Association of –2518A>G Promoter Polymorphism in the Monocyte Chemoattractant Protein-1 (*MCP-1*) Gene with Type 2 Diabetes and Coronary Artery Disease

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Aims: Inflammatory markers play an important role in the development of diseases related to metabolic syndrome, such as type 2 diabetes (T2D) and coronary artery disease (CAD). The present study evaluates the association of –2518A>G polymorphism in the monocyte chemoattractant protein-1 (*MCP-1*) gene with T2D and CAD. **Results:** The frequency of the G allele is greater in CAD cases (35%) as compared to T2D (24.6%) and controls (31%), while the frequency of the A allele is higher in T2D cases (75.4%) as compared to CAD cases (65%) and controls (69%). The analysis has revealed that in comparison to T2D cases, the G allele increases the risk of CAD by 1.9-fold ($p=0.008$; odds ratio [OR]=1.9, 1.18–3.06 at 95% confidence interval [CI]) but in comparison to controls the G-allele provided protection against T2D ($p=0.011$; OR=0.55, 0.35–0.87 at 95% CI), both under the dominant model (AG+GG vs. AA). **Conclusion:** Results of the present study suggests that G-allele of *MCP-1* –2518A>G polymorphism is associated with reduced risk of T2D and increased risk of CAD in the population of Punjab. The results indicate that there is a difference in the association of risk alleles with phenotypes of metabolic syndrome. Body mass index and waist circumference are important risk factors for T2D in the population of Punjab.

Introduction

THE INFLAMMATION OF coronary arteries has emerged as a critical process in the manifestation of atherosclerosis leading to coronary artery disease (CAD). Inflammation has also been recognized as an important factor in the pathogenesis of insulin resistance and type 2 diabetes (T2D). It has now been established that T2D increases the risk of cardiovascular mortality and morbidity by nearly two to fourfold. CAD has been recognized as one of the serious macrovascular complications associated with T2D (Feskens and Kromhout, 1992). T2D not only increases the risk of CAD but also accelerates its clinical course of development. The metabolic abnormalities associated with T2D, such as chronic hyperglycemia, dyslipidemia, and insulin resistance render arteries susceptible to atherosclerosis. In conditions related to T2D, the function of multiple cell types, such as endothelium, smooth muscle cells, and platelets is altered; thereby accelerating the rate of progression of atherosclerosis (Beckman *et al.*, 2002).

There is increasing evidence that inflammatory processes and specific immune mechanisms contribute to the development of atherogenesis. Levels of inflammatory markers are higher among subjects with insulin resistance and diabetes (Jialal *et al.*, 2004). Chemokines play an important role in the migration of neutrophils, lymphocytes, antigen-presenting

cells, including dendritic cells and cells of monocyte/macrophage lineage. These responses are activated as a result of an inflammatory insult in different phases of both innate and adaptive inflammatory responses (Rot and von, 2004; Deshmmane *et al.*, 2009). The initial phase of atherosclerosis is characterized by migration of monocytes into the vessel wall, which is mediated by chemokine ligands and their corresponding receptors (Eriksson, 2004). Monocyte chemoattractant protein-1 (*MCP-1*) is a member of the CC chemokine family that plays an important role in recruiting monocytes, memory T cells, and basophils; and in the development of atherogenesis (Yoshimura *et al.*, 1989; Charo and Taubman, 2004). The increased level of serum *MCP-1* in humans correlates with markers of the metabolic syndrome, including obesity, insulin resistance, T2D, hypertension, and increased serum triacylglycerol concentrations (Simeoni *et al.*, 2004).

A single nucleotide polymorphism in the promoter region of *MCP-1* gene has been reported to affect *MCP-1* transcription. Consequently, –2518A>G promoter polymorphism might dictate the *MCP-1* protein levels (Rovin *et al.*, 1999). –2518A>G polymorphism has also been associated with T2D, insulin resistance, type 1 diabetes and cardiovascular diseases (Szalai *et al.*, 2001; Simeoni *et al.*, 2004; Yang *et al.*, 2004). However, data on the association of this polymorphism with T2D and CAD is not available on the north Indian Punjab

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population. Besides, the data reported in other world populations is inconsistent with studies reporting both positive (Simeoni *et al.*, 2004; Mahgoub *et al.*, 2011) and negative associations (Kouyama *et al.*, 2007; Zhang *et al.*, 2009). Therefore, to fill in the existing lacunae, the present study is designed to evaluate the association of –2518A>G polymorphism of the MCP-1 gene with T2D and CAD in the population of Punjab.

Materials and Methods

In the present case control study, a total of 535 individuals comprising T2D without symptoms of secondary complications (211), CAD patients without T2D (150) and healthy controls above the age of 40 years (174) were enrolled from various hospitals and localities of Amritsar, Punjab with informed and written consent. The study has been approved by the institutional ethical committee. Individuals were diagnosed for T2D according to criteria of the American Diabetes Association (2011). Clinically confirmed CAD cases diagnosed according to the criteria given for Indian Population (Mohan *et al.*, 2001) were enrolled for the study. Normal healthy individuals at the age of 40 years or above matched for sex and ethnicity were included as controls for the present study. Clinical and demographic details of all the individuals were recorded. Anthropometric measurements like height, weight, waist circumference (WC) and hip circumference were measured for calculating body mass index (BMI) and waist-hip ratio (WHR) from each individual.

Genotyping

Genomic DNA was extracted from peripheral blood by the inorganic method of DNA isolation (Miller *et al.*, 1988). Genotyping of –2518A>G polymorphism in the MCP-1 gene was done with the allele specific amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method using primer sequences described by Moon *et al.* (2007). Amplification conditions included initial denaturation (94°C for 5 min) following 30 cycles of annealing with denaturation at 94°C for 50 s, touchdown annealing for 10 cycles from 67°C to 59°C followed by final annealing at 58°C for 50 s, extension at 72°C for 50 s each cycle and final extension at 72°C for 5 min. MCP-1 –2518A>G genotyping was based on the presence/absence of 175 bp PCR amplicons, specific to the particular alleles in a standard 2% agarose gel stained with ethidium bromide.

Statistical analysis

Continuous data is represented as the mean±standard deviation (SD). Difference between continuous variables is evaluated by the two-tailed Student's *t*-test. The power of the study has been calculated to be more than 0.8. The distribution of genotype (scored by gene counting method) and allele frequencies in cases and controls was compared using the chi-square (χ^2) test (significant $p < 0.05$). Association analysis was performed on different genetic models, and association was determined in terms of odds ratio (OR) at 95% confidence interval (CI). Corrected odds are obtained by binary logistic regression analysis after correction for age, sex, BMI, WC, and WHR. The analysis is performed at the significance level of 0.05 (5%). Statistical analyses have been performed by using SPSS software, version 16 (SPSS, Inc., Chicago, IL).

Results

Table 1 represents the comparison of various demographic, anthropometric and clinical characteristics between different groups (T2D, CAD and controls) using the *t*-test in total pooled and sex specified groups. It has been observed that all parameters except for the triglycerides had statistically significant differences in comparison among different groups. Interestingly, BMI and WHR were observed to be higher in control females as compared to T2D and CAD cases. Mean values of WC and random plasma glucose (RPG) were higher in individuals with T2D. Cholesterol and triglyceride mean values were higher in T2D cases in comparison to CAD patients although significant difference was observed only for cholesterol. The mean values for HDL levels were nearly similar in both groups.

Table 2 documents the genotype and allele frequency distribution of –2518A>G polymorphism of the MCP1 gene in the CAD, T2D and control groups. The frequency of the A allele is more in T2D (75.4%) cases as compared to CAD cases (65%) and controls (69%), and the frequency of the G allele is more in CAD cases (35%) as compared to T2D (24.6%) and controls (31%). A marginally significant difference is observed in the distribution of allele frequencies between T2D cases and controls ($p = 0.048$). However, in comparison between CAD and T2D cases, both genotype ($p = 0.006$) and allele frequency distribution showed significant difference ($p = 0.002$). Further analysis of various genetic models in comparison between different groups revealed that under the dominant model (AG+GG vs. AA), G allele increases the risk of CAD ($p = 0.008$, OR=1.9, 1.18–3.06 at 95% CI) after adjusting for confounding factors as age, sex, BMI, WC, and WHR. The additive model also suggests that the G allele increases the risk towards CAD by nearly 1.7-fold ($p = 0.002$, OR=1.73, 1.22–2.43 at 95% CI) in comparison to T2D cases. Interestingly, comparison of T2D cases with the control group revealed that under both additive ($p = 0.034$; OR=0.69, 0.49–0.97 at 95% CI) and dominant models ($p = 0.011$; OR=0.55, 0.35–0.87 at 95% CI), the G allele provided protection against T2D, while the A allele provided risk to T2D contrary to that observed in CAD cases. No association was observed in any of the models when CAD cases were compared with controls, although the frequency of G allele and GG genotype was observed to be higher in CAD cases.

WC, WHR, and BMI of all the study groups were categorized on the basis of normal cut-off values recommended for the Asian Indians (Snehalatha *et al.*, 2003; Misra *et al.*, 2009). Asian Indians tend to develop diabetes and cardiovascular diseases at lower levels of BMI as compared to the European population, which is attributed to the differences in body fat distribution. Therefore, lower cut offs of BMI have been recommended for Asian populations. In the present study, BMI <23 is taken as nonobese and BMI ≥23–24.99 as overweight and >25 as obese. Asian Indians also have higher tendency towards central obesity; therefore, WC and WHR are considered better indicators of progression to T2D in these ethnic groups, (Mohan *et al.*, 2003; Gautier *et al.*, 2010; Kamath *et al.*, 2011; Liao *et al.*, 2011). WC cut offs used in the present study are >85 for males and >80 for females; and for WHR the cut offs are >0.89 for males and >0.81 for females (Snehalatha *et al.*, 2003). χ^2 analysis for WC, WHR and BMI on males and females was performed separately (Table 3). The results revealed that a higher percentage of individuals with T2D had WC above the normal cut-off, both in male (92.2%) and female

TABLE 1. DEMOGRAPHIC, CLINICAL, AND ANTHROPOMETRIC CHARACTERISTICS OF CAD, T2D, AND CONTROL GROUPS

Variables	CAD (mean $\pm$ SD) (150)	T2D (mean $\pm$ SD) (211)	Control (mean $\pm$ SD) (174)	CAD vs. control (p-value)	T2D vs. control (p-value)	CAD vs. T2D (p-value)
Age (years)						
T	59.92 $\pm$ 11.75	54.52 $\pm$ 11.16	49.90 $\pm$ 9.62	1.12×10^{-15a}	2.18×10^{-5a}	1.28×10^{-5a}
M	60.24 $\pm$ 12.79	55.36 $\pm$ 11.85	53.15 $\pm$ 11.52	0.003 ^a	0.291	0.011 ^a
F	59.63 $\pm$ 10.79	53.73 $\pm$ 10.46	48.73 $\pm$ 8.59	7.60×10^{-14a}	7.55×10^{-5a}	2.28×10^{-4a}
BMI (kg/m ²)						
T	24.35 $\pm$ 7.10	27.82 $\pm$ 5.19	29.68 $\pm$ 7.75	1.68×10^{-9a}	0.006 ^a	1.66×10^{-7a}
M	23.04 $\pm$ 7.38	27.70 $\pm$ 4.79	25.93 $\pm$ 6.96	0.045 ^a	0.086	1.09×10^{-6a}
F	24.29 $\pm$ 8.59	27.94 $\pm$ 5.57	30.71 $\pm$ 8.10	3.09×10^{-7a}	0.003 ^a	0.001 ^b
WC (cm)						
T	91.19 $\pm$ 14.26	113.74 $\pm$ 65.03	96.70 $\pm$ 14.61	0.001 ^a	0.001 ^b	4.20×10^{-5a}
M	91.91 $\pm$ 13.75	107.99 $\pm$ 44.65	96.38 $\pm$ 15.59	0.113	0.095	0.004 ^a
F	90.56 $\pm$ 14.74	119.23 $\pm$ 79.60	96.81 $\pm$ 14.30	0.003 ^a	0.003 ^a	0.002 ^b
WHR						
T	0.93 $\pm$ 0.09	0.96 $\pm$ 0.14	0.93 $\pm$.11	0.615	0.013 ^a	0.031 ^a
M	0.92 $\pm$ 0.21	1.01 $\pm$ 0.11	0.94 $\pm$ 0.17	0.603	0.004 ^a	2.73×10^{-4a}
F	0.92 $\pm$ 0.08	0.92 $\pm$ 0.16	0.96 $\pm$ 0.08	0.382	0.698	0.830
SBP (mm/Hg)						
T	134 $\pm$ 23.19	129.68 $\pm$ 17.96	123.31 $\pm$ 15.29	5.15×10^{-6a}	0.001 ^a	0.052 ^a
M	133.94 $\pm$ 22.99	131.20 $\pm$ 18.96	124.76 $\pm$ 15.17	0.032 ^a	0.065	0.405
F	134.05 $\pm$ 23.52	128.24 $\pm$ 16.93	122.83 $\pm$ 15.36	1.16×10^{-4a}	0.014 ^a	0.055
DBP (mm/Hg)						
T	87.12 $\pm$ 15.54	84.40 $\pm$ 11.33	81.46 $\pm$ 10.93	3.83×10^{-4a}	0.015 ^a	0.061
M	89.09 $\pm$ 13.80	85.88 $\pm$ 11.11	83.27 $\pm$ 11.02	0.030 ^a	0.222	0.101
F	85.38 $\pm$ 16.82	83.00 $\pm$ 11.43	80.86 $\pm$ 10.88	0.027 ^a	0.157	0.259
RPG						
T	129.13 $\pm$ 32.71	199.54 $\pm$ 82.22	117.54 $\pm$ 31.06	0.012 ^a	7.73×10^{-17a}	9.94×10^{-16a}
M	129.96 $\pm$ 35.39	200.74 $\pm$ 75.47	119.25 $\pm$ 33.89	0.175	4.52×10^{-8a}	3.54×10^{-9a}
F	128.38 $\pm$ 30.39	198.34 $\pm$ 88.92	116.55 $\pm$ 29.56	0.038 ^a	9.73×10^{-10a}	5.39×10^{-8a}
Cholesterol						
T	130.61 $\pm$ 40.39	158.10 $\pm$ 51.12	167.60 $\pm$ 47.53	2.24×10^{-11a}	0.079	2.58×10^{-7a}
M	126.38 $\pm$ 36.69	163.00 $\pm$ 52.97	156.27 $\pm$ 40.84	3.32×10^{-4a}	0.495	3.06×10^{-6a}
F	134.42 $\pm$ 43.37	153.43 $\pm$ 49.09	171.37 $\pm$ 49.12	7.10×10^{-7a}	0.008 ^a	0.009 ^a
Triglyceride						
T	166.80 $\pm$ 104.52	269.27 $\pm$ 164.89	200.40 $\pm$ 106.96	0.009 ^a	0.575	0.008 ^a
M	156.41 $\pm$ 92.43	211.72 $\pm$ 140.71	209.44 $\pm$ 127.85	0.020 ^a	0.933	0.006 ^a
F	176.16 $\pm$ 114.19	206.93 $\pm$ 185.68	197.51 $\pm$ 99.70	0.189	0.645	0.215
HDL						
T	40.63 $\pm$ 19.59	40.48 $\pm$ 13.65	38.42 $\pm$ 10.96	0.245	0.135	0.935
M	41.49 $\pm$ 24.88	40.62 $\pm$ 14.87	38.75 $\pm$ 14.96	0.554	0.524	0.779
F	39.86 $\pm$ 13.28	40.35 $\pm$ 12.45	38.31 $\pm$ 9.38	0.365	0.178	0.801

^a $p < 0.05$ is significant.

T2D, type 2 diabetes; CAD, coronary artery disease; WC, waist circumference; BMI, body mass index; WHR, waist-hip ratio; SD, standard deviation; RPG, random plasma glucose.

(95.4%) groups. Significant association was observed in comparison of CAD and control group in females ($p = 0.007$), while comparison of CAD with T2D cases showed a highly significant association in both males ($p = 7.28 \times 10^{-8}$) and females ($p = 1.82 \times 10^{-5}$). WHR was observed to be higher in females of all the groups and males with T2D. The percentage of males with higher WC was 79.4% in CAD cases in comparison to 96.1% in T2D and the difference was statistically significant ($p = 5 \times 10^{-4}$). In females, however, no significant association was observed in comparison of any of the groups. On the basis of BMI, a higher percentage of males were found to be overweight (23.0–24.99) (19.4%) and obese (≥ 25) (66%) in T2D cases as compared to CAD (13.2%, 39.7%, respectively)

and controls (10.3%, 64.1%, respectively). In case of females, individuals with BMI in the range of overweight category (23.0–24.99) were greater in T2D cases (10.2%) followed by CAD cases (9.0%), whereas in controls a higher percentage of females (80.3%) was in the obese category (≥ 25). An overall higher percentage of T2D cases and controls were observed to have higher obesity profiles.

Discussion

There is considerable amount of evidence suggesting the role of inflammation in the pathophysiology of both atherosclerosis and T2D (Freeman *et al.*, 2002; Libby, 2006).

TABLE 2. GENOTYPE AND ALLELE FREQUENCY DISTRIBUTION WITH GENETIC MODEL ANALYSIS FOR MCP-1 –2518A>G POLYMORPHISM IN CASES (CAD, T2D) AND CONTROLS

MCP-1 –2518A>G	CAD (%) n=150	T2D (%) n=211	Control (%) n=174	CAD vs. Control	T2D vs. Control	CAD vs. T2D
Genotypes						
AA	40.7	55.0	43.7	$\chi^2=2.661$	$\chi^2=4.898$	$\chi^2=10.094$
AG	48.7	40.8	50.6	$p=0.264$	$p=0.086$	$p=0.006^a$
GG	10.7	4.3	5.7			
Alleles						
A	65.0	75.4	69.0	$\chi^2=1.148$	$\chi^2=3.903$	$\chi^2=9.142$
G	35.0	24.6	31.0	$p=0.284$	$p=0.048^a$	$p=0.002^a$
AG+GG	59.3	45.0	56.3	$\chi^2=0.299$	$\chi^2=4.87$	$\chi^2=7.18$
Dominant model (AG+GG vs. AA)				$p=0.58$	$p=0.011^b$	$p=0.008^b$
AA+AG	89.3	95.7	94.3	OR=1.13 (0.73–0.76)	OR=0.55 (0.35–0.87)	OR=1.9 (1.18–3.06)
Recessive model (GG vs. AA+AG)				$\chi^2=2.64$	$\chi^2=0.45$	$\chi^2=5.57$
Additive model (GG vs. AA+AG)				$p=0.10$	$p=0.50$	$p=0.17$
OR (GG vs. AG)				OR=1.96 (0.86–4.46)	OR=0.73 (0.29–1.84)	OR=1.94 (0.76–4.96)
=OR (AG vs. AA)				$\chi^2=1.31$	$\chi^2=4.50$	$\chi^2=9.84$
Codominant model AG vs. AA+GG				$p=0.25$	$p=0.034^a$	$p=0.002^a$
				OR=1.23 (0.86–1.75)	OR=0.69 (0.49–0.97)	OR=1.73 (1.22–2.43)
				$\chi^2=0.12$	$\chi^2=3.71$	$\chi^2=2.23$
				$p=0.73$	$p=0.05$	$p=0.136$
				OR=0.93 (0.60–1.43)	OR=0.67 (0.45–1.01)	OR=1.38 (0.90–2.10)

^a $p<0.05$ is significant, ^b p Corrected for age, sex, BMI, WC, WHR.
MCP-1, monocyte chemoattractant protein-1; OR, odds ratio.

Proinflammatory marker MCP-1 is a potent chemoattractant of monocytes that participates in various aspects of insulin resistance. Polymorphisms in the promoter region of MCP-1 have been considered important in dictating the levels of transcription and hence, influence disease progression. The

present study explored the effect of –2518A>G polymorphism in the MCP-1 gene on the development of T2D and CAD. In the present study, the G allele shows a protective association with T2D, the frequency of the G allele is lesser in T2D patients as compared to CAD cases (without T2D) and

TABLE 3. COMPARISON OF OBESITY PROFILES BASED ON CUT-OFF VALUES FOR BMI, WC, AND WHR IN CASES (CAD AND T2D) AND CONTROLS

Obesity profiles	CAD (150)	T2D (211)	Control (174)	CAD vs. control	T2D vs. control	CAD vs. T2D
Waist circumference (males)						
≤85	31.9%	7.8%	18.2%	$p=0.108$	$p=0.063$	$p=7.28 \times 10^{-8a}$
>85	68.1%	92.2%	81.8%			OR=22.23 (5.02–98.57)
Waist circumference (females)						
≤80	26.6%	4.6%	11.7%	$p=0.007^a$	$p=0.055$	$p=1.82 \times 10^{-5a}$
>80	73.4%	95.4%	88.3%			OR=7.46 (2.67–20.83)
WHR (males)						
≤0.89	20.6%	3.9%	10.3%	$p=0.169$	$p=0.142$	$p=5 \times 10^{-4a}$
>0.89	79.4%	96.1%	89.7%			OR=6.42 (2.01–20.46)
WHR (females)						
≤0.81	8.9%	8.3%	8.9%	$p=0.315$	$p=0.087$	$p=0.899$
>0.81	91.9%	91.7%	91.9%			
BMI (males)						
<23	47.1	14.6	25.6	1	1	1
23.0–24.99	13.2	19.4	10.3	$p=0.62$	$p=0.071$	$p=0.002^a$
				OR=1.4 (0.36–5.63)	OR=0.3 (0.08–1.14)	OR=4.74 (1.75–12.86)
≥25	39.7	66.0	64.1	$p=0.02^a$	$p=0.20$	$p=6.38 \times 10^{-6a}$
				OR=2.96 (1.21–7.25)	OR=0.55 (0.22–1.39)	OR=5.37 (2.52–11.47)
BMI (females)						
<23	48.7	21.3	12.8	1	1	1
23.0–24.99	9.0	10.2	6.8	$p=0.07$	$p=0.85$	$p=0.08$
				OR=2.89 (0.89–9.39)	OR=1.12 (0.36–3.42)	OR=2.59 (0.88–7.64)
≥25	42.3	68.5	80.3	$p=1.07 \times 10^{-8a}$	$p=0.07$	$p=7.2 \times 10^{-5a}$
				OR=7.22 (3.52–14.76)	OR=1.95 (0.95–3.99)	OR=3.71 (1.91–7.17)

^a $p<0.05$ is significant.

controls. However, the G-allele was observed to increase the risk of CAD by nearly 1.9-fold in comparison to T2D cases. The results suggest that there is a difference in the association of risk alleles with phenotypes of the metabolic syndrome. The results of the present study are in concordance with other studies conducted on the diabetic patients. In a study on a Caucasian population, the presence of the G allele was associated with a decreased prevalence of T2D, decreased prevalence of insulin resistance and decreased plasma MCP-1 levels. The decrease in G allele frequency was more pronounced among insulin-treated patients than among non-insulin-treated diabetic patients, suggesting that the role of the gene polymorphism may manifest at later stages of the disease (Simeoni *et al.*, 2004). In a study conducted on Korean T2D patients with progressive kidney failure it was observed that A-allele carriers showed a significant relation with disease progression (Moon *et al.*, 2007). On the contrary, consistent with the literature, a reverse trend was observed for the association of G-allele with CAD (without diabetes) in the present study. The first study associating MCP-1 –2518 A>G polymorphism with CAD also suggested higher frequency of G allele in CAD patients exclusively due to a twofold increase of GG homozygotes in Hungarian patients (Szalai *et al.*, 2001). A study conducted on an Egyptian population in acute myocardial infarction (MI) patients group showed a significantly higher frequency of the GA and GG genotypes as compared to the controls (Mahgoub *et al.*, 2011). Similar results were revealed by studies done on Slovakian men having episodes of MI (Bucova *et al.*, 2009). However, in a study done on MI patients from Tunisia population showed a significant but not independent association of MCP-1 –2518 G-allele with MI (Jemaa *et al.*, 2008). In the present study, although statistically significant association was not observed in comparison of CAD and controls, but a trend towards higher G allele in CAD cases, especially, the GG homozygotes has been observed. Probably, association analysis on a larger sample size is required to ascertain the risk allele in CAD cases with statistical significance.

Studies on the contribution of MCP-1 –2518 A>G polymorphism in T2D and CAD have also been inconsistent. Some of the studies have documented contradictory results failing to report the association of any of the MCP-1 –2518 alleles with T2D and MCP-1 levels in Caucasian and Japanese populations (Zietz *et al.*, 2005; Kouyama *et al.*, 2007). Studies done on Chinese Han and Czech populations have revealed no association of the polymorphism with CAD (Cermakova *et al.*, 2005; Zhang *et al.*, 2009). Complex diseases like T2D and CAD are substantially influenced by ethnicity, geographical area, inclusion criteria and confounding factors. Therefore, the differences observed in the association studies across different populations could be attributed to ethnic heterogeneity, which is a major obstacle in defining the genetic predisposition to complex diseases like T2D and CAD. In the present study even after applying correction for the confounding factors (age, sex, BMI, WC and WHR), G-allele provides protection to T2D, while risk to development of CAD suggesting that MCP-1 –2518 A>G alleles exhibit differential effects in pathophysiology of T2D and CAD.

Obesity has been proven to be an independent risk factor for CAD, (Hubert *et al.*, 1983; Manson *et al.*, 1990; Rosengren *et al.*, 1999) insulin resistance, hypertension, dyslipidemia, and T2D (Ginsberg, 2000; Kahn and Flier, 2000). BMI, WC and WHR are primary parameters to determine obesity. BMI pri-

marily reflects generalized obesity, while WC and WHR are related to central obesity, where body fat is primarily located in the abdomen (Sonmez *et al.*, 2003). Visceral fat is an important source of free fatty acids and inflammatory mediators, such as TNF- α , interleukins, and adipokines, which are directly delivered to the liver via the portal vein, affecting hepatic glucose and fat metabolism. Central obesity leads to free fatty acid influx to liver and contribute to the development of hepatic insulin resistance (Wajchenberg, 2000). The distribution pattern of lipid accumulation differs in women and men. Women more often develop peripheral adiposity, with gluteal fat accumulation, whereas men are more prone to central or android obesity (Williams, 2004). However, after menopause, concentrations of lipoproteins, as well as body fat distribution shift to a more male pattern due to hormonal imbalances (Wajchenberg, 2000). In the present study, central obesity (as represented by WC), WHR and BMI emerges as important risk factors for T2D in males, while central obesity and BMI increases the risk of T2D in females. Most of the CAD patients were on lipid lowering drugs and diet control; therefore, comparatively lesser CAD patients presented with higher WC, WHR, and BMI. Nonetheless, the obesity profiles in controls and T2D cases are suggestive of a higher trend towards generalized and central obesity in the population of Punjab probably due to consumption of high fat diet and transition to a sedentary life style.

In conclusion, the present study suggests that the G-allele of MCP-1 –2518 A>G polymorphism is associated with reduced risk of T2D and increased risk of CAD in the population of Punjab and the alleles exhibit differential risk pattern in T2D and CAD. Owing to the dietary habits, the profiles of obesity especially central obesity play an important role in susceptibility to T2D both in males and females. The trends suggest that those overweight and obese in the control population are at a higher risk of developing T2D and associated complications, if not controlled in time.

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Author Disclosure Statement

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