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A novel CYP7A1 polymorphism is associated with the low-density lipoprotein cholesterol response to atorvastatin

Zhi-Ke Liu[1], Wotan Zeng[2], Yan Zhang[3], Jia Jia[3], Jian-Ping Li[3], Yong Huo[3] and Da-Fang Chen[1*]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, Beijing 100191, China

[2]East Asian Gene Science and Technology Research Institute, Beijing, 100078, China

[3]Department of Cardiology, Peking University First Hospital, Beijing 100034, China

Abstract

Backgrounds and aims: Cholesterol 7α hydroxylase encoding by gene CYP7A1 is the initial and rate-limiting step in the classical bile acid synthesis pathway. Atorvastatin can markedly upregulate the mRNAs of bile acids synthetic enzymes CYP7A1 in the liver to increase fecal bile acid excretion. We tempt to investigate the association between a novel CYP7A1 polymorphism rs8192875 and reduction of lipid levels response to atorvastatin in Chinese patients with coronary artery disease.

Methods: Of 169 patients with coronary artery disease were treated with atorvastatin for one month. Lipid profiles, including triglycerides(TGs), total cholesterol(TC), low-density lipoprotein cholesterol(LDL-C), and high-density lipoprotein cholesterol(HDL-C) were determined before and after treatment. Rs8192875 genotypes were assayed with the iPLEX assay in conjunction with the MassARRAY platform. We performed independent sample t test or Kruskal-Wallis test to evaluate the effects of SNP.

Results: After one month of atorvastatin therapy, the lipid levels decreased significantly. Compared with AG genotype, the GG genotype of rs8192875 achieved a greater reduction of LDL-C level (0.694 ± 0.701 vs. 0.136 ± 0.401 mmol/l, p=0.0056; 24.090 ± 23.104% vs. 2.182 ± 20.809%, p=0.0031); and a similar pattern of efficacy appears to TC (0.808 ± 0.791 vs. 0.302 ± 0.381 mmol/l, p=0.0208; 16.410 ± 15.370% vs. 6.936 ± 9.711%, p=0.0341). The genotypes had no significant difference on TGs or HDL cholesterol-lowering response to atorvastatin.

Conclusions: A novel CYP7A1 exon variant rs8192875 is significantly associated with reducing LDL-C and TC level response to atorvastatin.

Introduction

Statins are most widely prescribed to lowering circulating concentrations of LDL cholesterol for the prevention of CVD [1]. Several meta-analyses from randomized clinical trials have manifested that a statin-mediated reduction of 1mmol/l in LDL cholesterol yield a proportional reduction of approximately 20% in major vascular events [2,3]. And in general, the greater reductions in LDL cholesterol level, the larger reductions in vascular disease risk.

Most of the patients are extremely well tolerated to the treatment of statins, but some others have intolerable muscle adverse drug reactions with symptoms ranging from mild myalgia to fatal rhabdomyolysis [4]. The risk tends to increase with the increasing dosage of statins. Such as myalgia, the reported incidence is 3-5% in clinical trials [5] and up to 10% among the patients of high-dose statins [6]. In addition, statin therapy has been insufficient in some patients, 40% of high-risk and 80% of very-high-risk individuals do not meet their LDL cholesterol targets [7]. Moreover, it is estimated that genetic determinants account for 20-95% of the variability in drug disposition and effects [8,9]. In the classical bile acid synthesis pathway, cholesterol 7α hydroxylase encoding by gene CYP7A1 is the initial and rate-limiting step for the removal of cholesterol into bile [10]. And then atorvastatin can markedly upregulate, covering 10-fold, the mRNAs of the bile acids synthetic enzymes CYP7A1 in the liver to increase fecal bile acid excretion [11]. Therefore, the gene CYP7A1 was chosen as a major candidate gene to investigate the individual sensitivity response to atorvastatin. At present, several studies have reported that CYP7A1 polymorphisms influence the LDL cholesterol-lowering response to atorvastatin [12-15], such as the CYP7A1 A-204C*A in Caucasians and rs8192870*T in Chineses have a greater reduction in LDL cholesterol level response to atorvastatin as compared with variant allele-carrying genotype. Above information have urged us to research optimal atorvastatin treatment options, which aims at maximizing reduction of LDL cholesterol at the risk of minimal adverse effects. In this study, we preliminarily tempt to investigate the association between a novel CYP7A1 polymorphism rs8192875 and reductions of lipid levels response to atorvastatin in Chinese patients with coronary artery disease.

Materials and methods

Subjects

This project was designed as a hospital-based prospective study and conducted by Peking University First Hospital (Beijing, China). Patients are eligible for the study who are scheduled for elective coronary

Correspondence to: Chen Da-fang, Department of Epidemiology and Biostatistics, Peking University Health Science Center, 38 Xueyuan Rd., Haidian District, Beijing 100191, P.R. China, Tel:+ 86-10-82802644; Fax: +86-10-82802644; E-mail: dafangchen@bjmu.edu.cn

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angiography and who have been clinically diagnosed with either NSTE-ACS (unstable angina or NSTE acute myocardial infarction) or stable angina pectoris. Patients with following conditions were excluded from the study: have LDL-C <1.8 mmol/L without statin therapy within 1 month; have uncontrolled clinically significant disease; have active hepatic disease or hepatic dysfunction are allergic or have experienced serious adverse reaction to HMG-CoA reductase; are participating in other interventional clinical trials using drugs or devices.

Between January 2010 and December 2011, a total of 165 consecutive unrelated adult patients in 20-75 years old with coronary artery disease were enrolled into the study. Of these subjects, 104 patients receive intensive atorvastatin therapy (atorvastatin 80mg/day for two consecutive days before and 40mg/day for 28 days after percutaneous coronary intervention); 65 patients receive routine atorvastatin therapy (less than atorvastatin 20mg/day daily for 30 days). The study was approved by the Ethics Committee of the Peking University Health Science Center, and all the subjects delivered written informed consent for participation.

In the first visit, demographic, behavioral information, clinical characteristics were collected from trained physicians, including age, gender, height, weight, smoking and drinking habits and medical history information. After one month, patients come back for a clinical follow-up. A clinical follow-up questionnaire was finished by a trained physician for monitoring the subjects' medication compliance and side effects.

Lipids tests

Over 8 hours fasting blood samples before and after one month of treatment with atorvastatin were collected for the measurement of lipid profiles. All biochemistry tests were performed by uniformly standard methods in the clinical laboratory of the Peking university First Hospital via an automatic biochemical analyzer.

DNA preparation and genotyping

Genomic DNA was extracted from the participants' peripheral blood leucocytes by using the protein precipitation method following standard procedures. Each DNA sample was quantified using a Nano-photometer (Implen, Germany) and was diluted to a final concentration of 50 ng/uL.

The rs8192875 genotypes were assayed with the iPLEX assay in conjunction with the MassARRAY platform (Sequenom, La Jolla, CA) according to the manufacturer's protocol. The SNP had a call rate of greater than 95%, 5% of the blind duplicate samples were genotyped repeatedly and the rate of consistency was 100%.

Statistical analysis

All statistical analyses were performed using SAS9.2 (SAS institute Inc., Cary, NC). A logarithmic transformation was applied to skewed distribution data before analysis. Means ± standard deviation (SD) are given for all continuous variables and absolute numbers and percentages for category variables. The $\chi 2$ test was used to examine the Hardy-Weinberg equilibrium. Differences between lipid parameters at baseline and after 30 days treatment were tested using paired-t-test. Category variables differences among the genotypes were analyzed using Pearson $\chi 2$ tests or Fisher's exact test of probabilities. The absolute reductions and relative changes of plasma lipid parameter levels among genotypes were tested using independent sample t-test or Kruskal-Wallis test. Two-sided tests were adopted and a p-value of <0.05 was considered statistically significant.

Results

Genotyping

A total of 169 patients with good adherence to atorvastatin treatment were included in the study. The genotypes were consistent with the Hardy-Weinberg equilibrium ($\chi 2$=0.01, p=0.935). Frequencies of the G and A alleles were 0.973 and 0.027, respectively. The variant allele was basically same, as compared to the population HAPMAP-CHB (0.049) (p=0.198 by Pearson $\chi 2$ test).

Baseline characteristics of participants

The mean age of the study subjects was 59.34 ± 8.16 years and 72.8% were male(n=123). The baseline characteristics of the study participants are shown in Table1. There were no significant differences in baseline demographic characteristics or clinical disease history between the rs8192875 genotype groups (Table1).

Lipid responses

Treatment with atorvastatin therapy daily for one month significantly reduced TC from 1.443 ± 0.219 to 1.264 ± 0.210 mmol/L (p<0.001), LDL-C from 0.913 ± 0.317 to 0.610 ± 0.335 mmol/L (p<0.001) and TG from 0.441 ± 0.478 to 0.190 ± 0.404 mmol/L (p<0.001), while increased HDL-C from 0.027 ± 0.239 to 0.088 ± 0.271 mmol/L (p=0.006) (Table 2).

Effect of the genetic polymorphisms on the lipids responses to atorvastatin

There was no statistically significant difference in all baseline lipid profiles, while significant differences in the absolute reduction and percentage change in TC and LDL-C level between rs8192875 genotype groups after one month of atorvastatin therapy. Patients with genotype GG/rs8192875 had a 21.9%(0.558 mmol/l) greater reduction in LDL-C level than those with genotype AG/rs8192875 (0.694 ± 0.701 vs. 0.136 ± 0.401 mmol/l, p=0.0056; 24.090 ± 23.104% vs. 2.182 ± 20.809%, p=0.0031). And the changes in TC appeared a similar pattern, a 9.47% or 0.506mmol/l greater reduction in TC in genotype GG/rs8192875 subjects as compared with genotype AG/rs8192875 subjects (0.808 ± 0.791 vs. 0.302 ± 0.381 mmol/l, p=0.0208; 16.410 ± 15.370% vs. 6.936 ± 9.711%, p=0.0341). The genotypes had no significant effects on TG or HDL-C response to atorvastatin (Table 3).

Table 1. Baseline demographic and clinical characteristics of patients between CYP7A1 rs8192875.

Variables†	CYP7A1 rs8192875			*P*
	All	GG (N=160)	AG (N=9)	
Intensive atorvastin	106(62.7)	102(63.8)	4(44.4)	0.244
Age(years)	59.34 ± 8.16	59.46 ± 8.21	57.22 ± 7.19	0.318
Male	123(72.8)	118(73.8)	5(55.6)	0.233
BMI(kg/m^2)	25.34 ± 3.16	25.30 ± 3.23	26.03 ± 1.30	0.166
Current Smoker	69(40.8)	67(41.9)	2(22.2)	0.1505
Chronic Statin	64(37.9)	60(37.5)	4(44.4)	0.676
Hypertension	104(61.5)	100(62.5)	4(44.4)	0.279
T2DM	43(25.4)	41(25.6)	2(22.2)	0.3055
Stroke	14(8.3)	12(7.5)	2(22.2)	0.1356
PVD	9(5.3)	8(5.0)	1(11.1)	0.3216

†: Continuous variables were presented as mean ± standard deviation, and categorical variables were presented as n (%); BMI: body mass index; T2DM: type 2 diabetes mellitus; PVD: Peripheral Vascular Diseases.

Table 2. Comparison of Plasma lipid parameters before and after treatment with atorvastatin.

Variables†	Baseline	30 Days	*P*
TC (mmol/l)	1.443 ± 0.219	1.264 ± 0.210	<0.001
LDL-C (mmol/l)	0.913 ± 0.317	0.610 ± 0.335	<0.001
TG (mmol/l)	0.441 ± 0.478	0.190 ± 0.404	<0.001
HDL-C (mmol/l)	0.027 ± 0.239	0.088 ± 0.271	0.006

†: variables were presented as mean ± standard deviation; *P*:analysed by paired t test.

Table 3. The association of rs8192875 with lipid lowering effects reponse to atorvastatin.

Variables†	CYP7A1 rs8192875		P
	GG (N=162)	AG (N=9)	
TC			
Baseline(mmol/l)	1.448 ± 0.219	1.367 ± 0.209	0.2838*
Reduction (abs)	0.808 ± 0.791	0.302 ± 0.381	0.0208
Reduction (per)	16.410 ± 15.370	6.936 ± 9.711	0.0341
LDL-C			
Baseline(mmol/l)	0.919 ± 0.318	0.816 ± 0.300	0.3416*
Reduction (abs)	0.694 ± 0.701	0.136 ± 0.401	0.0056
Reduction (per)	24.090 ± 23.104	2.182 ± 20.809	0.0031
TG			
Baseline(mmol/l)	0.436 ± 0.480	0.543 ± 0.457	0.5391*
Reduction (abs)	0.414 ± 0.580	0.523 ± 0.811	0.9189
Reduction (per)	17.648 ± 25.870	17.417 ± 24.669	0.6009
HDL-C			
Baseline(mmol/l)	0.029 ± 0.237	-0.006 ± 0.28	0.4752
Reduction (abs)	-0.058 ± 0.197	-0.004 ± 0.233	0.3207
Reduction (per)	-5.512 ± 18.889	-3.416 ± 22.208	0.5793

†: variables were presented as mean ± standard deviation; *P**: analysed by T test for two independent sample; *P*:analysed by Kruskal-Wallis Test.

Discussion

In the present study, the association of CYP7A1 rs8192875 polymorphism with lipid lowering effect respond to atorvastatin is statistically significant. There is a greater reduction in LDL cholesterol level and Total cholesterol level in subjects with genotype GG in contrast to the subjects with genotype AG, 21.9% (0.558 mmol/l) and 9.47% (0.506 mmol/l) respectively.

Previous pharmacogenetics studies aiming at evaluating the potential contribution of CYP7A1 polymorphisms to the observed variability in response to atorvastatin therapy have accumulated evidence of a causal relationship between them. In Caucasians, SNP rs3808607 (A-240C) has been reported to be associated with plasma LDL cholesterol level [16] and LDL cholesterol lowering reduction in response to atorvastatin [12,13]. Besides, three-loci interaction comprising of CYP7A1(rs8192871AA)/APOAI(*Pst*P1P1)/HMGCR (rs12916CT) was found to be associated with a maximum decrease in LDL cholesterol levels. Above associations about SNP rs3808607 are either inconsistent [14] or unobserved [15], but Wei et al. found the CYP7A1 -204A and ABCG8 1199A alleles appear to interact to affect lipid-lowering response to atorvastatin [14]. In addition, Jiang et al. applied a gene-wide tagging SNP strategy to study all common variants of CYP7A1 (minor allele frequency > 5%) on atorvastatin response, and the results show that genotype TT/rs8192870, in the first intron of CYP7A1 gene, had greater reduction of LDL cholesterol in response to atorvastatin [15]. Here, we report for the first time, that SNP rs8192875 is significantly associated with LDL cholesterol and total cholesterol-lowering response to atorvastatin. The National Center for Biotechnology Information (NCBI) annotate that SNP rs8192875 is a functional polymorphism located in the exon of CYP7A1, and its heterozygote or homozygote variant leads to missense mutation (Asp347Asn) [17], so we can assume that the variant may result in the decrease of the active site and enzyme function.

Cholesterol 7α hydroxylase (CYP7A1), which catalyzes the hydroxylation of cholesterol at the 7α position in the liver, the first reaction in the classic pathway of bile acid synthesis, and that represents the check-point of the whole process. Bile acid metabolism is strongly related to whole-body cholesterol homeostasis; bile acid synthesis and bile acid-facilitated biliary cholesterol secretion are the only significant pathways for cholesterol excretion from the body [18]. And in general, in humans, the major fraction of cholesterol is transported associated to LDL particles [19]. In 2002, Pullinger et al. elaborated the first and so far, only reported hypercholesterolemic phenotype of CYP7A1 deficiency in humans caused by frameshift mutations (L413fsX414) in exon 6 [20]. This information suggests that CYP7A1 enzyme activity profoundly influences cholesterol homeostasis.

There were some limitations in this study. First, the sample size of the study is small, the statistical power is not enough for further detecting the effect of interaction or analyzing the subgroup differences. A more large prospective study is warranted. Second, we did not test the level of lipoprotein(a). Lipoprotein(a) account for approximately 45% cholesterol, the lipoprotein(a) level may have a direct effect on the LDL cholesterol response to atorvastatin [21]. Third, compared with previous evidence, the one-month duration of the study was not long enough to observe a long-term effect of SNP rs8192875 on the efficacy of atorvastatin therapy. Despite the limitations mentioned above, our study has several strengths. The monotherapy of atorvastatin could avoid the concomitant medications influence. And prospective design could contribute to obtaining the information without recall bias. Moreover, the credibility of the associations of this research was enhanced by the similar pattern of lowering efficacy in LDL cholesterol and total cholesterol between genotypes of SNP rs8192875 response to atorvastatin. Above all, further functional research, as well as pharmacogenomic studies with good design and longer duration among other ethnic groups, is warranted.

Conclusion

The data presented here demonstrate that a CYP7A1 exon variant rs8192875 is significantly associated with response to atorvastatin in terms of LDL cholesterol and total cholesterol lowering. Patients with GG genotype yield a greater response to atorvastatin in terms of the reduction of LDL cholesterol and total cholesterol than those with AG genotype. These findings offer a new potential way to optimize the clinical therapy for individual patients in need of lipid-lowering.

Conflicts of interest

None of the authors has a conflict of interests with regard to the publication of this study.

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