ORIGINAL ARTICLE

Human C-reactive Protein (CRP) Gene 1059 G>C Polymorphism is Associated with Plasma CRP Concentration in Patients Receiving Coronary Angiography

*Dao-Fu Dai,[1] Fu-Tien Chiang,[1,2] Jiunn-Lee Lin,[1] Li-Ying Huang,[1] Chi-Ling Chen,[3] Chee-Jen Chang,[4] Ling-Ping Lai,[1] Kwan-Lih Hsu,[1] Chuen-Den Tseng,[1] Yung-Zu Tseng,[1] Juey-Jen Hwang[1]**

Background/Purpose: Elevation of C-reactive protein (CRP) level is associated with increased risk of cardiovascular events. The 1059 G>C polymorphism in exon 2 of the CRP gene has been shown to affect plasma concentration of CRP. We want to elucidate the effect of this polymorphism on the development of coronary artery disease (CAD) among the Chinese population in Taiwan.

Methods: We scrutinized 536 patients undergoing coronary angiography (365 patients with CAD and 171 controls with patent coronaries) and evaluated the association of CRP gene 1059 G>C polymorphism with CAD. Genotyping of the polymorphism was performed by polymerase chain reaction and *Mae*III restriction enzyme digestion.

Results: The CC genotype was associated with lower plasma CRP concentration (GG, 6.5±5.8; GC, 3.3±4.4; CC, 2.3±3.1 mg/L; $p=0.02$). Subjects with CAD or myocardial infarction (MI) had significantly higher plasma CRP concentration than that in controls (CAD *vs.* controls, 8.9±18.9 *vs.* 3.3±7.2 mg/L; $p<0.001$), while patients with MI showed higher CRP when compared to those with chronic stable angina (13.5±22.9 *vs.* 5.2±14.1 mg/L; $p<0.001$). However, this polymorphism was not associated with CAD in our population.

Conclusion: Our data suggest that human CRP gene 1059 G>C polymorphism is associated with plasma CRP concentration among Chinese in Taiwan receiving coronary angiography. [*J Formos Med Assoc* 2007; 106(5):347–354]

Key Words: C-reactive protein, CRP, coronary artery disease, CAD, genetic polymorphism, myocardial infarction, MI

It is well known that inflammation plays pivotal roles in the pathogenesis of atherosclerosis.[1,2] C-reactive protein (CRP), an acute-phase reactant indicating active inflammation has been shown to correlate with stable angina pectoris,[3] acute coronary syndrome,[4] stroke,[5,6] and peripheral vascular disease.[7] Epidemiologic surveys reveal that elevated high-sensitivity CRP concentrations are associated with an increased risk of future cardiovascular events among healthy men[8,9] and

[1]Cardiovascular Division, Department of Internal Medicine, [2]Department of Laboratory Medicine and [4]Department of Medical Research, National Taiwan University Hospital and [3]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

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***Correspondence to:** Dr Juey-Jen Hwang, Cardiovascular Division, Department of Internal Medicine, National Taiwan University Hospital, College of Medicine, National Taiwan University, 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail: juey@ha.mc.ntu.edu.tw

women.[10] CRP has also been shown to impair endothelium-dependent vasodilation via the nitric oxide pathway,[11–13] impair angiogenic function and attenuate survival of endothelial progenitor cells,[14,15] and induce proinflammatory and proatherogenic effects in several types of cells.[16–18] More and more evidence indicate the direct pathogenic effects of CRP in atherosclerosis, of which coronary artery disease (CAD) is the most critical disease.

The interplays between genetic and environmental factors are important in the phenotype development of complex trait diseases, such as CAD. Several single nucleotide polymorphisms of the CRP gene have been demonstrated to affect plasma CRP concentration, such as +1059 G>C and +1444 C>T in exon 2 and intronic T>A polymorphisms.[19,20]

Prior studies showed that 1059 G>C was associated with plasma concentrations of CRP.[19] The relationship persisted after adjustment for nongenetic confounders (e.g., age, sex and body mass index [BMI] etc.) and was present in subjects with and without prevalent coronary disease in the Caucasian population.[21] Another study showed that the CRP 1059 G>C polymorphism was neither associated with future myocardial infarction (MI) and stroke, nor with post-angioplasty restenosis.[22] Therefore, its correlation with the pathogenesis of CAD remains unknown. We thus intended to study the effect of this polymorphism on the risk of CAD in the Chinese population in Taiwan.

Materials and Methods

Study population

We recruited 536 patients undergoing coronary angiography at the National Taiwan University Hospital from April 2002 to October 2003. They consisted of 365 patients with CAD and 171 controls with patent coronaries, matched for sex and age in approximately 2:1 ratio. All of them are Chinese who mainly reside in Taipei and the northern part of Taiwan. Exclusion criteria included rheumatologic disorders, malignancy, active infection and overt heart failure. During hospitalization, all participants had a record on age, sex, BMI, classical risk factors of atherosclerosis, including diabetes, hypertension, smoking habit, hypercholesterolemia (total cholesterol ≥ 200 mg/dL and/or LDL-cholesterol ≥ 160 mg/dL) and hypertriglyceridemia (triglyceride ≥ 200 mg/dL). Baseline laboratory measurements included complete blood count, blood sugar level, biochemistry and lipid profiles. This study was approved by the hospital's ethics committee, and informed consent was obtained from all participants.

Phenotype definition in case control studies

CAD was defined as the presence of at least one significant coronary artery stenosis of more than 50% luminal diameter on coronary angiography. Patients with patent coronaries were enrolled as controls. In subgroup analysis of CAD patients, those with significant stenoses over two or more coronary arteries were classified into multi-vessel disease group, *vs.* patients with single-vessel disease. As this is a genetic association study, we recruited both new onset MI and historical MI to elucidate the genetic effect of this polymorphism on MI risk in CAD patients. New onset MI was defined according to the consensus criteria of ESC and ACC.[23] Historical MI that occurred before the time of enrollment was verified by medical record during hospitalization with the same criteria. For patients with significant history of chest pain, finding the pathologic Q wave on EKG or fixed perfusion defect on stress and redistribution thalium-201 myocardial perfusion scan, which is compatible with angiographic findings, was also defined as historical MI. Control patients who did not meet the above criteria were classified as having chronic stable angina.

Genotyping of CRP gene 1059 G>C polymorphism and measurement of plasma CRP concentration

Blood was withdrawn after hospitalization, centrifuged, DNA extraction was performed with standard phenol–chloroform method, and plasma

was stored in a −80°C freezer before analysis. Genotyping of CRP gene 1059 G/C polymorphism was performed by polymerase chain reaction (PCR) and further digestion by *Mae*III restriction enzyme, as previously described.[24–26] Briefly, PCR was performed by using the following primers: Forward-5′GATCTGTGTGATCTGAGAAACCTCT-3′ and Reverse-5′GAGGTACCAGAGACAGAGAC-GTG-3′. Target DNA was amplified using 94°C for 5 minutes, then 30 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds, followed by an extension step of 72°C for 10 minutes. Two percent agarose gel was used for visualization. Quality control for the experiments was evaluated by re-genotyping of 20 random samples blinded to the technician.

Plasma CRP concentration was measured by a high sensitivity immunonephelometry (Nephelometry, Behring Nephelometer II, Dade Behring Marburg GmbH, Germany) with the lowest detection limit of 0.16 mg/L. All laboratory analyses were performed blinded with respect to the diagnosis and patients' characteristics.

Statistical analyses

Baseline differences between cases and controls were compared by two-sample Student's *t* test and χ^2 test for continuous and categorical variables, respectively. Different genetic models were created to investigate the genotype distribution between cases and controls, including additive, dominant and recessive. We applied multiple logistic regression to adjust for age, sex, proportions of diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia and smoking habit for each genetic model.

Since plasma CRP concentration was not normally distributed, we used logarithmic transformation for normalization. Analysis of variance was applied to evaluate the differences in plasma CRP concentration among subjects with different genotypes, stratified by sex and altogether. Two-sample Student's *t* test was used to compare the differences in plasma CRP concentration between cases and controls for different clinical phenotypes. Then we created multiple regression models to predict plasma CRP concentration, including CRP gene 1059 C allele carrier, clinical phenotypes of CAD, age, sex, obesity (BMI ≥ 25 kg/m^2) and the aforementioned covariates. Statistical analyses were performed with Stata version 8.0. All tests are two-sided and p values less than 0.05 were considered statistically significant.

Results

A total of 365 cases (CAD) and 171 controls (patent coronaries) were recruited in our association study. The demographic characteristics are shown in Table 1. Cases and controls differed significantly in all conventional risk factors for CAD, including diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia and smoking. The allele frequency of G and C in our population was 89.3% and 10.7%, respectively, similar to those reported in Caucasian population.[19] The overall genotype distribution of CRP 1059 G > C polymorphism in our study population was in Hardy–Weinberg equilibrium.

Genotype data were analyzed under three genetic models: additive, dominant and recessive, to increase the possibility for detecting an association. Table 2 shows the genotype distributions between cases and controls. There was no significant association between CRP gene 1059 G > C polymorphism and CAD in all genetic models. In the subgroup analysis of patients with CAD, CRP gene 1059 G > C polymorphism was neither associated with MI nor multi-vessel disease when compared to subjects with chronic stable angina or single-vessel disease, respectively.

The results of plasma CRP concentration among different genotypes of CRP gene 1059 G > C polymorphisms are shown in the Figure. Subjects with CC and GC genotypes had significantly lower plasma CRP concentration than those with GG genotype (GG: 6.5 ± 5.8; GC: 3.3 ± 4.4; CC: 2.3 ± 3.1 mg/L; $p = 0.02$). No significant difference in plasma CRP concentration was noted between men and women for all genotypes. Table 3 shows that the mean plasma CRP concentration

Table 1. Baseline characteristics of cases and controls

	Patent coronaries ($n=171$)	CAD ($n=365$)	p
Age (yr)	60.9 ± 12.3	62.0 ± 11.5	0.3
Men, *n* (%)	94 (55)	228 (62.5)	0.1
BMI, kg/m^2	25.3 ± 4.3	25.0 ± 4.0	0.44
TC, mg/dL	197.8 ± 42.7	219.8 ± 99.9	0.007
TG, mg/dL	162.6 ± 122.8	192.9 ± 121.8	0.008
Risk factors, *n* (%)			
Diabetes	28 (16.4)	144 (39.4)	< 0.001
Hypertension	92 (53.8)	252 (69.0)	< 0.001
Smoking	59 (34.5)	166 (45.5)	< 0.001
Hypercholesterolemia	72 (42.1)	237 (64.9)	< 0.001
Hypertriglyceridemia	38 (22.2)	132 (36.2)	0.001

CAD = coronary artery disease; BMI = body mass index; TC = total cholesterol; TG = triglycerides.

in cases was significantly higher than those in controls for phenotypes of CAD *vs.* patent coronaries (8.9 ± 18.9 *vs.* 3.3 ± 7.2 mg/L, respectively, $p < 0.001$) and for MI *vs.* chronic stable angina (13.5 ± 22.9 *vs.* 5.2 ± 14.1 mg/L, respectively, $p < 0.001$). However, the mean plasma CRP concentration was not significantly different between subjects with multi-vessel disease and those with single-vessel disease (9.7 ± 20.6 *vs.* 7.1 ± 14.4 mg/L, respectively, $p = 0.12$).

To evaluate the predictors of plasma CRP concentration, we created a multiple regression model as shown in Table 4. The independent predictors for plasma CRP concentration were CRP gene 1059 C allele carriers ($\beta = -0.53$, $p = 0.004$), CAD ($\beta = 0.39$, $p = 0.02$), age ($\beta = 0.02$, $p = 0.001$) and the presence of diabetes ($\beta = 0.4$, $p = 0.006$). This model indicates that CRP 1059 C allele carrier is associated with lower plasma CRP concentration, while at an older age, the presence of CAD or diabetes is associated with higher plasma CRP concentration. All other covariates did not independently affect plasma CRP concentration.

Discussion

The critical roles of CRP in cardiovascular disease have made genetic studies of CRP an important issue. In this study, we investigated the association between CRP 1059 G > C polymorphism and the prevalence of CAD in the Chinese population in Taiwan. The CRP gene 1059 G > C polymorphism was neither associated with CAD nor MI after adjustment for covariates, although this polymorphism significantly affected plasma CRP concentration. We also demonstrated that subjects with CAD or MI had significantly higher plasma CRP concentration compared to controls. To the best of our knowledge, this is the first study to evaluate the association between CRP 1059 G > C polymorphism and CAD. Zee and Ridker[19] reported in prospective studies of Caucasian populations that the CRP gene 1059 G > C polymorphism, despite affecting plasma CRP concentration, was neither associated with future MI and stroke events, nor with post-angioplasty restenosis. On the contrary, a recent study by Chen et al[27] in a Chinese population revealed that the −717A > G polymorphism in the promoter region of CRP was associated with CAD, but they failed to show the functional effect of the polymorphism on plasma CRP concentration.

The concomitant correlation of plasma CRP concentration with the CRP genotype and the phenotype of CAD but the absence of association between this CRP gene 1059 G > C polymorphism and CAD in our study may provide some

Table 2. Genotype distribution between cases and controls for different phenotypes

Phenotype	*n*	GG (%)	GC (%)	CC (%)	OR, 95% CI, *p* value		
					Additive (GG = 1)	Dominant ([GG & GC] = 1)	Recessive (GG = 1)
Patent (controls)	171	80.7	17.0	2.3	CC: 0.30, (0.05–1.80), $p = 0.19$	OR: 0.59, (0.11–3.31), $p = 0.53$	OR: 0.76, (0.42–1.38), $p = 0.37$
CAD (cases)	365	83.8	15.3	1.8	GC: 0.84, (0.49–1.45), $p = 0.33$		
CSA (controls)	202	82.7	16.3	1.0	CC: 0.71, (0.1–5.33), $p = 0.74$	OR: 0.8, (0.11–5.94), $p = 0.83$	OR: 0.59, (0.3–1.03), $p = 0.06$
MI (cases)	163	85.3	14.1	0.6	GC: 0.55, (0.29–1.03), $p = 0.06$		
Single-vessel (controls)	107	88.8	10.3	0.9	CC: 0.39, (0.05–3.28), $p = 0.39$	OR: 0.35, (0.04–2.91), $p = 0.33$	OR: 1.66, (0.79–3.52), $p = 0.18$
Multi-vessel (cases)	258	81.8	17.4	0.8	GC: 1.94, (0.87–4.34), $p = 0.1$		

In the Additive model, GG is the reference genotype, versus GC and CC. In the Dominant model, (GG and GC) is the reference genotype, versus CC. In the Recessive model, GG is the reference genotype, versus (GC and CC). OR = odds ratio; CI = confidence interval; CSA = chronic stable angina.

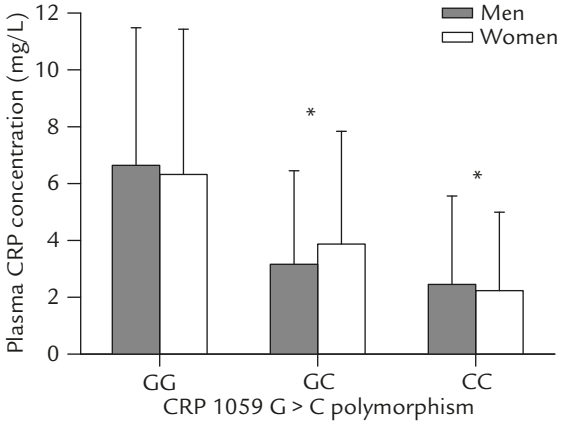

Figure. Mean plasma C-reactive protein (CRP) concentration stratified by CRP 1059 G > C polymorphism for both men and women. GC and CC genotypes have significantly lower plasma CRP concentration than GG genotype (*$p < 0.05$). No significant difference was noted between men and women for all genotypes.

argument that elevated plasma CRP level in CAD is merely an epiphenomenon. It implies that CRP is probably a surrogate marker in cardiovascular disease. However, this explanation is less likely due to increasing evidence of direct pathogenic effects of CRP on atherogenesis. CRP may cause the reduction of eNOS expression, inhibition of angiogenesis and attenuation of endothelial progenitor cell survival and may induce proinflammatory and proatherogenic effects in several types of cells.[11–18]

Besides, we proposed another explanation for this discrepancy. Other genetic and environmental factors also influence plasma concentration of CRP. According to the results of the NHLBI Family Heart Study, the heritability of CRP was 35–40%.[28] This fact implies that acquired factors are at least of the same importance as genetic factors in the determination of plasma CRP concentration. From the genetic perspectives, polymorphisms in the IL-1 gene cluster,[29,30] IL-6,[31] tumor necrosis factor alpha[32] and CRP genes[19–21,33] were shown to affect the expression of CRP. Since the 1059 G > C polymorphism is a silent mutation in exon 2 of the CRP gene,[24] its impact on plasma CRP concentration may be due to linkage disequilibrium with nearby genetic polymorphisms, especially with those in the promoter region.[33]

Table 3. Mean plasma C-reactive protein (CRP) concentration in cases versus control

	n	Mean hs-CRP (mg/L)	*p*
CAD	365	8.9 ± 18.9	< 0.001
Patent	171	3.3 ± 7.2	
MI	163	13.5 ± 22.9	< 0.001
CSA	202	5.2 ± 14.1	
Multi-vessel disease	258	9.7 ± 20.6	0.12
Single-vessel disease	107	7.1 ± 14.4	

CAD = coronary artery disease; MI = myocardial infarction; CSA = chronic stable angina.

Table 4. Multiple regression model to predict plasma C-reactive protein (CRP) concentration (log transformed)

	β	*p*	95% CI
CRP1059 C carrier (*vs.* non-carrier)	–0.53	0.004	–0.89, –0.18
Coronary artery disease	0.39	0.02	0.08, 0.70
Age, yr	0.02	0.001	0.01, 0.03
Male (*vs.* female)	0.32	0.25	–0.20, 0.73
Diabetes	0.40	0.006	0.12, 0.69
Hypertension	0.01	0.93	–0.26, 0.29
Obesity	0.13	0.35	–0.15, 0.41
Smoking	0.15	0.33	–0.15, 0.44
Hypercholesterolemia	–0.24	0.09	–0.51, 0.03
Hypertriglyceridemia	0.10	0.49	–0.19, 0.40

The other possible mechanism is the 1059 G > C polymorphism affecting the stability of CRP mRNA and hence changing the plasma CRP concentration. However, additional studies are needed to clarify the exact mechanism. From the environmental viewpoints, previous studies have indicated several acquired factors that influenced plasma CRP concentration, such as smoking, obesity, diet and chronic infection. Our regression model showed that the independent predictors of plasma CRP concentration are age, CRP gene 1059 C allele and the presence of diabetes and CAD.

As our study revealed negative findings, the issue of statistical power to address the research questions is very crucial. Based on the size of our study, we can detect, with 80% probability, at an alpha error of 0.05, an odds ratio of greater than 1.8 for an association between CRP1059 allele C and CAD, assuming an additive model. The limitation of this study is that our controls were chosen from subjects receiving coronary angiography who may not reflect the condition of the general population. Meantime, coronary angiography remains a gold standard in the diagnosis of CAD. The application of coronary angiography for all participants may avoid the misclassification of asymptomatic CAD or MI subjects, which has a prevalence of 18–40%, depending on the population studied.[34,35] The other advantage of our study is the homogeneous ethnicity of the Chinese population in Taiwan, which makes it suitable for genetic analysis.

On the other hand, one of the limitations was that we did not recruit truly normal people and analyze their CRP polymorphism. The control group in this study was a combination of normal subjects, patients with syndrome X, and patients with insignificant coronary lesions. It would be of interest to separate these heterogeneous groups and observe the differences. In addition, the regression model in this study might not be sufficient to demonstrate the influence and interactions

among a series of genetic and non-genetic factors. Our future goal will target at designing a decision tool to analyze these potential confounders as a whole.

In conclusion, our data suggest that human CRP gene 1059 G > C polymorphism is associated with plasma CRP concentration in the Chinese who live in Taiwan who receive coronary angiography. Higher plasma CRP concentration is also significantly correlated with CAD and MI. However, to better understand the contribution of genetic profiles *vs.* environmental effects on plasma CRP concentration and the risk of cardiovascular disease, further research of CRP together with related inflammatory genes, and by the application of haplotype analysis, intergenic interaction and gene–environment interaction analysis are necessary.

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