum VLDL-C levels; $37.03 \pm 5.67$ versus $28.97 \pm 4.96$, $P < 0.01$; $34.62 \pm 5.87$ versus $28.97 \pm 4.96$, $P < 0.05$). These results suggest that the HMGCR gene may serve as a modifier gene for hypercholesterolemia in Chinese diabetic patients.


*Keywords:* HMG-COA reductase inhibitor; Genetic polymorphism; Cholesterol; ScrF I

Type 2 diabetes is a common, multifactorial disease with genetic predisposition that is strongly influenced by environmental and behavioral factors, such as obesity and sedentary lifestyle. Previous studies have indicated that obesity, central obesity, physical inactivity, and a family history of diabetes are major risk factors for type 2 diabetes [1]. Furthermore, dyslipidemias related to insulin resistance, high total triglyceride level, low high-density lipoprotein cholesterol (HDL-C) level, and small dense low-density lipoprotein (LDL) particles, are risk factors for diabetes [2,3]. Therefore, variants in genes regulating dyslipidemias are potential risk factors for type 2 diabetes.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the formation of mevalonate from HMG-CoA and is thought to be the rate-limiting enzyme

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doi:10.1016/j.bbrc.2007.08.182

for cholesterol synthesis in liver. 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors, or statins, are potent inhibitors of cholesterol biosynthesis that are used extensively to treat patients with hypercholesterolemia [4–7]. Statins impair cholesterol biosynthesis by inhibiting activity of the enzyme HMG-CoA reductase, the rate-limiting step in cholesterol synthesis. This both decreases circulating lipoproteins and increases their hepatic uptake by up-regulating LDL receptors. The overall lipid-lowering effect of statins includes increased uptake and degradation of LDL, inhibition of LDL oxidation, a reduction in cholesterol accumulation and esterification, and decreased lipoprotein secretion and cholesterol synthesis [4–10].

Analyses of single nucleotide polymorphisms (SNPs) are useful to understand the pathogenesis of diseases as well as their pharmacogenetic traits [11]. A recent study has reported that the HMGCR gene polymorphism with levels of lipids [12]. The result prompted us to explore the clinical significance of the HMGCR gene polymorphism in lipid metabolism as well. To investigate whether ScrF I polymorphism in the 2nd intron of the HMGCR gene influences serum lipid levels in humans, we genotyped in Chinese Northern Han population with T2DM. Furthermore, we also studied the influence of this polymorphism on the effectiveness of cholesterol-lowering therapy by the HMG-CoA reductase inhibitor, simvastatin.

## Subjects and methods

*Subjects.* The study was approved by the Clinical Research Ethics Committee of Harbin Medical University, and all subjects gave written informed consent. A total of 168 T2DM patients with hyperlipidaemia were enrolled (91 females, 77 males; age range 56–73 years). None of the subjects had taken HMG-CoA reductase inhibitors (statins) previously and all strictly abstained from smoking, alcohol, and caffeine during treatment. Subjects with liver or renal failure, dropsical nephritis, liver or kidney transplantation were excluded from participation in the study. We also studied 78 patients with hypercholesterolemia, who took 5 mg simvastatin nightly [1]. Among subjects studied, there were neither patients with familial hypercholesterolemia nor familial combined hyperlipidemia (FCHL) [13,14]. Serum total cholesterol (TC), triglyceride (TG), very low-density lipoprotein cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were measured before and after 12 weeks of therapy. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula.

*Study design.* Investigators and participants were blind to genotype data during collection of the phenotypic data. Similarly, genotyping was performed blind to the phenotypic data. All subjects were asked to take low-fat diet and prospectively treated with an oral 20-mg daily dose of simvastatin at 21:00 h before bedtime. Morning fasting serum concentrations of TC, TG, LDL-C, HDL-C, and VLDL-C were measured before and after the 12-week treatment period with 20 mg simvastatin daily.

*Genotyping for ScrF I polymorphism of the HMGCR gene.* Genomic DNA was extracted from peripheral blood leukocytes. The ScrF I polymorphism of the HMGCR gene was determined using a PCR–RFLP based protocol as described by Eran Leitersdorf et al. [15].

*Statistical analysis.* Statistical analyses were performed with SPSS 13.0 software. Hardy–Weinberg equilibrium was assessed with a $\chi^2$ test of goodness-of-fit in the study sample. Paired Student's *t*-test was performed for the lipid concentrations before and after pravastatin treatment. An unpaired Student's *t*-test was used to compare the differences in the degree of reduction in plasma concentrations between the two HMGCR genotypic groups. A two-sided test with type error level ($\alpha$) set at 5% was used in all statistical analyses. Data were presented as mean ± SD. A two-tailed $P$-value $< 0.05$ was considered to be statistically significant for all analyses.

## Results

### *The relationship between distribution frequency of genotypes and alleles of HMGCR*

The genotype frequency of the ScrF I polymorphism of the HMGCR gene was as follows: AA homozygotes: 28.6%, Aa heterozygotes: 52.4%, aa homozygotes: 19.0%.The genotype frequency for this polymorphism was found to be in Hardy–Weinberg equilibrium. The frequency of the A allele was determined to be 54.8. The relationship between distribution frequency of genotypes and alleles of HMGCR is shown in Table 1. Lipid concentrations in patients treated with simvastatin are shown in Table 2. Subjects with the AA homozygotes had significantly higher serum VLDL-C levels than those with the Aa heterozygotes and aa homozygotes (VLDL: AA: $2.18 \pm 0.51$, Aa: $2.04 \pm 0.59$, aa: $1.86 \pm 0.43$, $P < 0.01$ for difference between AA and aa. There were no statistical differences in TG, TC, HDL-C, and LDL-C levels among these groups Table 3.

### *Influence of the ScrF I polymorphism of the HMGCR gene on the effectiveness of simvastatin treatment in patients with hypercholesterolemia*

To explore the potential impact of the ScrF I polymorphism on the effect of cholesterol-lowering therapy, we investigated the relationship between genotype and improvement in serum lipid levels by simvastatin treatment in 168 patients. The group of patients with a AA homozygotes genotype showed, on average, a $37.03 \pm 5.67\%$ (SD) reduction in VLDL-C, whereas patients with aa homozygous genotype displayed a $28.97 \pm 4.96\%$ reduction from baseline ($P < 0.01$) (Table 3). On the other hand, the degree of change in plasma LDL-C, HDL-C, TG, and TC concentrations did not show a significant difference among three genotypes (Table 3).

## Discussion

Type 2 diabetes mellitus is associated with an increased risk of cardiovascular disease (CVD) [16,17]. Elevated

Table 1
Distribution frequency of genotypes and alleles of HMG-CoA reductase gene between patients with type 2 diabetes mellitus and controls

| Group | *n* | Genotype | | | Allele | |
|---|---|---|---|---|---|---|
| | | AA (%) | Aa (%) | aa (%) | A (%) | a (%) |
| Controls | 144 | 37 (25.7) | 76 (52.8) | 31 (21.5) | 150 (52.1) | 138 (47.9) |
| T2DM group | 168 | 48 (28.6) | 88 (52.4) | 32 (19.0) | 184 (54.8) | 152 (45.2) |