



C-reactive protein + 1059 G>C polymorphism in type 2 diabetes and coronary artery disease patients[☆]

Ramandeep Kaur¹, Kawaljit Matharoo^{*}, Rubina Sharma², A.J.S. Bhanwer^{**}

Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab 143005, India

ARTICLE INFO

Article history:

Received 30 August 2013

Revised 11 October 2013

Accepted 23 October 2013

Keywords:

Association study

CRP

Dyslipidemia

Correlation

PCFA

Punjab

ABSTRACT

Human C-reactive protein (CRP) is an acute phase reactant involved in chronic and acute inflammation. CRP is associated with metabolic syndrome, obesity, atherosclerosis, unstable angina, insulin resistance and diabetes. The present study evaluates the association of + 1059 G>C silent polymorphism in exon 2 of *CRP* gene in 581 cases [CAD (206), T2D (266), T2D with CAD (109)] and 235 controls in the population of Punjab (North-West India). The frequency of + 1059 G allele is highest in CAD (98.3%) followed by T2D (98.1%), T2D + CAD cases (97.7%) and controls (94.7%). G-allele is associated with increased risk of T2D [$P = 0.003$, OR = 2.93 (1.39–6.17)] and CAD [$P = 0.004$, OR = 3.25 (1.39–7.60)] in comparison to controls. Recessive model shows that GG genotype increases the risk of CAD by 4 fold ($P = 0.003$, OR = 4.19, 1.62–10.80), T2D by 3 fold ($P = 0.008$, OR = 3.23, 1.36–7.60) and T2D + CAD by 3.5 fold ($P = 0.029$, OR = 3.64, 1.14–11.66). Factor analyses show that BMI, WC, and WHR are core

Abbreviations: ADA, American Diabetes Association; ARMS-PCR, amplification refractory mutation system-polymerase chain reaction; BMI, body mass index; CRP, C-reactive protein; CHO, total cholesterol; CI, confidence interval; CVDs, cardiovascular diseases; CAD, coronary artery disease; DNA, deoxyribonucleic acid; DBP, diastolic blood pressure; HWE, Hardy-Weinberg Equilibrium; HC, hip circumference; HDL, high density lipoprotein; IL-1, interleukin-1; IL-6, interleukin-6; LDL, low density lipoprotein; OR, odds ratio; PCFA, principal component factor analysis; RBS, random blood sugar; SNP, single nucleotide polymorphism; SD, standard deviation; SPSS, Statistical Package for Social Science; SBP, systolic blood pressure; TG, triglyceride; TNF- α , tumor necrosis factor- α ; T2D, type 2 diabetes; VLDL, very low density lipoprotein; WC, waist circumference; WHR, waist-hip ratio; WASP, web-based allele specific primer

[☆] This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

^{*} Corresponding author. Tel.: +91 95011 14487.

^{**} Corresponding author. Tel.: +91 98141 03474; fax: +91 183 2258819, +91 983 2258820.

E-mail addresses: ramandeepkaur0609@gmail.com (R. Kaur), matharookawal@gmail.com (K. Matharoo), rubina.genetics@gmail.com (R. Sharma), ajsbhanwer@gmail.com (A.J.S. Bhanwer).

¹ Tel.: +91 98766 32277.

² Tel.: +91 95011 14484.

predictors for CAD and T2D, whereas CHO, TG and VLDL for T2D + CAD. The present study concludes that GG genotype of CRP +1059 G>C polymorphism and clustering of obesity and dyslipidemia underlie the risk towards CAD, T2D and T2D + CAD in the North-West Indian population of Punjab.

© 2013 The Authors. Published by Elsevier B.V. All rights reserved.

1. Introduction

Human C-reactive protein (CRP) is an acute phase reactant involved in chronic and acute inflammation (Kushner, 1982). CRP production is rapidly stimulated in response to infection, tissue injury or inflammation. CRP is synthesized by hepatocytes and its expression is controlled by several trans-acting cytokines, like interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) (de Ferranti and Rifai, 2002; Li and Fang, 2004). Inflammation has a very diverse role from being an essential component in protection against pathogens and tissue damage to development of complex and autoimmune diseases (Loza et al., 2007). Chronic inflammation contributes to the development of atherosclerosis, cardiovascular diseases (CVDs) and type 2 diabetes (T2D) (Ross, 1999; Libby et al., 2002; Mazzone et al., 2008). Elevated blood CRP levels have been widely used as an ideal marker of inflammation and general health of the patient (Van Leeuwen and Van Rijswijk, 1994). Increased levels of CRP are reported to be associated with metabolic syndrome, obesity, atherosclerosis, unstable angina, insulin resistance and diabetes (Rizzello et al., 2007; Shankar and Li, 2008; Wee et al., 2008; Gelaye et al., 2010; Lai et al., 2010; Momiyama et al., 2010). Family and twin studies suggest that genetic factors account for 40% of the variance in plasma CRP levels (Pankow et al., 2001; Retterstol et al., 2003; MacGregor et al., 2004). +1059 G>C (rs1800947) is a single nucleotide polymorphism (SNP) in exon 2 of the CRP gene. +1059 G>C is a silent or synonymous polymorphism at the amino acid level (CTG \rightarrow CTC, Leu \rightarrow Leu at codon 184) which has been reported to affect the protein levels of CRP and contribute towards the progression of CAD (coronary artery disease) and T2D (Pasalic et al., 2009; Lange et al., 2006). Considering the paucity of data on the association of CRP +1059 G>C polymorphism with CAD and T2D from the North-West Indian population of Punjab, the present study aims to evaluate the association of +1059 G>C polymorphism with CAD, T2D and T2D patients with CAD from Punjab.

2. Materials and methods

2.1. Study design and subjects

Case-control association study design adopted for the present study enrolled a total of 816 individuals. There were three categories for the total cases that included 266 cases with only T2D (without CAD), 109 T2D cases with CAD as the secondary complication and 206 CAD patients without history of T2D. The control group included 235 normal healthy individuals without history of T2D and CAD. Samples were collected from various hospitals and localities of Punjab after obtaining written and informed consent. The study has been approved by the institutional ethical committee. All the cases recruited in the study were clinically diagnosed by the physician. Individuals were diagnosed for T2D according to the criteria given by the American Diabetes Association (ADA) 2011 i.e. fasting plasma glucose (FPG) \geq 126 mg/dl (7.0 mmol/l), or random plasma glucose 200 mg/dl (11.1 mmol/l), or 2-h plasma glucose \geq 200 mg/dl (11.1 mmol/l) (American Diabetic Association, 2011); CAD was diagnosed based on a past history of documented myocardial infarction (MI) or ECG changes suggestive of ST-segment depression (Minnesota codes 1-1-1 to 1-1-7) or Q-wave changes (Minnesota codes 4-1 to 4-2) or T-wave changes (Minnesota codes 5-1 to 5-3). Documented MI was diagnosed if an individual had a positive history of MI in the medical records (a summary report after discharge from a hospital) (Mohan et al., 2001). Ethnicity matched normal healthy individuals with age \geq 40 years were included as controls for the present study. Clinical and demographic details; and anthropometric measurements like height, weight, waist circumference (WC) and hip circumference (HC) were measured for calculating body mass index (BMI) and waist-hip ratio (WHR) from each individual.

2.2. Genotyping

Genomic DNA was isolated from intravenous blood, using salting out method of DNA isolation with some modifications according to the laboratory conditions (Miller et al., 1988). Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) which is based on allele specific amplification of desired fragment using primers corresponding to the alleles has been used for genotyping of +1059 G>C SNP in the *CRP* gene. Primer sequences were designed by WASP (web-based allele specific primer) designing tool (www.bioinfo.biotec.or.th/WASP). The sequences of specific primers used are: 1. Constant forward: 5' CATTGTACAAGCTGGGAGT 3', 2. Allele C specific reverse: 5' ATGGTGTAATCTCATCTGGTGGG 3', 3. Allele G specific reverse: 5' ATGGTGTAATCTCATCTGGTGGC 3'. The amplification was carried out in an Eppendorf Mastercycler Gradient thermal cycler. The conditions included initial denaturation (95 °C for 5 min) following a touchdown PCR with denaturation at 94 °C for 45 s, annealing at 66 °C to 62 °C with 1 °C decrease in temperature for the first 4 cycles followed by 29 cycles at 61 °C for 45 s, extension at 72 °C for 45 s each cycle and final extension at 72 °C for 10 min. *CRP* +1059 G>C genotypes were assessed from the presence/absence of PCR amplicon (237 bp), corresponding to the specific allele (C/G) on 2% agarose gel stained with ethidium bromide. 100 bp ladder was used as the molecular weight marker to determine the fragment size. To reduce the genotyping error, genotyping was repeated on 10–15% of the samples and positive controls for both the alleles were used in all the PCR reactions.

2.3. Statistical analysis

The statistical analysis was performed using Statistical Package for Social Science program (SPSS version 16.0; SPSS, Chicago, IL). Power of the study was calculated using the CaTS power calculator (Skol et al., 2006). Continuous data was expressed as mean \pm SD and continuous variables were compared with Student's t-test with Bonferroni correction for multiple testing (significant $P < 0.05$). Genotypes were tested for the Hardy–Weinberg Equilibrium (HWE) using chi square analysis. Categorical variables, genotype and allele frequencies between cases and controls were compared by chi square test. Unconditional binary logistic regression analysis was used to determine the association between the allelic and genotypic status of the studied polymorphism with CAD, T2D and T2D + CAD, after correction for age, sex, BMI, WC and WHR. Pearson's correlation was applied to see the relationship between various risk factors. Level of statistical significance was set at a P value < 0.05 . Principal components analysis was used to extract orthogonal components. The initial solution, principal component 1 explained the maximum variance, while successive components explained progressively smaller portions of the total variance. Principal components were simplified by orthogonal rotation (varimax). Relationships between components are explained by factor loadings, values greater than or equal to 0.4 were used to indicate meaningful correlations between the component and the variable.

3. Results

Data for the baseline characteristics of individuals investigated in the present study has been summarized in Table 1. In comparison to controls, CAD cases presented with significantly higher age, lower BMI, WC, CHO and LDL; T2D cases with CAD had higher age, WHR, SBP and RBS while lower values for BMI, CHO and LDL; and cases with T2D only had higher WC, WHR, SBP and RBS. In comparison against CAD cases, T2D cases with CAD (T2D + CAD) had significantly higher WHR and RBS; T2D cases without CAD were observed to have higher values for BMI, WC, WHR, RBS, CHO and LDL. On comparing T2D + CAD with T2D; age, BMI, WC, SBP, CHO and LDL show significant difference after applying the Bonferroni correction for multiple comparisons.

3.1. Association analysis of *CRP* (+1059 G>C) polymorphism

The genotype frequencies for the *CRP* (+1059 G>C) polymorphism were in HWE in the control group and patients with CAD. The genotype frequencies showed deviation from HWE in T2D patients with and without CAD. Power of the study was calculated to be 60%. The genotype and allele frequencies of the +1059 G>C polymorphism of *CRP* and the results of the genetic models which were observed to be

Table 1
Demographic, clinical and anthropometric characteristics of the study population.

Variables	CAD (Mean and S.D.)	T2D + CAD (Mean and S.D.)	T2D (Mean and S.D.)	Control (Mean and S.D.)	CAD vs. control (P value)	T2D + CAD vs. control (P value)	T2D vs. control (P value)	CAD vs. T2D + CAD (P value)	CAD vs. T2D (P value)	T2D + CAD vs. T2D (P value)
Age (years)	59.83 ± 11.839	62.10 ± 10.114	54.82 ± 10.848	51.94 ± 11.114	3.19 × 10 ^{-11*}	1.19 × 10 ^{-13*}	0.05	0.09	2.79 × 10 ^{-05*}	5.16 × 10 ^{-08*}
BMI (kg/m ²)	24.80 ± 5.294	25.64 ± 6.612	27.91 ± 5.260	28.07 ± 5.589	1.96 × 10 ^{-08*}	0.012*	0.74	0.229	1.09 × 10 ^{-08*}	0.012*
WC (cm)	91.87 ± 13.656	94.87 ± 13.128	102.00 ± 12.958	98.39 ± 13.601	1.11 × 10 ^{-06*}	0.32	0.04*	0.06	6.36 × 10 ^{-14*}	3.68 × 10 ^{-05*}
WHR	0.95 ± .086	0.99 ± .087	1.01 ± .081	0.95 ± .085	0.48	0.005*	6.69 × 10 ^{-11*}	7.81 × 10 ^{-04*}	8.68 × 10 ^{-12*}	0.07
SBP (mm/Hg)	134.82 ± 23.691	140.32 ± 30.897	134.84 ± 18.887	128.90 ± 17.179	0.06	0.012*	0.01*	0.09	0.99	0.04*
DBP (mm/Hg)	88.21 ± 18.246	89.50 ± 21.822	86.77 ± 10.322	85.50 ± 11.257	0.08	0.44	0.21	0.59	0.29	0.11
RBS	131.07 ± 38.218	202.41 ± 79.323	213.46 ± 81.503	123.98 ± 33.148	5.70	3.23 × 10 ^{-12*}	1.27 × 10 ^{-23*}	1.72 × 10 ^{-10*}	1.62 × 10 ^{-20*}	0.33
CHO	136.06 ± 47.356	130.17 ± 39.594	172.09 ± 53.186	180.26 ± 54.149	6.19 × 10 ^{-16*}	8.47 × 10 ^{-14*}	0.10	0.29	3.17 × 10 ^{-12*}	6.61 × 10 ^{-13*}
TG	164.35 ± 108.339	204.62 ± 169.241	182.63 ± 111.794	175.20 ± 112.059	0.32	0.07	0.47	0.18	0.08	0.16
HDL	43.49 ± 20.889	39.56 ± 19.197	40.12 ± 14.232	40.85 ± 10.989	0.11	0.46	0.54	0.12	0.54	0.77
LDL	61.046 ± 43.699	50.133 ± 35.641	95.604 ± 42.988	105.85 ± 52.311	6.35 × 10 ^{-18*}	2.90 × 10 ^{-22*}	0.24	0.41	1.01 × 10 ^{-14*}	5.57 × 10 ⁻²⁰
VLDL	32.701 ± 21.739	40.507 ± 33.926	36.526 ± 22.359	35.039 ± 22.412	0.28	0.09	0.47	0.22	0.07	0.20

BMI = body mass index; WC = waist circumference; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; RBS = random blood sugar; CHO = total cholesterol; TG = triglyceride; HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein.

* P < 0.05 is significant after the Bonferroni correction for multiple comparisons.

Table 2Genotype and allele frequency distribution with genetic model analysis for *CRP* 1059 G>C polymorphism in cases and controls.

Genotype	CAD (n = 206)	T2D + CAD (n = 109)	T2D (n = 266)	CN (n = 235)	CAD vs. CN	T2D + CAD vs. CN	T2D vs. CN	CAD vs. T2D + CAD	CAD vs. T2D	T2D + CAD vs. T2D
GG	96.6%	96.3%	97.0%	89.8%	$\chi^2 = 8.00$ P = 0.018*	$\chi^2 = 5.53$ P = 0.063	$\chi^2 = 13.14$ P = 0.001*	$\chi^2 = 1.98$ P = 0.371	$\chi^2 = 2.10$ P = 0.35	$\chi^2 = 0.11$ P = 0.95
GC	3.4%	2.8%	2.3%	9.8%						
CC	0%	0.9%	0.8%	0.4%						
Allele	98.3%	97.7%	98.1%	94.7%	$\chi^2 = 8.23$ P = 0.004*	$\chi^2 = 3.27$ P = 0.071	$\chi^2 = 8.76$ P = 0.003*	$\chi^2 = 0.27$ P = 0.603	$\chi^2 = 0.043$ P = 0.84	$\chi^2 = 0.13$ P = 0.71
G	1.7%	2.3%	1.9%	5.3%	OR = 3.25 (1.39–7.60)*		OR = 2.93 (1.39–6.17)			
C					P = 0.003 ^a	P = 0.029 ^a	P = 0.008 ^a	P = 0.90	P = 0.81	P = 0.74
Recessive model (GG vs. GC + CC)	-	-	-	-	OR = 4.19 (1.62–10.80)	OR = 3.64 (1.14–11.66)	OR = 3.23 (1.36–7.60)	OR = 1.08 (0.31–3.78)	OR = 0.88 (0.31–2.47)	OR = 0.81 (0.24–2.76)
Codominant model (GC vs. GG + CC)	-	-	-	-	P = 0.004 ^a	P = 0.016 ^a	P = 0.002 ^a	P = 0.76	P = 0.45	P = 0.77
Dominant model (GG + GC vs. CC)	-	-	-	-	OR = 0.24 (0.09–0.63)	OR = 0.20 (0.05–0.74)	OR = 0.23 (0.09–0.59)	OR = 1.24 (0.31–4.91)	OR = 1.52 (0.50–4.61)	OR = 1.23 (0.30–4.99)
					-	P = 0.58	P = 0.636	-	-	P = 0.87
						OR = 0.46 (0.03–7.4)	OR = 0.56 (0.05–6.26)			OR = 0.82 (0.07–9.12)

* P < 0.05 is significant.

^a P corrected for age, sex, BMI, WC, and WHR.

statistically significant after adjusting for confounding factors as age, sex, BMI, WC and WHR have been represented in Table 2. The frequency of G-allele was highest in CAD cases (98.3%) followed by T2D (98.1%), T2D + CAD cases (97.7%) and controls (94.7%), respectively. Both genotype and allele frequencies show significant difference in comparison to controls for CAD cases ($P = 0.018$, $P = 0.004$, $OR = 3.25$ (1.39–7.60) at 95% CI, respectively), and T2D cases ($P = 0.001$, $P = 0.003$, $OR = 2.93$ (1.39–6.17) at 95% CI, respectively). G allele appears to provide risk to both CAD and T2D in comparison to controls. Analysis under recessive model (GG vs. GC + CC) shows that in comparison to the control group, GG genotype increases the risk of CAD by 4 fold ($P = 0.003$, $OR = 4.19$, 1.62–10.80 at 95% CI), T2D by 3 fold ($P = 0.008$, $OR = 3.23$, 1.36–7.60 at 95% CI) and T2D + CAD by 3.5 fold ($P = 0.029$, $OR = 3.64$, 1.14–11.66 at 95% CI). However, under the codominant model (GC vs. GG + CC) OR less than 1 is observed in comparison of cases (CAD, T2D and CAD + T2D) with controls. The analysis suggests that GC genotype provides protection by 4.17 folds (1/0.24) in CAD ($P = 0.004$, $OR = 0.24$, 0.09–0.63 at 95% CI), 4.35 folds (1/0.23) in T2D ($P = 0.002$, $OR = 0.23$, 0.09–0.59 at 95% CI) and 5 folds (1/0.20) in T2D + CAD ($P = 0.016$, $OR = 0.20$, 0.05–0.74 at 95% CI). However, comparison of codominant model among the patient groups did not reveal any significant association. The result implies a heterozygote protection in comparison to controls of all the three patient groups, i.e. diabetic patients with or without CAD and patients affected only with CAD. No statistically significant difference in the distribution of genotype and allele frequencies was observed when CAD cases were compared against T2D cases with or without CAD. Similarly comparison between T2D cases with and without CAD did not reveal a statistically significant difference. The results of dominant model (CG + GG vs. CC) could not be computed in comparison of CAD cases with any of the groups as in CAD patients no individual with CC genotype was observed. Analysis in other groups (T2D, T2D + CAD and controls) did not reveal any statistically significant association under the dominant model.

3.2. Principle component analysis

Pearson's correlation among normally distributed variables in three case groups (CAD, T2D and T2D + CAD) is presented in Tables 3 and 4. In Table 3, the upper triangle correlations correspond to the T2D + CAD group; lower triangle refers to the CAD group, Table 4 shows results of the T2D group. For most variables among all three groups, correlations are of modest to moderate magnitude (0.135–0.734). Strongest positive correlation has been observed between SBP and DBP in the T2D group (0.734) followed by CAD (0.601) and T2D + CAD (0.517). Whereas CHO and TG were found to be strongly correlated in the T2D + CAD (0.573) group followed by T2D (0.534) and CAD (0.529).

The principal component factor analysis (PCFA) extracted 5 factors which explained nearly 75–81% of total variance of the 11 quantitative traits among CAD, T2D and T2D + CAD groups (Table 5). In CAD and T2D, factor 1 has high loadings for BMI and WC whereas T2D + CAD has high loading for CHO, TG and VLDL suggesting obesity as a strong indicator for CAD and T2D and dyslipidemia for T2D + CAD. In CAD and T2D, factors 2 and 3 are mainly loaded with traits representing dyslipidemia (TG, VLDL, CHO, LDL, HDL) whereas in T2D + CAD group factors 2 and 3 indicate obesity (WC and WHR) and hypertension

Table 3
Correlation matrix (bivariate) of various risk factors among CAD and T2D + CAD cases.

	BMI	WHR	SBP	DBP	CHO	Tri	HDL	RBS
BMI	1	0.015	0.016	0.050	−0.002	0.002	−0.039	0.023
WHR	0.341**	1	−0.107	0.206*	−0.148	0.014	0.003	0.072
SBP	0.121	0.143*	1	0.517**	0.075	−0.034	0.115	0.009
DBP	0.144*	0.180*	0.601**	1	0.072	−0.056	0.149	−0.009
CHO	0.007	0.046	0.175*	0.113	1	0.573**	0.044	−0.076
Tri	0.176*	0.138	0.020	0.064	0.529**	1	−0.178	0.083
HDL	0.104	0.055	0.245**	0.238**	0.128	−0.081	1	−0.053
RBS	0.037	0.086	−0.085	0.020	0.109	0.155	−0.112	1

BMI = body mass index; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; CHO = total cholesterol; TG = triglyceride; HDL = high density lipoprotein; RBS = random blood sugar.

Upper triangle corresponds to T2D + CAD, lower triangle to CAD.

** Correlation is significant at 0.01 level (2-tailed).

* Correlation is significant at 0.05 level (2-tailed).

Table 4

Correlation matrix of various risk factors among T2D cases.

	BMI	WHR	SBP	DBP	CHO	Tri	HDL	RBS
BMI	1							
WHR	0.250**	1						
SBP	0.135*	0.087	1					
DBP	0.054	0.038	0.734**	1				
CHO	0.088	0.051	0.076	0.143*	1			
Tri	−0.004	0.073	0.029	0.096	0.534**	1		
HDL	−0.078	−0.151*	−0.085	−0.072	0.229**	−0.135*	1	
RBS	−0.025	0.070	−0.063	−0.097	0.054	0.132	−0.065	1

BMI = body mass index; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; CHO = total cholesterol; TG = triglyceride; HDL = high density lipoprotein; RBS = random blood sugar.

** Correlation is significant at 0.01 level (2-tailed).

* Correlation is significant at 0.05 level (2-tailed).

(SBP and DBP) as risk factors. Factor 4 contains high loadings of SBP and DBP in the case of CAD and T2D and mainly with LDL in T2D + CAD. Factor 5 is predominantly loaded with WHR and RBS in CAD, WHR in T2D and of RBS in the case of T2D + CAD. A communality of 0.75 seems high and below 0.5 is considered as low communality. The common greater communality estimates (>0.9) have been found on WC, TG, VLDL among all the groups. Furthermore, CHO in T2D group and LDL in CAD and T2D + CAD have communalities above 0.9. Therefore, parameters related to obesity profiles and dyslipidemia may be considered as good predictors of these complications.

4. Discussion

Chronic low grade inflammation is known to play an important role in pathogenesis of T2D and progression to atherosclerosis. Therefore genes encoding for proteins involved in inflammatory pathways may be formulated as important candidates for T2D and CAD and genetic variations in these genes may define susceptibility to the disease (Ridker et al., 2002). CRP is a potential biomarker for prediction of future risk of cardiovascular disease (CVD) both in diabetic and non-diabetic individuals, because even a small rise in plasma CRP levels leads to cardiovascular events (Ridker et al., 2002; Anand et al., 2004; Toma et al., 2012). Genetic variations influence the inter-individual variations in CRP levels and thus the disease susceptibility. In the present investigation of the association of CRP + 1059 G>C polymorphism with the development of CAD, T2D and progression to CAD in T2D patients, we have observed that the frequency of G-allele is high in the North Indian population of Punjab (94.7%). Similarly high frequency for the G-allele has been reported in studies conducted on other world populations (90.0%–96.3%) (Pasalic et al., 2009; Balistreri et al., 2006; Tanja et al., 2008; Yazici et al., 2010). In the present study homozygosity of the risk allele i.e. the G-allele of the CRP + 1059 G/C polymorphism significantly increased the risk of T2D as well as CAD (with and without diabetes) by nearly 3.23–4.19 folds after correction for the confounding factors (OR = 3.23, 1.36–7.60 at 95% CI for T2D; OR = 3.64, 1.14–11.66 at 95% CI for T2D + CAD; OR = 4.19, 1.62–10.80 at 95% CI for CAD, respectively). Our results are in accordance with the study conducted on Croatian population that suggested that GG genotype provided risk (OR = 1.19, 1.06–3.72 at 95% CI) towards the susceptibility of CAD (Pasalic et al., 2009). There are very few studies pertaining to the association of CRP + 1059 G>C polymorphism with T2D and CAD in Indian and other world populations. Moreover, the results of the available association studies have yielded conflicting results. Balistreri and coworkers studied a homogeneous Caucasoid population from Sicily and suggested the protective role of GG genotype against AMI (OR = 0.27, 0.12–0.64 at 95% CI) while C + (CC + CG) associated with increased risk of AMI (OR = 3.59, 1.64–7.85 at 95% CI) (Balistreri et al., 2006). Data from some other studies failed to report any association between the studied polymorphism with either CAD or T2D (Tanja et al., 2008; Yazici et al., 2010; Dai et al., 2007; Ghattas et al., 2012). In a study conducted on Turkish T2D patients, CRP levels were reported to be higher in the patient group as compared to controls but no association of + 1059 G>C polymorphism was observed with either CRP levels or T2D in the study (Yazici et al., 2010). In Chinese population, association of higher CRP levels was observed with individuals carrying GG and GC genotypes. The CRP levels were also observed to be higher in cases with CAD or MI as

compared to chronic stable angina and controls. However, in their study also, no statistically significant association of +1059 G>C polymorphism was observed with CAD (Dai et al., 2007). Association study conducted on levels of CRP with +1059 G>C polymorphism in the Egyptian population reported higher CRP levels in carriers of GC and CC genotypes in both controls and AMI cases but no association of genotypes was observed with the risk of AMI (Ghattas et al., 2012). Therefore, most of the studies do suggest association of alleles with higher CRP levels but not with the disease.

Heterozygous carriers of C allele (GC) in the present studied population have shown protection towards the disease manifestation. The GC genotype is highest in controls (9.8%) as compared to cases (2.3%–3.4%) suggesting that some kind of selection pressure is operating on the GC genotype. This could probably explain the deviation observed in T2D and T2D + CAD cases from HWE. Considering the thrifty genotype hypothesis, the ancestral version of the alleles proves to be deleterious in the present day environment while the rarer alleles which may have protective effects against the disease have evolved lately (Sharma, 1998). Fernandez-Real and Ricart have implicated the role of genes encoding for cytokine synthesis as thrifty genes (Fernandez-Real and Ricart, 1999). The hypothesis suggested that higher secretion of cytokines and increased acute phase response were an evolutionary adaptation to phases of acute infections and trauma. In the ancestral period, outbreaks due to infections were higher. Simultaneously due to exposure to famines, metabolic pathways favored insulin resistance to survive in low food situations. It has been proposed that insulin resistance and cytokine responder genotypes were favorable adaptations to low fat, high fiber and high physical activity environment. The genomes of the present day human are still genetically adapted to ancestral conditions which are designed to fight against infection with minimal food intakes and high physical activity. However, environmental transition is more rapid, and evolution being a slow process, our genotypes have not modified according to the present day environments of lower infections, availability of surplus food and low physical activity. In the absence of the favorable conditions and with advancement of age, insulin resistance ensues which further activates inflammatory cascade that eventually results in atherosclerosis. Thus, in the presence of insulin resistance genotypes and western lifestyle, a high cytokine responder genotype would be more prone to develop T2D and atherosclerosis. Although +1059 G>C polymorphism is a silent polymorphism, yet the ancestral allele of the polymorphism (G-allele) has been associated with higher CRP levels in various studies as discussed above. Higher CRP levels are suggestive of inflammatory response which may get accentuated in the background of obesity and insulin resistance condition in T2D. The higher frequency of the G-allele in most of the populations and the protective effect of GC genotype in the present study could be viewed in the background of its role as a thrifty genotype or the polymorphism may be in LD with a more functionally relevant variant. The role of +1059 G>C polymorphism in modulating the levels of CRP have also been documented in a haplotype analysis using Tag SNPs in depressive patients (Carlson et al., 2005). The haplotype H1 having C allele at +1059 G>C locus was associated with lower CRP levels relative to H2 that had G allele at this locus. The study suggested a direct role of +1059 G allele with higher CRP levels probably through LD with a more functionally relevant SNP in the distal region of the gene. Thus, the CRP +1059 G>C polymorphism seem to have functional effects on protein production (Eklund et al., 2005). It has also been proposed that silent SNPs can lead to protein product with the same amino acid sequence, but with different structural and functional properties which might play an important role in defining the protein levels (Komar, 2007). The reason for discrepancies in association studies across populations may be explained by diverse ethnic background of different populations as different ethnic groups have different susceptibility towards the disease manifestation (Radha and Mohan, 2007).

In the limitations of the present study, firstly, the association of polymorphism with the protein levels needs to be established. Higher odds ratio observed in the present study could be explained in the background on the etiology of complex diseases where genetic heterogeneity plays a significant role. The OR observed due to association of individual SNP may be higher but when analyzed in the background of polymorphisms in other interacting genes and variants in LD within the gene, the OR values are pertinent to change. Secondly in case control studies larger the sample size lesser will be the range between lower and upper CI values. Hence, further studies relating the levels of CRP with studied polymorphism and other functionally relevant polymorphisms which may be in LD with the studied SNP are required on larger sample size to validate the findings of the present study with more power. However, results of the present study do indicate towards the role of CRP gene polymorphisms in the risk of T2D and CAD in the population of Punjab.

Table 5

Comparison of factor loading (rotated component), variances and communalities among CAD, T2D + CAD and T2D cases.

Variables	Factor 1			Factor 2			Factor 3		
	CAD	T2D + CAD	T2D	CAD	T2D + CAD	T2D	CAD	T2D + CAD	T2D
BMI	0.908	0.011	0.880	0.121	−0.029	0.020	−0.059	0.054	0.007
WC	0.900	0.033	0.879	0.070	0.983	0.016	−0.070	0.010	0.073
WHR	0.349	−0.052	0.261	0.032	0.656	−0.055	−0.120	0.312	−0.036
SBP	0.102	0.050	0.108	−0.004	−0.092	0.021	0.180	0.772	−0.024
DBP	0.139	−0.064	0.056	0.020	0.102	0.087	0.041	0.843	0.172
CHO	−0.037	0.745	0.148	0.485	−0.217	0.514	0.783	0.192	0.820
TGs	0.115	0.973	0.018	0.981	0.094	0.963	0.091	−0.060	0.115
HDL	−0.038	−0.075	−0.159	0.062	−0.336	−0.373	−0.282	0.290	0.611
LDL	−0.083	−0.154	0.222	−0.014	−0.156	0.271	0.984	0.118	0.794
VLDL	0.115	0.973	0.018	0.981	0.094	0.963	0.091	−0.060	0.115
RBS	−0.071	0.038	−0.149	0.072	−0.066	0.153	0.096	−0.056	0.013
Eigenvalues	3.277	2.571	3.444	2.398	2.205	2.247	1.571	1.598	1.634
% of variance	27.308	21.422	28.703	19.987	18.371	18.728	13.093	13.315	13.620
Cumulative %	27.308	21.422	28.703	47.294	39.793	47.431	60.387	53.108	61.051

BMI = body mass index; WC = waist circumference; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; CHO = total cholesterol; TG = triglyceride; HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; RBS = random blood sugar. Factor loadings, values greater than or equal to 0.4 are in bold.

In complex diseases like T2D and CAD, there are several modifiable and non-modifiable risk factors that modulate the effect of the disease. In addition to the effect of genotypes, other major risk factors for the population also need to be defined. The observation from Pearson's correlation in the present study showed that many risk factors for CAD and T2D are strongly inter-correlated. In the present study the most important multiple risk factors were identified through PCFA, with varimax rotation, to reduce 11 inter-correlated variables into groups of 5 independent factors. This data reduction method identifies 5 factors that explain 81% of variance among CAD, T2D + CAD and T2D groups (BMI, WC, TG and VLDL) that are strongly loaded (>0.7) and are strong independent predictors of these complications. The present findings were similar to other factor analysis studies related to CAD and T2D risk factors with high loadings of many other components (Meigs, 2000; Bellis et al., 2005; Badaruddoza et al., 2010). Present findings revealed different risk components among three different disease groups. BMI, WC, and WHR as core predictors for CAD and T2D, whereas CHO, TG and VLDL for T2D + CAD which is in concordance with other studies (Shmulewitz et al., 2001; Hanson et al., 2002; Kaur et al., 2012). The pattern of clustering of variables was interesting in the present study; factors representing obesity and dyslipidemia appear to load more than blood pressure, suggesting them to play a more important role than blood pressure in the occurrence of CAD, T2D and T2D + CAD.

The present study concludes that GG genotype of CRP +1059 G>C polymorphism increases the susceptibility towards CAD, T2D and T2D + CAD, while the GC genotype appears to provide protection against the disease. Clustering of obesity and dyslipidemia promotes the risk towards the disease in the population of Punjab. However, further association of serum levels of CRP with CRP +1059 G>C polymorphism and other functional polymorphisms should be analyzed for a better understanding of the role of polymorphisms in the CRP gene in modulating the disease pathophysiology.

Author disclosure statement

It is declared that there is no conflict of interest of authors, the research work is entirely for academic purpose and no competing financial interests exist.

Acknowledgments

The financial assistance to Kaur R by UGC is acknowledged. The financial assistance under the scheme of "Centre with Potential for Excellence in Particular Area" through grant no. F.8-2/2008 (NS/PE) (UGC, India) to Bhanwer AJS at Guru Nanak Dev University, Amritsar is humbly acknowledged.

Factor 4			Factor 5			Communalities		
CAD	T2D + CAD	T2D	CAD	T2D + CAD	T2D	CAD	T2D + CAD	T2D
0.048	0.104	0.026	0.084	0.411	−0.007	0.852	0.184	0.776
0.124	−0.052	0.086	0.338	−0.056	0.400	0.949	0.973	0.947
0.108	−0.115	−0.046	0.712	0.381	0.865	0.656	0.689	0.822
0.770	0.050	0.915	0.043	−0.031	−0.027	0.638	0.611	0.851
0.787	0.009	0.898	0.171	0.058	−0.019	0.670	0.729	0.846
0.218	0.461	0.139	−0.019	−0.194	0.051	0.898	0.889	0.981
0.021	−0.142	0.048	0.067	0.089	0.069	0.988	0.988	0.948
0.658	−0.559	−0.094	−0.153	−0.472	−0.151	0.542	0.738	0.570
−0.110	0.942	0.177	0.022	−0.054	0.072	0.989	0.952	0.790
0.021	−0.142	0.048	0.067	0.089	0.069	0.988	0.988	0.948
−0.018	−0.175	−0.006	0.728	0.663	0.552	0.550	0.479	0.351
1.281	1.446	1.316	1.066	1.168	1.051			
10.673	12.049	10.963	8.884	9.730	8.762			
71.060	65.157	72.014	79.944	74.887	80.775			

References

- American Diabetic Association, 2011. Diagnosis and classification of diabetes mellitus. *J. Diabetes Care* 34, S11–S61.
- Anand, S.S., Razak, F., Yi, Q., Davis, B., Jacobs, R., Vuksan, V., et al., 2004. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. *Arterioscler. Thromb. Vasc. Biol.* 24, 1509–1515.
- Badaruddoza, Barna, B., Bhanwer, A.J.S., 2010. Comparison of factor loadings for anthropometric and physiometric measures among type 2 diabetic males, pre and post menopausal females in North Indian Punjabi population. *Nat. Sci.* 2, 741–747.
- Balistreri, C.R., Vasto, S., Listi, F., Grimaldi, M.P., Lio, D., Colonna-Romano, G., et al., 2006. Association between +1059G/C CRP polymorphism and acute myocardial infarction in a cohort of patients from Sicily. *Ann. N. Y. Acad. Sci.* 1067, 276–281.
- Bellis, C., Hughes, R.M., Begley, K.N., Quinlan, S., Lea, R.A., Heath, S.C., et al., 2005. Phenotypical characterisation of the isolated Norfolk Island population focusing on epidemiological indicators of cardiovascular disease. *Hum. Hered.* 60, 211–219.
- Carlson, C.S., Aldred, S.F., Lee, P.K., Tracy, R.P., Schwartz, S.M., Rieder, M., et al., 2005. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am. J. Hum. Genet.* 77, 64–77.
- Dai, D., Chiang, F., Lin, J., Huang, L., Chen, C., Chang, C., et al., 2007. Human C-reactive protein (CRP) gene 1059 G > C polymorphism is associated with plasma CRP concentration in patients receiving coronary angiography. *J. Formos. Med. Assoc.* 106, 347–354.
- de Ferranti, S., Rifai, N., 2002. C-reactive protein and cardiovascular disease: a review of risk prediction and interventions. *Clin. Chim. Acta* 317, 1–15.
- Eklund, C., Lehtimäki, T., Hurme, M., 2005. Epistatic effect of C-reactive protein (CRP) single nucleotide polymorphism (SNP) + 1059 and interleukin-1B SNP + 3954 on CRP concentration in healthy male blood donors. *Int. J. Immunogenet.* 32, 229–232.
- Fernandez-Real, J.M., Ricart, W., 1999. Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. *Diabetologia* 42, 1367–1374.
- Gelaye, B., Revilla, L., Lopez, T., Suarez, L., Sanchez, S.E., Hevner, K., et al., 2010. Association between insulin resistance and C-reactive protein among Peruvian adults. *Diabetol. Metab. Syndr.* 2, 30.
- Ghaffar, M.H., Abo-Elmatty, D.M., El-Eraki, A.Z., 2012. C-reactive protein 1059G/C gene polymorphism, C-reactive protein levels and acute myocardial infarction. *J. Cardiovasc. Med.* 13, 716–719.
- Hanson, R.L., Imperatore, G., Bennett, P.H., Knowler, W.C., 2002. Components of the metabolic syndrome and incidence of type 2 diabetes. *Diabetes* 51, 3120–3127.
- Kaur, M., Doza, B., Kumar, R., 2012. Principal component factor analysis of cardiovascular risk factors among Punjabi female population. *World Appl. Sci. J.* 16, 616–621.
- Komar, A.A., 2007. Silent SNPs: impact on gene function and phenotype. *Pharmacogenomics* 8, 1075–1080.
- Kushner, I., 1982. The phenomena of acute phase response. *Ann. N. Y. Acad. Sci.* 389, 39–48.
- Lai, M.M., Li, C.L., Kardia, S.L., Liu, C.S., Lin, W.Y., Lee, Y.D., 2010. Sex difference in the association of metabolic syndrome with high sensitivity C-reactive protein in a Taiwanese population. *BMC Public Health* 10, 429.
- Lange, L.A., Carlson, C.S., Hindorf, L.A., Lange, E.M., Walston, J., Durda, J.P., et al., 2006. Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. *JAMA* 296, 2703–2711.
- Li, J.J., Fang, C.H., 2004. C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. *Med. Hypotheses* 62, 499–506.
- Libby, P., Ridker, P.M., Maseri, A., 2002. Inflammation and atherosclerosis. *Circulation* 105, 1135–1143.
- Loza, M.J., McCall, C.E., Li, L., Isaacs, W.B., Xu, J., Chang, B., 2007. Assembly of inflammation-related genes for pathway-focused genetic analysis. *PLoS ONE* 10, e1035.
- MacGregor, A.J., Gallimore, J.R., Spector, T.D., Pepys, M.B., 2004. Genetic effects on baseline values of C-reactive protein and serum amyloid A protein: a comparison of monozygotic and dizygotic twins. *Clin. Chem.* 50, 130–134.

- Mazzone, T., Chait, A., Plutzky, J., 2008. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet* 371, 1800–1809.
- Meigs, J.B., 2000. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am. J. Epidemiol.* 152, 908–911.
- Miller, S., Dykes, D., Polesky, H., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acids Res.* 16, 1215.
- Mohan, V., Shanthirani, S., Deepa, R., Premalatha, G., Sastry, N.G., Saroja, R., 2001. Intra-urban differences in the prevalence of the metabolic syndrome in southern India—the Chennai Urban Population Study (CUPS no. 4). *J. Diabetes Med.* 18, 280–287.
- Momiyama, Y., Ohmori, R., Fayad, Z.A., Kihara, T., Tanaka, N., Kato, R., 2010. Associations between plasma C-reactive protein levels and the severities of coronary and aortic atherosclerosis. *J. Atheroscler. Thromb.* 17, 460–467.
- Pankow, J.S., Folsom, A.R., Cushman, M., Borecki, I.B., Hopkins, P.N., Eckfeldt, J.H., et al., 2001. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis* 154, 681–689.
- Pasalic, D., Marinkovic, N., Grskovic, B., Ferencak, G., Bernat, R., Stavljenic-Rukavina, A., 2009. C-reactive protein gene polymorphisms affect plasma CRP and homocysteine concentrations in subjects with and without angiographically confirmed coronary artery disease. *Mol. Biol. Rep.* 36, 775–780.
- Radha, V., Mohan, V., 2007. Genetic predisposition to type 2 diabetes among Asian Indians. *Indian J. Med. Res.* 125, 259–274.
- Retterstol, L., Eikvar, L., Berg, K., 2003. A twin study of C-reactive protein compared to other risk factors for coronary heart disease. *Atherosclerosis* 169, 279–282.
- Ridker, P.M., Rifai, N., Rose, L., Buring, J.E., Cook, N.R., 2002. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N. Engl. J. Med.* 347, 1557–1565.
- Rizzello, V., Liuzzo, G., Giannuario, G.D., Trabetti, E., Salvatore, B., Matteo, S., et al., 2007. 1059G/C polymorphism within the exon 2 of the C-reactive protein gene: relationship to C-reactive protein levels and prognosis in unstable angina. *Coron. Artery Dis.* 18, 533–538.
- Ross, R., 1999. Atherosclerosis: an inflammatory disease. *N. Engl. J. Med.* 340, 115–126.
- Shankar, A., Li, J., 2008. Positive association between high-sensitivity C-reactive protein level and diabetes mellitus among US non-Hispanic black adults. *Exp. Clin. Endocrinol. Diabetes* 116, 455–460.
- Sharma, A.M., 1998. The thrifty-genotype hypothesis and its implications for the study of complex genetic disorders in man. *J. Mol. Med.* 76, 568–571.
- Shmulewitz, D., Auerbach, S.B., Lehner, T., Blundell, M.L., Winick, J.D., Youngman, L.D., et al., 2001. Epidemiology and factor analysis of obesity, type II diabetes, hypertension, and dyslipidemia (syndrome X) on the island of Kosrae, Federated States of Micronesia. *Hum. Hered.* 51, 8–19.
- Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication based analysis for two stage genome wide association studies. *Nat. Genet.* 38, 209–213.
- Tanja, B.G., Winfried, M., Wilfried, R., Bernhard, O.B., Michael, M.H., 2008. C-reactive protein genotypes associated with circulating C-reactive protein but not with angiographic coronary artery disease: the LURIC study. *Eur. Heart J.* 30, 170–182.
- Toma, L., Sanda, G.M., Stancu, C.S., Rogoz, D., Sima, A.V., 2012. Combined effect of inflammatory C-reactive protein and hyperglycemia on endothelial cell dysfunction. *Annals of RSCB.* 1, 29–36.
- Van Leeuwen, M.A., Van Rijswijk, M.H., 1994. Acute phase proteins in monitoring of inflammatory disorders. *Baillieres Clin. Rheumatol.* 8, 531–552.
- Wee, C.C., Mukamal, K.J., Huang, A., Davis, R.B., McCarthy, E.P., Mittleman, M.A., 2008. Obesity and C-reactive protein levels among white, black, and Hispanic US adults. *Obesity* 16, 875–880.
- Yazici, D., Dilek, G.Y., Meral, Y., Beste, O., Seda, S., Oguzhan, D., et al., 2010. C-reactive protein 1059 G/C gene polymorphism in type 2 diabetic patients. *Turk. J. Endocrinol. Metab.* 14, 85–88.