Int J Clin Exp Med 2016;9(2):4211-4221
www.ijcem.com /ISSN:1940-5901/IJCEM0016373

Original Article

The association of YKL-40 genetic polymorphisms with coronary artery disease in Taiwan population

Ke-Hsin Ting[1,2], Kwo-Chang Ueng[3,4], Shun-Fa Yang[1,5], Po-Hui Wang[1,4,6]

[1]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan; [2]Division of Cardiology, Department of Internal Medicine, Changhua Christian Hospital, Yunlin Branch, Yunlin, Taiwan; [3]Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan; [4]School of Medicine, Chung Shan Medical University, Taichung, Taiwan; [5]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan; [6]Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan

Received September 18, 2015; Accepted December 17, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Background: YKL-40, released by human activated macrophages, neutrophils and vascular smooth muscle cells, plays a role in the pathogenesis of endothelial dysfunction, atherosclerosis and abnormal angiogenesis. However, the association of single nucleotide polymorphisms (SNPs) of YKL-40 with coronary artery disease (CAD) has not been clear in the Taiwan population and needed to be investigated. Materials and methods: Five hundred and seventy-six unrelated Taiwanese patients (male 397, female 179), receiving coronary angiography because of chest pain at Chung Shan Medical University Hospital were recruited from April 2007 to March 2013. The blood samples were obtained for the analysis of YKL-40 SNPs rs6691378, rs10399805, rs4950928, rs880633 using real time PCR assay from CAD case group (373 patients) and non-CAD control group (203 controls). Results: In the female population, the frequencies of YKL-40 rs6691378 with GA/AA genotype [P=0.008, odds ratio (OR)=2.267] and rs10399805 with GA/AA genotype (P=0.004, OR=2.421,) were higher, as compared to their wild GG genotypes in CAD than non-CAD groups After multivariate analysis for YKL-40 SNPs and clinical features in the female group. In addition to, recent 24 hours severe angina and elevated cardiac enzyme, YKL-40 SNP rs10399805 GA/AA (P=0.009, OR=2.524, 95% confidence interval =1.254-5.078) was an independent factors for CAD. Conclusion: In the Taiwanese female, YKL-40 SNP rs6691378 (-1371G/A) with GA/AA genotype and SNP rs10399805 (-247G/A) with GA/AA genotype were associated with CAD. Based on multivariate analysis, YKL-40 SNP rs10399805 (-247G/A) however was an only independent genetic factor for CAD in the Taiwanese female.

Keywords: YKL-40, coronary artery disease, single-nucleotide polymorphism

Introduction

Coronary artery disease (CAD) is an increasing problem worldwide and the most common cause of death in Western countries. It is estimated that 132000000 Americans have CAD, of whom 650000 have angina pectoris and 7200000 have myocardial infarction. The World Health Organization has estimated that by 2020, the number of deaths worldwide from CAD will have risen from 7.2 million in 2002 to 11.2 million [1]. In Taiwan, ischemic heart disease has become the second most common etiology of all-cause mortality in the past 10 years.

YKL-40 is a 40-kDa heparin- and chitin-binding glycoprotein [2], and its abbreviation is based on the one-letter code for the first 3 N-terminal amino acids, tyrosine (Y), lysine (K), and leucine (L) and the apparent molecular weight of 40 kDa. YKL-40 is secreted in vitro by various cells and seems to be particularly involved in the activation of the innate immune system and in cell processes in relation to extracellular matrix remodeling [3]. In vivo YKL-40 protein expression is found in human vascular smooth muscle cells (VSMCs) in adventitial vessels and in subpopulations of macrophages and VSMCs in different tissues with inflammation and extracellular matrix remodeling, as in atherosclerotic plaques [4].

YKL-40 is also associated with both the early and late stages of the development of athero-

sclerosis. Macrophages in atherosclerotic plaques express YKL-40 mRNA, particularly macrophages that have infiltrated deeper into the lesion, with the highest expression of YKL-40 in macrophages in the early stage of atherosclerosis [5]. YKL-40 induces the maturation of monocytes to macrophages and is then secreted by macrophages during the late stages of differentiation and by activated macrophages [6]. An evidence-based trial proved that the relationship between the circulating YKL-40 level and CAD or cerebrovascular disease. In the CLARICOR trial [7], Kastrup et al. found that high serum YKL-40 levels are associated with myocardial infarction, cardiovascular death, and all-cause mortality in patients with stable CAD. In addition, genetic polymorphisms of YKL-40 seem to be correlated with the YKL-40 level but not CAD [8]. Zheng et al. found that YKL-40 -329G/A and YKL-40 -131C/G polymorphisms are associated with high serum YKL-40 levels but not with the prevalence or severity of CAD. However, the genetic polymorphisms of YKL-40 that are correlated with CAD or cardiovascular events have not been confirmed [9, 10]. Therefore, in our study, we investigated the correlation of YKL-40 single-nucleotide polymorphisms (SNPs) with CAD in the Taiwanese population.

Materials and methods

Study population

A total of 576 Taiwanese adult patients with angina pectoris were enrolled in this study between April 2007 and March 2013. All of them received coronary angiography after serial cardiovascular noninvasive examinations including a treadmill test, myocardial perfusion scan, or multislice cardiac computed tomography. Coronary angiography was performed via trans-radial or trans-femoral approach. The lumen stenosis of coronary artery was measured by quantitative coronary angiography (QCA). Angiographic criteria defining CAD cases or controls were as followings: 1) cases group (373 patients; male 275, female 98): lumen stenosis > 50% lumen narrowing on one or more major epicardial coronary arteries, 2) control group (203 controls; male 122, female 81): lumen stenosis < 50% lumen narrowing on one or more major epicardial coronary artery segments.

We collected information on demographic and clinical characteristics, including age, sex, height, weight, hypertension, diabetes, smoking history, family history, cholesterol level, and cardiac enzyme level. Acute coronary syndrome was defined as unstable angina, non-ST elevation myocardial infarction, and ST elevation myocardial infarction. The exclusion criteria were recent stroke, out-hospital cardiac arrest, and incomplete data. Elevated cardiac troponin I levels and positive coronary angiography indicated that the patients had acute coronary syndrome. All the patients provided informed consent. The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of Chung Shan Medical University Hospital.

Selection of YKL-40 gene polymorphisms

Three SNPs rs6691378 (-1371, G/A), rs10399805 (-247, G/A), and rs4950928 (-131, C/G) in the promoter region and one SNP rs880633 (+2950, T/C) in exon 5 were selected based on the Chinese HapMap (Han Chinese in Beijing, China) data and the studies of Thomsen et al. and Kjaergaard et al. [11, 12]. The minor allele frequencies (MAFs) of these SNPs were ≥ 5%.

DNA extraction

We collected whole blood samples from controls and patients with CAD in tubes containing EDTA, which were centrifuged and subsequently stored at -80°C. Genomic DNA was extracted using QIAamp DNA blood mini kits (Qiagen, Valencia, USA) according to the manufacturer's instructions, and the DNA was dissolved in TE buffer and then quantitated using absorbance. The final DNA prepared was stored at -20°C and used as templates for the following experiments.

Quantitative real-time PCR

Allelic discrimination of the YKL-40 SNPs rs6691378, rs10399805, rs4950928, rs880633 gene polymorphisms was assessed using an ABI StepOne™ real-time PCR system (Applied Biosystems), SDS V3.0 software (Applied Biosystems), and the TaqMan assay. The final volume for each reaction mixture was 5 μL, containing 2.5 mL TaqMan genotyping

Table 1. Demographic characteristics of patients with coronary artery disease (CAD; N=373) and without CAD (non-CAD; N=203)[1]

Demographic characteristics	Non-CAD (n=203)	CAD (n=373)	*P* value
Gender			
Male	122 (60.1%)	275 (73.7%)	0.001*
Female	81 (39.9%)	98 (26.3%)	
Age (years; mean ± SD)	66.32 ± 11.85	65.42 ± 11.21	0.366
Height (cm; mean ± SD)	160.43 ± 8.74	161.86 ± 8.41	0.055
Weight (kg; mean ± SD)	64.08 ± 13.08	67.08 ± 12.58	0.004*
Body mass index (kg/m^2; mean ± SD)	25.05 ± 3.79	25.58 ± 4.43	0.147
SBP (mmHg; mean ± SD)	131.36 ± 20.42	132.30 ± 21.04	0.611
DBP (mmHg; mean ± SD)	78.01 ± 14.84	79.06 ± 15.37	0.431

[1]Statistical analysis: Student's *t* test, chi-square test. *P value < 0.05.

master mix, 0.125 μL TaqMan probe mix, and 10 ng genomic DNA. The reaction conditions included an initial denaturation step at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. For each assay, appropriate controls (nontemplate and known genotypes) were included in each typing run to monitor reagent contamination and as quality control.

Statistical analysis

The Hardy-Weinberg equilibrium was assessed using a chi-square goodness-of-fit test for biallelic markers. The distributions of demographic characteristics and genotype frequencies for different genotypes between the study participants and controls were analyzed using the chi-square test, and Fisher's exact test was used for a small sample size for certain categories of variables. Student's *t*-test was used to evaluate the differences in the laboratory findings between the 2 groups. The odds ratios (ORs) and their 95% confidence intervals (CIs) of the association between the genotype frequencies and HCC were estimated using multiple logistic regression models by controlling for covariates. A *P* value of less than 0.05 was considered statistically significant. The data were analyzed using SPSS 12.0 statistical software.

Results

Patients, demographic data, and clinical features

Patients with CAD presented with the higher percentage in the demographic characteristics or clinical features when compared to subjects without CAD including male gender, height, body weight, obvious CAD risk factors, hypertension, diabetes mellitus, aspirin used in the past 7 days, severe angina in the recent 24 hours, elevated cardiac enzymes (**Tables 1** and **2**).

Association of CAD with YKL-40 genetic polymorphisms

Genotype or allele distributions of YKL-40 rs6691378, rs10399805, rs4950928, and rs880633 in the CAD and non-CAD groups are shown in **Table 3**. Odds ratios (ORs) for genotype or allele analyses were calculated for the CAD and non-CAD groups as a reference. No significant difference was observed in the frequency of YKL-40 rs6691378, rs880633 and rs10399805 genotypes or alleles ($P > 0.05$) between the groups. However, the frequency of the CG/GG genotype of YKL-40 rs4950928 in the CAD group (26.3%) was lower than that in the non-CAD group (34.5%), which reflected a lower OR for the CG or CG genotype in the CAD group (OR=0.677, 95% CI=0.468-0.980, P=0.039). The frequency of the G allele of YKL-40 rs4950928 in the CAD group (14.5%) was lower than that in the non-CAD group (19.2%), which indicated a lower OR for the G allele in the CAD group (OR=0.712, 95% CI=0.517-0.981, P=0.037). The clinical features of non-wild-type (CG/GG) and wild-type (CC) carriers of the YKL-40 rs4950928 genotype in the non-CAD group were compared (data not shown). A lower percentage of non-wild-type carriers (CG/GG, 27.1%) had elevated cardiac enzyme levels compared with wild-type carriers (CC, 43.6%). The OR obtained for the CG/GG genotype of YKL-40 rs4950928 was significantly lower than

Table 2. Clinical variables of patients with coronary artery disease (CAD; N=373) and without CAD (non-CAD; N=203)[1]

Variables	Non-CAD (n=203)	CAD (n=373)	Odds ratio (95% CI)	*P* value
CAD risks ≥ 3				0.011*
Negative	107	155	1.00	
Positive	96	218	1.568 (1.111-2.211)	
Age > 65 year				0.245
Negative	85	175	1.00	
Positive	118	198	0.815 (0.577-1.151)	
Family history				0.669
Negative	158	296	1.00	
Positive	45	77	0.913 (0.603-1.384)	
Hypertension				0.003*
Negative	78	99	1.00	
Positive	125	274	1.727 (1.200-2.486)	
Diabetes mellitus				0.015*
Negative	137	213	1.00	
Positive	66	160	1.559 (1.090-2.231)	
Active smoker				0.081
Negative	129	209	1.00	
Positive	74	164	1.368 (0.963-1.944)	
Cholesterol > 200				0.929
Negative	121	223	1.00	
Positive	82	150	0.977 (0.688-1.386)	
Aspirin use in the past 7 days				< 0.001*
Negative	150	226	1.00	
Positive	53	147	1.977 (1.355-2.885)	
Recent (< 24 h) severe angina				< 0.001*
Negative	86	103	1.00	
Positive	117	270	1.965 (1.371-2.817)	
Cardiac markers elevation				< 0.001*
Negative	126	156	1.00	
Positive	77	217	2.276 (1.604-3.231)	
Stroke				0.433
Negative	178	334	1.00	
Positive	25	39	0.807 (0.472-1.380)	

[1]Statistical analysis: Student's *t* test, Simple logistic regression. **P* value < 0.05.

that for the CC genotype (OR=0.482, 95% CI=0.257-0.903, *P*=0.023).

Associations among CAD, clinical characteristics, and YKL-40 genetic polymorphisms in female patients

Because multivariate analysis revealed no associations between YKL-40 SNPs and CAD in all patients and male patients (data not shown), we further analyzed the associations among CAD, clinical variables, and YKL-40 SNPs in the female patients. We found that hypertension, recent severe angina, and cardiac marker elevation were significantly associated with CAD (*P*=0.015, OR=2.294, 95% CI=1.178-4.469; *P*=0.009, OR=2.346, 95% CI=1.241-4.434; and *P*=0.001, OR=2.851, 95% CI=1.526-5.326, respectively; **Table 4**). Genotype or allele distributions of YKL-40 rs6691378, rs10399805, rs4950928, and rs880633 in the female patients in the CAD and non-CAD groups are shown in **Table 5**. No significant difference was observed in the frequency of YKL-40

Table 3. Genotype distributions of the single nucleotide polymorphisms of YKL-40 gene in patients with coronary artery disease (CAD) and those without CAD

Variables	Non-CAD (n=203) (%)	CAD (n=373) (%)	OR (95% CI)	*P* value
rs6691378				
GG	109 (53.7%)	184 (49.3%)	1.00	
GA	76 (37.4%)	161 (43.2%)	1.255 (0.874-1.801)	*P*=0.218
AA	18 (8.9%)	28 (7.5%)	0.921 (0.487-1.744)	*P*=0.802
GG	109 (53.7%)	184 (49.3%)	1.00	
GA/AA	94 (46.3%)	189 (50.7%)	1.191 (0.846-1.678)	*P*=0.310
GG/GA	185 (91.1%)	345 (92.5%)	1.00	
AA	18 (8.9%)	28 (7.5%)	0.834 (0.449-1.548)	*P*=0.565
G	294 (72.4%)	529 (70.9%)	1.00	
A	112 (27.6%)	217 (29.1%)	1.077 (0.823-1.409)	*P*=0.590
rs10399805				
GG	113 (55.7%)	194 (52.0%)	1.00	
GA	73 (36.0%)	156 (41.8%)	1.245 (0.867-1.788)	*P*=0.236
AA	17 (8.4%)	23 (6.2%)	0.788 (0.404-1.538)	*P*=0.485
GG	113 (55.7%)	194 (52.0%)	1.00	
GA/AA	90 (44.3%)	179 (48.0%)	1.158 (0.822-1.633)	*P*=0.401
GG/GA	186 (91.6%)	350 (93.8%)	1.00	
AA	17 (8.4%)	23 (6.2%)	0.719 (0.375-1.379)	*P*=0.321
G	299 (73.6%)	544 (72.9%)	1.00	
A	107(26.4%)	202 (27.1%)	1.038 (0.789-1.364)	*P*=0.791
rs4950928				
CC	133 (65.5%)	275 (73.7%)	1.00	
CG	62 (30.5%)	88 (23.6%)	0.686 (0.467-1.009)	*P*=0.056
GG	8 (3.9%)	10 (2.7%)	0.605 (0.233-1.567)	*P*=0.300
CC	133 (66.5%)	275 (73.7%)	1.00	
CG/GG	70 (34.5%)	98 (26.3%)	0.677 (0.468-0.980)	*P*=0.039*
CC/CG	195 (96.1%)	363 (97.3%)	1.00	
GG	8 (3.9%)	10 (2.7%)	0.671 (0.261-1.729)	*P*=0.409
C	328 (80.8)	638 (85.5%)	1.00	
G	78 (19.2%)	108 (14.5%)	0.712 (0.517-0.981)	*P*=0.037*
rs880633				
TT	81 (39.9%)	147 (39.4%)	1.00	
CT	96 (47.3%)	173 (46.4%)	0.993 (0.687-1.435)	*P*=0.970
CC	26 (12.8%)	53 (14.2%)	1.123 (0.653-1.931)	*P*=0.674
TT	81 (39.9%)	147 (39.4%)	1.00	
CT/CC	122 (60.1%)	226 (60.6%)	1.021 (0.720-1.448)	*P*=0.908
TT/CT	177 (87.2%)	320 (85.8%)	1.00	
CC	26 (12.8%)	53 (14.2%)	1.128 (0.681-1.866)	*P*=0.641
T	258 (63.5%)	467 (62.6%)	1.00	
C	148 (36.5%)	279 (37.4%)	1.041 (0.811-1.338)	*P*=0.751

The odds ratio (OR) with their 95% confidence intervals were estimated by simple logistic regression, chi-square or Fisher exact tests. *P value < 0.05.

rs4950928 genotypes or alleles ($P > 0.05$). No significant difference was observed in the frequency of YKL-40 rs880633 genotypes or alleles ($P > 0.05$). However, the frequency of

Table 4. Clinical variables of female patients with coronary artery disease (CAD; N=98) and without CAD (non-CAD; N=81)[1]

Variables	Non-CAD (n=81)	CAD (n=98)	Odds ratio (95% CI)	*P* value
CAD risks ≥ 3				0.076
Negative	48	45	1.00	
Positive	33	53	1.713 (0.945-3.107)	
Age > 65 year				0.912
Negative	25	31	1.00	
Positive	56	67	0.965 (0.511-1.821)	
Family history				0.883
Negative	66	79	1.00	
Positive	15	19	1.058 (0.499-2.244)	
Hypertension				0.015*
Negative	30	20	1.00	
Positive	51	78	2.294 (1.178-4.469)	
Diabetes mellitus				0.117
Negative	54	54	1.00	
Positive	27	44	1.630 (0.886-2.999)	
Active smoker				0.733
Negative	75	92	1.00	
Positive	6	6	0.815 (0.253-2.632)	
Cholesterol > 200				0.247
Negative	54	57	1.00	
Positive	27	41	1.437 (0.778-2.656)	
Aspirin use in the past 7 days				0.472
Negative	62	72	1.00	
Positive	19	26	1.285 (0.648-2.552)	
Recent (< 24 h) severe angina				0.009*
Negative	35	24	1.00	
Positive	46	74	2.346 (1.241-4.434)	
Cardiac markers elevation				0.001*
Negative	58	46	1.00	
Positive	23	52	2.851 (1.526-5.326)	
Stroke				0.096
Negative	76	84	1.00	
Positive	5	14	2.424 (0.832-7.064)	

[1]The odds ratio (OR) with their 95% confidence intervals were estimated by simple logistic regression, chi-square or Fisher exact tests. *P value < 0.05.

the GA/AA genotype of YKL-40 rs6691378 was higher in the CAD group than in the non-CAD group (P=0.008, 57.1% vs 37.1%), which revealed a higher OR in the CAD group than in the non-CAD group (OR=2.267, 95% CI=1.240-4.412). The frequency of the A allele of YKL-40 rs6691378 was higher in the CAD group than in the non-CAD group (P=0.023, 33.2% vs 22.2%). This reflected a higher OR in the CAD group than in the non-CAD group (OR=1.737, 95% CI=1.080-2.739). The frequency of the GA/AA genotype of YKL-40 rs10399805 was significantly different between the CAD and non-CAD groups (P=0.004, 56.0% vs 34.0%), with an increased OR obtained for developing CAD (OR=2.421, 95% CI=1.319-4.444). A statistically significant difference was observed in the frequency of the A allele between the CAD and non-CAD groups (P=0.024, 31.6% vs 21%), with an increased OR for developing CAD (OR=1.742, 95% CI=1.704-2.824). Based on multivariate analysis of the association of CAD with YKL-40 SNP and clinical features in the female patients, the serial independent risk factors were hypertension, severe angina in the previous 24 hours, and elevated cardiac troponin I levels (**Table 6**). The GA/AA genotype of YKL-40 rs10399805 was a strong independent risk factor for CAD in the female patients (P < 0.009, OR=2.524, 95% CI= 1.254-5.078). Moreover, regarding the associations of clinical features with YKL-40 SNPs, a significant difference was observed in elevated cardiac troponin I levels between patients with rs6691378 GA/AA and GG (P=0.01, 64.3% vs 38.1%, OR=2.925, 95% CI=1.277-6.699, **Table 7**).

Discussion

In our study, in the Taiwanese population, we found that the frequency of the G allele of YKL-

Table 5. Genotype distributions of the single nucleotide polymorphisms of YKL-40 gene in female patients with coronary artery disease (CAD) and those without CAD

Variables	Non-CAD (n=81) (%)	CAD (n=98) (%)	OR (95% CI)	*P* value
rs6691378				
GG	51 (63.0%)	42 (42.9%)	1.00	
GA	24 (29.6%)	47 (48.0%)	2.378 (1.255-4.506)	*P*=0.008*
AA	6 (7.4%)	9 (9.2%)	1.821 (0.600-5.531)	*P*=0.290
GG	51 (63.0%)	42 (42.9%)	1.00	
GA/AA	30 (37.0%)	56 (57.1%)	2.267 (1.240-4.142)	*P*=0.008*
GG/GA	75 (92.6%)	89 (90.8%)	1.00	
AA	6 (7.4%)	9 (9.2%)	1.264 (0.430-3.714)	*P*=0.670
G	126 (77.8%)	131 (66.8%)	1.00	
A	36 (22.2%)	65 (33.2%)	1.737 (1.080-2.793)	*P*=0.023*
rs10399805				
GG	53 (65.4%)	43 (43.9%)	1.00	
GA	22 (27.2%)	48 (49.0%)	2.689 (1.410-5.127)	*P*=0.003*
AA	6 (7.4%)	7 (7.1%)	1.438 (0.450-4.597)	*P*=0.540
GG	53 (65.4%)	43 (43.9%)	1.00	
GA/AA	28 (34.0%)	55 (56.0%)	2.421 (1.319-4.444)	*P*=0.004*
GG/GA	75 (92.6%)	91 (92.9%)	1.00	
AA	6 (7.4%)	7 (7.1%)	0.962 (0.310-2.984)	*P*=0.946
G	128 (79.0%)	134 (68.4%)	1.00	
A	34 (21.0%)	62 (31.6%)	1.742 (1.704-2.824)	*P*=0.024*
rs4950928				
CC	50 (61.7%)	69 (70.4%)	1.00	
CG	26 (32.1%)	25 (25.5%)	0.697 (0.361-1.346)	*P*=0.282
GG	5 (6.2%)	4 (4.1%)	0.580 (0.148-2.268)	*P*=0.433
C	50 (61.7%)	69 (70.4%)	1.00	
CG/GG	31 (38.3%)	29 (29.6%)	0.678 (0.363-1.265)	*P*=0.222
CC/CG	76 (93.8%)	94 (95.9%)	1.00	
GG	5 (6.2%)	4 (4.1%)	0.647 (0.168-2.493)	*P*=0.527
C	126 (77.8%)	163 (83.2%)	1.00	
G	36 (22.1%)	33 (16.8%)	0.709 (0.419-1.200)	*P*=0.200
rs880633				
TT	34 (42.0%)	42 (42.9%)	1.00	
CT	35 (43.2%)	43 (43.9%)	0.995 (0.527-1.877)	*P*=0.995
CC	12 (14.8%)	13 (13.0%)	0.877 (0.355-2.169)	*P*=0.776
TT	34 (42.0%)	42 (42.9%)	1.00	
CT/CC	47 (58.0%)	56 (57.1%)	0.965 (0.552-1.750)	*P*=0.905
TT/CT	103 (63.6%)	127 (64.8%)	1.00	
CC	59 (36.4%)	69 (35.2%)	0.948 (0.615-1.464)	*P*=0.811
T	103 (63.6%)	127 (64.8%)	1.00	
C	59 (36.4%)	69 (35.2%)	0.948 (0.615-1.464)	*P*=0.811

The odds ratio (OR) with their 95% confidence intervals were estimated by simple logistic regression, chi-square or Fisher exact tests. **P* value < 0.05.

40 rs4950928 (-131C/G) was lower in the CAD group than in the non-CAD group, indicating a lower OR for the G allele in the CAD group. YKL-40 SNP -131C/G (rs4950928) may protect Taiwanese patients from CAD. We also found that a lower percentage of carriers of the CG/GG genotype of YKL-40 rs4950928 had elevated cardiac enzyme levels (27.1%) compared with the carriers of the CC genotype (43.6%), as indicated by a lower OR for the CG or CG genotype in the event of acute coronary syndrome (*P*=.023, OR=0.482, 95% CI=0.257-0.903, data not shown). In the study on children with severe asthma [13], the YKL-40 -131C/G genetic polymorphism was strongly associated with high serum YKL-40 levels, because children homozygous for the C allele had higher levels of YKL-40 than those of CG carriers. The possible mechanism is that the G allele disrupts the binding of transcription factors and is associated with lower levels of messenger RNA, lower YKL-40 levels, and a reduced number of asthma-related hospital admissions [14]. In another study [13], Carole et al. showed that YKL-40 is a susceptibility gene for asthma, bronchial hyperresponsiveness, and reduced lung function, and that elevated circulating YKL-40 levels are biomarkers of asthma and lung function decline. In a study on the Chinese population and CAD, the YKL-40 -131G allele was significantly associated with reduced plasma YKL-40 levels in a recessive genetic model [15]. In addition, in another Taiwanese study on the association of YKL-40 SNP with peripheral artery disease [16], a lower YKL-40 level was observed in carriers of the CG/GG genotype of YKL-40 SNP -131C/G

Table 6. Multivariate analysis for the association of YKL-40 single nucleotide polymorphisms and clinical characteristics in the female patients with coronary artery disease (CAD; N=98) and without CAD (non-CAD; N=81)[1]

	Non-CAD (n=81)	CAD (n=98)	Odds ratio	*P* value
Hypertension			2.573 (1.185-5.590)	0.017*
Negative	30	20		
Positive	51	78		
Recent (< 24 h) severe angina			2.809 (1.340-5.890)	0.006*
Negative	35	24		
Positive	46	74		
Cardiac markers elevation			2.944 (1.441-6.015)	0.003*
Negative	58	46		
Positive	23	52		
rs10399805			2.524 (1.254-5.078)	0.009*
GG	53	43		
GA/AA	28	55		

[1]Statistical analysis: simple logistic regression, chi-square or Fisher exact tests. **P* value < 0.05.

(rs4950928) than in those of the CC genotype; therefore, the CG/GG genotype has protective effects against peripheral artery disease. Thus, YKL-40 SNP -131C/G (rs4950928) may have protective effects against CAD or acute coronary syndrome in the Taiwanese population.

In the CLARICOR trial [7], high serum YKL-40 levels were associated with myocardial infarction, cardiovascular death, and all-cause mortality in patients with stable CAD. The Copenhagen City Heart study [17] demonstrated that elevated plasma YKL-40 levels were associated with increased risks of ischemic stroke and ischemic cerebrovascular disease, independent of plasma C-reactive protein (CRP) levels. However, the association between genetic polymorphisms of YKL-40 and cardiovascular or cerebrovascular disease is unclear. The most crucial, novel findings of this study are the significant associations of YKL-40 SNPs rs6691378 (-1371G/A) and rs10399805 (-247G/A) with CAD in female Taiwanese patients. In addition to the CAD risk factors for hypertension, diabetes mellitus, aspirin use in the past 7 days, severe angina, and cardiac marker elevation, we found that sex was a critical risk factor for developing CAD based on univariate analysis. In female subgroup analysis, the frequency of the GA/AA genotype of YKL-40 SNP rs6691378 was higher in the CAD group than in the non-CAD group, and this genotype was associated with an increased risk of CAD. A significant difference was also observed in the frequency of the GA/AA genotype of YKL-40 SNP rs10399805 between the CAD and non-CAD groups. Therefore, YKL-40 -1371G/A (rs6691378) and YKL-40 -247G/A (rs10399805) were associated with the development of CAD.

Multivariate analysis revealed that YKL-40 -247G/A (rs10399805) was an independent risk factor for CAD in the female patients, in addition to hypertension, recent severe angina, and cardiac marker elevation. These results were also reported by several studies on different ethnicities and the disease. In a study on YKL-40 SNP and atopy in South Korea [18], the YKL-40 -247C/T (-247G/A) SNP in the promoter region was found to be associated with the risk of atopy. In another study on Taiwanese women, YKL-40 -1371G/A (rs6691378) and YKL-40 -247G/A (rs10399805) were correlated with the development of cervical precancerous lesions and invasive cancer [19]. In a nested case-control study on a prospective cohort of 23,294 initially healthy American women [9], genetic variation in YKL-40 SNP rs10399805 was associated with cardiovascular death or all-cause mortality. Moreover, 12 SNPs of YKL-40 were genotyped, and the serum YKL-40 level was measured in 2656 Danes representative of the general population in Denmark [10]; YKL-40 SNP rs6691378 -1371G/A and YKL-40 -131C/G were significantly associated with the YKL-40 level. The susceptibility to CAD resulting from YKL-40 SNPs (YKL-40 -1371G/A and YKL-40 -247G/A) was different in both sexes in our study. According to previous studies [20, 21], no significant difference has been observed in the YKL-40 level between men and postmenopausal women. Another study reported that the YKL-40 level was higher

Table 7. The associations of clinical variables with YKL-40 single nucleotide polymorphisms rs6691378 GG and GA/AA in female patients with CAD[1]

	YKL-40 rs6691378 GG (n=42)	YKL-40 rs6691378 GA/AA (n=56)	OR (95% CI)	*P* value
CAD risks ≥ 3				0.483
Negative	21	24	1.00	
Positive	21	32	1.333 (0.597-2.978)	
Age > 65 year				0.063
Negative	9	22	1.00	
Positive	33	34	0.421 (0.169-1.049)	
Family history				0.112
Negative	37	42	1.00	
Positive	5	14	2.467 (0.811-7.505)	
Hypertension				0.198
Negative	6	14	1.00	
Positive	36	42	0.500 (0.174-1.436)	
Diabetes mellitus				0.242
Negative	26	28	1.00	
Positive	16	28	1.625 (0.720-3.667)	
Active smoker				0.716
Negative	39	53	1.00	
Positive	3	3	0.736 (0.141-3.843)	
Cholesterol > 200				0.333
Negative	27	30	1.00	
Positive	15	26	1.502 (0.659-3.426)	
Aspirin use in the past 7 days				0.290
Negative	29	43	1.00	
Positive	13	13	0.610 (0.244-1.523)	
Recent (< 24 h) severe angina				0.735
Negative	11	13	1.00	
Positive	31	43	1.174 (0.465-2.964)	
Cardiac markers elevation				0.010*
Negative	26	20	1.00	
Positive	16	36	2.925 (1.277-6.699)	
Stroke				0.288
Negative	38	46	1.00	
Positive	4	10	1.975 (0.567-6.753)	

[1]The odds ratio (OR) with their 95% confidence intervals were estimated by simple logistic regression, chi-square or Fisher exact tests. **P* value < 0.05.

in the postmenopausal women than in the premenopausal women [22]. Therefore, the difference in the YKL-40 level between men and women implied that premenopausal women have a lower YKL-40 level. Postmenopausal women have an increased risk of CAD because of elevated YKL-40 levels and a higher influence of YKL-40 levels in postmenopausal women compared with that in premenopausal women [23, 24]. In summary, we found that YKL-40 -1371G/A (rs6691378) and YKL-40 -247G/A (rs10399805) increased the susceptibility of Taiwanese women to CAD. The association between YKL-40 SNP and CAD has not been observed in previous studies. Additionally, the significant higher elevated Troponin I cardiac enzyme was noted in YKL-40 rs6691378 GA/AA group than GG group in Taiwanese women with CAD. The same trend of elevated cardiac enzyme was also seen in YKL-40

rs10399805 GA/AA group. Therefore, the YKL-40 SNP rs6691378 and YKL-40 SNP rs10399805 implicated the higher susceptibility of acute coronary syndrome.

The limitations of our study are as follows: First, a relatively small sample size was used for invasive coronary angiography and YKL-40 SNP analysis. Particularly, we focused on the subgroup of Taiwanese women, further reducing the sample size. Second, selection or allocation bias may have occurred. These problems can be resolved using multivariate analysis. Third, in our study, serum YKL-40 levels were not measured for comparing the direct effects of YKL-40 SNPs. Although YKL-40 is a potential biomarker of CAD or cerebrovascular disease, many comorbidities can influence the YKL-40 level; for example, malignancy, infectious disease, asthma, or rheumatoid arthritis. Some medications can interfere with the serum YKL-40 level, including beta-receptor agonists or statins. Increasing serum YKL-40 levels have been significantly associated with age, hypertension, and diabetes mellitus, but not with sex, previous myocardial infarction, or smoking at entry [25]. Therefore, we focused on the association between YKL-40 SNPs and CAD without measuring the YKL-40 level.

Conclusion

YKL-40 -131C/G (rs4950928) SNP may decrease the genetic susceptibility to CAD, as shown in the study participants. In the female patients, YKL-40 SNP -1371G/A (rs6691378) with the GA/AA genotype or the A allele was associated with CAD. In addition, YKL-40 SNP -247G/A (rs10399805) with the GA/AA genotype or the A allele can increase the genetic susceptibility to CAD. More important, YKL-40 rs10399805 GA/AA may serve as an independent risk factor for CAD in Taiwanese women.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Po-Hui Wang, Institute of Medicine, Chung Shan Medical University, 110, Section 1, Chien-Kuo North Road, Taichung 40201, Taiwan. Tel: 886-4-24739595 Ext. 34253; Fax: 886-4-23248183; E-mail: wang082160@yahoo.com.tw

References

[1] Mann DL, Zipes DP, Libby P and Bonow RO. Braunwald's heart disease: a textbook of cardiovascular medicine. Elsevier Health Sciences 2014.

[2] Rathcke CN and Vestergaard H. YKL-40-an emerging biomarker in cardiovascular disease and diabetes. Cardiovasc Diabetol 2009; 8: 61-67.

[3] Rathcke C and Vestergaard H. YKL-40, a new inflammatory marker with relation to insulin resistance and with a role in endothelial dysfunction and atherosclerosis. Inflamm Res 2006; 55: 221-227.

[4] Nishikawa KC and Millis AJ. gp38k (CHI3L1) is a novel adhesion and migration factor for vascular cells. Exp Cell Res 2003; 287: 79-87.

[5] Rehli M, Niller HH, Ammon C, Langmann S, Schwarzfischer L, Andreesen R and Krause SW. Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. J Biol Chem 2003; 278: 44058-44067.

[6] Boot RG, van Achterberg TA, van Aken BE, Renkema GH, Jacobs MJ, Aerts JM and de Vries CJ. Strong induction of members of the chitinase family of proteins in atherosclerosis chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol 1999; 19: 687-694.

[7] Kastrup J, Johansen JS, Winkel P, Hansen JF, Hildebrandt P, Jensen GB, Jespersen CM, Kjøller E, Kolmos HJ and Lind I. High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. Eur Heart J 2009; 30: 1066-1072.

[8] Zheng JL, Lu L, Hu J, Zhang RY, Zhang Q, Chen QJ and Shen WF. Genetic polymorphisms in chitinase 3-like 1 (CHI3L1) are associated with circulating YKL-40 levels, but not with angiographic coronary artery disease in a Chinese population. Cytokine 2011; 54: 51-55.

[9] Ridker PM, Chasman DI, Rose L, Loscalzo J and Elias JA. Plasma Levels of the Proinflammatory Chitin-Binding Glycoprotein YKL-40, Variation in the Chitinase 3-Like 1 Gene (CHI3L1), and Incident Cardiovascular Events. J Am Heart Assoc 2014; 3: e000897.

[10] Rathcke CN, Thomsen SB, Linneberg A and Vestergaard H. Variations of CHI3L1, levels of the encoded glycoprotein YKL-40 and prediction of fatal and non-fatal ischemic stroke. PLoS One 2012; 2012: e43498.

[11] Zhang K, Hu S, Wu J, Chen L, Lu J, Wang X, Liu X, Zhou B and Yen Y. Overexpression of RRM2 decreases thrombspondin-1 and increases VEGF production in human cancer cells in vitro

and in vivo: implication of RRM2 in angiogenesis. Mol Cancer 2009; 8: 11.
[12] Liu X, Zhou B, Xue L, Yen F, Chu P, Un F and Yen Y. Ribonucleotide reductase subunits M2 and p53R2 are potential biomarkers for metastasis of colon cancer. Clin Colorectal Cancer 2007; 6: 374-381.
[13] Konradsen JR, James A, Nordlund B, Reinius LE, Söderhäll C, Melén E, Wheelock Å, Carlsen KCL, Lidegran M and Verhoek M. The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. J Allergy Clin Immunol 2013; 132: 328-335, e325.
[14] Cunningham J, Basu K, Tavendale R, Palmer CN, Smith H and Mukhopadhyay S. The CHI3L1 rs4950928 polymorphism is associated with asthma-related hospital admissions in children and young adults. Ann Allergy Asthma Immunol 2011; 106: 381-386.
[15] Tian CW and Liu SM. A promoter polymorphism (-131C > G) in the CHI3L1 gene is associated with circulating levels of YKL-40 but not with coronary artery disease in southern Han Chinese. Heart 2012; 98: E171-E171.
[16] Wu S, Hsu LA, Cheng ST, Teng MS, Yeh CH, Sun YC, Huang HL and Ko YL. Circulating YKL-40 Level, but not CHI3L1 Gene Variants, Is Associated with Atherosclerosis-Related Quantitative Traits and the Risk of Peripheral Artery Disease. Int J Mol Sci 2014; 15: 22421-22437.
[17] Kjaergaard AD, Bojesen SE, Johansen JS and Nordestgaard BG. Elevated plasma YKL-40 levels and ischemic stroke in the general population. Ann Neurol 2010; 68: 672-680.
[18] Sohn MH, Lee JH, Kim KW, Kim SW, Lee SH, Kim KE, Kim KH, Lee CG, Elias JA and Lee MG. Genetic variation in the promoter region of chitinase 3-like 1 is associated with atopy. Am J Respir Crit Care Med 2009; 179: 449-456.
[19] Lin YS, Liu YF, Chou YE, Yang SF, Chien MH, Wu CH, Chou CH, Cheng CW and Wang PH. Correlation of Chitinase 3-Like 1 Single Nucleotide Polymorphisms and Haplotypes with Uterine Cervical Cancer in Taiwanese Women. PLoS One 2014; 9: e104038.
[20] Qin W, Zhu W, Schlatter L, Miick R, Loy TS, Atasoy U, Hewett JE and Sauter ER. Increased expression of the inflammatory protein YKL-40 in precancers of the breast. Int J Cancer 2007; 121: 1536-1542.
[21] El-Mesallamy HO, Mostafa AM, Amin AI and El Demerdash E. The interplay of YKL-40 and leptin in type 2 diabetic obese patients. Diabetes Res Clin Pract 2011; 93: e113-e116.
[22] Johansen JS, Christensen IJ, Riisbro R, Greenall M, Han C, Price PA, Smith K, Brünner N and Harris AL. High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. Breast Cancer Res Treat 2003; 80: 15-21.
[23] Gordon T, Kannel WB, Hjortland MC and Mcnamara PM. Menopause and coronary heart disease: the Framingham Study. Ann Intern Med 1978; 89: 157-161.
[24] Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW and Wing RR. Menopause and Risk Factors for Coronary Heart Disease. N Engl J Med 1989; 321: 641-646.
[25] Kastrup J. Can YKL-40 be a new inflammatory biomarker in cardiovascular disease? Immunobiology 2012; 217: 483-491.