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Zinc Finger 259 Gene Polymorphism rs964184 is Associated with Serum Triglyceride Levels and Metabolic Syndrome

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Metabolic syndrome (MetS) is characterized by a cluster of cardiovascular risk factors that include: abdominal obesity, dyslipidaemia, hypertension, insulin resistance and impaired glucose tolerance. Recent genome wide association studies have identified several susceptibility regions involved in lipid metabolism that are also associated with MetS. We have explored the association of 9 genetic polymorphisms involved in lipid metabolism and hypertension, including: MTHFR C677T, SELE L554F, FGB - 455G>A, GNB3 C825T, ZNF259 C>G, PSRC-1 A>G, CETP I405V, LPL S447X and LPA C>T in 97 subjects with MetS and 96 individuals without MetS who were recruited randomly from Mashhad stroke and heart atherosclerotic disorder (MASHAD) study using a stratified cluster random sampling technique. Anthropometric parameters and biochemical measurements were determined in all the subjects. Genotyping was carried out followed by univariate and multivariate analyses. The subjects with MetS had a higher triglyceride and lower HDL- C. CG+ GG genotypes of ZNF259 polymorphism (rs964184 C>G) and TT+CT genotypes of MTHFR C677T (rs1801133) were associated with MetS, and individuals carrying the G allele for ZNF259 or the T allele for MTHFR polymorphisms were associated with MetS (e.g., odds ratio (OR) for CG+GG genotypes vs. CC wild type: 2.52, CI=1.33-4.77; P=0.005). However, after multiple comparison adjustment, this relationship remained significant only for CG+ GG genotypes of ZNF259 polymorphism. Moreover, the ZNF259 CG+ GG genotypes were associated with increased serum concentrations of triglycerides and LDL-C, compared to the wild type. These data support the necessity for further studies in larger multicenter settings.

Key words: Metabolic syndrome, gene polymorphisms, lipid pathway

Metabolic syndrome (MetS) is a common condition comprising a cluster of cardiovas-

cular risk factors including: abdominal obesity, dyslipidemia, hypertension, insulin resistance and

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impaired glucose tolerance. It is associated with an increased risk of cardiovascular disease (CVD) and diabetes mellitus (1). Over the past decades, the number of people with MetS has increased globally. The prevalence of MetS in the Iranian adult population (95% confidence interval) is approximately 32.1% (31.2-33.0) by the International Diabetes Federation (IDF) definition, 33.2% (32.3-34.1) by the adult treatment panel III report (ATPIII) and 18.4% (17.6-19.2) according to the WHO definition (2).

Genome wide association studies (GWAS) have identified several susceptibility loci and genes related to MetS and CVD; these include: Lipoprotein(a) (LPA), lipoprotein lipase (LPL) and Cholesteryl ester transfer protein (CETP) (3, 4). Kraja et al. identified 29 unique variants in or near 15 genes. These genes were mainly related to lipid metabolism pathway, which has been shown to have an important role in the genetic background of MetS. Consistent with these observations, several other studies had shown that the most important variants in the correlation among traits of MetS were in or near LPL, CETP and ZNF259 genes, which are known to play a key role in lipid metabolism (4-8). Lipoprotein (a) consists of a cholesterol-laden low-density lipoprotein (LDL)like particle bound to a plasminogen-like glycoprotein, apolipoprotein (a). Lipoprotein (a) has been shown to be associated with thrombosis and atherosclerosis, and genetic data support a role for lipoprotein (a) in atherosclerotic stenosis and MetS (9-11).

Lipoprotein lipase (LPL) plays an important role in lipid metabolism and is expressed in the myocardium, adipose tissue and skeletal muscle (12). It catalyzes the hydrolysis of triacylglycerol present in chylomicron particles and VLDL (13). This reaction provides free fatty acids and monoacylglycerol for use by skeletal, cardiac muscle and adipose tissue. The LPL gene is located on the short

(p) arm of chromosome 8 at position 22. A common variant S447X (rs328) was reported at carrier frequencies of approximately 10 to 25% or higher frequencies in some populations (14-15).

CETP has been reported to have an association with CVD and its exact role in disease pathogenesis is unclear. CETP plays a key role in cholesteryl ester transfer from HDL-C to TG-rich lipoproteins. *CETP* polymorphisms are also associated with MetS (16) and increased level of TG and lower HDL- C levels (3). The CETP gene is located on the long (q) arm of chromosome 16 at position 21. It has been shown previously that polymorphisms in the *CETP* gene are related to increased risk for CAD (17-18).

High plasma concentration of homocysteine may predispose individuals to atherosclerosis by injuring the vascular endothelium, which might result in hypertension (19-21). The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3. Another candidate gene, which might be involved with hypertension, is fibrinogen. Fibrinogen is a soluble glycoprotein, which is synthesized in the hepatocyte. Plasma fibrinogen is a dimer composed of three polypeptide chains, α , β and γ that are coded by three genes FGA, FGB and FGG, respectively (22, 23). It has been shown that plasma fibrinogen levels are associated with CVD, however, the role of genetic variation in the etiology of MetS still remains conflicting (22, 23). The FGB gene is located on the long (q) arm of chromosome 4 at position 28. The rs1800790 (-455 G/A) polymorphism within the promoter of FGBgene has been shown to be related with increasing plasma fibrinogen concentration (22, 24). Furthermore, circulating markers of systemic inflammation such as SELE were shown to predict an increased risk of CVD. Endothelial leukocyte adhesion molecule-1 (selectin E) is a cell adhesion molecule that mediates the interaction of circulating leukocytes with vascular endothelium in various pathological and physiological settings (26). Leukocyte- endothelial interactions contribute to a variety of vascular disease processes such as atherosclerosis and chronic inflammation in metabolic disease. The SELE gene is located on the long (q) arm of chromosome 1 at position 24.2. Several studies have been reported that the L554F allele (rs5355) is associated with a higher risk of developing atherosclerosis and increased blood pressure risk in overweight individuals (27-28). In 1998, a common single nucleotide polymorphism (SNP), rs5443 (C825T) (thymidine to cytosine change), located on exon 10 of the GNB3 gene was identified to be associated with CVD (29, 30). Several studies have shown that the T allele of the GNB3 rs5443 SNP is associated with a number of health outcomes and other features of MetS including obesity, insulin resistance, dyslipidemia, hypertension (29, 30). SORT1 is involved in the uptake of LDL by the liver. Several studies have illustrated a significant positive correlation between PSRC1 and serum LDL concentrations (31-33). The risk allele of this variant on chromosome 11q23.3 (ZNF259, APOA5-A4-C3-A1 gene region) was associated with increased LDL cholesterol and decreased HDL cholesterol (and previously, with increased triglycerides) (34).

In the present study, we investigated the association of nine polymorphisms involved in lipid metabolism and hypertension, including *MTHFR* rs1801133, *SELE* rs5355, *FGB* rs1800790, *GNB3* rs5443, *PSRC1* rs599839, *LPL* rs328, *Lp(a)* rs3798220, *ZNF259* rs964184 *and CETP* rs5882 in 193 subjects with and without MetS.

Materials and Methods

Phenotypic definition of metabolic syndrome

We used the IDF criteria to define MetS. Accordingly, a person with MetS would have central obesity (waist circumference (WC) in males ≥94 cm and in females≥80 cm) plus any two of the following four factors: TG level≥1.7 mmol/L (150

mg/dL) or specific treatment for this lipid abnormality; HDL-cholesterol< 1.03 mmol/L (40 mg/dL) in males and <1.29 mmol/L (50 mg/dL) in females or specific treatment for this lipid abnormality; systolic blood pressure (SBP)≥130 or diastolic blood pressure (DBP)≥85 mmHg or treatment of previously diagnosed hypertension; fasting plasma glucose (FPG)≥ 5.6 mmol/L (100 mg/dl) or previously diagnosed type 2 diabetes) (35).

Population

In the current study, 193 individuals, including 97 patients with MetS, and 96 healthy controls were recruited randomly from Mashhad stroke and heart atherosclerotic disorder (MASHAD) study, who were drawn from three regions in Mashhad, located in the north-eastern Iran, using a stratified cluster random sampling technique (36). Participants had no family history of stroke, myocardial infarction, and diabetes mellitus. Informed consent was obtained from all participants using study protocol approved by the Ethics Committee of Mashhad University of Medical Sciences.

Anthropometric and Biochemical Measurements

Anthropometric parameters (height, body weight, waist and hip circumference) were measured as described previously (36, 37). Body mass index (BMI) was calculated as body weight (kg) divided by squared height in meters (m²), and BMIs of 20-25, 25-30 or >30 were considered as normal, over-weight or obese, respectively. SBP and DBP were measured in duplicate by sphygmomanometer. Serum total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein(LDL) and triglyceride (TG), and fasting blood glucose (FBG) concentrations were evaluated by standard enzymatic techniques, while serum Creactive protein(CRP) levels were determined by polyethylene glycol-enhanced immunoturbidimetry, as mentioned beforehand (37).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp® DNA Mini-Kit

(Qiagen, San Diego, CA) according to the manufacturer's protocol at the VU university medical center Amsterdam. The concentration and purity of DNA samples was measured with the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of GNB3, FGB, MTHFR, SELE, PSRC1, LPL, Lp(a), ZNF259 and CETP polymorphisms was performed using Taqman®-probes-based assay; PCR reactions were carried out in 12.5 µl total volume, using 20 ng of DNA in TaqMan® universal master mix with specific primers and probes (C-2184734-10, C-7429790-20, C-1801133-10, C-11975323-20, C-972962-10, C-901792-10, C-25930271-10, C-890762-10, C-790057-10; Applied Biosystems Foster City, CA). The ABIPRISM-7500 instrument equipped with the SDS version-2.0 software was utilized to determine the allelic content of the samples.

Statistical analysis

Data were analyzed using SPSS-20 software (SPSS Inc., IL, USA). The normality of distribution was determined using the Kolmogorov-Smirnov statistics Descriptive including test. frequency, and standard deviation (SD) were determined for all variables and were expressed as mean± SD for normally distributed variables (or as the median and IQR for not normally distributed variables). For normally distributed variables, the student's t-test was used to compare the clinical characteristics and baseline demographics between the groups. A Bonferonni correction was applied for multiple comparisons. The Mann-Whitney U test was used for continuous variables if they were not normally distributed. Logistic regression analysis was used to calculate association of polymorphisms and MetS in the presence of confounders such as age, sex and smoking. All analyses were two- sided and statistical significance was set at p < 0.05.

Results

Characteristics of the population

The baseline characteristics of the individuals with and without MetS are summarized in Table 1. Not surprisingly, subjects with MetS had a significantly higher triglyceride (TG), WC, SBP, smoking, HDL cholesterol (HDL-C); p< 0.05), while no differences were found for age, gender, BMI, weight, height, hip circumference (HC), serum TC, LDL- C, high-sensitivity CRP (HsCRP), DBP, FBG between the groups (Table 1).

Polymorphisms and risk of MetS

To investigate whether there was an association between MTHFR C677T, SELE L554F, FGB-455G>A, GNB3 C825T, ZNF259 C>G, PSRC1 A>G, CETP 1405V, LPL S447X and LPA C>T polymorphisms and MetS, we carried out genotyping using genomic DNA extracted from peripheral blood samples. Genotyping was successfully performed in the vast majority of DNA samples, and no discrepancies were found in the samples analyzed in duplicate (approximately less than 10%).

As shown in Table 2, the wild- type *MTHFR*-rs1801133 genotype (CC) had a frequency of 49%, whereas the CT and TT genotypes were found in 43.8% and 7.3% of the control group, respectively, while these frequencies in the MetS group were 40.4% (CC), 41.5% (CT), 18.1% (TT). Moreover, individuals with the MTHFR- rs1801133-TT genotype or those who carried the T allele of the *MTHFR*-rs1801133 polymorphism were more likely to have MetS (p< 0.05, respectively).

Furthermore, 5.2% and 35.1% of MetS patients had the *ZNF259* GG or CG genotype respectively, whilst CC genotype was found in 59.8% of the patients. However, these frequencies in the control group were 3.2%, 17.7 and 78.9 for GG, CG and CC genotypes (Table 2). The G allele of *ZNF259* variant increased the risk of MetS (OR=2.58, 95%CI=1.31-5.08; P=0.006). These

findings were tested based on the ORs and their 95% CI for the association of the SNP with MetS using logistic regression. Furthermore, no significant differences were identified between the *MTHFR* and other polymorphic genotypes and groups after adjustment for age, sex, and smoking status (Table 2). All polymorphisms were consistent with the Hardy–Weinberg equilibrium, as calculated using the SNP analyzer software (http://snp.istech21.com/snpanalyzer/2.0/; Table 2) and their allelic frequencies were comparable to the reported population in the NCBI and NCI-SNP500 databases.

Additionally, we evaluated the association of the emerging genotypes with the components of MetS including WC, blood pressure (BP), HDL-C, FPG and TG levels. These analyses showed that CG+GG genotypes of *ZNF259 C>G* polymorphism were associated with an increased serum of LDL-C and TG, compared to the CC wild-type genotype (Figure 1). Other analyses related to MetS components such as WC, HDL - C, FPG and BP were not significant (data not shown). Additionally,

we evaluated the association of the emerging genotypes with the components of MetS including WC, blood pressure (BP), HDL-C, FPG and TG levels. These analyses showed that CG+GG genotypes of *ZNF259 C>G* polymorphism were associated with an increased serum of LDL-C and TG, compared to the CC wild-type genotype (Figure 1). Other analyses related to MetS components such as WC, HDL-C, FPG and BP were not significant (data not shown).

Discussion

This is the first study evaluating the association of *MTHFR*- rs1801133, *SELE*-rs5355, *FGB*-rs1800790, *GNB3*- rs5443, *PSRC1*- rs599839, *LPL*- rs328, *Lp(a)*- rs3798220, *ZNF259*-rs964184 and *CETP*-rs5882 polymorphisms and MetS in Iranian patients. We demonstrated that GG genotype of *ZNF259* was markedly associated with increased risk of MetS in our population. Moreover, consistent with several studies, no statistically significant association was detected for other SNPs with MetS (20, 25, 38-40).

Table 1. Baseline characteristics of the individuals with and without metabolic syndrome					
Characteristics	Without MetS (n=96)	With MetS (n=97)	P value		
Age (y)	50.1±10.5	51.3±9.6	0.412		
Gender, N(%) Male	31(32.3)	37(38.1)	0.395		
Non- smokers, N (%)	13(13.5)	66(68)	< 0.001		
Weight (Kg)	71.9±11.1	75.1±11.9	0.062		
Height (cm)	160±10	160±0.9	0.876		
BMI (Kg/m^2)	28.3±4.3	29.4 ± 4.0	0.082		
WC (cm)	91.8±11.2	98.6±8.4	< 0.001		
HC (cm)	102.1±7.7	105.0±8.2	0.132		
SBP (mmHg)	124.2±20.8	130.4±19.8	0.036		
DBP (mmHg)	81.6±11.819	84.5±12.3	0.103		
TC (mg/dl)	199.2±40.9	165.9±37.2	0.563		
LDL (mg/dl)	122.9±39.5	120.4±33.9	0.649		
hsCRP (mg/dl)	1.8(1.1-3.5)	1.54(1.1-3.0)	0.584		
HDL (mg/dl)	43.5±9.1	38.0±8.6	< 0.001		
FBG (mg/dl)	88.7±30.1	89.6±24.9	0.817		
TG (mg/dl)	128.0(99.5-171.0)	177.5(135.0-230.5)	< 0.001		

Values are expressed as mean ±SD, median and interquartile range for normally and non-normally distributed variables, respectively. BMI: body mass index; WC: waist circumference, TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood glucose; HC: hip circumference, SBP: systolic blood pressure; DBP: diastolic blood pressure; high-sensitivity C-reactive protein: HsCRP; Metabolic syndrome: MetS

	Control	MetS	Odds ratio	P value	*Odds ratio	*P
	Control	Mets	(95%CI)	P value	*Odds rau0 (95%CI)	*P value
Rs5355 SELE	96	97				
CC	77(80.2)	84(86.6)	Ref Cat			
CT	19(19.8)	13(13.4)	0.62(0.29-1.35)	0.235	0.37(0.13-0.99)	0.058
C	173(90)	181(93)	Ref Cat			
T	19(10)	13(7)	0.66(0.24-1.83)	0.435		
rs1800790 FGB	93	97				
GG	58(62.4)	50(51.5)	Ref Cat			
GA	27(29)	41(42.3)	1.76(0.95-3.26)	0.071	1.57(0.43-5.70)	0.493
AA	8(8.6)	6(6.2)	0.87(0.28-2.67)	0.808	0.51(0.25-1.04)	0.064
G	143(77)	141(73)	Ref Cat		***************************************	
A	43(23)	53(27)	1.25(0.78-1.98)	0.347		
rs5443 <i>GNB3</i>	89	84	1120 (01/10 1190)	0.0 . /		
CC	55(61.8)	44(52.4)	Ref Cat			
CT	25(28.1)	29(34.5)	1.45(0.74-2.82)	0.274	0.88(0.26-2.97)	0.849
TT	9(10.1)	11(13.1)	1.52(0.58-4.01)	0.390	1.12(0.53-2.34)	0.762
C	135(76)	11(13.1)	Ref Cat	0.570	1.12(0.33-2.34)	0.702
T	43(24)	51(30)	1.37(0.69-2.70)	0.360		
rs1801133 <i>MTHFR</i>	96	94	1.37(0.09-2.70)	0.500		
CC	47(49)	38(40.4)	Ref Cat			
CT		38(40.4)	1.14(0.62-2.11)	0.657	0.74(0.35-1.58)	0.446
TT	42(43.8)	` ′	,		` ,	0.446
	7(7.3)	17(18.1)	3.00(1.12-7.99)	0.028	2.06(0.63-6.69)	
TT+CT	89(92.7)	77(81.9)	2.80(1.10-7.12)	0.030	2.37(0.77-7.24)	0.129
C	136(71)	115(61)	Ref Cat	0.047		
T CHEEN	56(29)	73(39)	1.54(1.00-2.36)	0.047		
rs5882 CETP	95	97	D.CG.			
GG	44(46.3)	41(42.3)	Ref Cat	0.44	0.67(0.21.1.42)	0.200
GA	40(42.1)	43(44.3)	1.15(0.63-2.11)	0.643	0.67(0.31-1.43)	0.303
AA	11(11.6)	13(13.4)	1.26(0.51-3.14)	0.608	0.88(0.29-2.65)	0.823
G	128(67)	125(64)	Ref Cat			
A	62(33)	69(36)	1.14(0.74-1.73)	0.544		
rs328 LPL	96	96				
CC	77(80.2)	81(84.4)	Ref Cat			
CG	17(17.7)	15(15.6)	0.83(0.39-1.79)	0.651	1.41(0.58-3.45)	0.443
GG	2(2.1)	0(0)	< 0.001	0.999	< 0.001	0.999
C	171(89)	177(92)	Ref Cat			
G	21(11)	15(08)	0.69(0.34-1.38)	0.296		
rs599839 <i>PSRC1</i>	96	96				
AA	78(81.2)	77(80.2)	Ref Cat			
AG	15(15.6)	18(18.8)	1.21(0.57-2.58)	0.612	0.84(0.33-2.15)	0.728
GG	3(3.1)	1(1)	0.33(0.03-3.31)	0.352	0.14(0.01-2.02)	0.153
A	(89)171	172(90)	Ref Cat		. ,	
G	21(11)	20(10)	0.94(0.49-1.81)	0.869		
rs964184 ZNF259	95	97				
CC	75(78.9)	58(59.8)	Ref Cat			
CG	17(17.9)	34(35.1)	2.15(0.49-9.39)	0.307	3.25(1.43-7.38)	0.005
GG	3(3.2)	5(5.2)	2.58(1.31-5.08)	0.006	1.42(0.23-8.62)	0.701
CG+GG	20(21.1)	39(40.2)	2.52(1.33-4.77)	0.005	2.92(1.34-6.36)	0.007
C C	167(88)	150(77)	Ref Cat	0.005	2.72(1.57 0.50)	0.007
G	23(12)	44(23)	2.13(1.22-3.69)	0.007		
rs3798220 <i>LPA</i>	88	91	2.13(1.22-3.09)	0.007		
			Pof Cot			
TT	75(85.2)	80(87.9)	Ref Cat	0.500	0.06(0.24.2.71)	0.065
TC	13(14.8)	11(12.1)	0.79(0.33-1.87)	0.599	0.96(0.34-2.71)	0.965
T	163(93)	171(94)	Ref Cat	0.415		
C	13(7)	11(6)	0.80(0.35-1.85)	0.612		

Ref Cat: Reference category, CI: Confidence interval; Logistic regression analysis was used to calculate association of polymorphisms and metabolic syndrome.* After correction for age, sex and smoking

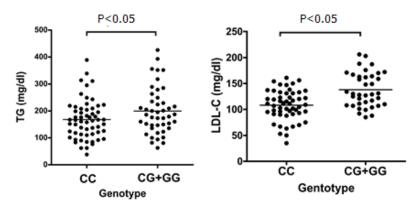


Fig. 1. Association of ZNF259 polymorphism with TG and LDL-C. Association of (A) TG and (B) LDL-C in MetS subjects with T-CG+GG genotypes versus control group.

MetS and its components, including hypertension, hyperlipidemia, obesity, insulin resistance or glucose intolerance, and fatty liver diseases, significantly contribute to the increased risk of cardiovascular diseases and diabetes (1). In recent years, genome-wide association studies identified (GWASs) have many candidate polymorphisms that may be associated with metabolic-related traits (3, 4). These studies revealed the association of several polymorphisms in lipid metabolism pathway with MetS. In particular they identified LPL, CETP and ZNF259 as the most significant influential variants related to the traits of MetS (4-8). In addition, another recent GWAS has identified LPA variants as the most significant factor related to the CVD (9). Furthermore, Clarke et al. demonstrated a significant association between LPA variants with both an increased level of lipoprotein (a) and an increased risk of coronary disease (9). Clee et al. showed that S447X variant carriers had a trend toward decreased vascular disease, decreased triglyerides and decreased DBP compared to noncarriers (15). Previous studies reported that LPL variants are associated with individual components of MetS (5-7), as well as with insulin resistance and CVD (6, 7). In a case-control study of Ashkenazi Jewish families with exceptional longevity, an increased frequency in homozygosity for the V405

allele (rs5882) (VV genotype) was found. Longlived individuals had lower serum CETP concentrations and increased lipoprotein sizes. They suggested that the inherited large lipoprotein particle sizes promoted a healthy aging phenotype (18). Wang et al. established a GWAS study in a case-control cohort and detected the association between myocardial infarction and four SNPs, including rs599839 near PSRC1 and sortilin 1 (SORTI) gene on the chromosomal region of 1p13.3 (31). Furthermore, an intra-genic variant, rs964184, near ZNF259 is found to be related to serum TG level and hyperlipidemia (33). By contrast, several other studies could not replicate these findings (20, 25, 38). In particular, Yamada and collaborators evaluated the association of candidate gene polymorphisms, e.g., GNB3 1429C>T, related to lipid metabolism in 2417 Japanese subjects, including 1522 with MetS and 895 controls. This study failed to show any relationship between GNB3 and MetS (38). Consistent with this data, we found that this polymorphism was not associated with the disease in our population. Similarly, a recent meta- analysis by Povel et al. could not demonstrate a correlation between the C825T polymorphism and MetS (pooled OR of 825T vs. C: 1.03, 95% CI 0.94-1.12) (41). Additionally, Albert et al. examined the relationship between candidate polymorphisms in the fibrinogen gene and its association with plasma levels in 565 white, 476 African-American, 277 Hispanic and 370 Asian women participating in the Women's Genome Health Study. They illustrated that the -455 G/A polymorphism was significantly associated with baseline plasma levels of fibrinogen and with increased CVD risk (24). However, several other studies evaluating functional polymorphisms in the fibrinogen gene and relationship with CVD events have generally showed a weak or no relationship (25), which is in line with our observation.

A common genetic variant in MTHFR gene, rs1801133 (677 C>T), leads to the reduction in the activity of the MTHFR enzyme, and increased plasma total homocysteine (tHcy) levels. MTHFR 677C>T polymorphism is shown to modulate total tHcy and folate metabolism (19) and has been shown to be the most frequent genetic causes for mild hyperhomocysteinemia (19). More recently, Yang et al. evaluated the association of 23 SNPs located within 17 candidate genes in 2014 subjects with overweight and obesity, diabetes, metabolic phenotypes. This study showed that MTHFR had a strong association with hypertension with an odd ratio (OR) similar to the result of a previous metaanalysis among Asian population (20, 21). Accumulating evidence has shown the relation between MTHFR rs1801133 polymorp-hisms with reduced MTHFR enzyme activity, CVD risk (42) and hypertension (21). Our analysis showed a statistically significant association of MTHFR with MetS. We found that subjects with the TT genotype had approximately 3-fold higher OR for MetS compared to the wild-type MTHFR-rs1801133 genotype (CC). However adjustment for age, sex and other confounding factors did change the statistical significance and the magnitude of the association, indicating that the association may be dependent on these confounding factors. We also analyzed the relationship between rs1800790,

rs5355, rs599839, rs328, rs3798220 and rs5882 polymorphisms and MetS, adjusting for multiple covariates. However, we did not detect a statistically significant association between these polymor-phisms and MetS, which might be possible because of the low statistical power due to a small sample size or small genetic effect sizes. On the other hand, several candidate gene studies had been investigated and showed inconsistent results (25, 38). This lack of correlation could be explained by several factors, including variations in the life style, diet, severity of the disease, small sample size, ethnic origin and/or medications. The findings of this stndy is in agreement with, several studies that also demonstrated inconsistent data (39-41).

To the best of our knowledge, this is the first study showing the association of ZNF259 with lipid profile and MetS. Our data illustrated that individuals carrying G allele for ZNF259 were at an increased risk of having MetS with OR of 2.52 (95% CI= 1.33- 4.77; P= 0.005). The results of our stndy, are consistent with several recent studies that showed its association with hyperlipidaemia and CVD (43). Aung et al. explored the association of the ZNF259 with hypercholesterolaemia and hypertriglyceridaemia. They showed that ZNF259 rs964184 SNP was associated with serum lipid levels and the presence of hyperlipidaemia (43). Braun et al. reported an association between the BUD13-ZNF259 and serum TG level (44). We also found that patients carrying G genotype had a significantly higher level of LDL-C and TG, compared to the CC wild-type.

The main limitation of this study is its case control study design and small sample size or small genetic effect sizes. Additionally, we cannot exclude the heterogeneity which might be present in Iranian population, future studies in this population, and using more carefully defined ethnic groups are needed.

In conclusion, we demonstrated a significant association of ZNF259 C>G polymorphism with MetS and showed that patients with GG genotype or those who carried the G allele had approximately 2.5- fold increased risk for developing MetS, further studies on evaluating the role of genetic markers in MetS are recommended.

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Conflict of interest

The authors declared no conflict of interests.

References

- 1. Padmalayam I, Suto M. Role of adiponectin in the metabolic syndrome: current perspectives on its modulation as a treatment strategy. Current pharmaceutical design 2013;19:5755-63.
- 2. Zabetian A, Hadaegh F, Azizi F. Prevalence of metabolic syndrome in Iranian adult population, concordance between the IDF with the ATPIII and the WHO definitions. Diabetes research and clinical practice 2007;77:251-7.
- 3. Kraja AT, Vaidya D, Pankow JS, et al. A bivariate genomewide approach to metabolic syndrome: STAMPEED consortium. Diabetes 2011;60:1329-39.
- 4. Kristiansson K, Perola M, Tikkanen E, et al. Genome- wide screen for metabolic syndrome susceptibility Loci reveals strong lipid gene contribution but no evidence for common genetic basis for clustering of metabolic syndrome traits. Circulation Cardiovascular genetics 2012;5:242-9.
- 5. Mead JR, Ramji DP. The pivotal role of lipoprotein lipase in atherosclerosis. Cardiovascular research 2002;55:261-9.
- 6. Goodarzi MO, Guo X, Taylor KD, et al. Lipoprotein lipase is a gene for insulin resistance in Mexican Americans. Diabetes 2004;53:214-20.
- 7. Goodarzi MO, Guo X, Taylor KD, et al. Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans. Genetics in medicine: official journal of the American College of Medical Genetics 2003;5:322-7.
- 8. O'Brien PJ, Alborn WE, Sloan JH, et al. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low

- concentrations compared with other apolipoproteins. Clinical chemistry 2005;51:351-9.
- 9. Clarke R, Peden JF, Hopewell JC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. The New England journal of medicine 2009;361:2518-28.
- 10. Kamstrup PR, Tybjaerg-Hansen A, Nordestgaard BG. evidence that lipoprotein(a) associates atherosclerotic stenosis rather than venous thrombosis. Arteriosclerosis. thrombosis. vascular and biology 2012;32:1732-41.
- 11. Kamstrup PR, Tybjaerg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and improved cardiovascular risk prediction. Journal of the American College of Cardiology 2013;61:1146-56.
- 12. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. Journal of lipid research 2002;43:1997-2006.
- 13. Mead JR, Irvine SA, Ramji DP. Lipoprotein lipase: structure, function, regulation, and role in disease. Journal of molecular medicine 2002;80:753-69.
- 14. Fisher RM, Humphries SE, Talmud PJ. Common variation in the lipoprotein lipase gene: effects on plasma lipids and risk of atherosclerosis. Atherosclerosis 1997;135:145-59.
- 15. Clee SM, Loubser O, Collins J, et al. The LPL S447X cSNP is associated with decreased blood pressure and plasma triglycerides, and reduced risk of coronary artery disease. Clinical genetics 2001;60:293-300.
- 16. Chasman DI, Pare G, Zee RY, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high- density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and Apolipoprotein B among 6382 white women in genome-wide analysis with replication. Circulation Cardiovascular genetics 2008;1:21-30.
- 17. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese- American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. The Journal of clinical investigation 1996;97:2917-23.
- 18. Barzilai N, Atzmon G, Schechter C, et al. Unique lipoprotein phenotype and genotype associated with exceptional longevity. Jama 2003;290:2030-40.
- 19. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk for vascular disease: a common mutation in factor

- methylenetetrahydrofolate reductase. Nature genetics 1995:10:111-3
- 20. Yang J, Liu J, Liu J, et al. Genetic association study with metabolic syndrome and metabolic-related traits in a cross-sectional sample and a 10-year longitudinal sample of chinese elderly population. PloS one 2014;9:e100548.
- 21. Markan S, Sachdeva M, Sehrawat BS, et al. MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians. Molecular and cellular biochemistry 2007;302:125-31.
- 22. Carty CL, Heagerty P, Heckbert SR, et al. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the Cardiovascular Health Study. Annals of human genetics 2010;74:1-10.
- 23. Theodoraki EV, Nikopensius T, Suhorutsenko J, et al. Fibrinogen beta variants confer protection against coronary artery disease in a Greek case-control study. BMC medical genetics 2010;11:28.
- 24. Albert MA, Pare G, Morris A, et al. Candidate genetic variants in the fibrinogen, methylenetetrahydrofolate reductase, and intercellular adhesion molecule-1 genes and plasma levels of fibrinogen, homocysteine, and intercellular adhesion molecule-1 among various race/ethnic groups: data from the Women's Genome Health Study. American heart journal 2009; 157:777-83 e1.
- 25. Morgan TM, Krumholz HM, Lifton RP, et al. Nonvalidation of reported genetic risk factors for acute coronary syndrome in a large-scale replication study. Jama 2007;297:1551-61.
- 26.Khazen D, Jendoubi- Ayed S, Aleya WB, et al. Polymorphism in ICAM-1, PECAM-1, E-selectin, and L-selectin genes in Tunisian patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2009;21:167-75.
- 27. Wenzel K, Felix S, Kleber FX, et al. E-selectin polymorphism and atherosclerosis: an association study. Human molecular genetics 1994;3:1935-7.
- 28. Marteau JB, Sass C, Pfister M, et al. The Leu554Phe polymorphism in the E-selectin gene is associated with blood pressure in overweight people. Journal of hypertension 2004;22:305-11.
- 29. Hsiao TJ, Hwang Y, Liu CH, et al. Association of the C825T polymorphism in the GNB3 gene with obesity and metabolic

- phenotypes in a Taiwanese population. Genes & nutrition 2013:8:137-44.
- 30. Guo L, Zhang LL, Zheng B, et al. The C825T polymorphism of the G-protein beta3 subunit gene and its association with hypertension and stroke: an updated meta-analysis. PloS one 2013:8:e65863.
- 31. Wang AZ, Li L, Zhang B, et al. Association of SNP rs17465637 on chromosome 1q41 and rs599839 on 1p13.3 with myocardial infarction in an American caucasian population. Annals of human genetics 2011;75:475-82.
- Schadt EE, Molony C, Chudin E, et al. Mapping the genetic architecture of gene expression in human liver. PLoS biology 2008:6:e107.
- 33. Braun TR, Been LF, Singhal A, et al. A replication study of GWAS-derived lipid genes in Asian Indians: the chromosomal region 11q23.3 harbors loci contributing to triglycerides. PloS one 2012;7:e37056.
- 34. Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nature genetics 2011;43:333-8.
- 35. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabetic medicine: a journal of the British Diabetic Association 2006;23:469-80.
- 36. Ghayour-Mobarhan M, Moohebati M, Esmaily H, et al. Mashhad stroke and heart atherosclerotic disorder (MASHAD) study: design, baseline characteristics and 10-year cardiovascular risk estimation. International journal of public health 2015;60:561-72.
- 37. Mirhafez SR, Avan A, Pasdar A, et al. Association of tumor necrosis factor-alpha promoter G-308A gene polymorphism with increased triglyceride level of subjects with metabolic syndrome. Gene 2015;568:81-4.
- 38. Yamada Y, Ichihara S, Kato K, et al. Genetic risk for metabolic syndrome: examination of candidate gene polymorphisms related to lipid metabolism in Japanese people. Journal of medical genetics 2008;45:22-8.
- 39. Ehret GB, O'Connor AA, Weder A, et al. Follow-up of a major linkage peak on chromosome 1 reveals suggestive QTLs associated with essential hypertension: GenNet study. European journal of human genetics: EJHG 2009;17:1650-7.

- 40. Zintzaras E, Zdoukopoulos N. A field synopsis and metaanalysis of genetic association studies in peripheral arterial disease: The CUMAGAS-PAD database. American journal of epidemiology 2009;170:1-11.
- 41. Povel CM, Boer JM, Reiling E, et al. Genetic variants and the metabolic syndrome: a systematic review. Obesity reviews : an official journal of the International Association for the Study
- of Obesity 2011;12:952-67.
- 42. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a metaanalysis. Bmj 2002;325:1202.
- 43. Aung LH, Yin RX, Wu DF, et al. Association of the variants in the BUD13-ZNF259 genes and the risk of hyperlipidaemia. Journal of cellular and molecular medicine 2014;18:1417-28.