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RESEARCH ARTICLE

ASSOCIATION OF G1359A POLYMORPHISM OF THE ENDOCANNABINOID TYPE 1 RECEPTOR (*CNR1*) WITH CORONARY ARTERY DISEASE (CAD) WITH TYPE 2 DIABETES MELLITUS CNR 1 IN CAD AND T2DM

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ABSTRACT

Emerging evidence that the cannabinoid type 1 receptor (CB1) and its endogenous ligands, endocannabinoids, involved in regulation of feeding behavior and body weight. Over-activation of ECS is associated with metabolic diseases as dyslipidemia and insulin resistance involved in CAD and diabetes. The aim was to determine whether G1359A polymorphism of CNR1 associated with CAD with and without T2DM, and with T2DM patients free of CAD and elucidate the association of CNR1 polymorphism with CAD risk factors. The study was carried on 50 patients with CAD (25 patients with and 25 patients without T2DM), 25 patients with T2DM free of CAD and a group of 20 healthy subjects as a control group. Coronary artery angiography for patient group, serum lipid profile (TG, TC, LDL and HDL) and assessment of G1359A polymorphism of CNR1 by RFLP method were done. CAD patients with and without T2DM had significantly higher age, fasting blood glucose, systolic and diastolic blood pressure, male gender, smoking, and body mass index (BMI) compared with control. GG genotype and G allele of G1359A polymorphism were significantly associated with CAD patients with T2DM ($p<0.05$). G allele increased risk of occurrence of CAD with diabetes by 5.22 (OR) 95% CI (1.32-20.54). GG genotype was significantly associated with higher TC ($p<0.01$), LDLc ($p<0.001$) and BMI ($p=0.001$). Association of G1359A polymorphism with BMI and disordered lipid may explain in part its association with CAD patients with T2DM and may encourage use of cannabinoid receptor antagonist in treatment of these disorders.

INTRODUCTION

Cardiovascular disease is the principal threat to health in Africa and Middle East countries, as elsewhere (WHO, 2012; Almahmeed *et al.,* 2012). The epidemiology of coronary heart disease in Egypt was provided by The Egyptian National Hypertension Project (Sharraf *et al.,* 2003; Ibrahim *et al.,* 1995; Ibrahim *et al.,* 2001) in addition to data from the WHO (WHO, 2012). This nationally found an adjusted overall prevalence of coronary heart disease of 8.3% (Almahmeed *et al.,* 2012; Sharraf *et al.,* 2003; Ibrahim *et al.,* 1995; Ibrahim *et al.,* 2001). Obesity and insulin resistance are associated with cardiovascular risk factors, including altered levels of inflammatory markers and adipocytokines. In this topic area, the important role played by endocannabinoid system is emerging: Endocannabinoids are endogenous lipid mediators with wide range of biological effects. It controls food intake, energy balance and glucose metabolism through both central and peripheral effects, and stimulated lipogenesis and fat accumulation (Ameri, 1999; Brown *et al.,* 2002). The two most widely studied endocannabinoids are arachidonoyl ethanolamide or anandamide (AEA) and 2- arachidonoylglycerol (2-AG) (Mechoulam *et al.,* 1998) but several other similar endogenous substances have also been identified (Pacher *et al.,* 2006; Milman *et al.,* 2006). Endocannabinoids exert their biological effects via two main G protein coupled cannabinoid receptors, the CB1 and CB2 (Pacher *et al.,* 2006; Howlett, 2005). Previously, it was thought that CB2 receptors are mainly expressed in immune and hematopoietic cells mediating various immunomodulatory effects, while CB1 receptors are primarily distributed in the central nervous system. However, recent studies have also demonstrated CB1 receptors in various peripheral tissues (e.g., myocardium) (Bonz *et al.,* 2003; Batkai *et al.,* 2004; Mukhopadhyay *et al.,* 2007) human coronary artery endothelial and smooth muscle cells (Rajesh *et al.,* 2007; Rajesh *et al.,* 2008) adipose tissue (Cota *et al.,* 2003; Engeli *et al.,* 2005) and the liver (Engeli *et al.,*2005; Osei-Hyiaman *et al.,* 2005; Mallat and Lotersztajn, 2008).

The endocannabinoid receptor type 1 gene (CNR1), located on 6q 14–6q15, It is considered that ECS is "on demand" remaining inactive in repose physiologic conditions and connects physical and emotional responses to stress with appetite and energy balance (Dinu *et al.,* 2009) A common polymorphism (G1359A) of the CNR1 gene was reported to be significantly associated with lower body mass index (BMI) (Gazzerro *et al.,* 2007) abnormal lipid homeostasis (Baye *et al.,* 2008). Recent studies showed that the mutant genotype of CNR1 is associated with a better cardiovascular profile including triglyceride, high density lipoprotein cholesterol, insulin, and homeostasis model assessment (HOMA-IR) levels than the wild-type group (Luis *et al.,* 2009). The obese-hypertensive patients with the 3813G allele had a relatively lower prevalence of metabolic syndrome compared with subjects with the 3813A allele (Bordicchia *et al.,* 2010). It has already been stated that anandamide and other cannabinoids relax the coronary blood vessels of the heart. These responses in the rat are sensitive to CB1 receptor blockade but not to CB2 receptor antagonism (Ford *et al.,* 2002; Hiley, 2009). Vascular smooth muscle cell migration and proliferation are pivotal events in the pathogenesis of atherosclerosis and are directly implicated in the failure of clinical interventions used

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to treat patients with coronary heart disease (Gerthoffer, 2007). As recently demonstrated in vitro, CB1 antagonism (with rimonabant) dose-dependently inhibited PDGF induced proliferation, migration and signal transduction of human coronary artery smooth muscle cells (Rajesh *et al.,* 2008; Nissen *et al.,* 2008; Di Marzo, 2008). The Stradivarius trial studied the effect of rimonabant on atherosclerosis progression in patients with abdominal obesity and coronary artery disease (Nissen *et al.,* 2008) proving a non statistical difference was observed in the percent atheroma volume (PAV). However, the total atheroma volume, was significantly improved (Di Marzo, 2008). Emerging evidence suggests a crucial implication of the endocannabinoid system in the regulation of insulin sensitivity, glucose homeostasis, and lipid profile (Scheen *et al.,* 2006). The pathophysiology of type 2 diabetes is closely related to these disorders. Clinical studies investigated that, treatment of overweight type 2 diabetic patients with rimonabant (CB1 antagonism) (20 mg/day for 1year) significantly reduced body weight and improved glycemic control and HbA1C levels in comparison to placebo (Scheen *et al.,* 2006). The aim of this study was to determine whether G1359A polymorphism of CNR1 is associated with coronary artery disease (CAD) with and without type 2 diabetes mellitus (T2DM) and with T2DM patients free of CAD and to elucidate the association of G1359A polymorphism of CNR1 with lipid profile and other cardiovascular risk factors.

SUBJECTS AND METHODS

Subjects

Patients were selected from internal medicine department and cardiac catheterization unit of cardiology department in Menoufiya University Hospital. Full history, general and heart clinical examination were done before selection. Ethical approval for this research was obtained from the Research Ethics Committee, Faculty of Medicine, Menoufiya University, and informed consent was obtained from all participants. This study was carried on 50 CAD patients (25 patients with T2DM and 25 patients without T2DM), 25 patients with T2DM free of CAD and a group of 20 healthy subjects as a control group. CAD patients were diagnosed if they had coronary artery stenosis in at least one of the major epicardial coronary vessels, left main coronary artery, left anterior descending artery (LAD), left circumflex artery (LCA) or the right coronary artery (RCA) or a major branch vessel. T2DM was defined under these criteria as fasting plasma glucose (FPG) was ≥126 mg/dl (≥7.0mmol/l) or symptoms of hyperglycemia and a random plasma glucose ≥ 200 mg/dl (≥11mmol/l) (Goldstein *et al.,* 2007) the control group consisted of 20 healthy individuals free of CAD and T2DM. The excluding criteria of the study included diabetic nephropathy, cancer, renal disease, and any other chronic illnesses.

Lipid profiles analysis

5 ml venous blood were taken after an overnight fasting for determination of serum total cholesterol (TC), triglycerides (TG) and HDL-C levels. Lipid profiles were measured by the standard enzymatic colorimetric kits (SPINREACT, Spain). The serum LDL-c was calculated by Friedewal formula as TG level was not exceed 400 mg/dl: LDL-c = total cholesterol - (TG/5 + HDL-c) (Wallach, 1996).

DNA analysis

5ml venous blood was drained slowly into vacunated EDTA tube for isolation of peripheral blood mononuclear cells (PBMCs) using Lymphoflot solution *(Bio Test AG, Dreieich, Germany)* (Sirchia *et al.,* 1972). Genomic DNA was extracted from PBMCs using Gene JET Whole Blood DNA Mini Kits (Thermo Scientific, Sigma), for yielding pure DNA and stored at – 20°C for direct amplification. CNR1 1359 G/A polymorphism was detected by the polymerase chain reaction (PCR) using Perkin Elmer thermal cycler 2400 (USA). Genotyping of G1359A of CNR1 gene was done as described in previous studies (Gadzicki *et al.,* 1999). 25-ul PCR product was performed to genotype CNR1 gene using 200 ng of genomic DNA and 20 pmol each of the following primers (Invitrogen, USA) 5`-GAAAGCTGCATCAAGA GCCC-3`(forward) and5`-TTTTCCTGTGCTGCCAGGG- 3` (reverse), 1.5 mM MgCl2, 400 mM of each dNTP, 1.25 U Taq polymerase, and 1x Tag buffer (New England Biolabs, Beverly, MA, USA). DNA amplification (111bp) was performed with 40 cycles of denaturation for 1min at 94°C, annealing for 1:30 min at 60°C, and extension for 1:30 min at 72°C, preceeded by a single cycle of initial denaturation for 5 minutes at 94°C and followed by a single cycle of final extension for 5 minutes at 72°C. The resulting 111 bp PCR product were then digested overnight with 10 U of Mspl (400 unit) (Fermentaz, USA) at 37°C. This resulted in fragments of 92 and 19 bp, when a G was present at nucleotide position 385, while the fragment remained uncut (111bp), when an A was present. Restriction bands were analyzed by gel electrophoresis in a 2% agarose gel stained with ethidium bromide and visualized under UV light as AA at 111bp, GA at 111bp and 92bp and GG at 92 bp (Figure 1a,b).

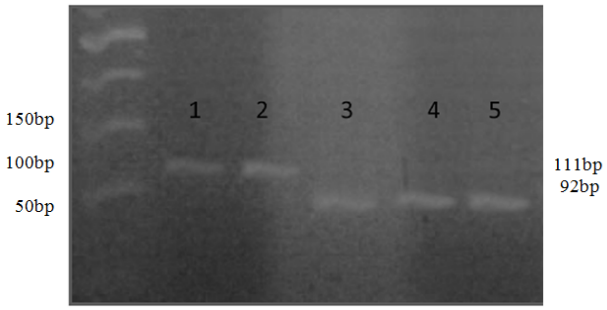

Figure 1a shows different G1359A genotypes of CNR1 polymorphism using 50 bp DNA ladder. Lane 1, 2 shows AA genotypes, lanes 3-5 shows GG genotype

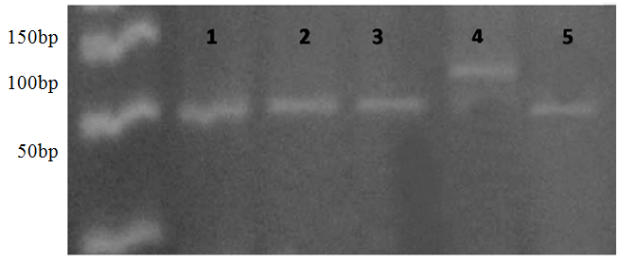

Figure 1b shows different G1359A genotypes of CNR1 polymorphism using 50 bp DNA ladder. Lane 1-3 and 5 shows GG genotypes, while lane 4 shows GA genotype

Statistical analysis

Results were statistically analyzed by statistical SPSS programs version 16. Two types of statistics were done: *Descriptive statistics:* e.g. percentage, mean and standard deviation (SD). *Analytic statistics:* Genotypes and allele frequencies were compared between cases and controls using Chi- Square ($\chi 2$) test and Mann-Whitney test. All odds ratios involving genotypes and alleles were calculated by logistic regression. A two-tailed Student's *t*-test was used to compare quantitative data. Statistical significance was considered significant at the P value < 0.05.

RESULTS

Demographic and clinical data regarding age, male gender [64%; 92% vs. 30%], smoking, systolic [141.2±16.4; 136.40 ±14.98 vs. 121.00 ± 14.1 and diastolic blood pressure [90.00 ±5.75; 87.20 ± 4.1 vs. 79.00 ± 9.67], body mass index (BMI) [26.41±3.71; 25.76±3.26 vs. 23.81±2.77] were significantly higher in CAD patients with and without type 2 DM compared with control (Table 1). Moreover, fasting blood glucose was significantly higher in CAD patients with and without type 2 DM and in T2DM patients compared with control [158.16±19.28; 84.60± 12.48; 137.6±11.16 vs. 78.40± 6.57] (Table 1). Regarding gender [68%Type 2 DM were male vs. 32% were female] as compared to [30% control were male vs. 70% were female]. In addition T2DM patients had significantly higher systolic and diastolic blood pressure [134.0 ±13.54 and 85.4±6.11 vs. 121.00 ± 14.1and 79.00 ± 9.67] and BMI [26.29±3.29 vs. 23.81±2.77] as compared to

Table 1. Demographic and clinical paremeters in CAD Patients with and without Diabetes, Type 2 diabetes patients and Controls

	Controls n = 20	CAD with diabetes n=25	CAD without diabetes n = 25	T2DM n= 25	P value
Age (years) X±SD	47.80 ± 7.43	55.08±5.57	54.12 ± 6.98	52.44±14.42	0.001* <0.01** >0.05***
Systolic pressure (mmHg)	121.00 ± 14.1	141.2±16.4	136.40 ±14.98	134.0 ±13.54	<0.001*,** <0.01***
Diastolic pressure (mmHg)	79.00 ± 9.67	90.00 ±5.75	87.20 ± 4.1	85.4±6.11	<0.001* <0.001** 0.01***
Sex no. (%)					
Male	6 (30%)	16(64%)	23(92%)	17(68%)	<0.05*
Female	14 (70%)	9(36%)	2 (8%)	8(32%)	<0.001** <0.05***
Smoking no. (%)					
Positive	2 (10%)	5(20%)	7 (28%)	8(32%)	<0.05*
Negative	18 (90%)	10(40%)	8 (32%)	14(56%)	<0.05**
Ex-smoker	0 (0.0%)	10(40%)	10 (40%)	3(12%)	>0.05***
BMI X±SD	23.81±2.77	26.41±3.71	25.76±3.26	26.29±3.29	0.01* <0.05**,***
Fasting blood glucose (mg/dl)	78.40± 6.57	158.16±19.28	84.60± 12.48	137.6±11.16	<0.001* <0.05** <0.001***
Cholesterol (TC) (mg/dl) X±SD	146.08 ± 39.4	179.72±40.42	167.18 ±46.03	191.03±38.1	<0.01*,** <0.001***
Triglyceride(TG) (mg/dl)X±SD	86.86 ±68.37	188.89±115.49	175.7 ± 97.72	208.2±115.4	0.001*,** <0.001***
HDLc (mg/dl) X±SD	50.43 ± 9.63	37.55±10.74	31.88 ± 8.08	32.18±7.66	<0.05* <0.001**,***
LDLc (mg/dl) X±SD	78.32 ± 36.21	102.55±29.77	100.12 ± 40.51	114.81±32.6	<0.05* >0.05** 0.001***

* comparison between CAD with diabetes and control, ** comparison between CAD without diabetes and control, *** comparison between Type 2 DM and control

Table 2. G1359A genotypes and alleles in CAD patients with and without Diabetes, Type 2 DM and Control

	Control n = 20	CAD with diabetes n = 25	CAD without diabetes n=25	T2DM n=25	P value
G1359A Genotypes no. (%)					
GG	12 (60%)	23 (92%)	19(76%)	20(80%)	<0.05*
GA	6 (30%)	1 (4%)	4(16%)	3(12%)	>0.05**
AA	2 (10%)	1 (4%)	2 (8%)	2(8%)	>0.05*** >0.05****
G1359A Allele no. (%)					
G	30 (75%)	47 (94%)	42 (84%)	43(86%)	<0.05*
A	10 (25%)	3 (6%)	8 (16%)	(14%) 7	>0.05** >0.05*** >0.05****
Odds Ratio for G allele		5.22	1.75	2.04	
95% CI		[1.32-20.54]	[0.61-4.95]	[0.70-5.98]	

control group (Table 1), while age and smoking were not significantly differ between T2DM patients and control groups. CAD patients with and without type 2 DM and T2DM patients groups had significantly higher cholesterol (TC) [179.72±40.42; 167.18 ±46.03; 191.03±38.1 vs. 146.08 ± 39.4], triglycerides (TG) [188.89±115.49; 175.7 ± 97.72; 208.2±115.4 vs. 86.86 ±68.37], and lower HDLc [37.55±10.74; 31.88 ± 8.08; 32.18±7.66 vs. 50.43 ± 9.63] than control. Although LDLc [102.55±29.77; 114.81±32.6 vs. 78.32 ± 36.21] was significantly higher in CAD patients with type 2 DM and T2DM patients, however CAD patients without diabetes had no significant difference regarding LDLc as compared to control group (Table 1). GG genotype and G allele were significantly associated with CAD patients with type 2 DM ($p<0.05$) while CAD patients without type 2 DM and type 2 DM had no significant association with GG genotype or G allele ($p>0.05$) when compared with control (Table 2). Also GG genotype and G allele were not significant between CAD with diabetes and CAD without diabetes ($p>0.05$). G allele increased risk of occurrence of CAD with diabetes by 5.22 odds ratio (OR), 95% CI (1.32-20.54) while OR in CAD without diabetes was 1.75 95% CI (0.61-4.95) and in type 2 DM was 2.04 95% CI (0.70-5.98). OR of G allele in CAD with T2DM 2.98 95% CI (0.74-11.99) when compared with CAD without diabetes (Table 2). GG genotype of G1359A polymorphism was not associated with age, fasting blood glucose, systolic and diastolic blood pressure, lipid profile and BMI in control group and CAD patients with type 2 DM (Table 3 and Table 4). In CAD without DM, GG genotype was significantly associated with higher TC ($p<0.01$), LDLc ($p<0.001$) and BMI ($p=0.001$) (Table 5). In diabetes mellitus group, GG genotype

was significantly associated with higher TG ($p<0.05$) while not significantly associated with other clinical parameters (Table 6).

Table 3. Relation of different G1359A genotypes with clinical parameters in control group

Control group	G/A Genotypes		P value
	GG	GA+AA	
Age (years)	45.17 ±8.8	51.75±6.80	>0.05
Systolic pressure	116.67 ±7.78	127.5 ±19.08	>0.05
Diastolic pressure	75.83 ±5.14	83.75 ±13.02	>0.05
BMI	23.46±3.16	24.30±2.14	>0.05
Fasting bloodglucose	76.83±7.46	80.75±4.36	>0.05
Tc mg/dl	154.68 ±44.34	133.18 ±28.41	>0.05
Tg mg/dl	92.91 ±82.83	77.77 ±21.08	>0.05
LDL mg/dl	85.69 ±43.46	67.26 ±18.89	>0.05
HDL mg/dl	50.45 ±8.75	50.40 ±11.48	>0.05

Table 4. Relation of different G1359A genotypes with clinical parameters in CAD patients with DM group

CAD patients with DM group	G/A Genotypes		P value
	GG	GA+AA	
Age (years)	55.0 ±5.68	56.00±5.65	>0.05
Systolic pressure	141.30 ±16.87	168.0±11.31	>0.05
Diastolic pressure	90.0 ±6.03	90.0 ±0.0	>0.05
BMI	30.1±2. 93	24.63±7.7	>0.05
Fasting bloodglucose	176.0±19.76	140.0 ±14.14	>0.05
Tc mg/dl	239.0 ±42.21	210.25 ±40.65	>0.05
Tg mg/dl	283.0 ±141.0	276.2 ±75.51	>0.05
LDL mg/dl	101.58 ±29.88	113.75±25.52	>0.05
HDL mg/dl	38.15 ±11.00	30.6 ±0.0	>0.05

Table 5. Relation of different G1359A genotypes with clinical parameters in CAD patients without DM group

CAD patients without DM	G/A Genotypes		P value
	GG	GA+AA	
Age (years)	53.16 ±7.19	57.17±5.74	>0.05
Systolic pressure	135.76 ±13.46	138.33 ±20.41	>0.05
Diastolic pressure	87.37 ±4.20	86.67 ±4.08	>0.05
BMI	29.73±1.46	25.35±3.59	0.001
Fasting bloodglucose	85.42±13.28	82.0±10.1	>0.05
Tc mg/dl	213.83 ±31.67	152.45 ±39.91	<0.01
Tg mg/dl	181.5 ±104.00	157.96 ±79.93	>0.05
LDL mg/dl	148.75 ±24.26	84.76 ±31.42	<0.001
HDL mg/dl	31.38 ±8.89	33.46 ±5.01	>0.05

Table 6. Relation of different G1359A genotypes with clinical parameters in T2DM group

T2DM patients	G/A Genotypes		P value
	GG	GA+AA	
Age (years)	49.80 ±15.33	56.67±0.50	>0.05
Systolic pressure	134.0 ±15.00	134.0 ±5.75	>0.05
Diastolic pressure	85.00 ±6.68	87.0 ±2.73	>0.05
BMI	26.99±2.2	26.1±4.14	>0.05
Fasting blood glucose	138.5±11.33	135.8±11.60	>0.05
Tc mg/dl	188.96 ±38.69	199.32 ±39.11	>0.05
Tg mg/dl	288.65 ±50.37	181.78 ±112.4	<0.05
LDL mg/dl	119.22 ±32.55	97.16 ±29.70	>0.05
HDL mg/dl	30.38 ±6.54	39.04 ±8.21	>0.05

DISCUSSION

Coronary artery disease is a major vascular complication in patients with T2DM. Up to 30% of patients with T2DM with myocardial ischemia are symptomatic and are associated with worse prognosis compared to non-diabetic subjects. Therefore, it is important to assess the risk of CAD in patients with T2DM earlier and then to target strategies to prevent CAD for patients with T2DM. Both genetic and environmental factors are thought to play an important role in the etiology of T2DM and CAD (Prudente *et al.,* 2009; Padmanabhan *et al.,* 2010). This study revealed that GG genotype and G allele of G1359A polymorphism of CB receptor 1 gene were significantly associated with CAD patients with type 2 DM ($p<0.05$) while CAD patients without T2DM and T2DM had no significant association with GG genotype or G allele ($p>0.05$) when compared with control. In line with our results, previous studies suggested association of GG genotype with CAD where GG genotype frequency of CNR1 was significantly higher in patients with CAD (94.63%) than in controls (86.05%, $p = 0.019$). Logistic analysis indicates that the GG genotype was associated with a significantly increased risk for developing CAD compared with the GA and AA genotypes ($p = 0.013$; OR 2.857; 95.00% CI 1.249 - 6.533). (Liu and Zhang, 2011), and also with CAD with T2DM where GG genotype frequency of CNR1 in patients with CAD was higher (91.9%) than in controls (80.9%, $p = 0.009$), GG genotype was significantly associated with the presence of CAD in the patients with T2DM compared with GA and AA genotypes (odds ratio, 2.632; 95% confidence interval, 1.481-4.678; $P < 0.001$) (Wang *et al.,* 2012). The results of our study supported the results that provide important evidences to support G1359A polymorphism of CNR1 to serve as a genetic risk marker in the assessment of cardiovascular risk and establishment of primary prevention strategies for CAD (Wang *et al.,* 2012). Cannabinoid receptor-1activation could induce there active oxygen species–mitogen-activated protein kinase activation Y cell death pathway and thereby contribute to the development of endothelial dysfunction and pathophysiology of multiple cardiovascular diseases (Rajesh *et al.,* 2010).

Enhanced insulin receptor signaling and increased B-cell proliferation and mass were found after genetic and pharmacologic blockade of CNR1. Furthermore, CNR1 antagonism treatment in mice resulted in reduced blood glucose and increased B-cell proliferation and mass coupled with enhanced insulin receptor signaling in B cells (Kim *et al.,* 2011). However Luis DA found lack of association of G1359A polymorphism of CB receptor 1 gene with obesity, cardiovascular risk factors and adipocytokines. The inconsistencies between association studies may reflect the complex interactions between multiple population-specific genetic and environmental factors (Luis *et al.,* 2010). Our study found association of GG genotype with higher TC, LDLc and BMI in CAD without DM. In diabetes mellitus group, GG genotype was significantly associated with higher TG. This may prove relation of G1359A polymorphism of CNR1 gene with dyslipidemia and obesity which are major risk factors of cardiovascular diseases and diabetes. Liu R and Zhang Y found that with the GG genotype of the CNR1 in patients with CAD compared with GA and AA genotypes had relatively lower levels of body mass index, homeostasis model assessment of insulin resistance and serum triglycerides, and elevated levels of high-density lipoprotein cholesterol and reported that these findings suggest that the G1359A polymorphism of the CNR1 gene may be associated with the risk for developing CAD (Liu and Zhang, 2011). Wang *et al.* reported that GG genotype of G1359A polymorphism was significantly associated with elevated levels of BMI, SBP, and HOMA-IR and decreased levels of HDL-C and demonstrated that polymorphism of CNR1was associated with multiple compounding factors of metabolic syndrome and this suggested that this polymorphism may play a role in the mechanism of metabolic syndrome (Wang *et al.,* 2012). Hu and Feng (2010). Suggested that, among metabolic syndrome subjects, those with mutant CNR1 genotype have relatively lower TG levels and BMI than subjects with wild-type CNR1 and proposed that the G1359A polymorphism of CNR1 gene is a predisposing factor for metabolic syndrome. This indicates that this specific variant of CNR1 affects an individual's genetic susceptibility to metabolic syndrome. Therefore, it is possible to hypothesize that mutation in the CNR1 gene may cause decreased expression or activity of CNR1, leading to reduced stimulation of the endocannabinoid receptor by endocannabinoid and thereby a decreased risk of developing metabolic syndrome through a direct effect on adipose tissue (Hu and Feng 2010). This polymorphism has also been associated with decreased

weight, BMI, fat mass and WC (Luis *et al.,* 2011) Earlier study observed that obese patients carrying at least one A allele in CNR1 lost more weight than wild-type patients after a 3-month low-fat calorie-restricted diet (Aberle *et al.,*2008). However, there are also inconsistent studies showing that the G1359A polymorphism of CNR1 is not associated with obesity (Jaeger *et al.,*2008; Aberle *et al.,*2007). This discrepancy may have resulted from environmental and ethnic differences between populations or differences in sample collection. Common polymorphism of CNR1 is associated with several clinical lipid dysfunctions known to accompany metabolic syndrome such as high TG and low HDL levels (Baye *et al.,* 2008). Other studies found no association between CNR1 polymorphism and dyslipidemia (Luis *et al.,* 2009; Luis *et al.,* 2010). This discrepancy may be attributed to the use of lipid-lowering drugs, which may have had an effect on the association between this polymorphism and dyslipidemia in patients (Hu and Feng 2010). In conclusion, we found significant association of G1359A polymorphism with CAD patients with T2DM and with dyslipidemia and BMI supporting an emerging body of in vitro and in vivo findings elucidated a key role for endocannabinoid-mediated CB1 signaling in the pathogenesis of atherosclerosis and diabetes suggesting that CB1 antagonism may represent a promising therapeutic strategy for the treatment of this life-threatening disease.

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