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ORIGINAL ARTICLES

Identification of a *CYP19* Gene Single-Nucleotide Polymorphism Associated with a Reduced Risk of Coronary Heart Disease

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Objective: An imbalance in sex hormone ratios has been identified in coronary heart disease (CHD), and as a key enzyme in the conversion of androgen to estrogen, aromatase plays an important role in the balance of sex hormone levels. However, there is a paucity of research into the potential roles of aromatase in CHD. In this study, we investigated associations between single-nucleotide polymorphisms (SNPs) in the *CYP19* gene, which encodes aromatase, and CHD. *Methods:* We collected 1706 blood samples from CHD patients and control participants and used propensity score matching techniques to match case and control groups with respect to confounding factors. In a final study population, including 596 individuals, we conducted a case–control study to identify associations between three SNPs in *CYP19* and CHD using χ^2 or Fisher exact tests, and binary logistic regression analysis. Differences in lipid levels and parameters of echocardiography among individuals with different genotypes were assessed by one-way analysis of variance. *Results:* The distributions of rs2289105 alleles in the *CYP19* gene differed significantly between the CHD and control groups ($p = 0.014$), and the heterozygote CT genotype was associated with a significantly lower risk of CHD compared to the homozygous wild-type CC genotype ($p = 0.0063$ and odds ratio $= 0.575$). However, blood lipid levels and echocardiographic parameters among individuals with different genotypes did not differ between the CHD and control groups. *Conclusions:* The CT genotype of the rs2289105 polymorphism in the *CYP19* gene is associated with a decreased risk of CHD and may be a genetic marker of protection from CHD.

Introduction

Coronary heart disease (CHD) is the most common form of heart disease and the leading cause of mortality and morbidity worldwide. Accordingly, clinical complications of CHD are a main source of rising healthcare costs. Thus, more effective strategies to prevent CHD are needed urgently. Well-established risk factors for CHD include advanced age, personal history of cardiac dysfunction, family history of CHD, hyperlipidemia (Assmann *et al.*, 1999), high blood pressure, high cholesterol (Wilson *et al.*, 1998), and others. In addition, the difference in the incidence of CHD between genders has caught the interest of many clinical researchers.

The average age at the onset of symptomatic CHD in women is reported to be about 10 years older than that in men (Wenger, 1997), and a delay in the occurrence of menopause is associated with a decrease in the cardiovascular mortality rate for postmenopausal woman (Van der Schouw *et al.*, 1996). Incredibly, after menopause, the risk of cardiovascular disease among women increases rapidly and eventually is equivalent to that of men (Barrett-Connor and Bush, 1991; Isles *et al.*, 1992; Davis *et al.*, 1994; Mendelsohn and Karas, 2005).

Unfortunately, the roles of sex steroids in myocardial pathophysiology remain uncharacterized, and the adverse effects of hormone replacement therapy are thought to be outweighed by the advantages (Grodstein *et al.*, 1996; Grodstein and Stampfer, 1998). Dai *et al.* (2012) described a negative correlation between the estradiol/testosterone ratio and aromatase, as well as imbalance of the serum estradiol/testosterone ratio in women with CHD. Recently, Konstantian *et al.* found that a genetic variant in *CYP19* shows a correlation with CHD (Bampali *et al.*, 2015). In addition, aromatase deficiency has been observed in a number of hyperandrogenic patients (Harada *et al.*, 1992). Other studies demonstrated that aromatase suppression may increase the development of atherosclerotic plaques, and aromatase knockout mice exhibit abnormal glucose tolerance, insulin

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resistance, and hypercholesterolemia, which may be lead to the onset of CHD (Scott *et al.*, 2012; Verma *et al.*, 2012; Gagliardi *et al.*, 2014). Based on the current literature, androgen and estrogen have received the most attention in studies of hormones in cardiovascular pathologies, whereas aromatase, which is a key enzyme in the conversion of androgen to estrogen in specific tissues, has received far less attention.

Aromatase is encoded by the *CYP19* gene, and previous research indicated that aromatase is expressed predominantly in the coronary vasculature. This evidence of cardiac aromatase expression suggests that the local cardiac androgen–estrogen system likely affects heart function and structural modeling (Jazbutyte *et al.*, 2012). In addition, genetic variations in the *CYP19* gene have been shown to result in the alteration of blood levels of sex hormones (Wang *et al.*, 2011; Koudu *et al.*, 2012; Zhang *et al.*, 2012b). When Ma *et al.* (2005) resequenced all coding exons, all upstream untranslated exons indicated that genetic variations in *CYP19* might contribute to variations in the pathophysiology of estrogen-dependent diseases.

In the present study, we hypothesized that *CYP19* gene polymorphisms might lead to an imbalance between androgen and estrogen, and thus, one or more such polymorphisms may have an impact on the coronary vascular pathology. We conducted a case–control study to examine the associations between polymorphisms in *CYP19* and CHD among a Chinese population.

Methods

Study population

From 2010 to 2013, 1706 individuals were recruited from the Department of Cardiovascular Medicine at First Affiliated Hospital of XinJiang Medical University. Although our study population contained individuals of both genders (983 men and 723 women) and different ethnicities (Table 1), we used propensity score matching techniques to match case and control groups to eliminate the effect of confounding factors. Height, weight, and blood pressure were measured, and body–mass index (BMI) was calculated. Participants completed a study survey regarding their personal medical history (hypertension, diabetes mellitus, etc.), familial medical history, reproductive history, menopausal status, and lifestyle habits (smoking, drinking, etc.) Blood samples were drawn for routine analysis of blood levels, biochemical tests, coagulation function, and genetic analyses. Written informed consent was obtained from all participants, and ethics approval was granted by the medical ethics committee of First Affiliated Hospital of XinJiang Medical University.

All patients had received a differential diagnosis for chest pain or pressure and tightness in the chest after examination in the Cardiac Catheterization Laboratory of the First Affiliated Hospital of XinJiang Medical University, and all coronary angiography procedures were performed by experienced and skilled physicians using the Judkins technique. The findings of coronary angiography were interpreted by at least two knowledgeable imaging specialists, who were blinded to the clinical date, and the final diagnosis of CHD was made according to the angiography report and the standard 15-segment model established by the American Heart Association in 1975 (Austen *et al.*, 1975). All patients were evaluated by cardiac ultrasound, which was performed by doctors with more than 10 years of experience. Similarly, the results of cardiac ultrasound were analyzed by two specialists together.

The study population included 962 patients with CHD (331 from the Han population and 631 from the Uygur population), whose coronary angiographic examination showed at least one significant coronary artery stenoses of more than 50% the luminal diameter. The control population included 744 individuals (404 from the Han population and 340 from the Uygur population). These participants did not have coronary vessel stenosis, and the exclusion criteria included obvious clinical, electrocardiographic, or echocardiography evidence of myocardial ischemia, myocardial infarction, valvular disease, cardiomyopathy, and previous stent deployment or bypass surgery. Patients, also, were excluded if they exhibited impaired renal function, malignancy, or plaque formation beginning in the neck vessels. Hypertension was diagnosed according to guidelines established by the World Health Organization and the International Society of Hypertension in 1999 (Chalmers *et al.*, 1999), and diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association (Mellitus, 2002).

Biochemical analyses

The main blood indices that have been previously associated with CHD were measured in the Clinical Laboratory Department of the First Affiliated Hospital of Xinjiang Medical University using standard methods. These indices included prothrombin time and levels of hemoglobin (Lawler *et al.*, 2013), platelets, fibrinogen (O'Connor *et al.*, 1984), glucose (Kannel and McGee, 1979), triglyceride (Hulley *et al.*, 1980; Do *et al.*, 2013), total cholesterol, high-density lipoprotein, low-density lipoprotein (May *et al.*, 2012), apolipoprotein A, apolipoprotein B (Boekholdt *et al.*, 2012), lipoprotein (Tsimikas and Hall, 2012), and the total protein.

Genotyping

We selected three single-nucleotide polymorphisms (SNPs) of the *CYP19* gene that had a minor allele frequency >0.03 in the Chinese population according to the National Center for Biotechnology Information (NCBI) SNP database (www.ncbi.nlm.nih.gov/projects/SNP), considering prior resequencing data and functional studies (Ma *et al.*, 2005) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/gtmb).

Genomic DNA was isolated from peripheral blood leukocytes using the phenol–chloroform method (Gross and Rotzer, 1998). DNA was dissolved in 200 μL sterile distilled water. Then, the DNA concentration was quantified by ultraviolet/visible (UV/Vis) spectrophotometry (http://chem247.files.wordpress.com/2007/09/chem-247-dna-lab.pdf), and samples were stored at −80°C. Finally, we analyzed the genotype with pure, integrated, and qualified DNA samples.

Genotyping was conducted using the TaqMan SNP Genotyping Assay (ABI 7900) following the manufacturer's instructions. Briefly, polymerase chain reaction (PCR) amplification was conducted in a total volume of 6 μL containing 2.5 μL Master mix, 0.15 μL SNP mix, 0.5 μL TE buffer, 1.85 μL double-distilled water, and 1 μL of DNA sample. The primers and FAM/VIC-labeled probes were designed by Applied Biosystems (http://

Table 1. Characteristics of Study Participants

Independent variable	*Control*	*CHD*	*Total*	p *value*
Sex				
Men	395 (53.09%)	588 (61.01%)	983 (57.62%)	<0.0001
Women	349 (46.91%)	374 (38.99%)	723 (42.38%)	
Race				
Han	404 (54.30%)	331 (34.41%)	735 (43.08%)	0.0002
Uygur	340 (45.70%)	631 (65.59%)	971 (56.92%)	
Age (years)	53.73 ± 10.59	57.33 ± 10.30	55.64 ± 10.59	<0.0001
BMI (kg/m^2)	26.29 ± 3.84	26.38 ± 3.75	26.33 ± 3.79	0.6711
SMOKE (mM)	553 (71.17%)	500 (57.01%)	1053 (63.66%)	<0.0001
SBP (mmHg)	126.13 ± 18.22	128.49 ± 19.88	127.39 ± 19.16	0.0154
DBP (mmHg)	78.65 ± 12.04	78.83 ± 12.04	78.75 ± 12.03	0.7673
EH	455 (58.56%)	418 (47.66%)	873 (52.78%)	<0.0001
DM	688 (88.66%)	683 (77.88%)	1371 (82.94%)	<0.0001
HB (g/L)	137.44 ± 16.16	137.55 ± 14.77	137.50 ± 15.42	0.8893
PLT 10^9/L	211.11 ± 59.60	215.01 ± 64.00	213.23 ± 62.04	0.2137
PT(S)	10.42 ± 1.38	10.66 ± 1.56	10.54 ± 1.48	0.0039
Fg (g/L)	3.34 ± 0.69	3.60 ± 0.82	3.48 ± 0.77	<0.0001
Glu (mM)	5.51 ± 2.17	6.26 ± 2.58	5.91 ± 2.43	<0.0001
TG (mM)	1.95 ± 1.46	2.16 ± 2.96	2.05 ± 2.36	0.0167
TC (mM)	4.19 ± 1.12	4.22 ± 1.66	4.20 ± 1.42	0.072
HDL (mM)	1.10 ± 0.42	0.93 ± 0.32	1.01 ± 0.38	<0.0001
LDL (mM)	2.56 ± 0.82	2.56 ± 1.06	2.56 ± 0.96	0.8871
apoA (g/L)	1.20 ± 0.27	1.18 ± 0.42	1.19 ± 0.38	0.4128
apoB (g/L)	1.33 ± 0.89	0.95 ± 0.47	1.10 ± 0.69	<0.0001
LP(a) (mg/L)	183.54 ± 183.97	212.33 ± 188.12	198.92 ± 186.69	0.0021
TP (g/L)	65.55 ± 5.65	66.13 ± 5.50	65.82 ± 5.59	0.0441

Continuous variables are expressed as mean ± standard deviation. Continuous variables were compared by independent sample *t*-tests. Differences in categorical variables were analyzed using χ^2 test or Fisher exact test.

$p < 0.05$.

BMI, body–mass index; CHD, coronary heart disease; SBP, systolic pressure; DBP, diastolic pressure; EH, essential hypertension; DM, diabetes mellitus; HB, hemoglobin; PLT, platelet; PT, prothrombin time; Fg, fibrinogen; Glu, glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; apoA, apolipoprotein A; apoB, apolipoprotein B; LP(a), lipoprotein; TP, total protein.

tools.lifetechnologies.com/content/sfs/brochures/cms_040597. pdf). The assay IDs of the selected assays were C__8234946_20(rs12050772), C__15880593_10(rs2289105), and C__27892984_20(rs4774585).

Statistical methods

Chi-square tests for genotype distribution were conducted to evaluate the deviation from Hardy–Weinberg equilibrium for the three SNPs. Data are shown as mean ± standard deviation (SD), and baseline characteristics were compared by independent sample *t*-tests or Chi-square tests. Statistical significance was established at $p < 0.05$. Spearman and Hoeffding correlations and multiple logistic regression analyses were used to identify correlations between the independent variables and CHD. Differences in categorical variables were analyzed using Fisher exact test. The distribution of genotypes between CHD and control participants was tested using χ^2 tests or Fisher exact test and binary logistic regression analysis, and differences in lipids and the parameters of echocardiography among individuals with different genotypes were assessed by one-way analysis of variance (ANOVA). Again, a p value <0.05 was considered statistically significant. Analyses were performed using SAS software (Cary, NC).

Results

Identification of clinical variables associated with CHD

Table 1 shows the clinical characteristics of the study participants, and the mean values of some variables differed significantly between CHD patients and control participants. Notably, more CHD patients had high protein levels than the controls. We then used the Spearman rank correlation and Hoeffding D measurement methods to reject variables that showed no correlation with our final variable, CHD, to reduce the number of variables to be matched in subsequent analyses (Supplementary Tables S2 and S3).

Genotyping of study groups

According to the genotype and allele distribution data presented in Table 2, among the Han population, the distribution of rs2289105 differed significantly between CHD patients and control participants among the total population and among male participants ($p = 0.0019$ and $p = 0.027$, respectively). In the Uygur population, which are a Eurasian (mixed ancestry) population with Eastern and Western Eurasian anthropometric and genetic traits, independent of gender, the distribution of rs4774585 differed significantly between CHD patients and control participants ($p = 0.0424$ for the total population, $p = 0.0485$ for men, and $p = 0.0025$ for women).

Table 2. Genotypes and Allele Distributions in Patients with Coronary Heart Disease and Control Participants

	Total			Men			Women		
	CHD	*Control*	p	*CHD*	*Control*	p	*CHD*	*Control*	p
Han population									
N	331	404		211	208		120	196	
rs12050772									
Allele									
G	331 (0.500)	358 (0.444)	0.034	210 (0.498)	187 (0.452)	0.1836	121 (0.504)	171 (0.436)	0.096
T	331 (0.500)	448 (0.556)		212 (0.502)	227 (0.548)		119 (0.496)	221 (0.564)	
Genotype									
GG	82 (0.248)	75 (0.186)		54 (0.256)	40 (0.193)		28 (0.233)	35 (0.179)	
GT	167 (0.505)	208 (0.516)	0.0852	102 (0.483)	107 (0.517)	0.3037	65 (0.542)	101 (0.515)	0.2236
TT	82 (0.248)	120 (0.298)		55 (0.261)	60 (0.290)		27 (0.225)	60 (0.306)	
rs2289105									
Allele									
C	384 (0.584)	401 (0.498)	0.001	242 (0.579)	176 (0.421)	0.2223	142 (0.592)	194 (0.495)	0.0189
T	274 (0.416)	405 (0.502)		207 (0.500)	207 (0.500)		98 (0.408)	198 (0.505)	
Genotype									
CC	114 (0.347)	93 (0.231)		70 (0.335)	45 (0.217)		44 (0.367)	48 (0.245)	
CT	156 (0.474)	215 (0.533)	0.0019	102 (0.488)	117 (0.565)	0.027	54 (0.450)	98 (0.500)	0.053
TT	59 (0.179)	95 (0.236)		37 (0.177)	45 (0.217)		22 (0.183)	50 (0.255)	
Paired comparisons									
CC/CT			0.0198			0.013			
CC/TT			0.0016			0.0287			
rs4774585									
Allele									
A	9 (0.014)	18 (0.022)	0.2233	6 (0.014)	10 (0.024)	0.3081	3 (0.013)	8 (0.020)	0.4607
G	649 (0.986)	790 (0.978)		412 (0.986)	406 (0.976)		237 (0.988)	384 (0.980)	
Genotype									
AA									
AG	9 (0.027)	18 (0.045)	0.2189	6 (0.029)	10 (0.048)	0.3033	3 (0.025)	8 (0.041)	0.4567
GG	320 (0.973)	386 (0.955)		203 (0.971)	198 (0.952)		117 (0.975)	188 (0.959)	
Uygur population									
N	591	312		337	187		254	153	
rs12050772									
Allele									
G	465 (0.395)	247 (0.396)	0.9639	277 (0.411)	153 (0.409)	0.9525	188 (0.373)	118 (0.386)	0.7198
T	713 (0.605)	377 (0.604)		397 (0.589)	221 (0.591)		316 (0.627)	188 (0.614)	
Genotype									
GG	96 (0.163)	42 (0.135)		58 (0.172)	25 (0.134)		38 (0.151)	21 (0.137)	
GT	273 (0.463)	163 (0.522)	0.2165	161 (0.478)	103 (0.551)	0.244	112 (0.444)	76 (0.497)	0.5925
TT	220 (0.374)	107 (0.343)		118 (0.350)	59 (0.316)		102 (0.405)	56 (0.366)	
rs2289105									
Allele									
C	561 (0.478)	291 (0.468)	0.6861	321 (0.476)	178 (0.478)	0.9448	240 (0.480)	143 (0.467)	0.7264
T	613 (0.522)	331 (0.532)		353 (0.524)	194 (0.522)		260 (0.520)	163 (0.533)	
Genotype									
CC	140 (0.239)	62 (0.199)		80 (0.237)	36 (0.194)		60 (0.240)	33 (0.216)	
CT	281 (0.479)	167 (0.537)	0.219	161 (0.478)	106 (0.570)	0.1303	120 (0.480)	77 (0.503)	0.84
TT	166 (0.283)	82 (0.264)		96 (0.285)	44 (0.237)		70 (0.280)	43 (0.281)	
rs4774585									
Allele									
A	134 (0.114)	59 (0.095)	0.2179	62 (0.092)	43 (0.116)	0.224	72 (0.143)	20 (0.065)	0.0008
G	1044 (0.886)	563 (0.905)		612 (0.908)	329 (0.884)		432 (0.857)	286 (0.935)	
Genotype									
AA	5 (0.008)	6 (0.019)		1 (0.003)	5 (0.027)		4 (0.016)	1 (0.007)	
AG	124 (0.211)	47 (0.151)	0.0424	60 (0.178)	33 (0.177)	0.0485	64 (0.254)	18 (0.118)	0.0025
GG	460 (0.781)	258 (0.830)		276 (0.819)	148 (0.796)		184 (0.730)	134 (0.876)	
Paired comparisons									
GG/AG			0.0355			0.9157			0.001
GG/AA			0.2025			0.0139			0.3193

p values were calculated by χ^2 test or Fisher exact test.

$p < 0.05$.

Table 3. Genotype and Allele Distributions in Patients with Coronary Heart Disease and Control Participants After Matching

	Alleles G (freq) T (freq)	p	Genotypes G/G (freq)	G/T (freq)	T/T (freq)	p
rs12050772						
case	253 (0.427) 339 (0.573)	0.388	60 (0.203)	133 (0.449)	103 (0.348)	0.009
control	241 (0.403) 357 (0.597)		37 (0.124)	167 (0.559)	95 (0.318)	
	C (freq) T (freq)		*C/C (freq)*	*C/T (freq)*	*T/T (freq)*	
rs2289105						
case	295 (0.505) 289 (0.495)	0.385	84 (0.288)	127 (0.435)	81 (0.277)	0.014
control	287 (0.482) 309 (0.518)		62 (0.208)	163 (0.547)	73 (0.245)	
	A (freq) G (freq)		*A/A (freq)*	*A/G (freq)*	*G/G (freq)*	
rs4774585						
case	38 (0.064) 558 (0.936)	0.426	2 (0.007)	34 (0.114)	262 (0.879)	0.656
control	45 (0.076) 551 (0.924)		4 (0.013)	37 (0.124)	257 (0.862)	

p values were calculated by χ^2 test or Fisher exact test.
$p < 0.05$.

Genotype comparison between CHD patients and control participants after matching

We used the SAS ''pscore'' command to generate propensity scores, and the code and output produced by the ''pscore'' command have been described previously (Coca-Perraillon, 2007). This procedure automatically tests for balance between the case and control groups on covariates used to predict the propensity score, and when we controlled the differences in pscores from 0 to 0.1, our total of 596 samples (298 control participants and 298 CHD patients) remained to the end. The Chi-square (χ^2) test was used to compare the independent variables (previously segmented) between the cases and controls after matching, and the analysis confirmed that there were no differences ($p > 0.05$; Supplementary Table S4).

The data in Table 3 show that in the 596 study subjects, the *CYP19* rs12050772 genotypic distributions for CHD patients (0.203 for GG, 0.449 for GT, and 0.348 for TT) differed from those for the controls (0.124, 0.559, and 0.318, respectively), but unfortunately, the genotypic distribution for control participants was not in Hardy–Weinberg equilibrium. In contrast, the distributions of the *CYP19* gene rs2289105 and rs4774585 polymorphisms were in Hardy–Weinberg equilibrium for both groups. The *CYP19* rs2289105 genotypic distributions for CHD patients (0.288 for CC, 0.435 for CT, and 0.277 for TT) were significantly different from those for the controls (0.208, 0.547, and 0.245, respectively), whereas the genotypic distributions of *CYP19* rs4774585 for CHD patients (0.007 for AA, 0.114, for AG, and 0.879 for GG) did not differ from those of the control participants (0.013, 0.124, and 0.862, respectively).

Binary logistic regression analysis (Table 4) showed that compared to the GG genotype of the distribution of rs12050772, the GT genotype was associated with a significantly lower risk of CHD ($p = 0.003$ and odds ratio OR = 0.491), but again, unfortunately, the genotypic distribution of rs12050772 was not in Hardy–Weinberg equilibrium in control participants. We also observed that the rs2289105 heterozygote GT was associated with a significantly lower risk of CHD than the homozygous wild-type GG ($p = 0.0063$ and OR = 0.575).

The data in Table 5 show that the blood lipid levels and other parameters of echocardiography among individuals with different genotypes did not differ between CHD patients and control participants ($p > 0.05$). In addition, multiple logistic regression analysis (Table 6) showed that after adjustment for the risk factors of CHD, the associations between rs4774585 SNPs and CHD in the Uygur population were no longer statistically significant.

Table 4. Binary Logistic Regression Analysis for Genotype and Coronary Heart Disease After Matching

Variable	*Estimate*	*StdErr*	*WaldChiSq*	*ProbChiSq*	*Effect*	*OddsRatioEst*	*LowerCL*	*UpperCL*
Intercept	0.1122	0.0928	1.4634	0.2264				
rs12050772	–0.0314	0.1239	0.0641	0.8002	SNP1NC 4–2	0.669	0.407	1.097
rs12050772	**–0.3399**	**0.1145**	**8.8126**	**0.003**	**SNP1NC 4–3**	**0.491**	**0.307**	**0.785**
Intercept	0.0527	0.087	0.3672	0.5446				
rs2289105	0.0513	0.1275	0.1619	0.6874	SNP2NC 4–2	0.819	0.519	1.292
rs2289105	**–0.3023**	**0.1106**	**7.4676**	**0.0063**	**SNP2NC 4–3**	**0.575**	**0.385**	**0.86**
Intercept	–0.2285	0.3015	0.5741	0.4486				
rs4774585	–0.4646	0.5839	0.6331	0.4262	SNP3NC 4–2	0.496	0.09	2.733
rs4774585	0.2285	0.3333	0.4697	0.4931	SNP3NC 4–3	0.992	0.595	1.656

rs12050772, 2:TT; 3:GT; 4:GG; rs2289105, 2:TT; 3:CT; 4:CC; rs4774585, 2:AA; 3:AG; 4:GG.
$p < 0.05$, there is significance between two genotypes, and the OR value is between 0 and 1, the latter is a protect factors.

TABLE 5. DIFFERENCES IN LIPIDS AND PARAMETERS OF ECHOCARDIOGRAPHY AMONG INDIVIDUALS WITH DIFFERENT GENOTYPES ACCORDING TO ANALYSIS OF VARIANCE

	rs2289105				
Variables	*TT*	*CT*	*CC*	F	p
HDL (mM)	1.01 ± 0.33	0.99 ± 0.34	1.01 ± 0.36	0.14	0.869
LDL (mM)	2.57 ± 0.98	2.56 ± 0.78	2.51 ± 0.78	0.26	0.771
apoA(gl/L)	1.19 ± 0.25	1.19 ± 0.25	1.20 ± 0.25	0.151	0.86
apoB(g/L)	1.08 ± 0.65	1.08 ± 0.68	1.05 ± 0.64	0.129	0.879
LP(a)(mg/L)	204.25 ± 157.40	185.41 ± 176.96	209.78 ± 205.05	1.095	0.335
TP(g/L)	66.46 ± 4.88	65.75 ± 5.71	65.62 ± 5.34	1.133	0.323
LVDd(mm)	50.48 ± 6.08	49.25 ± 5.36	50.41 ± 7.21	2.087	0.125
LVDs(mm)	33.93 ± 6.86	32.51 ± 6.54	33.09 ± 5.46	1.866	0.156
IVS(mm)	9.15 ± 1.28	9.20 ± 1.73	9.33 ± 1.37	0.404	0.668
PW(mm)	9.04 ± 1.30	9.18 ± 1.79	9.46 ± 2.24	1.554	0.213
RVOT(mm)	27.04 ± 2.91	26.99 ± 3.05	27.37 ± 2.63	0.629	0.534
RV(mm)	18.57 ± 1.94	18.58 ± 2.38	18.75 ± 2.84	0.197	0.821
RA(mm)	33.73 ± 3.46	33.19 ± 3.21	33.04 ± 2.75	1.527	0.218
PA(mm)	22.77 ± 3.42	22.13 ± 2.73	22.64 ± 2.76	2.126	0.121
FS(%)	33.33 ± 6.61	33.62 ± 5.03	33.39 ± 4.39	0.14	0.869
EF(%)	60.48 ± 10.81	61.53 ± 9.69	61.96 ± 6.64	0.755	0.47
SV(mL)	73.63 ± 15.78	70.84 ± 12.90	74.79 ± 18.72	2.71	0.068
CO(L/min)	5.60 ± 1.54	5.27 ± 1.20	5.34 ± 1.32	2.318	0.1

$p > 0.05$.
LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; IVS, interventricular septum; PW, posterior wall; PVOT, right ventricular outflow tract; RV, right ventricle; RA, right atrium; PA, pulmonary artery; FS, fractional shortening; EF, ejection fraction; SV, stroke volume; CO, cardiac output.

Discussion

We identified a significant association between rs228105 in *CYP19* and CHD, and to the best of our knowledge, this is the first investigation of such an association. Our interest in *CYP19* (aromatase) in relation to CHD was derived from studies implying that sex hormones may play complex roles in cardiac functions, such as a study suggesting the existence of both estrogen and androgen receptors on endothelial cells and vascular smooth muscle cells (Oparil *et al.*, 1996) and another study proposing that sex hormone ratios influence coronary health (He *et al.*, 2007). The *CYP19* gene located on chromosome 15q21.1 codes for a single CYP19 protein known as aromatase. The aromatase activity affects both androgen and estrogen metabolism. Moreover, aromatase is a key enzyme in the conversion of androgen to estrogen and plays an important role in the balance of sex hormone levels in different tissues (Belgorosky *et al.*, 2009; Santen *et al.*, 2009). Aromatase has been found to be produced in the ovary, adipose tissue, bone, and brain (Simpson *et al.*, 2002), and notably, aromatase expression has been observed in vascular cell types such as smooth muscle cells (Harada *et al.*, 1999), endothelial cells (Sasano *et al.*, 1999), and immature heart cells/cardiomyocytes (Price *et al.*, 1992; Grohé *et al.*, 1998).

TABLE 6. ADJUSTED ASSOCIATIONS BETWEEN RS4774585 AND CORONARY HEART DISEASE IN UYGUR POPULATION

	Exp (B)	*95% CI*	p
SNP3			
*GG			Reference
AA	<0.0001		0.999
AG	0.839	(0.4, 1.76)	0.642
PT	1.11	(0.91, 1.354)	0.302
Fg	1.423	(0.902, 2.245)	0.13
Glu	1.047	(0.918, 1.193)	0.495
TG	0.958	(0.735, 1.247)	0.749
HDL	0.446	(0.164, 1.212)	0.113
LDL	1.129	(0.733, 1.737)	0.582
apoA	1.383	(0.518, 3.69)	0.517
apoB	0.755	(0.185, 3.089)	0.696
LP(a)	1.001	(1, 1.003)	0.126
EH	0.942	(0.522, 1.699)	0.842

$p > 0.05$.

Human aromatase deficiency was first reported in 1995, and in this condition, the basal concentrations of plasma androgen were elevated, whereas plasma estradiol levels were low (Morishima *et al.*, 1995). These results indicated that a lack of aromatase leads to a disturbance in the balance of sex hormones and also indicates that a single base change in exon 9 of *CYP19* may be directly responsible for these changes. Based on several studies that have comprehensively evaluated associations between SNPs in the *CYP19* gene and levels of sex hormones (Haiman *et al.* 2007; Cai *et al.*, 2008; Kidokoro *et al.* 2009), we believe that significant *CYP19* gene polymorphisms may alter hormone levels to varying degrees. In particular, imbalance of the estrogen/androgen ratio, rather than individual levels of estragon or androgen, has been associated with the development of CHD (Dai *et al.*, 2012), and Seruga *et al.* (2014) reported that the use of aromatase inhibitors might be associated with an increased risk for CHD. Interaction between *CYP19* polymorphisms and estrogen-dependent diseases such as polycystic ovary syndrome (PCOS) and osteoporosis also have been reported. In PCOS patients, Zhang *et al.* (2012a) found that an SNP in *CYP19*

might inhibit the aromatase activity and be associated with the estradiol/testosterone ratio. Considering these previous study results and the lack of research investigating associations between *CYP19* polymorphisms and CHD, we sought to directly determine whether specific *CYP19* gene polymorphisms correlate with the risk of CHD. We identified rs12050772 and rs2289105 within the NCBI database because the minor allele frequencies for both were close to 0.5 in the Chinese population. We identified rs4774585 based on a previous cohort study that reported this mutation may be related to the outcomes of cardiovascular disease. Thus, we considered that these mutations are likely to be protection factors in humans, although the relevant literature is lacking.

CHD is an extremely complicated disease, for which certain clinical parameters such as glucose and blood lipid levels are known to differ significantly between patients and health controls. To eliminate the effects of the major confounding factors for CHD, we used propensity score matching techniques to match the case and control groups directly, and the same propensity score indicated the same distribution of measured baseline covariates (Rosenbaum and Rubin, 1983; Frisco *et al.*, 2007). We believe this strengthens the ability of our study to identify potential effects of *CYP19* polymorphisms. In the present case–control study, we found that compared to the GG genotype of rs12050772, the GT genotype is associated with a significantly lower risk of CHD. However, unfortunately, the genotypic distribution of rs12050772 was not in Hardy–Weinberg equilibrium in our control group. We confirmed the results of genotyping, and because all 96-well plates included one blank well as a control, we consider the results to be valid and choose to control according to the strict criteria selection. *De novo* mutations, selection, genetic drift, and gene flow can all theoretically bias the allele and genotype frequencies and thus the Hardy–Weinberg equilibrium. We believe that we can only temporarily ignore the effect of rs12050772 in CHD, and we will continue to explore the relationship between rs12050772 and CHD by increasing the sample size in our control group.

Our results did reveal that the rs2289105 genotypic distributions in CHD patients differed significantly from those in control participants, and the heterozygote CT genotype was associated with a significantly lower risk of CHD than the homozygous wild-type CC genotype. In addition, no significant difference in CHD risk was found between the homozygous mutant and the homozygous wild type. Thus, we propose that the CT genotype of rs2289105 in *CYP19* may be a protective genetic marker for CHD. Furthermore, we observed that the rs4774585 genotype distributions did not differ significantly between CHD patients and controls. Initially, we observed an association between the rs4774585 polymorphism and CHD in the Uygur population that was independent of gender and because the sample size was not large enough to use propensity score matching techniques for group matching, we used multiple logistic regression analysis. After adjustment for the risk factors, these associations were no longer statistically significant. This outcome may indicate that the role of the confounding factors is more important compared with the SNP, but it may also simply be the result of our sample size being too small. Thus, we will strengthen the power in future analyses by increasing the sample sizes in the Uygur population groups.

A previous cohort study investigating associations between *CYP19* and cardiovascular disease found that the SNP3 G>A variant allele was associated with a 78% increase in mortality in men, and in their hypertensive CHD group, the variant allele was associated with a 65% increase in death, myocardial infarction, or stroke in men and a 69% decrease in these outcomes in women. To summarize, they showed that the rs4774585 polymorphism and outcomes of CHD and hypertension are closely related (Beitelshees *et al.*, 2010). However, in our case–control study, we did not observe a statistically significant relationship between rs4774585 and CHD. One disadvantage in case–control studies is that control individuals may become patients in the future. Although we used propensity score matching to estimate the analogous probability of CHD development in the samples and matched the case and control groups, we believe further cohort studies are necessary. In addition, based on limitations in time and manpower, we did not have access to hormone levels in our study populations. Thus, we cannot further investigate whether our results are associated with imbalances in sex hormone ratios. Interestingly, a study of hypertension in PCOS patients showed that the estrogen-to-androgen ratio was lower among patients with hypertensive PCOS, and although this study did not describe mutations of *CYP19*, subcutaneous *CYP19* mRNA expression was shown to be significantly higher in patients with hypertensive PCOS. Moreover, the study reported that serum estradiol levels in these patients were similar to those in the normotensive PCOS and control groups (Lecke *et al.*, 2011). They speculated that the synthesized estrogens were only partially secreted into the circulation and acted on tissues through intracrine, autocrine, or paracrine mechanisms (Harada *et al.*, 1999; Simpson, 2003; Czajka-Oraniec and Simpson, 2010). These effects are worth further consideration. In our study, ANOVA indicated that blood lipid levels and parameters of echocardiography among individuals with different genotypes did not differ between CHD patients and control participants. These results suggest that aromatase may affect the heart function through mechanisms other than those involving lipid metabolism, and recent studies in animals have shown that testosterone and estrogen have contrasting inotropic actions and modulate Ca(2+) handling and transient characteristics (Bell *et al.*, 2013). We intend to further investigate feasible mechanisms underlying the effects of aromatase in the cardiovascular system through studies in cells and animal models.

In conclusion, this is the first case–control study to examine the associations between rs12050772, rs2289105, and rs4774585 in *CYP19* and CHD. The results show that the heterozygote CT genotype of rs2289105 is associated with a reduced risk of CHD and may be a marker for protection from CHD susceptibility. However, further research into the mechanisms by which aromatase affects the cardiovascular system is needed.

Author Disclosure Statement

No competing financial interests exist.

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