Original Research Article

Effect of CYP2C9*3 gene polymorphism on lipid-lowering efficacy of fluvastatin in a Chinese hyperlipidemic population

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Abstract

Purpose: To investigate the frequency of gene CYP2C9*3 in Chinese populations, and to analyze the impact of CYP2C9*3 genetic polymorphism on the cholesterol-lowering effect of fluvastatin in a Chinese hyperlipidemic population.

Methods: CYP2C9 genotype was determined by polymerase chain reaction - restriction fragment length polymorphism (PCR - RFLP) in 270 unrelated hyperlipidemic patients who were treated with 80 mg fluvastatin monotherapy daily for 4 weeks and 250 healthy controls. Clinical data were collected prior to treatment with fluvastatin and 4 weeks after.

Results: In 270 hyperlipidemic patients, the frequency of CYP2C9*3 was 3.70 % which is significantly higher than in 250 healthy controls (2.60 %) (p < 0.01). After oral intake of fluvastatin 80 mg daily for 4 weeks, CYP2C9*1/*3 genotype was associated with a decrease in LDL-C levels (by 33.9 % in CYP2C9*1/*3 versus 24.5 % for CYP2C9*1/*1, p < 0.05) and with reduction of TC (by 36.4 % in CYP2C9*1/*3 versus 19.4 % in CYP2C9*1/*1, p < 0.05).

Conclusion: The frequency of CYP2C9*3 is 3.17 % in Chinese populations, and those who carry CYP2C9*3 mutation have a high risk of hyperlipidemia. CYP2C9*3 seems to increase the lipid-lowering effects of fluvastatin.

Keywords: Hyperlipidemia, Gene, CYP2C9, Polymorphism, Polymerase chain reaction, Restriction fragment length polymorphism, Fluvastatin

INTRODUCTION

Hypercholesterolemia plays a key role in the development of atherosclerotic disease. Statins are known to reduce cardiovascular morbidity and mortality. However, there is a wide inter-individual variation in response to statin therapy. The underlying causes of this phenomenon have been extensively debated, but remain uncertain [1-3]. Statins are substrates of CYP450 enzymes. Genetic polymorphisms of CYP enzymes are an important source of inter-individual variation in drug metabolism [4]. CYP2C9 is an important enzyme, and several drugs are metabolized by it, e.g., fluvastatin, simvastatin, losartan and warfarin [4-5]. Therefore, the polymorphisms of CYP2C9 may be have an effect on the above drugs' therapeutic efficacy. Any predictions of biological response of individuals to statins would thus be very valuable for more efficacious, personalized treatments. Several single-nucleotide polymorphisms (SNPs) of CYP2C9 genes have been identified. The most common mutation site in

CYP2C9 is *CYP2C9*3.* Individuals who carry *CYP2C9*3* gene may have reduced enzyme activity [6]. Fluvastatinis metabolized primarily by *CYP2C9* [7], therefore there may be an association between *CYP2C9* polymorphisms and the lipid-lowering efficacy of fluvastatin.

However, there is no report associated with *CYP2C9* genotype with the efficacy of fluvastatin in Chinese peoples. Therefore, the aim of this study was to investigate the distribution of gene *CYP2C9*3* relates to drug therapy and to analyze the impact of *CYP2C9* gene variants on hyperlipidemia in Chinese populations.

METHODS

Subjects

From January to December, 520 non-related Chinese were randomly recruited at the Hospital of Hubei, including 270 hyperlipidemic patients (140 females, 130 males; mean age 50.35 ± 5.85 years) and 250 healthy volunteers (130 females, 120 males; mean age 48.21 ± 6.35 years). Hyperlipidemia was defined as cholesterol > 5.60 mmol/L, triglycerides > 1.70 mmol/L or low - density lipoprotein (LDL) > 3.20 mmol/L.

Exclusion criteria were: patients with diabetes mellitus, cancer, severe endocrine diseases, hepatic disease and renal disease and those that were on therapy for any chronic inflammatory disease. This study followed the guidelines of Declaration of Helsinki [8]. The inclusion criterion for enrolment in the trial was the initiation of treatment for hypercholesterolemia with fluvastatin (80 mg monotherapy) daily. All subjects involved gave written informed consent, and the study was approved by the Ethical Committee of the Hospital of Hubei (approval ref. no. 20160101).

Genotyping

Peripheral blood samples (2 mL) for *CYP2C9*3* genotyping were collected from all subjects. DNA was extracted from blood cells using standard protocols. *CYP2C9*3* was genotyped using PCR-RFLP. The pair of primers used were 5' - TGCACGAGGTCCAGAGGTAC - 3' and 5' - AAACATGGAGTTGCAGTGTAG - 3'. PCR reaction was performed using 50 ng DNA as template; the conditions of PCR reaction were: 94 °C for 5 min, following by 35 cycles of 94 °C for 50 s, annealing at 60 °C for 60 s, and extension at 72 °C for 1 min, with a final 5 min extension at 72 °C. The amplified DNA fragment was digested with restriction enzyme *Kpn* I at 37 °C for 12 h. The digestion product of *CYP2C9*3* was separated with electrophoresis on a 4 % agarose gel and analysed by ethidium bromide staining.

Statistical analysis

The distribution of genotypes in the control and hyperlipidemia groups was obtained by Chi-square test. $P < 0.05$ was considered statistically significant. All statistical analyses were processed using SPSS15.0 software.

RESULTS

Demographic characteristics of the study population

The characteristics of the subjects in the hyperlipidemia and control groups are shown in Table 1. Compared with control group, Age and BMI in the hyperlipidemia group were significantly different in the case group ($p < 0.05$), while no significant difference in gender, systolic blood pressure (SBP) and diastolic blood pressure (DBP) in the two groups.

Table 1: Characteristics of study population (mean ± SD)

Variable	Hyperlipidemic patients (n = 270)	Healthy control (n = 250)
Gender		
Male	133	122
Female	137	128
Mean age (years)	58.15 ± 8.02*	46.59 ± 6.23
BMI (kg/m^2)	28.03 ± 3.28*	23.56 ± 3.01
SBP (mmHg)	128.52 ± 13.28	121.52 ± 10.35
DBP (mmHg)	89.36 ± 10.64	82.23 ± 9.65
TC (mmol/L)	4.89 ± 1.16*	4.12 ± 1.02
TG (mmol/L)	2.85 ± 1.06*	1.25 ± 0.56
HDL-C (mmol/L)	0.99 ± 0.56*	1.38 ± 0.29
LDL-C (mmol/L)	2.58 ± 1.03*	2.02 ± 0.86

*Compared with control, * p < 0.05*

Table 2: Frequency of genotypes and alleles of *CYP2C9*3* in hyperlipidemia patients *(n, %)*

Genotype & alleles	Hyperlipidemia			Control		
	Male (133)	*Female (137)*	*n=270*	*Male (122)*	*Female (128)*	*n=250*
CYP2C9*3						
AA	123 (92.49)	127 (92.70)	250 (92.59)	116 (95.08)	121 (94.53)	237 (94.80)
AC	10 (7.51)	10 (7.30)	20 (7.41)	6 (4.92)	7 (5.47)	13 (5.20)
CC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
C	3.76	3.65	3.70*	2.50	2.73	2.60

Compared with control, *p* < 0.01

Table 3: Effect of fluvastatin (80 mg/day, 4 weeks) on TG, LDL and TC for different genotypes of *CYP2C9* subjects (mean±SD, mmol/L)

Variable	*CYP2C9*1*1*			*CYP2C9*1*3*		
	TG	*TC*	*LDL-C*	*TG*	*TC*	*LDL-C*
Before fluvastatin	2.56±1.63	6.28±1.36	3.64±1.02	2.34±1.59	6.89±1.51	3.92±1.35
After fluvastatin	1.65±1.32	5.06±0.84	2.75±0.58	1.42±1.28	4.38±0.75	2.59±0.58
Change (%)	35.6	19.4	24.5	39.3	36.4*	33.9*

*Compared with CYP2C9*1*1, p < 0.05*

Genotype distribution of *CYP2C9*3* loc

The genotype distributions are shown in Table 2. There was no significant deviation from Hardy-Weinerg equilibrium in each group at *CYP2C9*3* locus. As to *CYP2C9*3* locus, the observed genotype distribution and allele frequencies differ between the hyperlipidemia and control groups (p < 0.01). Compared with the control group, the frequency of T allele was higher in the hyperlipidemia group (p < 0.01).

Association between genotype of *CYP2C9*3* and hyperlipidemia

In all subjects, 33 individuals carrying heterozygote for *CYP2C9*3*, the frequency of *CYP2C9*3* was 0.0317, among whom were 20 subjects from 270 hyperlipidaemic patients, the allelic frequency of *CYP2C9*3* was 0.037, and 13 subjects from 250 healthy control, the allelic frequency of *CYP2C9*3* was 0.026. None was identified as homozygote. The frequencies of *CYP2C9*3* showed significant difference between healthy control and hyperlipidaemic patients (p < 0.01). This indicates that *CYP2C9*3* is associated with hyperlipidaemia.

Lipid-lowering efficacy of fluvastatin

Fasting serum concentrations of blood lipids (inluding triglycerides, total cholesterol, and LDL cholesterol) were measured before and after the 4-week treatment with 80 mg fluvastatin daily. In Table 3, after oral intake of fluvastatin 80 mg daily for 4 weeks, the *CYP2C9*1/*3* genotype was associated with a decrease in LDL-C levels (by 33.9% in *CYP2C9*1/*3* verse 24.5% for

*CYP2C9*1/*1*, p < 0.05) and with the reduction of TC (by 36.4% in *CYP2C9*1/*3* versus 19.4% in *CYP2C9*1/*1*). Nobody took any other lipid-lowering drug. No side effects were reported among all the participants.

DISCUSSION

In this study, the frequency of *CYP2C9*3* was 0.0317, which is similar to other literature reports for the Chinese [9-11]; the frequency of *CYP2C9*3* was 0.043 in African-Americans, 0.10 in Spaniards [12], 0.095 in Croats [13], 0.09 in Italians [14], 0.085 in Britons [15], 0.08 in French [16], 0.067 in Russians [17], 0.066 in Swedes [18] and 0.059 in Czech population [1]. A significant difference in the distributions of *CYP2C9*3* was observed between hyperlipidaemic patients and healthy individuals, but there was no statistically significant difference between male and female participants, which suggests that there is no gender difference in the distribution of *CYP2C9*3* in the total population.

A previous study showed that there was only a significant difference in the frequency of *CYP2C9*3* between patients and healthy controls in the female group, thus strongly indicating that gender is associated with *CYP2C9*3* in patients with hyperlipidaemia [10]. Our results were not consistent with the above, and it is possibly due to the diversity of the study population. However, we found that heterozygous CYP2C9*1/*3 carriers showed a greater reduction in plasma LDL-C or TC levels than wild-type subjects, which is consistent with a previous study [1].

CYP2C9 is a key CYP enzyme which accounts for approximately 20 % of hepatic enzymes. Approximately 15 % of drugs are metabolized by CYP2C9. Overall, CYP2C9 metabolizes more than 100 drugs including antidiabetic, antihypertensive, antiepileptic, anti-inflammatory drugs. Until now, over 60 different types of gene polymorphism of CYP2C9 have been reported. This suggests that individuals who carry different genotypes of CYP2C9 exhibit varying response to drugs metabolized by CYP2C9 [19,20]. Previous studies reported that *CYP2C9* accounts for 50 – 80 % of fluvastatin metabolism, which suggests that *CYP2C9* gene mutation may be associated with the lipid-lowering efficacy of fluvastatin in healthy volunteers [21]. *CYP2C9*3* allele is the most common mutation, which result in decreased metabolic enzyme activity and impaired metabolism of substrate drugs [22].

CYP2C9 genetic polymorphisms are an important variation in drug metabolism which metabolized including losartan, S-warfarin, phenytoin, hypoglycemic drugs and anti-inflammatory drugs [4]. A previous study showed that patients carrying *CYP2C9*3* showed a higher lipid-lowering efficacy against TC and LDL compared with wild-type of *CYP2C9*, but no significant difference exist in the frequency of adverse drug reactions in patients who carry different *CYP2C9* genotypes; this suggests that CYP2C9 polymorphism associated with the lipid-lowering efficacy of fluvastatin in hyperlipidemic patients [23]. Another study showed that subjects carrying *CYP2C9*3* have increased the risk of adverse effects of 2.5-times, while patients who carry CYP2C9*3 and are receiving CYP2C9 inhibitors have a risk of adverse effects of 6-times compared with those who were treated with the inhibitor [24].

A study reported that *CYP2C9*3* and gender contribute to inter-subject variability in pitavastatin pharmacokinetics and that personalized medicine should be necessary for hypercholesterolaemic patients receiving pitavastatin [25]. Patients who carried *CYP2C9* heterozygous for *CYP2C9*3* allele had higher plasma levels of fluvastatin, which can increase the incidence of adverse events.

SNP causes changes in the functions of CYP enzyme which results unexpected and serious adverse drug reactions. Therefore, CYP gene polymorphism can be used as a genome biomarker for predicting adverse drug reactions. Thus, compared with healthy controls, the frequency of *CYP2C9*3* is significantly higher in hyperlipidemic patients, which illustrates that

those with *CYP2C9*3* mutation show a high risk for hyperlipidemia. Thus, *CYP2C9*3* polymorphism is closely related to the lipid-lowering efficacy of fluvastatin in patients with hyperlipidemia, suggesting that people with CYP2C9*3 mutation should exercise caution in order to avoid developing hyperlipidemia, and personalized medicine should be necessary for such hyperlipidemia patients.

Limitations of the study

In this study, the sample size of participants was small, and therefore, the results may be biased. Hence, a larger-sample study needs to be undertaken.

CONCLUSION

The frequency of *CYP2C9*3* in Chinese populations is 3.17 %, and those with *CYP2C9*3* mutation have high risk of hyperlipidemia. *CYP2C9*3* seems to enhance the lipid-lowering effects of fluvastatin.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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