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Common variants in the *CRP* gene are associated with serum C-reactive protein levels and body mass index in healthy individuals in Mexico

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ABSTRACT. Variants in the C-reactive protein (*CRP*) gene have been found to be associated with various phenotypic traits. We evaluated the effect of four SNPs in the *CRP* gene on serum levels of protein and body mass index (BMI) in 150 unrelated Mexican subjects from 18 to 25 years old, without hypertension, non-overweight, and without inflammatory diseases, non-smoking and non-consumers of alcohol. Subjects were measured for BMI, waist circumference, blood pressure, and serum glucose and triglycerides. The identification of SNPs was

performed by PCR-RFLP. Three of the four SNPs were associated with variation in serum levels of CRP, increased in TT (rs1130864) and GG (rs2794521) genotypes, and decreased in the AA genotype of rs1205. The TT genotype was associated with a significant increase in BMI (β = 1.1 kg/m^2, P = 0.04). Two haplotypes were significantly associated with increased serum levels of CRP, but not with BMI. We conclude that variation in the *CRP* gene affects serum protein levels.

Key words: C-reactive protein; *CRP* gene; Body mass index; Haplotypes

INTRODUCTION

Various biological and genetic markers, such as proinflammatory cytokines and C-reactive protein (CRP), secreted by the liver in response to the stimulation of interleukins 1 and 6 and tumor necrosis factor alpha, have been proposed for the prediction and early assessment of cardiovascular disease (CVD) risk (Black et al., 2004). Elevated blood levels of CRP have been associated with atherosclerosis (Momiyama et al., 2010), unstable angina (Rizzello et al., 2007), diabetes (Shankar and Li, 2008), metabolic syndrome (Lai et al., 2010), insulin resistance (Gelaye et al., 2010), and obesity (Wee et al., 2008). Prospective studies have shown that increased blood CRP levels are associated with a greater risk of ischemic heart and cerebrovascular diseases (Sabatine et al., 2007; Oudi et al., 2010).

CRP mediates phagocytosis of low-density lipoprotein by macrophages, induces apoptosis of endothelial cells, inhibits angiogenesis, and increases activation of nuclear factor kappa β. CRP is also involved in endothelial activation and the survival and differentiation of endothelial progenitor cells. The capability of CRP to inhibit progenitor endothelial cells may be an important mechanism in the inhibition of angiogenesis in chronic ischemia. The concentration of CRP may be an indicator of endothelial dysfunction as well as a mediator of insulin resistance and arterial injury (Sanchez-Recalde and Carlos, 2001). Several studies have reported that variants in the *CRP* gene are associated with variations in blood levels of CRP in cardiovascular and other diseases such as diabetes, microangiopathic stroke, metabolic syndrome, and hypertension (Brull et al., 2003; Chen et al., 2005; Szalai et al., 2005; Lange et al., 2006; Komurcu-Bayrak et al., 2009; Hsu et al., 2010; Kuhlenbaeumer et al., 2010).

The majority of studies examining the relationship between various *CRP* polymorphisms and serum protein or the risk of CVD have been conducted in white populations. Only one study has reported an association of the polymorphism rs1130864 in the *CRP* gene with increased serum levels of CRP in adolescents of western Mexico (Guadalajara, Jalisco) with Spanish high genetic component (INAFED, 2005; Mendoza-Carrera et al., 2010). However, these results may vary owing to the genetic diversity of the Mexican population. Previous studies in a population of the State of Guerrero (southwest Mexico) have shown a higher percentage of Native American ancestry (66%; Silva-Zolezzi et al., 2009). Another study in Mexico City showed 65% Native American ancestry (Martínez-Marignac et al., 2007). This value was modified when the study was conducted in individuals from the northwest, in which a 56% Amerindian component was determined (Martinez-Fierro et al., 2009). In this context, our interest was to evaluate the relationship between *CRP* variants and blood levels of CRP and body mass index (BMI) in subjects stratified by risk of coronary heart disease (CHD) in Guerrero State, Mexico.

PATIENTS AND METHODS

Study type and subjects

A genetic association study was conducted in 150 healthy, genetically unrelated individuals aged 18 to 25 years without overweight or hypertension. All participants were born in Guerrero State, Mexico, as were their parents and grandparents. Individuals who were overweight or obese, smoked, drank alcohol, had a diagnosis of CVD, presented with evidence of infectious disease within the previous 15 days, or had received any treatment that could influence the biochemical parameters were excluded from the study. The subjects received written and oral information before giving their consent to participate. The study was approved by the Ethics Committee of the University of Guerrero. Each of the participants answered a questionnaire to provide socio-demographic data, family history, and history of diseases. Weight (kg), height (m), waist circumference (cm), and blood pressure (mmHg) were also measured. A fasting blood sample was obtained from each subject.

Laboratory measurements

Serum glucose and triglyceride levels were obtained using routine biochemical analysis. High-sensitivity CRP levels were measured via immunonephelometry with an automated BN-100 system (Dade Berhing). The intra- and interassay coefficients of variation of CRP were <4.2 and <5.5%, respectively, and the analytical sensitivity was 0.175 mg/L. To evaluate the risk of CHD, we used cut points for CRP levels at low risk (<1.0 mg/L), average risk (1.0 to 3.0 mg/L), and high risk (>3.0 mg/L) based on the criteria of the Centers for Disease Control and Prevention and the American Heart Association (Pearson et al., 2003).

Genotyping

DNA was extracted from peripheral blood leukocytes using a salting-out procedure. Gene fragments containing the +1444 C>T (rs1130864), +1846 G>A (rs1205), -717 A>G (rs2794521), and -409 G> A (rs3093062) variant sites in *CRP* were amplified using polymerase chain reaction (PCR). The amplification products were digested with the appropriate restriction enzymes under the conditions recommended by the manufacturers (New England Biolabs, USA) according to protocols described elsewhere (Brull et al., 2003; Russell et al., 2004; Chen et al., 2005; Szalai et al., 2005). The digested fragments were then separated using electrophoresis on 4% agarose gels, followed by ethidium bromide staining and visualization under ultraviolet light. To improve genotyping accuracy, we used samples with known genotypes in each batch as positive controls to evaluate the completeness of the PCR product. The primers and restriction enzymes used are described in Table 1.

Statistical analyses

Comparisons between genders and risk of CHD by CRP level were performed using

the chi-square test, the Student *t*-test, the Mann-Whitney U-test, or analysis of variance. The Spearman correlation coefficient was used to assess the relationship between CRP and other variables. Departures from Hardy-Weinberg equilibrium were verified using the chi-square test with one degree of freedom. The effects of the various genotypes and haplotypes of the *CRP* gene on the concentration of CRP and BMI were evaluated with multiple linear regression models. Statistical analyses were performed using STATA (v.10.1) and $P < 0.05$ was reported as statistically significant. Haplotypes were constructed using the genetic data analysis program SNPStats (http://bioinfo.iconcologia.net/SNPstats).

Table 1. Primers and restriction enzymes used.

SNP	Primers		Enzyme
	Forward	Reverse	
rs1130864	5'-AGCTCGTTAACTATGCTGGGGCA-3'	5'-CTTCTCAGCTCTTGCCTTATGAGT-3'	*Hpy*CH_4 III
rs1205	5'-GGAGTGAGACATCTTCTTG-3'	5'-CTTATAGACCTGGGCAGT-3'	*Hpy*CH_4 III
rs2794521	5'-GCCGTCATTTAGTGCCAAC-3'	5'-ATGCTCCTCCCAGAGCCATGG-3'	*Bst*u1
rs3093062	5'-TTTGGGCTAAGTAGGTGTTG-3'	5'-AGGGCTCCACTTTGGCTATC-3'	*Apa*I

SNP = single nucleotide polymorphism.

RESULTS

Clinical and demographic characteristics

The average age of subjects was 20.5 years, and 65.3% were women. The average BMI, waist circumference, systolic and diastolic blood pressures, and serum triglycerides were higher in men. The serum CRP level was higher in women (0.70 *vs* 0.51 mg/L). Of the participants, 69.4% reported having an ancestral origin (grandparents and parents) in the central area of Guerrero State, 7.3% in the north, 5.3% in the mountains, and smaller proportions in other regions of the state. The largest proportion of women reported having a family history of diabetes and CVD (Table 2). CRP was positively correlated with BMI ($r = 0.22$, $P = 0.026$; data not shown). This relationship is shown in Table 3, which shows a significant trend in average BMI increasing with increasing CRP levels stratified according CHD risk.

Genotype and allele frequencies

Individuals carrying the TT genotype for SNP rs1130864 included 14.7% of subjects; the AA genotype of rs1205 included 22.7%, and the GG genotype of rs2794521 composed 2%. The SNP rs3093062 AA genotype was not observed in the population analyzed. None of the polymorphisms showed deviation from Hardy-Weinberg equilibrium. Serum CRP levels were increased through the genotypes of rs1130864 and rs2794521 polymorphisms, from the highest to the lowest genotype frequency. Trends were observed in the frequencies of risk genotypes, TT and GG, across the CRP risk strata (Table 4).

Effect of variation in the *CRP* gene on CRP levels and BMI

We found an average increase of 0.6 mg/L in serum CRP in individuals carrying

the TT variant of SNP rs1130864 compared with genotype CC, and the GG rs2794521 variant displayed an increase of 1.4 mg/L compared with the AA genotype. Conversely, in carriers of the rs1205 AA variant, serum CRP was decreased by 0.5 mg/L. Differences were adjusted for gender, BMI, region of origin, and family history of CVD (Table 5). Compared with individuals carrying the CC genotype, only the TT genotype of rs1130864 polymorphism had an effect on the average increase in BMI (β coefficient = 1.1 kg/m^2; 95% confidence interval = 0.1-2.1; P = 0.04) in a model adjusted for gender, region of origin, and family history of CVD (data not shown). This effect was not seen with any other clinical variable.

Table 2. Clinical and biochemical characteristics of study participants.

Characteristic	Total N = 150	Men N = 52 (34.7)	Women N = 98 (65.3)	P value
Age (years)	20.5 ± 1.7	20.6 ± 1.6	20.5 ± 1.7	0.713†
BMI (kg/m^2)	21.5 ± 2.1	22.1 ± 1.9	21.1 ± 2.2	0.008†
Waist circumference (cm)	75.5 ± 7.4	80 ± 7.0	73.1 ± 6.5	<0.001†
Systolic BP (mmHg)	103.8 ± 11.5	109.9 ± 10.3	100.6 ± 10.8	<0.001†
Diastolic BP (mmHg)	66.6 ± 8.1	68.7 ± 9.0	65.6 ± 7.4	0.025†
Region of origin [Guerrero State, N (%)]				
Center	104 (69.4)	37 (71.1)	67 (68.4)	0.535‡
North	11 (7.3)	6 (11.5)	5 (5.1)	
Mountain	8 (5.3)	2 (3.9)	6 (6.1)	
Costa Grande	8 (5.3)	2 (3.9)	6 (6.1)	
Other	11 (7.4)	4 (7.7)	7 (7.2)	
Not reported	8 (5.3)	1 (1.9)	7 (7.1)	
FH of diabetes (yes), N (%)	91 (60.7)	25 (48.1)	66 (67.3)	0.021‡
FH of CVD (yes), N (%)	55 (36.7)	13 (25.0)	42 (42.9)	0.031‡
Exercise (yes), N (%)	93 (62.0)	44 (84.6)	49 (50.0)	<0.001‡
Glucose (mg/dL)	87.9 ± 12.1	88.8 ± 10.7	87.4 ± 12.9	0.582†
Triglycerides (mg/dL)*	100.6 (5.5)	116.8 (11.7)	92.9 (5.5)	0.002§
HsCRP (mg/L)*	0.63 (0.08)	0.51 (0.09)	0.70 (0.11)	0.053§
HsCRP from CVD risk, N (%)				
<1 mg/L	98 (65.4)	40 (76.9)	58 (59.2)	0.075‡
1-3 mg/L	44 (29.3)	11 (21.2)	33 (33.7)	
>3 mg/L	8 (5.3)	1 (1.9)	7 (7.1)	

Data are reported as means ± standard deviation or N (%). *Geometric mean (SE); †Student *t*-test, χ^2 test; ‡,§Mann-Whitney test; BMI = body mass index; BP = blood pressure; FH = family history; CVD = cardiovascular disease; HsCRP = high sensitivity C-reactive protein.

Table 3. Factors and their relationship with cardiovascular disease (CVD) risk C-reactive protein (CRP).

Factor	CRP (mg/L)			
	Low (<1)	Average (1-3)	High (>3)	P value
Gender, N (%)				
Male	40 (40.8)	11 (25.0)	1 (12.5)	0.082†
Female	58 (59.2)	33 (75.0)	7 (87.5)	
BMI (kg/m^2)	21.1 ± 2.1	22.0 ± 2.0	22.2 ± 2.2	0.040‡
Waist circumference (cm)	74.7 ± 7.7	77.1 ± 7.0	76.3 ± 5.5	0.200‡
Systolic BP (mmHg)	103 ± 12.0	104 ± 11.0	104 ± 9.2	0.770‡
Diastolic BP (mmHg)	66 ± 7.6	68 ± 9.1	66 ± 7.4	0.275‡
FH of diabetes (yes), N (%)	59 (60.2)	26 (59.1)	6 (75.0)	0.746†
FH of CVD (yes), N (%)	34 (34.7)	17 (38.6)	4 (50.0)	0.616†
Exercise (yes), N (%)	59 (60.2)	30 (68.2)	4 (50.0)	0.517†

†Fisher exact test, ‡Analysis of variance. For abbreviations, see legend to Table 2.

Table 4. C-reactive protein (CRP) levels according to genotypes and alleles of the *CRP* gene polymorphisms.

Polymorphism	Frequencies, N (%)	Geometric mean CRP (mg/L)	CRP for CVD risk, N (%)			
			Low (<1)	Average (1-3)	High (>3)	P
rs1130864						
CC	54 (36.0)	0.5 (0.10)	43 (43.9)	10 (22.7)	1 (12.5)	0.022
CT	74 (49.3)	0.7 (0.12)	45 (45.9)	25 (56.8)	4 (50.0)	
TT	22 (14.7)	1.0 (0.24)	10 (10.2)	9 (20.5)	3 (37.5)	
C	182 (0.61)	0.6 (0.11)	131 (0.67)	45 (0.51)	6 (0.38)	
T	118 (0.39)	0.8 (0.18)	65 (0.33)	43 (0.49)	10 (0.62)	
HWE: χ^2 (P)	0.17 (0.68)					
rs1205						
GG	45 (30.0)	0.8 (0.20)	24 (24.5)	15 (34.1)	6 (75.0)	0.020
GA	71 (47.3)	0.6 (0.10)	46 (46.9)	23 (52.3)	2 (25.0)	
AA	34 (22.7)	0.4 (0.09)	28 (28.6)	6 (13.6)	0	
G	161 (0.54)	0.7 (0.15)	94 (0.48)	53 (0.60)	14 (0.88)	
A	139 (0.46)	0.5 (0.09)	102 (0.52)	35 (0.40)	2 (0.12)	
HWE: χ^2 (P)	0.35 (0.55)					
rs2794521						
AA	115 (76.7)	0.6 (0.09)	81 (82.7)	28 (63.6)	6 (75.0)	0.012
AG	32 (21.3)	0.7 (0.18)	17 (17.3)	14 (31.8)	1 (12.5)	
GG	3 (2.0)	2.2 (0.92)	0	2 (4.6)	1 (12.5	
A	262 (0.87)	0.6 (0.13)	179 (0.91)	70 (0.80)	13 (0.81)	
G	38 (0.13)	1.5 (0.55)	17 (0.09)	18 (0.20)	3 (0.19)	
HWE: χ^2 (P)	0.19 (0.66)					
rs3093062						
GG	148 (98.7)	0.6 (0.08)	97 (99.0)	43 (97.7)	8 (100)	0.575
GA	2 (1.3)	0.9 (0.68)	1 (1.0)	1 (2.3)	0	
G	298 (0.99)	0.8 (0.38)	195 (0.99)	87 (0.99)	16 (1.00)	
A	2 (0.01)	0.9 (0.68)	1 (0.01)	1 (0.01)	0	
HWE: χ^2 (P)	0.01(0.93)					

HWE = Hardy-Weinberg equilibrium. CVD = cardiovascular disease.

Table 5. Effect of genotypes on serum C-reactive protein levels.

SNP	Genotype	β (95%CI)[a]	P value	β (95%CI)[b]	R^2	P value
rs1130864	CC	Ref.		Ref.		
	CT	0.3 (-0.03, 0.6)	0.083	0.3 (0.01, 0.6)	0.16	0.048
	TT	0.7 (0.2, 1.2)	0.003	0.6 (0.1, 1.0)		0.011
	CT+TT	0.4 (0.1, 0.7)	0.017	0.4 (0.1, 0.7)		0.013
rs1205	GG	Ref.		Ref.		
	GA	-0.2 (-0.6, 0.1)	0.189	-0.3 (-0.6, 0.06)	0.15	0.109
	AA	-0.6 (-1.0, -0.1)	0.002	-0.5 (-0.9, -0.1)		0.010
	GA+AA	-0.3 (-0.7, -0.01)	0.044	-0.4 (-0.7, -0.04)		0.026
rs2794521	AA	Ref.		Ref.		
	AG	0.2 (-0.2, 0.6)	0.320	0.3 (-0.1, 0.6)	0.16	0.151
	GG	1.3 (0.3, 2.4)	0.016	1.4 (0.3, 2.4)		0.010
	AG+GG	0.3 (-0.1, 0.6)	0.120	0.3 (0.01, 0.7)		0.047
rs3093062	GG	Ref.		Ref.		
	GA	0.4 (-1.0, 1.7)	0.576	0.2 (-1.1, 1.5)	0.11	0.795

SNP = single nucleotide polymorphism; β = regression coefficient; 95%CI = confidence interval. [a]Unadjusted; [b]Adjusted by gender, body mass index, region of origin, and family history of cardiovascular disease.

Relationship of *CRP* gene haplotypes and CRP concentration

Haplotypes 3 (TGAG) and 5 (CGGG) of *CRP* had significant effects on the average increase in concentration of CRP (β = 0.6 mg/L; 95% confidence interval = 0.3-0.9; P < 0.001)

compared to the most frequent haplotype (Table 6). We did not observe the same effect on the increase in BMI.

Table 6. Effect of haplotype on serum C-reactive protein (CRP) levels.

Haplotype	SNP					CRP (mg/L)	
	rs1130864	rs1205	rs2794521	rs3093062	Frequency	β (95%CI)[a]	P value
1	C	A	A	G	0.285	Reference	
2	C	G	A	G	0.225	0.1 (-0.3, 0.4)	0.630
3	T	G	A	G	0.192	0.6 (0.3, 0.9)	<0.001
4	T	A	A	G	0.165	0.2 (-0.2, 0.6)	0.280
5	C	G	G	G	0.087	0.9 (0.3, 1.2)	0.001
6	T	G	G	G	0.026	-0.04 (-0.7, 0.7)	0.910
Rare					0.020	0.6 (-0.3, 1.4)	0.210

SNP = single nucleotide polymorphism; 95%CI = confidence interval. [a]Adjusted by gender, body mass index, region of origin, and family history of cardiovascular disease.

DISCUSSION

Increased serum CRP levels have been reported in subjects with obesity, metabolic syndrome, and type 2 diabetes (T2D), indicating that these individuals present a state of subclinical, low-grade inflammation that promotes the development of atherosclerosis mediated by a process of endothelial dysfunction, increasing the risk of ischemic heart disease (Wee et al., 2008; Hu et al., 2009). Serum CRP levels vary widely among individuals and, in the absence of disease, are influenced by socio-demographic factors, lifestyle, and significantly, gender, and obesity (Flores-Alfaro et al., 2008; Wee et al., 2008; Tsai and Tsai, 2010). It has been shown that variation in *CRP* influences the concentration of protein (Carlson et al., 2005).

We designed a genetic association study to assess the effect of 4 *CRP* polymorphisms on CRP levels and BMI, minimizing the influence of factors that modify CRP, such as overweight and obesity, disease involving inflammation, smoking, and alcohol consumption. We found that increased BMI, elevated serum CRP levels, and average protein concentration are higher in women than in men. These results indicate an indirect effect of the increase in adipose tissue on the secretion of pro-inflammatory cytokines (interleukins 1 and 6, and tumor necrosis factor α), which regulate the synthesis and secretion of CRP (Blake and Ridker, 2001).

The minor allele frequencies reported for the rs1130864 SNP (T) in different populations worldwide vary from 13 to 34.6%. The genotype frequencies of SNPs rs1130864 and rs3093062 revealed in this study did not differ significantly from those reported in other studies (Kim et al., 2008; Kolz et al., 2008; Almeida et al., 2009; Grammer et al., 2009; Schumacher et al., 2009; Mendoza-Carrera et al., 2010). Differences did arise, however, in the frequencies of SNP rs1130864 found in this study and those from a study conducted in Africa (Israelsson et al., 2009), which may be due to genetic differences between subjects. Conversely, we found minimal differences in the frequencies of the minor allele of SNP rs1205 (A) reported in other populations, which vary from 33 to 35%. We also found this variation with the minor allele frequencies of rs2794521 (24.0 to 28.6%; Kim et al., 2008; Kolz et al., 2008; Grammer et al., 2009). These differences can be explained by the genetic diversity among populations.

We found significant differences in the distribution of individuals carrying the genotypes of the SNPs rs1130864, rs1205, and rs2794521 throughout the strata of CRP in CHD risk. Evaluating the effect of the four SNPs on serum protein with co-dominant and dominant

inheritance models clearly shows a significant trend in the increase in CRP across the genotypes of SNP rs1130864, a decreasing trend across the genotypes of SNP rs1205, and an additive effect in the dominant models. This trend was not observed with the rs2794521 SNP. The effects of the SNPs on the variation in CRP levels have been reported in various populations around the world (Kolz et al., 2008; Almeida et al., 2009; Grammer et al., 2009; Schumacher et al., 2009; Teng et al., 2009; Mendoza-Carrera et al., 2010), demonstrating that the effect of *CRP* SNPs on CRP occurs independent of ethnicity.

We found that individuals carrying the TT genotype of SNP rs1130864 displayed an increase in average BMI of 1.1 kg/m^2 compared with that in CC carriers. A genome scan study found 2 regions linked to T2D in chromosomes 1 and 6 (1q21-q24 and 6q21-q23; Xiang et al., 2004), which is the locus of *CRP* on chromosome 1 (1q21-q23; http://www.genenames.org/data/hgnc_data.php?hgnc_id=2367). In addition, an association among T2D and several *CRP* SNPs has been reported (Zee et al., 2008), suggesting that they may be candidate genes associated with T2D, a disease that is usually preceded by obesity or metabolic syndrome, disorders related to insulin resistance, and CVD. Meanwhile, Teng et al. (2009) reported an obesity-SNP interaction with increased serum levels of CRP and the SNPs rs2794521 and rs1800947 ($P = 0.034$ and $P = 0.020$, respectively).

Several studies have found an association between various haplotypes of the gene and variation in CRP levels (Miller et al., 2005; Kathiresan et al., 2006; Teng et al., 2009). In our study, two haplotypes (TGAG, CGG) had a significant effect on serum CRP levels, a relationship not observed with BMI. This result indicates a clear effect of genetic variability on serum levels of CRP.

In conclusion, our results demonstrated the independent effect of variants in *CRP* on protein levels as well as a possible relationship between the TT genotype of SNP rs1130864 and increased BMI. Future studies are needed to evaluate the relationship between *CRP* variants and the presence of obesity.

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