Association of *CD36* Gene Variants and Metabolic Syndrome in Iranians

Azita Zadeh-Vakili¹, Bita Faam¹, Maryam S. Daneshpour¹, Mehdi Hedayati¹, and Fereidoun Azizi²

Aims: The *CD36* gene encodes for a membrane receptor that facilitates fatty-acid uptake and utilization. Genetic variants of the *CD36* gene have been associated with metabolic syndrome (MetS). We aimed to evaluate the association between the rs10499859A>G and rs13246513C>T polymorphisms and MetS components. **Methods:** For this case–control study, 140 MetS and 187 normal subjects were randomly selected from the Tehran Lipid and Glucose Study participants. Biochemical and anthropometrical variables were measured. Genotyping for both single nucleotide polymorphisms (SNPs) was performed by polymerase chain reaction–restriction fragment length polymorphism. **Results:** Case and control groups were not different in allele and genotype frequencies for these SNPs. However, the A and T alleles of these SNPs were significantly associated with elevated levels of high-density lipoprotein cholesterol (HDL-C) before age and sex adjustment ($p=0.027$ and 0.016 , respectively). Association between the A allele and body mass index (BMI) was also significant after adjustment for MetS under the dominant model ($p=0.009$, $\beta^2=0.68$). **Conclusions:** Based on our results, these polymorphisms do affect HDL-C level and BMI (MetS components), although the effect may be slight and restricted specifically to an environment-genotype.

Introduction

METABOLIC SYNDROME (MetS) is a group of risk factors that increase susceptibility to atherosclerotic cardiovascular disease and type 2 diabetes (Klein *et al.*, 2002; Malik *et al.*, 2004). Both genetic and environmental factors influence all components of the syndrome which include hypertension, obesity, insulin resistance, and dyslipidemia (Ford *et al.*, 2002; Love-Gregory *et al.*, 2008). Prevalence of the MetS is increasing to epidemic proportions not only in developed countries but also in developing nations. Thus, improved understanding of the genetic variations that increase susceptibility to the MetS is important and requisite for effective prevention and intervention. Several recent genome-wide linkage scans identified the 7q11.2–7q21.11 region of chromosome 7 as strongly linked to MetS components (Arya *et al.*, 2002; An *et al.*, 2005; Malhotra *et al.*, 2007; Love-Gregory *et al.*, 2008). This region contains the *CD36* gene that encodes a glycoprotein of the class B scavenger receptor family. *CD36* is an 88-kDa glycoprotein, which is expressed in many cell types such as cardiac and skeletal muscles, adipocytes, macrophages, and microvascular endothelial cells (Coburn *et al.*, 2000; Collet-Teixeira *et al.*, 2007). *CD36* binds with many ligands including native lipoproteins, oxidized low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs), and long-chain fatty acids (Calvo *et al.*, 1998; Connelly *et al.*, 1999; Feng *et al.*, 2000;

Hirano *et al.*, 2003; Nassir *et al.*, 2007). Further, *CD36* plays an important role in cholesterol uptake by enterocytes (Nassir *et al.*, 2007). The presented functions suggest a critical role for *CD36* in lipid metabolism and insulin resistance that is the major condition linked with the MetS. This role of *CD36* has been documented by the results of *CD36*-deficient patients and knock-out mice studies (Febbraio *et al.*, 1999; Hirano *et al.*, 2003). The results of subsequent studies on *CD36* gene polymorphisms suggested the association between these genetic variants and altered serum lipids and also impaired glucose metabolism (Lepretre *et al.*, 2004; Meex *et al.*, 2005; Morii *et al.*, 2009).

Because of the prevalence of MetS in Iran (32.1% in adults) (Zabetian *et al.*, 2007) and the absence of association studies on *CD36* gene in our population, this study was designed to examine the association of rs10499859A>G and rs13246513C>T polymorphisms with the MetS and its components. These single nucleotide polymorphisms (SNPs) were chosen because of their frequencies (minor allele frequency ≥ 0.05) and based on the results from a previous study, which suggested association between these SNPs and MetS components.

Materials and Methods

For this case–control study, 327 subjects, aged 19–70 years, were randomly selected from the Tehran Lipid and Glucose

¹Obesity Research Center and ²Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

Study (TLGS). In brief, the TLGS is a longitudinal study, which is designed to determine the major risk factors for noncommunicable diseases among the Tehran urban population and to develop population-based measures to change lifestyles in order to prevent the rising trend of diabetes mellitus, dietary disorders, and dyslipidemia. In TLGS, the study sample was selected using multistage stratified cluster sampling from the population of Tehran. TLGS subjects were interviewed privately. Information on age; demographic and biochemistry data; physical activity status; education levels; and medication usage for the treatment of diabetes, hypertension, and lipid disorders were collected in the TLGS questionnaire. For the present study, cases and controls were randomly selected from the TLGS participants with and without MetS (based on participant information and MetS definition), respectively. A detailed description of the methodology, rationale, and design of this study has been published elsewhere in detail (Azizi *et al.*, 2002).

This study was approved by the Medical Ethics Committee of the Research Institute for Endocrine Sciences at Shahid Beheshti University of Medical Science and informed consent was given by each participant.

The protocols for collection of clinical data have been described previously (Azizi *et al.*, 2003; Daneshpour *et al.*, 2010). Fasting serum glucose, triglycerides (TG), total cholesterol, and HDL-cholesterol (HDL-C) were measured using the enzymatic colorimetric method (Pars Azmon) by a Selectra 2 auto-analyzer (Vital Scientific). The Friedewald equation was used to calculate LDL-cholesterol; samples with TG greater than 400 mg/dL were assayed by the direct method. HDL subfractions were separated by differential polyanion precipitation. In all of these biochemical analyses, the coefficients of variation were less than 5%. For CD36 polymorphism analysis, genomic DNA was extracted as described previously (Truett *et al.*, 2000; Daneshpour *et al.*, 2008). The polymerase chain reaction (PCR) was used to amplify a 555-bp fragment for rs13246513C>T and 453 bp for rs10499859A>G in the CD36 gene using the oligonucleotide primers F: 5'-TCT ACC ACT TTG TTC TAG GCT AAT TTT T-3', R: 5'-CAA ATA TCC TAA TGC AAA TAG AAT AAA CC-3', F: 5'-CTT ATT TGG ATG GTA GGT TTG ACA CAG G-3', and R: 5'-GAG GAA AGA AAG CAA CAT TTC AAG ACT TC-3', respectively. Amplification was performed using the following program after initial denaturation at 95°C for 3 min: 95°C for 1 min, 60°C (for rs10499859A>G) or 55°C (for rs13246513C>T) for 45 s, at 72°C for 45 s for 30 cycles (Corbett). Each amplification was performed using 200 ng of total genomic DNA in a final volume of 15 µL containing 40 pmol of each oligonucleotide, 0.2 mM of each deoxyribo-nucleotide triphosphate (dNTP), 1.5 mM magnesium chloride, 10 mM Tris (pH 8.4), and 0.25 units of Taq polymerase (Fermentase). The PCR products were subjected to restriction digestion analysis. For the rs10499859A>G polymorphism, digestion with HincII (Fermentase) resulted in 284-bp and 169-bp fragments for G allele and a 453-bp fragment for A allele. For rs13246513C>T polymorphism, digestion with TaqI (Fermentase) resulted in 294-bp and 261-bp fragments for C allele and a 555-bp fragment for the T allele. The fragments were separated by electrophoresis on 2% agarose gels and DNA fragments were visualized by gel documentation (Optigo). Ten percent of the PCR samples were directly sequenced to confirm the polymerase chain reaction-restriction fragment length polymorphism results.

Definition

MetS was defined according to the joint interim statement criteria (Alberti *et al.*, 2009) with the presence of any three of five risk factors of the following: (1) abdominal obesity as waist circumference ≥ 95 cm in men and women according to population- and country-specific cutoff point for Iranians (Azizi *et al.*, 2010); (2) fasting blood sugar (FBS) ≥ 100 mg/dL or drug treatment; (3) fasting TG ≥ 150 mg/dL or drug treatment; (4) fasting HDL-C < 50 mg/dL in women and < 40 mg/dL in men or drug treatment; (5) raised blood pressure defined as systolic blood pressure ≥ 130 mmHg, diastolic blood pressure (DBP) ≥ 85 mmHg, or antihypertensive drug treatment. Those subjects with two or fewer risk components were assigned as controls.

Statistical analysis

Continuous variables are presented as mean with standard deviation (SD), ordinal variables as median with range, and categorical variables as frequency and percentage. Genotype and allele frequencies between the groups were assessed by the χ^2 -test for Hardy-Weinberg equilibrium ($p < 0.05$). A general linear model was used to compare mean values of anthropometric markers. In this case, the adjustment variables were age and gender. The mean values and SDs of biochemical variables in different groups (case, control, and genotype groups) were obtained and compared using an independent sample *t*-test. Logarithmic and Cox transformations were performed to normalize the error distribution and stabilize the error variance. The Mann-Whitney U test was used by considering five genetic models: a codominant model (three genotype groups separated), a dominant model (heterozygotes grouped with the homozygotes for the minor allele), a recessive model (heterozygotes grouped with the homozygotes for the major allele), a log-additive model (a score was assigned counting the number of minor alleles: score 0 for the homozygotes of the major allele, score 1 for the heterozygotes, and score 2 for the homozygotes of the minor allele), and an over-dominant model (homozygotes for the major allele grouped with the homozygotes for the minor allele). The Akaike information criterion was used to choose the genetic model that best fits the data. Multivariable linear regression was used to control the effect of potential confounders, including MetS, sex, age, and body mass index (BMI). All statistical analyses were performed using SPSS version 16 (SPSS). The statistical significance threshold was set to $p \leq 0.05$ (two-tailed test).

Results

The clinical characteristics and genotype frequencies of the 339 selected subjects are shown in Table 1. As expected, cases were significantly different from controls with regard to the variables that have been defined as MetS criteria (all $p < 0.05$). Significant differences in HDL subfractions (HDL-2 and HDL-3) were also seen between case and control groups. Genotype distributions of the two CD36 SNPs in MetS and normal subjects are shown in Table 2. Genotypes and allele distributions for rs10499859A>G polymorphism were in Hardy-Weinberg equilibrium but for rs13246513C>T they deviate from the equilibrium ($\chi^2 = 4.93$; $p = 0.0264$). Genotype and

TABLE 1. CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF THE STUDY SUBJECTS

Variables	Total (n=327)	Controls (n=187)	Cases (n=140)	p Value
Age (years)	42.1 ± 19.4 ^a	33.6 ± 18.5	52.9 ± 14.3	< 0.001
BMI (kg/m ²)	27.1 ± 5.41	24.5 ± 4.73	30.3 ± 4.36	< 0.001
Waist (cm)	89.8 ± 14.7	81.6 ± 13.5	101 ± 7.40	< 0.001
Fasting blood sugar (mg/dL)	98.9 ± 30.4	89.4 ± 15.9	111 ± 39.4	< 0.001
Total cholesterol (mg/dL)	190 ± 41.1	178 ± 39.9	207 ± 36.4	< 0.001
Triglycerides (mg/dL)	157 ± 104	115 ± 60.4	213 ± 122	< 0.001
HDL-cholesterol (mg/dL)	46.3 ± 11.4	49.2 ± 11.4	42.6 ± 10.4	< 0.001
HDL-2 (mg/dL)	17.1 ± 8.33	19.4 ± 8.69	13.9 ± 6.73	< 0.001
HDL-3 (mg/dL)	29.5 ± 5.95	30.2 ± 5.89	28.6 ± 5.95	0.02
LDL-cholesterol (mg/dL)	112 ± 33.4	106 ± 33.4	119 ± 31.9	0.001
C reactive protein (ng/mL)	1996 ± 2380	1650 ± 2268	2453 ± 2455	0.003
Diastolic blood pressure (mmHg)	73.9 ± 10.9	70.2 ± 9.55	78.8 ± 10.7	< 0.001
Systolic blood pressure (mmHg)	117 ± 21.7	108 ± 17.2	129 ± 21.1	< 0.001

^aMean ± SD.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

allele frequencies for the studied SNPs did not differ between subjects with and without MetS.

Differences between MetS components, except HDL-C, were not significant between different allele carriers (Table 3). The data show that HDL-C level was significantly higher in A allele carriers of rs10499859 (A carrier: 47.1 ± 1.26 mg/dL vs. G carrier: 43.9 ± 1.27 mg/dL; $p=0.027$) and T allele carriers of rs13246513 (T carrier: 47.2 ± 11.9 mg/dL vs. C carrier: 43.8 ± 9.61 mg/dL; $p=0.016$; HDL). These associations were not retained following covariates adjustment for MetS, sex, age, and BMI by dominant model ($p=0.098$ and $p=0.751$, respectively). The means of the biochemical variables and BMI in genotype groups for each of the SNPs, under different genetic models for confounders' analysis, are presented in Table 4. The association of rs13246513C>T polymorphism with BMI, TG, and cholesterol before and after adjustment was not significant. No significant association was observed between rs10499859A>G alleles and BMI before adjustment for MetS (A carrier: 27.5 ± 5.71 kg/m² vs. G carrier: 26.6 ± 5.16 kg/m²; $p=0.208$). This relationship became significant ($p=0.009$) after adjustment for MetS, as a common confounding factor. DBP was significantly increased in the G carriers (A carrier: 73.5 ± 1.15 mmHg vs. G carrier: 96.2 ± 1.21 mmHg; $p=0.021$) under recessive model. This association was no longer significant after adjustment for MetS, sex, and age ($p=0.133$). The association of rs10499859A>G polymorphism with other variables was not significant.

Discussion

The goal of this study was to evaluate the relation between rs10499859A>G in the 5' untranslated region (5'UTR) and rs13246513C>T in the 3' flanking region of *CD36* gene with MetS components. The results showed that these variants, although slightly, do affect MetS components, particularly HDL-C level. Carriers of A and T alleles of these SNPs had increased level of HDL-C, although the differences in levels were not statistically significant after adjustment for sex and age. This is, to our knowledge, the first report for the correlation between the *CD36* gene polymorphism and MetS in Iranians. To establish this correlation other polymorphisms in this gene should be assessed.

There is a large amount of evidence that suggest low HDL-C is one of the major determinants of the MetS and an independent risk factor for cardiovascular disease (Coon *et al.*, 2001). Therefore research has been carried out to investigate the association between variation in certain genes, like *CD36*, and HDL-C concentration. A 2008 study of 2020 African-American subjects by Love-Gregory *et al.* (2008) showed that some *CD36* variants, including rs10499859A>G, were related to the levels of HDL-C and their minor alleles were accompanied by higher levels of HDL-C. Their results implied that the magnitude of the increase in HDL-C was positively associated with the number of minor alleles present. They reported an increase of 2.5 mg/dL in HDL-C per G allele of rs10499859 SNP. They also showed that SNP rs13246513 increased the odds for the MetS by 32%.

Although our results reveal the relationship between aforementioned SNPs and HDL-C levels too, but indicate the role of the major A allele at rs10499859 in HDL-C increase and also a similar role for minor T allele at rs13246513. The divergent results between our study and those reported in African-American population could be due on one hand to the difference in the genetic background and on the other hand to the small sample size of our study.

The results of studies that assessed the relationship between *CD36* gene SNPs and BMI, as a measure of obesity, revealed a positive relation and suggest that genetic variation within the *CD36* locus may contribute to metabolic disease via its effect on body adiposity (Bokor *et al.*, 2010; Heni *et al.*, 2011). The reported result by Yun *et al.* (2007) on a Korean population showed

TABLE 2. GENOTYPE FREQUENCIES FOR *CD36* GENE SNPs IN THE CASE AND CONTROL GROUPS

	Controls	Cases	Total
rs13246513C>T			
CC (%)	45 (26.6)	44 (27.8)	89 (27.2)
CT (%)	89 (52.7)	82 (51.9)	171 (52.3)
TT (%)	35 (20.7)	32 (20.3)	67 (20.5)
rs10499859A>G			
AA (%)	52 (34.9)	42 (29.6)	94 (32.3)
AG (%)	82 (55)	77 (54.2)	159 (54.6)
GG (%)	15 (10.1)	23 (16.2)	38 (13.1)

TABLE 3. CHARACTERISTICS OF THE STUDY SUBJECTS

Variables	<i>rs13246513C>T</i>			<i>rs10499859A>G</i>		
	C Carriers	T Carriers	p	A Carriers	G Carriers	p
BMI (kg/m ²)	27.3±5.35 ^a	26.1±5.61	0.137	27.5±5.71	26.6±5.16	0.208
Fasting plasma glucose (mg/dL)	94.1 (69, 238) ^b	90.1 (75, 286)	0.052	91 (69,264)	88 (70,286)	0.385
Waist circumference (cm)	93.5 (52, 135)	91.1 (48, 123)	0.145	93 (60,135)	93 (48,118)	0.801
Triglycerides (mg/dL)	142±1.61	133±1.76	0.303	134±1.73	154±1.65	0.162
Total cholesterol (mg/dL)	190±40.8	189±42.3	0.867	189±40.2	199±52.9	0.186
HDL cholesterol (mg/dL)	43.8±9.61	47.2±11.9	0.016	47.1±1.26	43.9±1.27	0.027
LDL cholesterol (mg/dL)	111±32.9	116±35.2	0.204	111±33.4	114±41.8	0.644
Diastolic blood pressure (mmHg)	71.7±1.18	73.7±1.15	0.142	73.5±1.15	96.2±1.21	0.020
Systolic blood pressure (mmHg)	112 (68, 193)	113 (80, 187)	0.277	113 (80,187)	111 (68,166)	0.441

^aMean±SD.^bMedian (CI 25%).

SD, standard deviation; CI, confidence interval.

association of a polymorphism in intron 3 of the CD36 gene with an increase in BMI. Our results are in concordance with their results that the mean of BMI in A allele carriers was significantly greater than the respective value in G allele carriers.

Although we found no significant differences in allele frequency between cases and controls, and this may indicate that there is no association between these polymorphisms and MetS, we should bear in mind that the MetS is a multifactorial disorder in which each involved genetic factor has a slight and cumulative effect, and then the results are not unexpected. Also we should remember that a certain amount of the observed variation is in fact due to the different effect of environmental factors on different age groups, or the differences in lipid profile caused by hormonal background of the two genders. In small samples, the above effects will efficiently minimize and mask the true genetic differences between subjects.

This study has certain limitations. First, the small size of our study population might not have had sufficient power to detect direct evidence. Second, in spite of many polymorphisms that are detected, only two SNPs were evaluated in this region. Finally, only the genotype and allele distributions of rs10499859A>G SNP were in Hardy–Weinberg equilibrium, therefore haplotype analysis was not possible.

In conclusion, this study provides some evidence for identification of an effect of CD36 gene polymorphisms on MetS components and is a step on a path toward recognition of underlying genetic factors, the identification of which could lead to the prevention of MetS and therefore cardiovascular

disease. Further genetic studies in a larger population should be carried out to confirm this observation.

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Disclosure Statement

No competing financial interests exist.

References

- Alberti KG, Eckel RH, Grundy SM, *et al.* (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 120:1640–1645.
- An P, Freedman BI, Hanis CL, *et al.* (2005) Genome-wide linkage scans for fasting glucose, insulin, and insulin resistance in the National Heart, Lung, and Blood Institute Family Blood Pressure Program: evidence of linkages to chromosome 7q36 and 19q13 from meta-analysis. *Diabetes* 54:909–914.
- Arya R, Blangero J, Williams K, *et al.* (2002) Factors of insulin resistance syndrome—related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic Mexican-Americans. *Diabetes* 51:841–847.
- Azizi F, Emami H, Salehi P, *et al.* (2003) Cardiovascular risk factors in the elderly: the Tehran Lipid and Glucose Study. *J Cardiovasc Risk* 10:65–73.
- Azizi F, Khalili D, Aghajani H, *et al.* (2010) Appropriate waist circumference cut-off points among Iranian adults: the first report of the Iranian National Committee of Obesity. *Arch Iran Med* 13:243–244.
- Azizi F, Rahmani M, Emami H, *et al.* (2002) Cardiovascular risk factors in an Iranian urban population: Tehran Lipid and Glucose Study (phase 1). *Soz Praventivmed* 47:408–426.
- Bokor S, Legry V, Meirhaeghe A, *et al.* (2010) Single-nucleotide polymorphism of CD36 locus and obesity in European adolescents. *Obesity* 18:1398–1403.

TABLE 4. RELATION BETWEEN CD36 GENE POLYMORPHISMS AND METS PARAMETERS AFTER ADJUSTMENT

Variable	SNP	Genetic model	p ^a	p ^b	p ^c
BMI	A>G	Dominant	0.009		
HDL-cholesterol	A>G	Dominant			0.098
	C>T	Dominant			0.715
Diastolic blood pressure	A>G	Recessive		0.133	

p^a, adjusted for MetS; p^b, adjusted for MetS, sex, and age; p^c, adjusted for MetS, sex, age, and BMI.

MetS, metabolic syndrome.

- Calvo D, Gomez-Coronado D, Suarez Y, *et al.* (1998) Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. *J Lipid Res* 39:777–788.
- Coburn CT, Knapp FF Jr., Febbraio M, *et al.* (2000) Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem* 275:32523–32529.
- Collet-Teixeira S, Martin J, McDermott-Roe C, *et al.* (2007) CD36 and macrophages in atherosclerosis. *Cardiovasc Res* 75:468–477.
- Connelly MA, Klein SM, Azhar S, *et al.* (1999) Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein-cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake. *J Biol Chem* 274:41–47.
- Coon H, Leppert MF, Eckfeldt JH, *et al.* (2001) Genome-wide linkage analysis of lipids in the Hypertension Genetic Epidemiology Network (HyperGEN) Blood Pressure Study. *Arterioscler Thromb Vasc Biol* 21:1969–1976.
- Daneshpour MS, Hedayati M, Eshraghi P, *et al.* (2008) Association of apolipoprotein E gene polymorphism and lipid level in an Iranian population: Tehran Lipid and Glucose Study. *Iranian J Diabet Lipid* 7:399–405.
- Daneshpour MS, Hedayati M, Eshraghi P, *et al.* (2010) Association of Apo E gene polymorphism with HDL level in Tehranian population. *Eur J Lipid Sci Technol* 112:810–816.
- Febbraio M, Abumrad NA, Hajjar DP, *et al.* (1999) A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 274:19055–19062.
- Feng J, Han J, Pearce SF, *et al.* (2000) Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR- γ . *J Lipid Res* 41:688–696.
- Ford ES, Giles WH, Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 287:356–359.
- Heni M, Mussig K, Machicao F, *et al.* (2011) Variants in the CD36 gene locus determine whole-body adiposity, but have no independent effect on insulin sensitivity. *Obesity* 19:1004–1009.
- Hirano K, Kuwasako T, Nakagawa-Toyama Y, *et al.* (2003) Pathophysiology of human genetic CD36 deficiency. *Trends Cardiovasc Med* 13:136–141.
- Klein BE, Klein R, Lee KE (2002) Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam. *Diabetes Care* 25:1790–1794.
- Lepretre F, Linton KJ, Lacquemant C, *et al.* (2004) Genetic study of the CD36 gene in a French diabetic population. *Diabetes Metab* 30:459–463.
- Love-Gregory L, Sherva R, Sun L, *et al.* (2008) Variants in the CD36 gene associate with the metabolic syndrome and high-density lipoprotein cholesterol. *Hum Mol Genet* 17:1695–1704.
- Malhotra A, Elbein SC, Ng MC, *et al.* (2007) Meta-analysis of genome-wide linkage studies of quantitative lipid traits in families ascertained for type 2 diabetes. *Diabetes* 56:890–896.
- Malik S, Wong ND, Franklin SS, *et al.* (2004) Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation* 110:1245–1250.
- Meex SJ, van der Kallen CJ, van Greevenbroek MM, *et al.* (2005) Up-regulation of CD36/FAT in preadipocytes in familial combined hyperlipidemia. *FASEB J* 19:2063–2065.
- Morii T, Ohno Y, Kato N, *et al.* (2009) CD36 single nucleotide polymorphism is associated with variation in low-density lipoprotein-cholesterol in young Japanese men. *Biomarkers* 14:207–212.
- Nassir F, Wilson B, Han X, *et al.* (2007) CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. *J Biol Chem* 282:19493–19501.
- Truett GE, Heeger P, Mynatt RL, *et al.* (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29:52–54.
- Yun YM, Song EY, Song SH, *et al.* (2007) CD36 polymorphism and its relationship with body mass index and coronary artery disease in a Korean population. *Clin Chem Lab Med* 45:1277–1282.
- Zabetian A, Hadaegh F, Azizi F (2007) Prevalence of metabolic syndrome in Iranian adult population, concordance between the IDF with the ATPIII and the WHO definitions. *Diabetes Res Clin Pract* 77:251–257.

Address correspondence to:
Fereidoun Azizi, M.D.

Professor of Internal Medicine and Endocrinology
Research Institute for Endocrine Sciences
Shahid Beheshti University of Medical Sciences
Tehran 19395-4763
Iran

E-mail: azizi@endocrine.ac.ir