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Single nucleotide polymorphisms at five loci are associated with C-reactive protein levels in a cohort of Filipino young adults

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Abstract

C-reactive protein (CRP) is a component of non-specific immune defense and is a reliable marker of low-grade inflammation involved in obesity, type 2 diabetes and cardiovascular disease. Genome-wide association studies (GWAS) in middle-aged and elderly populations, predominantly of European descent, demonstrated associations of CRP levels with SNPs at several loci. To examine whether the variants identified are replicated in Filipino young adults, we applied Tobit regression models to study the association of plasma CRP with 12 SNPs at seven loci in a cohort of 1,691 Filipino young adults (aged 21.5 ± 0.3 years) from the Cebu Longitudinal Health and Nutrition Survey (CLHNS). SNPs in or near *CRP* ($P = 3.2 \times 10^{-11}$), *HNF1A, IL6R, APOE-APOC1* and *LEPR* showed significant associations ($P < 0.05$) and together explained 4.8% of the total variation in CRP. Modest interactions were observed between *LEPR* rs1892534 and waist circumference (uncorrected $P_{interaction} = 0.020$) and between *APOE* rs769449 and pathogen exposure (uncorrected $P_{interaction} = 0.0073$) in models predicting CRP. Our results demonstrated that variants in several loci are significantly associated with plasma CRP in Filipino young adults, suggesting shared genetic influences on circulating CRP across populations and age groups.

Keywords

C-reactive protein (CRP); Filipinos; genetic association; interaction; SNP; young adults

INTRODUCTION

C-reactive protein (CRP) is a component of the acute-phase response and a marker of chronic inflammation, which plays important roles in the co-morbidities that accompany obesity, dyslipidemia, type 2 diabetes and cardiovascular diseases[1-4]. In a large US-based study, an estimated 35-40% of the variability in circulating CRP was explained by genetic

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variation[5]. Studies using a candidate gene strategy have described associations linking circulating CRP with several SNPs and haplotypes in the gene regions of *CRP*, *LEPR* and *APOE*[6-8]. A prior genome-wide association (GWA) study based on individuals of European ancestry from the women's genome health study (WGHS) has provided evidence of associations between CRP and SNPs at seven genetic loci, including *CRP*, *HNF1A*, *LEPR*, *IL6R*, *APOE*, *GCKR* and 12q23.2, together accounting for 10.1% of the total variation in circulating CRP in this population[9]. Similar findings of association were reported by subsequent GWA studies conducted in individuals of middle-aged and elderly European descent[10-11] and in Japanese[12]. A recent meta-analysis confirmed the reported 7 loci and identified 11 additional loci associated with CRP levels[13].

Quantitative traits such as CRP concentrations are known to be influenced by both genetic and environmental factors, and interactions between them are likely to be important predictors as well[14]. Evidence exists that an interaction between adiposity and *CRP* gene variants influences CRP levels. An interaction analysis conducted in Taiwanese noted that the association between *CRP* haplotypes and CRP level predominantly occurred in obese subjects[15], and a *CRP* variant by adiposity interaction analysis in Europeans showed that the correlated increase in CRP levels with adiposity was accentuated by the presence of the *CRP* rs1205 G allele[16].

Genetic background may vary across populations, and the genetic contribution of identified variants on circulating CRP remains unclear in populations of non-European descent. The purpose of this study is to assess whether the CRP-associated loci identified in Europeans are associated with plasma CRP in a cohort of Filipino young adults living in the Metro Cebu area of the Philippines. In light of the roles of infectious agents[17] and adipose tissue[18] in up-regulating inflammatory pathways, prior work in this population demonstrated that CRP levels were influenced by a dual burden of environmental pathogenicity and central adiposity[19-21]. Thus, we also aim to determine whether measures of individual adiposity and household-level pathogenicity modify any effects of these polymorphisms on CRP levels.

MATERIALS AND METHODS

Samples and data come from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an on-going community-based cohort study of births between May 1, 1983 and April 30, 1984. The CLHNS is based in Metro Cebu, Philippines, which encompasses three central cities within Cebu province as well as contiguous peri-urban and rural municipalities. The original study design and recruitment protocols have been previously described in detail[22]. Written informed consent was obtained from all participants, and study protocols were approved by the University of North Carolina Institutional Review Board for the Protection of Human Subjects. In the 2005 survey, the available sample included 1,779 Filipino young adults. A pathogen exposure score was constructed based on the mean value of five interviewer-assigned variables, each scored on a three point scale (0=low exposure, 1=moderate, 2=high): cleanliness of the food preparation area, means of garbage disposal, presence of excrement near the house, level of garbage and excrement present in the neighborhood surrounding the household[19]. In addition, to control for any effects of active infection on CRP levels, participants were asked whether they were experiencing any symptoms of infection at the time of blood collection. Reported symptoms included runny nose, cough, fever, diarrhea, sore throat and the more general categories of flu, cold and sinusitis[20].

Overnight fasting blood samples were obtained at the 2005 survey and collected into EDTA-coated tubes. Plasma CRP concentration was determined using a high sensitivity immunoturbidimetric method (Synchron LX20 with lower detection limit of 0.1mg/L;

Beckman Coulter, Fullerton, CA) with between-assay CVs < 7.6 across the assay range[19]. Following AHA/CDC guidelines, participants with CRP level > 10 mg/L were interpreted as having an acute infection[23] and were therefore excluded from analyses. The final sample set for analysis included 1,691 unrelated CLHNS young adults with complete data on plasma CRP, all genotypes and covariates.

Selection of SNPs for analyses was based on published data regarding their association with CRP concentrations[9-10]. At the seven loci (*CRP*, *LEPR*, *IL6R*, *GCKR*, 12q23.2, *HNF1A* and *APOE-APOC1*) for which we have > 80% power to detect the reported variance explained by SNPs, we selected SNPs with the strongest evidence of association at a given locus or with potential functional significance based on annotation[24-25]. The nine SNPs included rs1205 (*CRP*), rs3091244 (*CRP*), rs1892534 (*LEPR*), rs2228145 (*IL6R*), rs1260326 (*GCKR*), rs10778213 (12q23.2), rs1169288 (*HNF1A*), rs7310409 (*HNF1A*) and rs769449 (*APOEAPOC1*). Three additional SNPs in *APOE* or *APOC1* were selected to better characterize this locus. SNPs rs429358 and rs7412 were selected to construct the reported *APOE* haplotypes $\varepsilon 2$ (rs429358-rs7412: T-T), $\varepsilon 3$ (rs429358-rs7412: T-C), and $\varepsilon 4$ (rs429358-rs7412: C-C)[8]. SNP rs4420638 in *APOC1* was selected based on reproducible evidence of association with CRP level in previous reports[11-13].

Genotyping was performed using TaqMan fluorogenic 5' nuclease assays (Applied Biosystems) using an ABI PRISM® 7900 system. The triallelic SNP rs3091244 was genotyped based on two separate assays (C/T and C/A)[26], and the final genotypes were assigned as shown in Supplementary Table 1. Genotyping success rates for all assays were > 96.5%, and all SNPs were in agreement with Hardy-Weinberg equilibrium ($P > 0.1$). SNP rs4420638 in *APOC1* was imputed using MACH[27] from MetaboChip (Illumina, San Diego, CA, USA) SNP genotypes in CLHNS young adults [Damien C. Croteau-Chonka, *et.al.*, manuscript submitted] based on CEU, CHB and JPT haplotypes created from the 1000 Genomes Project pilot (June 2011 data release). Based on the high imputation quality ($r^2 = 0.96$) of rs4420638, we used the posterior expected genotypes in statistical analyses.

A total of 574 (33.9%) individuals among the 1,691 CLHNS young adults had CRP concentrations below the detectable level (0.1 mg/L), and log-CRP concentrations produced a left-censored distribution. Given the markedly skewed distribution of CRP concentrations and the presence of many values < 0.1, we used Tobit regression that applies linear regression to a mixture of censored discrete data and normally distributed continuous distributions[28] to test association between natural log-transformed CRP (left-censored at value of -3, corresponding to natural log of 0.05) and genotype in the CLHNS young adults. In addition, we applied linear regression, in which CRP values were natural log-transformed after adding the constant 0.10. In initial analysis for main effects, an additive genetic model was assumed. Sex, pathogenic score and infection status were included as covariates. In secondary analyses, both models were further adjusted for waist circumference to examine whether the associations of SNPs and plasma CRP were mediated by central adiposity. Interactions between SNPs and waist circumference and between SNPs and pathogen exposure score were evaluated in Tobit regression models, accounting for the same covariates used in the main effect analyses. Waist circumference and pathogenic score were treated as dichotomous variables, using the medians (waist circumference < 69 or ≥ 69 cm and pathogen exposure score <0.5 or ≥ 0.5) as the threshold to stratify the study samples.

We further assessed the combined effect of multiple SNPs on plasma CRP using a cumulative genotype score as an independent variable. The cumulative genotype score was calculated based on the number of alleles associated with elevated CRP level, weighted by the fitted linear regression model coefficients. A total of five SNPs (*CRP*-rs1205, *LEPR*-rs1892534, *IL6R*-rs2228145, *HNF1A*-rs7310409 and *APOE*-rs429358) representing each of

the five CRP-associated loci in the CLHNS were used for the combined effect estimation. Individuals with genotypes available for all the 5 selected SNPs (n = 1,592) were categorized into quintiles based on the cumulative genotype score.

All analyses for association with single SNPs were performed with SAS version 9.2 (SAS Institute, Cary, NC, USA). The 'haplo.score' and 'haplo.glm' functions implemented in the 'haplo.stats' R package were applied for haplotype analysis.

RESULTS

Twelve SNPs at seven loci were investigated in 1,691 CLHNS young adults (Table 1). Based on the results from Tobit regression models, we observed evidence of significant association with plasma CRP for SNPs at five loci, including *CRP* (P for rs1205 = 3.2×10^{-11} and P for rs3091244 = 5.6×10^{-10}), *HNF1A* (P for rs7310409 = 4.1×10^{-5} and P for rs1169288 = 1.0×10^{-4}), *IL6R* (P for 2228145 = 6.2×10^{-4}), *APOE-APOC1* (P for rs769449 = 0.0068, P for rs429358 = 0.0060, and P for rs4420638 = 0.044), and *LEPR* (P for rs1892534 = 0.018), all showing the same direction of effect as previously reported[9, 13] (Table 2). The associations for variants at *CRP*, *HNF1A* and *IL6R* remained significant after Bonferroni correction for multiple testing ($P < 0.0042$, 0.05/12 tests). No evidence for association was observed for variants at *GCKR* ($P = 0.46$ for rs1260326) or the gene desert region of 12q23.2 ($P = 0.73$ for rs10778213). A third SNP in the *APOE* gene, rs7412, showed no association with CRP level in CLHNS young adults ($P = 0.51$). Consistent results for association were observed using linear regression models (data not shown). We further examined whether these associations with CRP level were affected by adjustment for waist circumference. None of the associations substantially changed, suggesting that the genetic associations with plasma CRP were independent of central adiposity (Supplementary Table 2).

An association between CRP level and *APOE* haplotype consisting of the SNPs rs429358 and rs7412 was previously reported in middle-aged and elderly Caucasians[8]. Our results provided confirmatory evidence of the haplotype association in this cohort of Filipino young adults (Table 3). Haplotype analysis based on score statistic revealed an overall association between haplotypes and CRP levels (global P value = 0.018). Specifically, the *APOE* ε4 haplotype with a frequency of 0.099 was significantly associated with lower CRP level ($P = 0.0047$). Complementary analysis that assessed the effect on CRP level of each additional copy of the specific haplotype compared to the homozygote reference haplotype found that the *APOE* ε4 haplotype was significantly associated with decreased plasma CRP ($\beta = -0.210$, $P = 5.4 \times 10^{-3}$) compared to the most common *APOE* ε3 haplotype. We next performed conditional analysis at *APOE-APOC1* to examine whether the associated SNPs represent a single signal. Reciprocal conditional analysis on *APOC1*-rs4420638 and *APOE*-rs429358 (LD $r^2 = 0.51$) showed that the effect of association with rs4420638 was substantially attenuated ($P_{\text{conditional}} = 0.76$) whereas the association with rs429358 remained significant ($P_{\text{conditional}} = 0.0093$). Therefore, the SNPs at the *APOE-APOC1* cluster appear to represent a single signal best represented by *APOE*-rs429358.

We next investigated whether the genetic associations with plasma CRP are modified by waist circumference or pathogen exposure (Supplementary Table 3), both of which are predictors of baseline CRP level. Modest evidence was detected for the interaction of waist circumference with rs1892534 at *LEPR* (uncorrected $P_{\text{interaction}} = 0.020$). In a secondary analysis stratifying by waist circumference above or below the median value, the estimated increase in log-CRP level for each additional C allele of rs1892534 was 0.232 ($P = 0.0062$) in individuals with smaller waist circumference and 0.015 ($P = 0.86$) in those with larger waist circumference. We also observed evidence for interaction between *APOE* variant

rs769449 and pathogen exposure on plasma CRP (uncorrected $P_{interaction} = 0.0073$). When stratified analysis was conducted, we found that the significant and strong association between CRP level and the *APOE* variant was predominantly observed in individuals with lower exposure to a pathogenic environment ($\beta = 0.419$, $P = 6.8 \times 10^{-5}$) compared to those with higher exposure ($\beta = 0.017$, $P = 0.88$). Given that the observed interaction was not significant after Bonferroni correction (corrected $P_{interaction} > 0.0045$, 0.05/ 11 tests), further studies in larger populations are warranted.

We observed no evidence of multiplicative SNP-SNP interaction among variants *CRP*-rs1205, *LEPR*-rs1892534, *IL6R*-rs2228145, *HNF1A*-rs7310409 and *APOE*-rs429358 (all uncorrected $P_{interaction} > 0.12$). In assessing the combined effects of multiple SNPs on plasma CRP, we observed a dose response. Individuals carrying a greater number of alleles associated with elevated level of CRP had increased circulating CRP level (P for trend = 1.0×10^{-17}, Figure 1). The geometric mean of plasma CRP in the group of individuals in the highest quintile (Q5) was 0.648 mg/L (SE = 0.048), a 2.4-fold increase compared to that in the lowest quintile group (geometric mean = 0.273, SE = 0.019).

DISCUSSION

In this study of Filipino young adults, we replicated associations for plasma CRP with several variants previously identified in middle-aged or elder Europeans[9]. SNPs at five loci of *CRP*, *HNF1A*, *LEPR*, *IL6R*, *APOE-APOC1* were significantly associated with CRP level and together explained 4.8% of the variance in CRP in this population. In addition, modest interactions were observed between *LEPR* rs1892534 and waist circumference and between *APOE* rs769449 and pathogen exposure in models predicting plasma CRP, suggesting that the impact of these variants may be contingent upon environmental exposures.

In past studies, variants at the *CRP* locus, which encodes the CRP protein, were reproducibly reported to show significant association with circulating CRP not only in populations of European ancestry, but also in Asians such as Chinese and Japanese[15, 29]. Specifically, the triallelic SNP rs3091244 was suggested to be functionally relevant[30]. In our Filipino young adult sample, the SNPs rs1205 and rs3091244 at the *CRP* gene region showed the strongest associations with plasma CRP, consistent with the previous findings from candidate gene and GWAS[9-10, 15]. We also observed a strong association between plasma CRP and *HNF1A* variants rs7310409 and rs1169288. Because the protein HNF1A is involved in modulating *CRP* gene expression by directly binding to its promoter[31], the gene region of *HNF1A* had convincing prior evidence of association with CRP as a candidate gene and was further confirmed by GWAS in European-based populations[9-10]. We also replicated associations with CRP related variants at *IL6R*[32], *APOE*[8], *APOC1*[13], and *LEPR*[7], providing the first confirmation of genetic association for these loci in a young Asian population.

We did not find evidence for an association between plasma CRP and the SNP in *GCKR* (rs1260326) or the intergenic SNP 12q23.1-rs10778213, despite our >95% power to detect the previously reported variance explained by these SNPs[9]. Similar to our results, the association with these SNPs failed to reach significant levels in a prior GWAS conducted in the Pharmacogenomics and Risk of Cardiovascular Disease (PARC) study[10], thus suggesting that the association for variants at these loci might be population-specific. We did not test additional CRP associated loci reported by a recent meta-analysis of GWA studies[13] because our study was poorly powered (< 35%) to detect the reported variance explained by those SNPs. In addition, given the relatively younger age of our study sample compared to those previously reported[9], the potential interactive influence between gene and age may also partially explain the discrepancy. Furthermore, because the environmental

factors and behavior such as infection, smoking, diet pattern and physical activity may contribute to baseline CRP variation[33], we cannot exclude the possibility that the long-term exposure to certain environmental factors may accentuate the genetic susceptibility on CRP level in middle-aged or elderly populations.

In conclusion, our results demonstrate that SNPs at *CRP*, *HNF1A*, *IL6R*, *APOE-APOC1* and *LEPR* are associated with plasma CRP in a sample of Filipino young adults. This study expands our understanding of the genetic associations with CRP level to a younger population of mostly non-European ancestry and suggests a shared genetic influence between ethnic groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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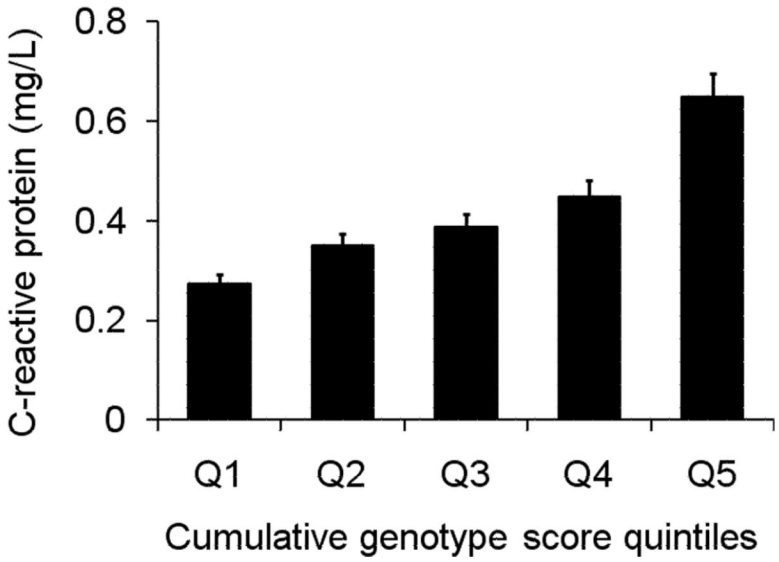

Figure 1.

CRP level increases with genotype score in 1,592 CLHNS Filipino young adults. The cumulative genotype score was calculated based on the number of alleles associated with elevated CRP level, weighted by the fitted linear regression model coefficients. Individuals with genotypes available from five selected SNPs (*CRP*-rs1205, *LEPR*-rs1892534, *IL6R*-rs2228145, *HNF1A*-rs7310409 and *APOE*-rs429358) were categorized into quintiles based on the cumulative genotype score.

Table 1

Characteristics of the CLHNS young adult cohort

	Females (n=799)	Males (n=892)	Total (n=1,691)
Age (years)	21.5 ± 0.3	21.5 ± 0.3	21.5 ± 0.3
CRP (mg/L)	0.3 (0, 1.0)	0.2 (0, 0.8)	0.2 (0, 0.9)
Waist circumference (cm)	68.0 ± 7.6	72.2 ± 7.5	70.3 ± 7.8
BMI (kg/m^2)	20.4 ± 3.2	21.1 ± 3.1	20.8 ± 3.2
Symptoms of infection (%)	13.9	12.8	13.3
Pathogen exposure score	0.54 ± 0.39	0.55 ± 0.40	0.54 ± 0.40

Data are mean ± SD, median (25th percentile, 75th percentile) or %.

Table 2

Candidate SNP association with CRP levels in 1,691 CLHNS young adults

SNP	Locus	Annotation	Reported Effect Allele	Effect Allele	Non-effect Allele	β (SE)	*P*
rs1205	*CRP*	3'UTR	C	C	T	0.518 (0.077)	3.2E-11
rs3091244	*CRP*	Promoter	A or T	A or T	C	0.575 (0.092)	5.6E-10
rs1892534	*LEPR*	Downstream	C	C	T	0.258 (0.109)	0.018
rs2228145	*IL6R*	Asp358Ala	A	A	C	0.313 (0.091)	6.2E-04
rs7310409	*HNF1A*	Intron	G	G	A	0.347 (0.084)	4.1E-05
rs1169288	*HNF1A*	Ile27Leu	A	A	C	0.324 (0.083)	1.0E-04
rs1260326	*GCKR*	Pro446Leu	T	T	C	0.060 (0.080)	0.46
rs10778213	12q23.2	Intergenic	T	T	C	0.034 (0.097)	0.73
rs769449	*APOE*	Intron	G	G	A	0.376 (0.139)	0.0068
rs429358	*APOE*	Cys130Arg	T	T	C	0.377 (0.137)	0.0060
rs7412	*APOE*	Arg176Cys	N.A.	T	C	0.083 (0.125)	0.51
rs4420638	*APOC1*	3'UTR	A	A	G	0.260 (0.129)	0.044

Adjusted for sex, infectious status, and pathogen score. Individuals with CRP > 10mg/L were excluded from analyses. Reported effect allele: the allele that is associated with elevated CRP levels in previously reported GWAS; Effect Allele: the allele associated with elevated CRP level in CLHNS young adults.

Table 3

CRP association with *APOE* haplotypes

Haplotype	rs429358-rs7412	Freq	Haplo.score		Haplo.glm	
			score	P	β (SE)	P
APOE ε2	T-T	0.018	0.58	0.56	0.018 (0.070)	0.79
APOE ε3	T-C	0.794	1.59	0.11	reference	reference
APOE ε4	C-C	0.099	-2.83	0.0047	-0.210 (0.075)	5.4×10^{-3}

Using the “haplo.score” function implemented in the “haplo.stats” R package, the global *P* values for association between haplotypes and CRP level were 0.018. The “haplo.glm” function implemented in the “haplo.stats” R package was used to calculate the coefficient β and *P* value for each haplotype compared with the reference haplotype (APOE ε3). The same covariates used for genotype analysis were applied in haplotype analysis.