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Lack of Association between *FTO* Gene Variations and Metabolic Healthy Obese (MHO) Phenotype: Tehran Cardio-Metabolic Genetic Study (TCGS)

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Abstract

Background: Obesity is currently an international epidemic and metabolic derangements pose these individuals at greater risk for future morbidity and mortality. Genetics and environmental factors have undeniable effects and among genetic risk factors, *FTO/CETP* genes are important. The current study examines the interaction between obesity phenotypes and *FTO/CETP* SNPs and their effects on lipid profile changes.

Material and Methods: We selected 954 adult subjects from TCGS (47.9% male). Participants were stratified according to their BMI and presence of metabolic syndrome according to the Joint Interim Statement (JIS) definition. Nine selected polymorphisms from *FTO/CETP* genes were genotyped using Tetra ARMS-PCR method. After age and sex adjustment the interaction of 9 markers with lipid profiles among phenotypes were tested by PASW.

Results: In three main groups, HDL-C level had a strong significant association with CETP markers: (rs3764261, β(95%CI) -0.48(-0.61-0.35), P=1.0 × 10^{-11}), (rs1800775, β(95%CI) 0.5(0.36;0.65), P=1.0 × 10^{-6}) and (rs1864163, β(95%CI) 0.3(0.16;0.43), P=9.1 × 10^{-5}). This association was also seen in rs7202116 within the total population. In only unhealthy metabolic obese (MUHO) subgroups four new FTO markers (rs1421085, rs1121980, rs1558902 and rs8050136) (P-value<0.01) demonstrated significant association, even after lipid profile adjustment.

Conclusion: In the present study, we investigated the association between obesity phenotypes and some variations in *FTO/CETP* genes for the first time. Our study showed that four markers in the first intron of the *FTO* gene should be the risk marker in MUHO participants.

Abbreviation

MetS: Metabolic Syndrome; FTO: Fat Mass and Obesity Associated Gene; CETP: Cholesteryl Ester Transfer Protein; SNP: Single Nucleotide Polymorphisms; TCGS: Tehran Cardio-metabolic Genetics Study; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist Circumference; HC: Hip Circumference; FPG: Fasting Plasma Glucose; HDL-C: High-Density Lipoprotein Cholesterol; TG: Triglycerides; TC: Total Cholesterol; CV: Coefficients of Variation; MHNW: Normal Weight without Mets as Metabolically Healthy and Normal Weight; MHOW: Overweight Without Mets; MUHOW: Overweight With Mets; MHO: Obese Without Mets; MUHO: Obese With Mets; OR: Odds Ratios; GWAS: Genome-Wide Association Studies; JBTS: Joubert Syndrome Type 7

Introduction

Along with the epidemic of obesity, concomitant metabolic derangements pose obese individuals at greater risk for future morbidity and mortality [1,2]. Metabolic syndrome (MetS) is a disorder of energy utilization and storage and could increase the risk of developing cardiovascular disease and diabetes. Abdominal obesity, insulin resistance, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) and hypertension are important clinical traits for this syndrome [3]. A combination of obesity and metabolic components leads to the evolvement of different obesity phenotypes that may have different risks for future health outcomes [2,4].

Human population genetic associations have shown a strong and significant association between the fat mass and obesity associated gene (*FTO*) polymorphisms and obesity [5-8]. However, limited studies are available on the effect of FTO markers on lipid concentration in overweight and obese individuals [9]. An association study between FTO and the cholesteryl ester transfer protein (*CETP*) gene variations in relation to lipid profile concentration showed significant association [5,9,10].

FTO is responsible for production of 2-oxoglutarate-dependent nucleic acid demethylase in various tissues and is most abundant in the hypothalamus - the control center of energy balance [11]. This gene is known as one of the most effective genes in human metabolic pathways with nearly 10,000 variations. The *CETP* gene codes a protein that is involved in the transfer of neutral lipids like cholesteryl ester and triglyceride among lipoprotein particles. It also allows the net

movement of cholesteryl ester from high-density lipoproteins/HDL to triglyceride-rich very low-density lipoproteins/VLDL, and the equimolar transport of triglyceride from VLDL to HDL [12,13].

Given the scarcity of data in genetic studies on different obesity phenotypes, we aimed to examine the interaction of 9 remarkable single nucleotide polymorphisms (SNP) in FTO and CETP with lipid profiles among these mentioned phenotypes in the Tehran Cardio-metabolic Genetics Study (TCGS).

Materials and Methods

Population

Subjects were selected from the ongoing Tehran Cardio-metabolic Genetics Study (TCGS) which is an ongoing genetic study involving a cohort designed to determine the risk factors for major non-communicable disorders in the Tehran population referred to as the Tehran lipid and glucose study [14,15]. Written consent was obtained from each subject and the research council of the Research Institute of Endocrine Sciences of the Shahid Beheshti University of Medical sciences approved the study.

Demographic information and biochemical analysis

Information for age, sex and history of using medication for diabetes, hypertension and lipid disorders were collected with a standardized questionnaire. Weight and height were recorded using standard protocols [16]. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. Systolic blood pressure (SBP), Diastolic blood pressure (DBP) and anthropometric variables such as Waist circumference (WC) and Hip circumference (HC) were measured as described previously [17]. Fasting plasma glucose (FPG), Triglycerides (TG), Total cholesterol (TC) and High-density lipoprotein cholesterol (HDL-C) levels were measured by Pars AzmunCo (Iran); in addition, Coefficients of variation (CV) for total cholesterol, HDL-C and triglyceride measurements were below 5% [18]. Non-HDL-C was calculated by subtracting HDL-C from TC [19]. LDL-C concentrations were calculated using modified Friedewald's equation [20].

Genetic analysis

Genomic DNA from 954 subjects was extracted from peripheral blood using the standard Proteinase K, salting-out method [21]. Nine selected polymorphisms (FTO polymorphisms located in intron: rs6499640, rs1421085, rs1558902, rs1121980, rs8050136, rs7202116; CETP polymorptahisms located in upstream and intron: rs3764261, rs1800775, rs1864163) were studied with the T-ARMS assay. In each assay, there were two different inner allele-specific primers to produce allele-specific PCR products. Two outer primers produced a PCR product to use as an internal control for reaction. For all studied SNPs, the PCR reaction was optimized in a 12.5 μl total volume containing 1.5 μl DNA template, 6.25 μl Master Mix containing $MgCl_2$, Smart Taq polymerase (CinnaGene co; Iran) and BSA 0.1% (TaKaRa; Japan) and 2 μl primer containing (outers and inners) and 2.75 μl water. Details of the primers information and final fragments size are mentioned in Table 1. The PCR products were separated by size via agarose gel electrophoresis so each genotype generated a special band. Accuracy of results was confirmed by direct sequencing of 10% samples using outer primers.

SNP	Alleles	-	Primers	TM	length	Homozygote (bp)	Heterozygote (bp)	Homozygote (bp)
rs1421085	T>C	OF	GTTGCATCGCCAGACTGTCTCTAAG	63.6	25	TT: 357, 190	TC: 357, 223, 190	CC: 357, 223
		OR	AATGCTTCTGGACAGTGCGTAGACTA	63.9	26			
		IF	AGCAGTTCAGGTCCTAAGGCATCAT	63.9	25			
		IR	CCTACAAATTCTCATCAGACACTTAATCACTG	62.4	32			
rs1558902	T>A	OF	TATAGTAACCACCACTGAGCATTGTTATG	63.1	29	TT:376, 256	TA: 376, 256,175	AA: 376, 175
		OR	CCTACCACCCTGTTTACCTACTCATTAC	63.1	28			
		IF	TGTCTAGCACTGTGGGTTTACATTTGA	64.3	27			
		IR	GTACGTTGCAGCAATAACCTACCTTAA	63.4	27			
rs7202116	A>G	OF	TATGGATATCCCTGTTGGTTGAAGT	59.6	25	AA: 707,249	AG: 707,249,513	GG:707,513
		OR	GAAGAAGATGCATCAGATTATAATTTC	55.4	27			
		IF	CTGGTATCTCTAACTAATCATATAAGCG	57.4	28			
		IR	ACATGCTACACAGTCTAAGATGAAATAT	58.9	28			
rs1121980	G>A-C/T (REV)	OF	TATTGCCTCATGACTATGTTGCCTGCA	64.8	27	GG:621,238	GA:621,436,238	AA:621,436
		OR	GGAGCACAGTGGAAGGATGTTTGTTAT	63.5	27			
		IF	TTCCTAGTCACGTGTCTTGGTACTGTG	64.1	27			
		IR	GGTAGGCGGGTGGATCTGAAATCTTAT	64.2	27			

rs17817449	T>G	OF	ACGGTGAAGAGGAGGAGATTGTGTAACT	66.5	28	TT:568,128	TG:568,489,128	GG:568,489
		OR	TGTAGTAGTAGTGACAGAAGTGGAGAAA	58.7	28			
		IF	GTTTCAGCTTGGCACACAGAATCG	65.4	24			
		IR	AGGAGCGGGACTGTTAAATTAAAGCA	66.5	26			
rs8050136	C>A	OF	CCAACCAAGGTCATTATAGGAAGAGCT	62.5	27	CC:530,342	CA:530,342,237	AA:530,237
		OR	TACATCCTGAGCTCTGCCACTATACCA	64.6	27			
		IF	ATGCAAGTTGACCACTGTGGCTATC	63.6	25			
		IR	GCAAAAACCACAGGCTCAGATACTT	62	25			
rs9939609	T>A	OF	GGTGGTACGCTGCTATGGTTCTACA	64.4	25	TT:455,306	TA: 455,306,200	AA: 455,200
		OR	TCAGCCTCTCTACCATCTTATGTCCAA	62.9	27			
		IF	GGTTCCTTGCGACTGCTGTGAATATA	63.3	26			
		IR	AACAGAGACTATCCAAGTGCATCGCA	64.4	26			
rs9939973	G>A	OF	CTCAAGTGATTTACCCATTTCAGTGCTCCAA	65.5	31	GG:479,227	GA:479,227,301	AA:479,301
		OR	CTGGCTCATGGTGTGTGTCATCTCCTG	67	27			
		IF	AGCACCCAAGGGACCATCAAACAGA	66.2	25			
		IR	CTTCGCATTCCCTCTCCACAACTGC	66	25			
rs6499640	G>A	OF	ATCTGCTCTTAATGTGGAAACTGTGG	61.5	26	GG:577,206	GA:577,206	AA:577,424
		OR	ATATTCAAACCCTCAACTCTACCAGCT	62	27			
		IF	TGTGTAAGGAACAGGGTTTATCTAAAG	59.1	27			
		IR	CTGATGGTAGAGTATTTCAAAGATGCT	59.3	27			

OF: Outer Forward Primer; OR: Outer Reverse Primer; IF: Inner Forward Primer; IR: Inner Reverse Primer.

Table 1: Specific information for selected markers.

Definition

The metabolic syndrome was defined according to the joint interim statement (JIS) definition as the presence of at least three of the following criteria [22]. a) Abdominal obesity (increased WC ≥ 91 cm in females and males) based on national cut-offs [23], b) TG ≥ 150 mg/dl or receiving treatment for hyper triglyceridemia, c) HDL<50/40 mg/dl in F/M, d) SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or receiving treatment for hypertension, e) FPG ≥ 100 mg/dl or previously diagnosed type 2 diabetes.

Statistical analysis

All participants were classified into 3 categories according to their BMI: normal weight (<25 kg/m²), overweight (25 to 29.9 kg/m²) and obese (≥ 30 kg/m²). Then, subjects in BMI groups were classified to five subgroups: 1) normal weight without MetS as metabolically healthy and normal weight (MHNW) as a reference group; 2) overweight without Mets (MHOW); 3) overweight with Mets (MUHOW); 4) obese without Mets (MHO) and 5) obese with Mets (MUHO). Given limited study subjects in metabolically unhealthy and normal weight subgroups (n=17), they were not included in the study.

All continuous variables for describing population characteristics were expressed as mean and standard deviation, whereas categorical variables were summarized as frequencies and percentages. The mean differences were examined by one-way ANOVA. Differences comparing between two groups were calculated using the chi-square test and odds ratios (OR). Logistic regression analyses in the entire population were performed under an additive model to estimate the associations of each SNP with phenotypic parameters related to obesity and lipid profile. The lipid concentration was calculated with mean of valid present measurements after age and sex adjustment. The significance of deviations of observed genotype frequencies from those predicted by the Hardy-Weinberg equation were evaluated with χ^2 test. Statistical significance was considered at the level of $p<0.05$. Allelic analysis were done by Power Marker v.3.25 and the remainder were done by PASW statistics software (Ver18) [24,25].

Results

The baseline characteristics and allelic frequency in the general population (n=954) and obesity phenotype subgroups are presented in Table 2. The present population with 47.9% men and the mean ± SD of the age in the total population 43 ± 16 were examined. Among all participants 15.7% were smoker and 14.4% were under blood lipid

treatment. Sub-group analysis of overweight with and without MetS (MHOW, n=247; MUHOW, n=210, respectively) and obese subjects with and without MetS (MHO, n=94; MUHO, n=195, respectively) and versus reference group (MHNW, n=208) showed significant differences in lipid profile and anthropometric parameters except in SPB, TG and HDL-C in MHOW and HDL-C in MUHOW. None of the studied variations deviated from Hardy-Weinberg equilibrium in the general

population (p>0.05). Minor allele frequency (MAF) for FTO and CETP results showed the lowest frequency in rs1864163. The association between genetic markers and obesity phenotype subgroups were analyzed. The comparison between reference group and sub-groups showed the presence of four significant risk alleles in the *FTO* gene (rs1421085, rs1121980, rs1558902 and rs8050136) in only the MUHO group (P-value<0.01) (Table 2).

Statics	Total Population	Normal weight Non MetS	Overweigh(n=457)		Obese (n=289)	
	(n=945)	(n=208)	MetS (n=247)	Non MetS(n=210)	MetS (n=94)	Non MetS (n=195)
Age (Year)	43 ± 16	36 ± 16	40 ± 15	53 ± 16	44 ± 14	49 ± 14.7
Male (%)	47.9	48.7	52.9	53.3	35.9	41.7
Smoker (%)	15.2	14.8	16	14.1	9.4	14.7
Lipid lowering drug user (%)	14.4	2.5	6.2	17.8	7	17.8
Systolic blood pressure	116 ± 18	108 ± 15	112 ± 16	128 ± 19[†]	114 ± 13[†]	128 ± 20[†]
Diastolic blood pressure	77 ± 10	72 ± 9	74 ± 8[†]	83 ± 11[†]	76 ± 8[†]	84 ± 10[†]
Waist circumference (cm)	94 ± 12	82 ± 8	92 ± 7[†]	98 ± 6[†]	104 ± 9[†]	107 ± 9[†]
Hip circumference (cm)	101 ± 8	93± 5	100 ± 4[†]	99 ± 5[†]	109 ± 6[†]	108 ± 6[†]
Triglyceride (mg/dl)	152 ± 132	112 ± 58	124 ± 63	232 ± 286[†]	132 ± 62[†]	213 ± 97[†]
Cholesterol (mg/dl)	188 ± 40	178 ± 36	186 ± 38[*]	199 ± 49[†]	193 ± 40[†]	195 ± 39[†]
LDL-C (mg/dl)	46 ± 12	105 ± 31	114 ± 33[†]	117 ± 40[*]	117 ± 35[†]	114 ± 36[*]
HDL-C (mg/dl)	113 ± 34	50 ± 12	48 ± 11	38 ± 9	49 ± 11[†]	41 ± 10[†]
non-HDL-C (mg/dl)	142 ± 40	128 ± 37	138 ± 36[†]	160 ± 49[†]	144 ± 38[†]	155 ± 38[†]
SNP	**Minor Allele Frequency**					
rs6499640 (Intronic)	0.57 (A)	0.35	0.37	0.34	0.36	0.36
rs1421085 (Intronic)	0.39 (C)	0.35	0.38	0.39	0.4	0.48[†]
rs1558902 (Intronic)	0.47 (A)	0.37	0.38	0.38	0.39	0.45[*]
rs1121980 (Intronic)	0.40 (A)	0.26	0.28	0.3	0.29	0.35[†]
rs8050136 (Intronic)	0.36 (A)	0.23	0.26	0.3	0.27	0.33[†]
rs7202116 (Intronic)	0.42 (G)	0.57	0.55	0.56	0.56	0.56
rs3764261 (Upstream)	0.33 (A)	0.24	0.27	0.19	0.29	0.26
rs1800775 (Upstream)	0.53 (A)	0.52	0.55	0.48	0.58	0.56
rs1864163 (Intronic)	0.26 (A)	0.21	0.21	0.21	0.21	0.25
Data are presented as mean ± standard deviation, [*]p-value<0.05, [†]p-value<0.005						

Table 2: Descriptive table and Allelic distribution of the FTO and CETP studied polymorphisms.

Selected lipid profile and anthropometric indices in three main groups (total population, overweight and obese) were compared in relation to all markers and then were presented in Table 3. The most allelic significant associations were related to the HDL-C and TG concentration among FTO and CETP markers. In addition, WC and HC in the total population demonstrated significant association with

some SNPs. The HDL_C level in all all groups showed very strong association with CETP markers, especially with the up-stream gene variations: total population (rs3764261, β (95% CI) -0.48(-0.61, -0.35), P=1.0×10^{-11}), (rs1800775, β (95% CI) 0.5(0.36;0.65), P= 1.0×10^{-6}) and (rs1864163,β (95% CI)0.3(0.16;0.43), P= 9.1×10^{-5}) (Table 3).

Statistic		HDL-C (mg/dl)	LDL-C (mg/dl)	non-HDL-C (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Hip circumference	Waist circumference
		SE,β (95% CI)	SE,β (95% CI)	SE,β (95% CI)	SE,β (95% CI)	SE,β (95% CI)	SE,β (95% CI)	SE,β (95% CI)
rs6499640	Total population	0.09,-0.05(-0.22;0.12)	0.09,-0.11(-0.28;0.07)	0.09,-0.08(-0.25;0.09)	0.09,-0.1(-0.28;0.07)	0.08,0.08(-0.08;0.25)	0.08,0.05(-0.11;0.21)	0.08,0.09(-0.07;0.24)
	Overweight	0.13,0.01(-0.25;0.26)	0.13,-0.13(-0.39;0.14)	0.13,-0.08(-0.33;0.17)	0.13,-0.08(-0.34;0.19)	0.12,0.13(-0.12;0.37)	0.09,0.04(-0.14;0.21)	0.08,0.06(-0.1;0.22)
	Obese	0.16,0.1(-0.22;0.41)	0.18,-0.24(-0.59;0.11)	0.16,-0.22(-0.53;0.1)	0.17,-0.2(-0.54;0.15)	0.15,0.04(-0.25;0.33)	0.12,0.12(-0.11;0.35)	0.12,0.2(-0.04;0.44)
rs1421085	General population	0.07,0.07(-0.08;0.21)	0.07,0.01(-0.13;0.16)	0.07,-0.04(-0.17;0.1)	0.07,-0.02(-0.16;0.13)	0.07,-0.09(-0.22;0.05)	0.07,-0.12(-0.25;0.01)	0.07,-0.16(-0.29;-0.03)*
	Overweight	0.11,-0.02(-0.22;0.19)	0.11,-0.01(-0.22;0.2)	0.1,-0.08(-0.28;0.12)	0.11,-0.07(-0.28;0.14)	0.1,-0.1(-0.3;0.1)	0.07,0.04(-0.1;0.18)	0.07,-0.01(-0.14;0.12)
	Obese	0.14,0.09(-0.18;0.36)	0.15,0.03(-0.27;0.33)	0.14,-0.07(-0.34;0.2)	0.15,-0.05(-0.35;0.25)	0.13,-0.23(-0.47;0.02)	0.1,0.14(-0.06;0.33)	0.11,-0.03(-0.24;0.18)
rs1558902	General population	0.1,-0.03(-0.22;0.17)	0.1,-0.04(-0.24;0.17)	0.1,-0.11(-0.31;0.09)	0.1,-0.12(-0.33;0.08)	0.1,-0.2(-0.4;0)*	0.1,-0.25(-0.44;-0.05)*	0.1,-0.22(-0.41;-0.03)*
	Overweight	0.15,-0.21(-0.5;0.08)	0.15,-0.03(-0.32;0.26)	0.15,-0.04(-0.33;0.25)	0.15,-0.11(-0.4;0.18)	0.14,-0.01(-0.29;0.26)	0.1,-0.12(-0.32;0.08)	0.1,-0.11(-0.3;0.08)
	Obese	0.19,-0.05(-0.43;0.33)	0.22,-0.02(-0.45;0.42)	0.2,-0.28(-0.68;0.12)	0.21,-0.3(-0.72;0.12)	0.21,-0.59(-1;-0.18)*	0.16,0.05(-0.27;0.37)	0.16,0.05(-0.26;0.37)
rs1121980	General population	0.07,0.06(-0.08;0.21)	0.07,0.04(-0.11;0.18)	0.07,-0.02(-0.16;0.12)	0.07,0(-0.15;0.14)	0.07,-0.09(-0.23;0.05)	0.07,-0.14(-0.28;-0.01)*	0.07,-0.18(-0.31;-0.05)*
	Overweight	0.11,-0.07(-0.28;0.14)	0.11,0.01(-0.2;0.22)	0.1,-0.04(-0.24;0.16)	0.11,-0.05(-0.26;0.16)	0.1,-0.04(-0.24;0.16)	0.07,0.04(-0.11;0.18)	0.07,-0.01(-0.14;0.12)
	Obese	0.14,0.16(-0.11;0.44)	0.16,0.08(-0.23;0.38)	0.14,-0.06(-0.34;0.22)	0.15,-0.02(-0.32;0.29)	0.13,-0.3(-0.55;-0.05)	0.1,0.08(-0.12;0.28)	0.11,-0.06(-0.28;0.15)
rs805036	General population	0.07,0.06(-0.08;0.2)	0.07,-0.05(-0.19;0.09)	0.07,-0.1(-0.24;0.04)	0.07,-0.08(-0.22;0.06)	0.07,-0.11(-0.24;0.02)	0.07,-0.14(-0.27;-0.01)*	0.06,-0.17(-0.3;-0.05)*
	Overweight	0.1,-0.02(-0.23;0.18)	0.11,-0.07(-0.27;0.14)	0.1,-0.12(-0.32;0.08)	0.11,-0.12(-0.33;0.08)	0.1,-0.08(-0.28;0.11)	0.07,0.05(-0.08;0.19)	0.06,-0.02(-0.15;0.11)
	Obese	0.13,0.08(-0.18;0.35)	0.15,-0.02(-0.31;0.28)	0.13,-0.14(-0.4;0.12)	0.15,-0.11(-0.4;0.18)	0.12,-0.29(-0.53;-0.04)*	0.1,0.06(-0.13;0.25)	0.1,-0.04(-0.25;0.16)
rs7202116	General population	0.09,0.2(0.02;0.38)*	0.1,-0.04(-0.23;0.15)	0.1,-0.14(-0.32;0.05)	0.1,-0.08(-0.27;0.11)	0.1,-0.28(-0.47;-0.09)*	0.09,0.03(-0.15;0.21)	0.09,-0.01(-0.19;0.17)
	Overweight	0.15,0.32(0.03;0.61)*	0.14,-0.06(-0.35;0.22)	0.14,-0.16(-0.44;0.12)	0.15,-0.08(-0.36;0.21)	0.14,-0.3(-0.57;-0.03)*	0.1,-0.01(-0.2;0.19)	0.1,-0.06(-0.24;0.13)
	Obese	0.15,0.24(-0.05;0.53)	0.18,-0.03(-0.38;0.32)	0.16,-0.2(-0.52;0.12)	0.17,-0.13(-0.47;0.2)	0.16,-0.48(-0.8;-0.16)*	0.13,0.13(-0.13;0.38)	0.12,0.02(-0.23;0.26)
rs3764261	General population	0.07,-0.48(-0.61;-0.35)*	0.07,-0.04(-0.18;0.1)	0.07,0.02(-0.12;0.15)	0.07,-0.11(-0.25;0.03)	0.07,0.13(0;0.26)	0.07,-0.06(-0.19;0.07)	0.06,-0.06(-0.18;0.06)
	Overweight	0.1,-0.49(-0.68;-0.3)*	0.1,-0.06(-0.26;0.14)	0.1,0.02(-0.17;0.21)	0.1,-0.1(-0.3;0.11)	0.1,0.11(-0.08;0.3)	0.07,-0.14(-0.28;-0.01)*	0.06,-0.13(-0.25;-0.01)*
	Obese	0.12,-0.51(-0.75;-0.26)*	0.14,-0.23(-0.51;0.05)	0.13,-0.17(-0.42;0.09)	0.14,-0.3(-0.57;-0.03)*	0.12,0.09(-0.14;0.33)	0.09,0.02(-0.17;0.2)	0.1,0.07(-0.12;0.27)
rs1800775	General population	0.07,0.5(0.36;0.65)*	0.08,0.09(-0.06;0.24)	0.08,0(-0.15;0.15)	0.08,0.13(-0.02;0.29)	0.07,-0.16(-0.3;-0.02)*	0.07,-0.01(-0.15;0.13)	0.07,-0.02(-0.15;0.12)

	Overweight	0.11,0.34(0.12;0.56)*	0.11,0.25(0.03;0.47)	0.11,0.15(-0.07;0.36)	0.11,0.23(0;0.45)*	0.11,-0.09(-0.3;0.12)	0.08,0.01(-0.14;0.16)	0.07,-0.03(-0.17;0.1)
	Obese	0.13,0.51(0.25;0.77)*	0.15,0.15(-0.15;0.44)	0.14,0.05(-0.22;0.32)	0.15,0.19(-0.1;0.48)	0.13,-0.1(-0.35;0.15)	0.1,-0.02(-0.21;0.18)	0.11,0(-0.21;0.2)
rs1864163	General population	0.07,0.3(0.16;0.43)*	0.07,0.04(-0.11;0.18)	0.07,-0.03(-0.17;0.1)	0.07,0.05(-0.09;0.19)	0.07,-0.15(-0.28;-0.02)*	0.07,-0.16(-0.29;-0.03)*	0.06,-0.08(-0.21;0.04)
	Overweight	0.1,0.11(-0.09;0.31)	0.11,0.01(-0.2;0.22)	0.1,-0.05(-0.25;0.15)	0.11,-0.03(-0.23;0.18)	0.1,-0.11(-0.3;0.08)	0.07,-0.16(-0.3;-0.02)*	0.06,-0.08(-0.21;0.04)
	Obese	0.12,0.5(0.26;0.74)*	0.14,0.18(-0.1;0.46)	0.13,0.08(-0.18;0.33)	0.14,0.21(-0.06;0.49)	0.12,-0.19(-0.42;0.05)	0.1,0.01(-0.18;0.19)	0.1,-0.03(-0.23;0.16)

*p<0.01

Table 3: Association of SNPs with lipid profile and anthropometric indices in three main groups.

Table 4 presents the results of comparison between MUHO subgroups and the reference group. As mentioned above the presence of risk alleles in four FTO is higher in MUHO group significantly even after adjustment for lipid profile (HDL-C, LDL, NHDL, Chol and TG). In addition, these associations remained after lipid profile adjustment.

Three SNPs rs1421085, rs1121980 and rs8050136 showed strong association (P-value<0.001) with HDL-C, LDL-C, NHDL, TC and TG. However, the rs1558902 had remarkable association with LDL-C, NHDL and TC (P-value<0.01). Conversely, the CETP markers did not show any significant association.

Statistics	Just SNP	HDL-C (mg/dl)		LDL-C (mg/dl)		non-HDL-C (mg/dl)		Cholesterol (mg/dl)		Triglyceride (mg/dl)	
	OR (95% C.I)	Mean ± SD	OR (95% C.I)	Mean ± SD	OR (95% C.I)	Mean ± SD	OR (95% C.I)	Mean ± SD	OR (95% C.I)	Mean ± SD	OR (95% C.I)
rs6499640	0.94(0.54-1.63)	41.79 ± 6.41	0.71(0.4-1.27)	109.63 ± 33.83	0.79(0.46-1.37)	149.42 ± 35.54	0.75(0.43-1.33)	191.21 ± 36.79	0.8(0.46-1.39)	204.63 ± 71.47	0.69(0.37-1.28)
rs1421085	0.47(0.29-0.76)*	40.09 ± 10.46	0.44(0.27-0.73)*	120.87 ± 33.23	0.42(0.26-0.67)*	159.47 ± 36.65	0.41(0.25-0.66)*	199.56 ± 35.82	0.41(0.26-0.67)*	193 ± 57.51	0.36(0.21-0.62)*
rs1558902	0.32(0.13-0.75)*	45.43 ± 12.39	0.46(0.21-1.02)	107.6 ± 30.09	0.36(0.17-0.78)*	139.14 ± 39.58	0.37(0.17-0.83)*	184.57 ± 36.92	0.36(0.16-0.78)*	157.71 ± 55.27	0.5(0.21-1.17)
rs1121980	0.42(0.26-0.69)*	40.41 ± 10.95	0.4(0.24-0.67)*	123.36 ± 32.35	0.37(0.23-0.6)*	161.93 ± 35.51	0.36(0.22-0.59)*	202.34 ± 34.27	0.37(0.22-0.6)*	192.86 ± 58.44	0.34(0.2-0.6)*
rs8050136	0.44(0.28-0.71)*	39.51 ± 11.26	0.37(0.23-0.62)*	120.24 ± 31.7	0.37(0.23-0.59)*	158 ± 35.44	0.37(0.23-0.6)*	197.51 ± 35.24	0.37(0.23-0.59)*	193.49 ± 78.73	0.33(0.19-0.57)*
rs7202116	0.84(0.44-1.63)	46.41 ± 11.03	0.95(0.48-1.87)	125.72 ± 23.74	0.83(0.44-1.6)	162.18 ± 31.22	0.88(0.45-1.74)	208.59 ± 31	0.86(0.45-1.67)	191.88 ± 104.12	1.21(0.56-2.6)
rs3764261	0.97(0.63-1.5)	38.73 ± 8.55	0.48(0.29-0.8)*	109.87 ± 33.24	0.83(0.54-1.29)	149.29 ± 34.81	0.79(0.5-1.24)	188.02 ± 34.88	0.87(0.56-1.34)	199.21 ± 87.18	0.69(0.42-1.13)
rs1800775	0.83(0.51-1.33)	43.46 ± 9.79	1.65(0.95-2.88)	115.75 ± 35.68	0.91(0.56-1.48)	153.94 ± 40.96	1(0.6-1.66)	197.4 ± 40.04	0.88(0.54-1.44)	195.06 ± 74.78	1.25(0.71-2.2)
rs1864163	0.65(0.42-1)	43.07 ± 10.04	1.22(0.75-1.99)	114.87 ± 34.64	0.76(0.49-1.17)	155.53 ± 37.29	0.75(0.48-1.18)	198.6 ± 35.99	0.73(0.47-1.13)	214.57 ± 100.57	0.91(0.55-1.5)

*p<0.05

Table 4: Association of SNPs with lipids profile in metabolic unhealthy and obese (MUHO) population.

Discussion

In present study the association between rs1421085, rs1558902, rs1121980 and rs8050136 in *FTO* gene and lipid profiles in metabolic unhealthy obese (MUHO) phenotypes were reported for the first time, while the healthy metabolic obese subgroup has not demonstrated any significant association. According to the pervious publications the association between HDL-C concentration and all studied *CETP* gene markers were shown in the general population, overweight and obese subgroups in our study [26-29]. In addition, one of the *FTO* gene (rs7202116) markers has presented this kind of association. This interesting and new finding inspired deeper inquiry into this kind of association and desire to make clear the role of the *FTO* gene in metabolic pathways.

Thus far, genome-wide association studies (GWAS) have identified approximately 75 obesity-susceptibility loci [30,31]. Fat mass and obesity associated gene (*FTO*) was the first obesity-susceptibility gene identified through GWAS and continues to be the locus with the largest effect on BMI and obesity risk factors, most widely replicated with a variety of obesity traits throughout the life course [31].

FTO located on 16q12.2 and RPGRIP1L is adjacent to and coded for on the opposite DNA strand to FTO [32]. RPGRIP1L is involved in Joubert syndrome type 7 (JBTS), which presents clinically with cerebellar and brainstem malformation and renal failure. These patients do not present with any obvious body weight-related phenotypes [33]. Some studies believe there is evidence for co-regulatory mechanisms between FTO and RPGRIP1L, with a possible overlapping regulatory region within FTO intron that contains at least two putative transcription factor binding sites (CUX1). As mentioned earlier, one gene overlaps with other obesity associated SNPs and it remains a possibility that the association between FTO SNPs and body weight regulation is mediated through changing the expression of both FTO and RPGRIP1L [32,34].

Some GWASs reported FTO to be an obesity susceptibility gene and each identified a different SNP in the first intron as the most significantly associated with BMI; i.e. rs99396098, rs99305069. Three large-scale GWAS in East Asian populations (Korean (27), Chinese (29), and Japanese (28)) identified different FTO SNPs (rs9939609, rs17817449, rs12149832, respectively) as the most significantly associated with BMI. Nonetheless, these studies did not report the metabolic effects of FTO on the obese population.

In 2014, a Chinese research group reported the association analysis of FTO markers among adolescents who are overweight and normal weight. They found that BMI was higher in wild TT genotypes (rs9939609: P=0.038; rs1558902: P=0.038), CC genotypes (rs8050136: P=0.024) and GG genotypes (rs3751812: P=0.024) but after the adjustment for multiple testing no significance was shown. Also, they reported in case-control studies and haplotype analyses that the mentioned SNPs were not significantly associated with being overweight [26]. However, based on our results we believe that it is better to use obesity phenotypes in future studies to replicate this finding in order to shed light on the role of the *FTO* gene on weight gaining and metabolic pathways.

The major limitation of this study was the limited number of subjects in our subgroups due to cost and time limitation. Moreover, we focused on only on a few polymorphisms in intronic region of *FTO* gene and promoter area of the *CETP* gene, so we cannot comment with absolute certainty about the performance and function of those genes. On the other hand, strengths of our analysis include the examination and assessment of different overweight and obese phenotypes based on MetS in genetic association.

In conclusion, this is the first study which investigates the association between obesity phenotypes and some variations in *FTO* and *CETP* genes in the Middle East region. Our study showed the risk alleles of some FTO markers in the first intron have effects on only unhealthy metabolic obese (MUHO) participants and not metabolic healthy obese (MHO) participants. Although, for further evaluation of the associations between the polymorphisms and obesity risk, a larger sample size of various ethnic populations is indicated. In addition, investigation of this chromosomal region is essential to clarify the role of the *FTO* gene.

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