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Gender specific association of *CYP2C9*3* with hyperlipidaemia in Chinese

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Aims
To investigate the association of *CYP2C9*3* and **6* with hyperlipidaemia in Chinese.

Methods
Four hundred and seventy-six Chinese participated in the study, including 211 uncomplicated hyperlipidaemic patients and 265 healthy controls. PCR-RFLP was used to identify *CYP2C9*3* and **6*.

Results
*CYP2C9*6* was not detected in this study. The allelic frequency of *CYP2C9*3* was 0.039 (95% CI 0.022, 0.056). A nonsignificant difference existed in *CYP2C9*3* frequencies between males and females ($P = 0.605$, OR = 1.194, 95% CI 0.610, 2.336), patients and controls ($P = 0.063$, OR = 0.506, 95% CI 0.244, 1.049) in the total population. However, in the female group, *CYP2C9*3* frequency in patients with hyperlipidaemia was significantly lower than that in controls ($P < 0.0001$, OR = 0.062, 95% CI 0.008, 0.476).

Conclusions
The association of *CYP2C9*3* with hyperlipidaemia was specific for females in this Chinese population.

Introduction

It is generally acknowledged that oestrogens are protective factors for hyperlipidaemia. By increasing low-density lipoprotein (LDL) receptor expression, oestrogens decrease blood LDL cholesterol and progestogens decrease triglycerides and high-density lipoprotein (HDL) cholesterol [1]. Therefore, premenopausal women run a lower risk of hyperlipidaemia than men of corresponding age, while postmenopause, LDL cholesterol concentrations in females increase as the concentration of oestrogens decreases [2].

CYP2C9, known for abundant expression in liver, participates in the disposition of oestrogens. At high substrate concentrations, CYP2C9 can catalyze 2- and 4-hydroxylation of estradiol and estrone and 21-hydroxylation of progesterone. Furthermore, it is a major enzyme catalyzing 17β-hydroxy dehydrogenation of estradiol at low substrate concentration [3, 4]. This suggests that genetic polymorphisms of *CYP2C9* may affect oestrogen concentrations.

To date, 11 different variations of *CYP2C9* have been reported, namely *CYP2C9*2* to **12*, respectively, with

*CYP2C9*1* as a wild type. Among these functional mutations, *CYP2C9*2, *4,* and **5* have been rarely detected in Chinese [6, 7], and the distribution of *CYP2C9*6* in Chinese is unclear. Little information about *CYP2C9*7* to **12* has been obtained [5]. Only *CYP2C9*3* (95% CI 0.017, 0.065) is common in Chinese [6, 7]. This study aimed to investigate the frequencies of *CYP2C9*3* and **6* in Chinese and their association with hyperlipidaemia.

Methods

Study protocol

In total, 476 nonrelated Chinese (225 females, 251 males) were randomly recruited, including 211 hyperlipidaemic patients (98 females, 113 males; 32–73 years of age, mean age 54.0 ± 9.6 years) and 265 healthy volunteers (127 females, 138 males; 18–62 years of age, mean age 25.8 ± 11.5 years). Physical examination, medical history and serum biochemical tests were performed to identify healthy or hyperlipidaemic subjects (LDL > 3.70 mmol l^{-1}, or TG > 1.69 mmol l^{-1}, or total cholesterol >5.70). Most of the patients were outpatients with uncomplicated primary hyperlipidaemia. Hyperlipidaemia secondary to other serious systemic disease such as diabetes, hypothyroidism or systemic lupus erythematosus (SLE) was excluded. This study had ethical approval and all subjects gave informed consent to participate.

Genomic DNA was extracted from 5 ml peripheral blood, and genotyping of *CYP2C9*3* and **6* was done by polymerase chain reaction followed by digestion with restriction enzymes as described previously [8, 9].

Statistical analysis

Results are expressed as mean ± SD. Allelic frequencies were compared by using Pearson Chi-Square, as well as the test for Hardy–Weinberg equilibrium. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated using SPSS software (version 11.0; SPSS, Chicago, IL).

Results

Genotyping of each specimen was replicated. In the total population, 37 individuals (7.8%; 18 male, 19 female) were identified as heterozygote for *CYP2C9*3,* among whom were 11 subjects (10 male, 1 female) from 211 hyperlipidaemic patients giving an allelic frequency of 0.026 (95% CI 0.012, 0.040), and 26 subjects (8 male, 18 female) from 265 healthy volunteers giving an allelic frequency of 0.049 (95% CI 0.030, 0.068). None was identified as homozygote. The allelic frequency in our whole population was 0.039 (95% CI 0.022, 0.056). A non-significant difference was observed in the frequencies of *CYP2C9*3* between healthy subjects and hyperlipidaemic patients ($\chi^2 = 3.464$, $P = 0.063$; OR = 0.506, 95% CI 0.244, 1.049). In the female group, the frequency of *CYP2C9*3* in hyperlipidaemic patients was significantly lower than that in healthy volunteers, whereas in the male group, this difference was not significant (Table 1). This indicated a gender specific association of *CYP2C9*3* with hyperlipidaemia.

*CYP2C9*6* was not detected in the 148 hyperlipidaemic patients.

Discussion

To our knowledge, this is the first report of CYP2C9 in hyperlipidaemic patients. *CYP2C9*6* was not detected,

Table 1
Distribution of *CYP2C9*3* in our study

	Total (*n* = 476)		Total (*n* = 476)		Male (*n* = 251)		Female (*n* = 225)	
Genotype	Male (*n* = 251)	Female (*n* = 225)	Hyperlipidaemic (*n* = 211)	Healthy (*n* = 265)	Hyperlipidaemic (*n* = 113)	Healthy (*n* = 138)	Hyperlipidaemic (*n* = 98)	Healthy (*n* = 127)
CYP2C9*1*1	233	206	200	239	103	130	97	109
CYP2C9*1*3	18	19	11	26	10	8	1	18
χ^2	0.268		3.464		0.870		12.377	
P	0.605*		0.063**		0.351**		<0.0001**	
OR	1.194		0.506		0.634		0.062	
(95% CI)	(0.610, 2.336)		(0.244, 1.049)		(0.241, 1.664)		(0.008, 0.476)	

**Male vs. female. **Patients vs. healthy volunteers. P < 0.05 was considered as significant.*

which suggested that it might not contribute to hyperlipidaemia in Chinese. The frequency of *CYP2C9*3* in our study was 0.039, which is similar to other reports in Chinese [6, 7]. In the total population, no significant difference in *CYP2C9*3* allelic distributions was observed between hyperlipidaemic and healthy, or male and female subjects, which suggested that the distribution of *CYP2C9*3* in the total population was similar in males and females.

The frequency of *CYP2C9*3* only showed a significant difference between patients and controls in the female group. Moreover, most female (18 out of 19) *CYP2C9*3* carriers were healthy. This strongly indicated a gender specific association of *CYP2C9*3* with hyperlipidaemia. The finding of no significant difference in the frequency of *CYP2C9*3* between patients and controls in the total population might be due to the higher incidence of *CYP2C9*3* in males with hyperlipidaemia (10 out of 18). Many other studies have also revealed some genetic association with disease which was specific for females, such as the correlation of the 5-HT_{2A} receptor gene with essential hypertension and the glutathione S-transferases gene (*GSTP1*) with Hodgkin's lymphomas [10, 11]. A possible mechanism in the present study might be the involvement of oestrogen. A previous study in our laboratory showed that at physiological concentration *in vitro*, 17β-hydroxy dehydrogenation dominated estradiol metabolism and CYP2C9 had high catalyzing activity [3]. Thus, *CYP2C9*3* female carriers might catalyze less oestrogen and maintain higher oestrogen concentrations for longer and thereby have a longer and stronger protective effect from hyperlipidaemia, especially postmenopause. In this study, most female hyperlipidaemic patients were postmenopausal (mean age 55.0 ± 7.1 years) and highly predisposed to hyperlipidaemia. Since no participant was receiving oestrogen replacement therapy, we argued that CYP2C9 played a role in hyperlipidaemia pathophysiology in females, with the *CYP2C9*3* variant showing a secondary protective effect. In contrast, lack of genetic association in males could be explained by the fact that androgens are not metabolized by CYP2C9 and cause an opposite effect on serum lipids [12]. Alternative explanations involving other mechanisms are also possible. Even though the mean ages of patients and controls were not similar, age will not affect genotype. Our study has not only provided new information on hyperlipidaemia pathogenesis to physicians, but has also implied a lower possibility of drug interaction and side-effects when CYP2C9 catalyzing drugs are co-administered to hyperlipidaemic female patients because of the lower incidence of *CYP2C9*3*.

In conclusion, we studied *CYP2C9*3* and **6* in Chinese healthy volunteers and hyperlipidaemic patients. *CYP2C9*6* was not identified. The association of *CYP2C9*3* with hyperlipidaemia was specific for females. Further studies are required to determine whether *CYP2C9*3* is a marker for females not susceptible to hyperlipidaemia.

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Competing interests: None to declare.

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