The Effect of Interactions of Single Nucleotide Polymorphisms of APOA1/APOC3 with Food Group Intakes on the Risk of Metabolic Syndrome

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Abstract

Background: The aim of this study was to examine the interaction of dietary food groups and genetic variants of APOA1/APOC3, relative to Metabolic Syndrome (MetS) risk in adults.

Methods: In this matched nested case-control study, 414 MetS subjects and 414 controls were selected from among participants of Tehran Lipid and Glucose Study. Dietary intake was assessed with the use of a valid and reliable semi-quantitative food frequency questionnaire. Single Nucleotide Polymorphisms (SNPs), APOA1 (rs670, -75G>A and rs5069, +83C>T/APOC3 rs5128 C3238>G) were genotyped by the conventional polymerase chain reaction and restriction fragment length polymorphism.

Results: The mean (SD) of age was 40.7 (13) and 41.2 (13) years in male cases and controls versus 44.0 (11) and 44.0 (12) years in female case and controls. A significant interaction between intake quartiles of the sugar group and APOA1 combined group (GA+AA/CT+TT) SNPs was found; The ORs for these genotype carriers were (1, 0.44, 0.36, 0.23; P trend<0.001) in quartiles of intake, relative to other combined genotypes (P interaction=0.02). MetS risk appeared to be increased significantly in higher quartiles of sweet beverages and fish intakes in the GA+AA/CT+TT/CC genotypes of APOA1/APOC3 SNPs, compared to other genotypes (P interaction=0.01). The combined effect of genotypes of APOC3/APOA1 showed further decrease in MetS risk in higher quartiles of sugar group intakes (OR: 1, 0.24, 0.26, 0.14, P trend=0.001) relative to other combinations (P interaction=0.008).

Conclusion: Results obtained demonstrate that some dietary food groups (sugar, fish, and sweet beverages) modulate the effect of APOA1/APOC3 SNPs in relation to MetS risk.

Keywords: Food, Genetic polymorphisms, Metabolic Syndrome

Introduction

The worldwide increase in the incidence of the Metabolic Syndrome (MetS) and diabetes mellitus over the last few decades is a salient example of how the interaction between lifestyle and genotype can dramatically impact health. Some of good reasons for this interaction are the familial nature of MetS, the obvious difference in the prevalence of MetS within various racial groups and the different rates of MetS prevalence among monozygotic twins. Interactions between genes and diet play important roles in the development of MetS, although contribution of each per se can be different. For example, in a study conducted on 303 elderly pairs of twins, it was observed that glucose intolerance, obesity and low concentrations of High Density Lipoprotein Cholesterol (HDL-C) are significantly higher among monozygotic twins than among dizygotic twins, indicating the outstanding role of genetics in this area. On the other hand, in the case of
insulin, triglyceride levels and blood pressure, the role of environmental factors including diet were more important than genetics. 

Decreased HDL-C concentration is a hallmark of the dyslipidemic profile associated with the development of MetS. Apolipoprotein A1 (APOA1) is one of the major structural and functional protein constituents of HDL-C and an in vivo activator of Lecithin-Cholesterol-Acyltransferase (LCAT). An inverse relationship has been reported between apoA1 and HDL-C and CVD in general populations. The gene encoding APOA1 is highly polymorphic and several Single Nucleotide Polymorphisms (SNPs) have been identified in the apoA1 gene located on the long arm of chromosome 11, in association with plasma lipid and lipoprotein concentrations. Among these, a common G/A substitution located at −75 bp upstream from the apoA1 transcription start site (rs670) and a C/T substitution at position +83 bp (rs5069) from the first intron were reported to differentially affect apoA1 expression. Some studies have shown that allele A is strongly associated with higher HDL-C and APOA1. Moreover, MetS risk was exacerbated among individuals who had high intakes of fat (>35% energy) and monounsaturated fat (>14% energy) and GG homozygote for the APOA1 rs670. The relevance of APOC3 to human triglyceride (TG) rich lipoprotein metabolism comes primarily from genetic studies, which have consistently shown associations between the APOC3 locus and TG concentration. APOC3 inhibits lipolysis of very low-density lipoproteins via non-competitive inhibition of lipoprotein lipase and hepatic triglyceride lipase activity, and by interfering with lipoprotein binding to glycosaminoglycans on cell membranes. Of several variants within APOC3, a transversion from C to G in the 3′ untranslated region (3′UTR) of exon 4 results in an rs5128 polymorphism (also known as SstI, sacI, 3238C>G or 3175C>G) in the 3′ untranslated region (3′UTR) of exon 4. One study showed that higher levels of Low Density Lipoprotein Cholesterol (LDL-C) and total cholesterol were associated with a higher saturated fatty acid intake for the G allele of APOA1 G-75A (rs670) and higher LDL-C was associated with a higher saturated fat and total fat intake for allele C of APOC3 3238C>G (rs5128). Due to the fact that until now, no study has investigated interactions between food groups and APOA1/APOC3 SNPs, the main goal of present study was to examine the interaction of dietary food groups and some genetic variants of APOA1/APOC3, relative to MetS risk in adults.

**Materials and Methods**

**Study population**

This nested case-control study was a part of the Tehran Lipid and Glucose Study (TLGS), an ongoing population based cohort study being conducted to determine the risk factors of non-communicable diseases on a sample of residents of district 13 of Tehran, the capital of Iran. The baseline examination survey was conducted from 1999 to 2001 in 15005 subjects, aged ≥3 years, and follow-up examinations have been conducted every 3 years (2002-2005; 2006-2008; 2008-2011) to update health related measurements of baseline characteristics and to identify any newly developed diseases.

Of 11001 and 9807 individuals, aged ≥18 years, who participated in baseline and second follow-up surveys respectively, 5280 were excluded because of having MetS at either baseline or second follow-up survey. In the current study, 503 cases were randomly selected from among participants who developed MetS at the third (n=918) and fourth (n=827) follow-up surveys. After excluding individuals with a history of cardiovascular events, weight loss or gain >5 kg in the last 6 months, pregnant or lactating women, or those taking any CVD/anticoagulant/steroid or hormonal medication (n=20), 483 cases were included in the study. Controls were defined as participants with ≤1 MetS components at the time that the corresponding case developed MetS. Each case was individually pair matched with a control, randomly by age (±5 years) and sex. After excluding cases/controls lacking DNA purification in the range of 1.7<A260/A280<2(n=108), and those whose reported energy intake divided by the predicted energy intake did not qualify for the ±3 SD range (n=30), finally, 828 (414 pairs) MetS and matched controls remained for the analysis. The study protocol was approved by the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Written informed consent was obtained from each participant.

**Dietary intakes**

Dietary intake was assessed with the use of a valid and reliable, 168-item, semi-quantitative food frequency questionnaire (FFQ), which included a list of foods with standard serving sizes. Based on macronutrient composition and using available literature, 25 food groups were categorized including different kinds of green, red-yellow, starchy and other vegetables (squash, eggplant, onions, garlic, cabbages, peppers and chilli), fruits (all kinds of fruits and dried fruits), nuts and seeds, legumes, eggs (all kinds of preparations), liquid oils, solid oils (butter and hydrogenated fats), low fat dairy, high fat dairy, whole grains (whole wheat breads, flours and biscuits, barley, wheat, corn and sprouts), refined grains (rice, macaroni, white bread and flours), fast foods (processed meats, fried potato and pizza), soft drinks (all kinds of sugar added beverages), salty snacks (salty biscuits, pickles and chips), red meats, organ meats, fish (all kinds of preparations, tuna fish), poultry (all kinds of preparations), sweets (cakes, donut, biscuits, desserts and chocolates), sweetened fruit juices, sugar (sugar, jam, honey, candy), tea and coffee.

**Anthropometric measurements and blood pressure**

While the subjects were minimally clothed and not
and 1.6% and 0.6% for TG, respectively. Met the acceptable criteria. In ter- and intra-assay coef-
samples were analyzed when internal quality control
analyzer for each day of laboratory analyses, and all
Lipid standard (Cfas, Boehringer Mannheim; catalog
no. 1446070 for Percinorm and 171778 for Percipath).
applicable (Boehringer Mannheim, Germany; catalog
ence range), and Percipath (pathologic range) wherever
20 tests, using lipid control serum, Percinorm (refer-
ting of assay performance was performed once every
measured after precipitation of apolipoprotein B con-
(Par Azmoon, Tehran, Iran) by enzymatic colorimetric
Nutrigenetics, Metabolic Syndrome
wearing shoes, weight was measured using digital
Body Mass Index (BMI) was calculated. Waist Cir-
circles (Vital Scientific, Spankeren, the Nether-
participants sat for 15 min and the mean BP of the 2
measurements recorded was reported as the subject’s
BP.
Physical activity
Physical activity level was assessed using the Per-
sian translated Modifiable Activity Questionnaire
(MAQ) in Tehranian adults, for which high reliability
and relatively moderate validity have been reported.
The frequency and time spent on light, moderate, hard
and very hard intensity activities according to the list
of common activities of daily life over the past year
were obtained. Physical activity levels were expressed
as metabolic equivalent hours per week (METs/h/wk)
22-24.
Laboratory assays
Between 7:00 and 9:00 AM, after 12 to 14 hr of
overnight fasting, blood samples were drawn into
vacutainer tubes in a sitting position from all study
participants 18. Samples were centrifuged within 30 to
45 min of collection according to standard protocols.
All biochemical analyses were performed at the TLGS
research laboratory on the same day of blood collec-
and analyses were conducted using a Selectra 2
autoanalyzer (Vital Scientific, Spankeren, the Nether-
lans). Fasting Blood Glucose (FBG) was measured by
the enzymatic colorimetric method using glucose oxi-
dase. Triglycerides (TG) were measured using TG kits
(Pars Azmoon, Tehran, Iran) by enzymatic colorimetric
tests and with glycerol phosphate oxidase. HDL-C was
measured after precipitation of apolipoprotein B con-
taining lipoproteins with phosphotungstic acid. Moni-
toring of assay performance was performed once every
20 tests, using lipid control serum, Percinorm (refer-
ence range), and Percipath (pathologic range) wherever
applicable (Boehringer Mannheim, Germany; catalog
no. 1446070 for Percinorm and 171778 for Percipath).
Lipid standard (Cfas, Boehringer Mannheim; catalog
no. 759350) was used to calibrate the Selectra 2 auto
alyzer for each day of laboratory analyses, and all
samples were analyzed when internal quality control
met the acceptable criteria. Inter- and intra-assay coef-
cients of variation were both 2.2% for serum glucose
and 1.6% and 0.6% for TG, respectively.
Genetic analysis
For genotyping the APOA1/APOC3 polymor-
phisms, buffy coats were separated from the non-coag-
ulated whole blood samples and stored at -70°C until
processing, when genomic DNA was extracted by the
Protease K, salting out method. The Polymerase
Chain Reaction followed by Restriction Fragment
Length Polymorphism technique (PCR-RFLP) was
employed to investigate polymorphisms in the gene
fragments 25,26.
PCR was done using the following primer; APOA1
(rs670, -75G>A and rs5069,+83C>T) (F: 5’-AGG GAC
AGA GCT GAT CCTGA ACT CTT AAG-3’; R: 5’-
TTA GGG GCA CCT AGCCCT CAG GAA GAG
AGC A-3’/APOC3 (rs5128, C3238>G) (F: 5’-GGT
GAC CGA TGG CTT CAG TT-3’; R: 5’-CAGAAG
GTG GAT AGA GCG CT-3’); hybridization was car-
ried out in a DNA thermal cycler (Corbett co. Aus-
tralia). Amplified DNA was digested with MspI (HpaII)
and SacI restriction enzymes (Fermentas) at 37 °C for
overnight 27; fragments were separated by electropho-
resis on a 2% agarose gel. DNA fragments were visual-
ized by gel documentation (Optigo Co. Optigo, Ijssel-
stein, The Netherlands); 5% of samples were randomly
replicated and genotyped with ≥99% concordance. Five
percent of the PCR samples were directly sequenced to
confirm the PCR-RFLP results.
Definitions
MetS was defined as participants with three or more
of the following conditions by the modified National
Cholesterol Education Program/Adult Treatment panel
III (ATP III) definition 28,29: 1) TG ≥150 mg/dl or drug
treatment, 2) Low density lipoprotein cholesterol
(LDL-C) <40 mg/dl in men and <50 mg/dl in women or
drug treatment; 3) Elevated BP ≥130/85 mmHg or
drug treatment for a previous diagnosis of hypertension, and
4) FBG ≥110 mg/dl or drug treatment of hyperglyce-
mia. WC was coded according to the newly-introduced
cut-off points for Iranian adults, i.e. ≥95 cm for both
genders.
Statistical analysis
Statistical analyses were performed using the Statis-
tical Package for Social Sciences (Version 16.0; SPSS,
Chicago, IL) and STATA (Statistics/Data analysis
12.0). A 2-sided p-value <0.05 was considered signifi-
cant. Pearson’s chi-square statistic was used to test the
Hardy-Weinberg Equilibrium for the SNP and to test
the differences in percentages, using Power-Marker
software. TG concentration was log-transformed for
the statistical analysis. Multiplicative interactions be-
tween quartiles of food group intakes and polymor-
phisms (dominant model) in relation to MetS were
examined using conditional logistic regression analy-
sis, after adjustment for baseline BMI and using the
likelihood ratio test; a comparison of the likelihood
scores of the 2 models with and without the interaction
terms. Conditional logistic regression was used to ex-
amine the joint role of quartiles of dietary pattern
scores and genotypes of rs5128 (CC/CG+GG), rs670
(GG/GA+AA) and rs5069 (CC/CT+TT) in predicting MetS risk, using the lowest quartile of food group intakes and homozygote group with the major allele as the reference category. For combined analyses, the risk genotypes, which comprised the rs670 (GA+AA) and rs5069 (CT+TT) were included in the analysis. When APOC3 rs5128 was included in the analysis, the risk genotype combination comprised rs670 (GA+AA), rs5069 (CT+TT) and rs5128 (CC). Conditional logistic regression was used to examine the joint role of quartiles of food group intakes and genotype combinations in predicting MetS risk, which were adjusted for baseline BMI. To determine the p-value for trend across the quartiles of food group intakes, logistic regression was used with the median of each quartile of food group intakes used as a continuous variable.

## Results

The mean (SD) age of study participants was 42.1 (12) and 42.4(13) years in cases and controls at baseline, respectively. Cases had higher BMI in comparison with controls, 26.1(4) vs. 22.9(4) Kg/m² (p<0.05) at baseline. In MetS subjects, the mean (SD) of MetS risk factors were different with controls at baseline; the low HDL-C concentration (78.5%) and abdominal obesity (74.6%) had the highest prevalence among MetS risk factors. There were no statistical differences in the mean energy and macronutrient intakes, depending on MetS status (Table 1).

No statistically significant differences were observed in genotype frequencies of APOA1 rs670/rs5069 and APOC3 rs5128 between MetS subjects and controls. Genotype frequency did not deviate from Hardy-Weinberg equilibrium expectations, except for rs670 (Table 2).

Adjusted odds ratio for MetS according to quartile classification of food groups by the dominant model of APOA1/APOC3 genotypes are presented in table 3. The risk of MetS was not homogenous in APOA1 rs670 genotypes, across quartiles of sugar and nut group intakes; A allele carriers of rs670 had lower odds of MetS in higher quartiles of sugar group intake (OR: 3.0, 1.60, 1.52, 1.30, P trend<0.01) (P interaction=0.02), compared to the GG genotype (OR: 1, 1.70, 1.31, 2.20, P trend=0.46). The GG genotype group of rs670 and high nut group intake had the highest MetS risk, compared with minor allele carriers and the low nut group intake (P interaction=0.0006). MetS risk appeared to be significantly increased in higher quartiles of fast food (P trend=0.04) and salty snack (P trend=0.006) group intakes in A (allele carriers of APOA1 rs670). Examination of other food groups did not identify a gene-food group interaction with APOA1 rs670 genotypes (data not shown).

| Baseline Age (y)          | With MetS (n=414) | without MetS (n=414) |
|---------------------------|-------------------|----------------------|
| Men (n=470)               | 42.1(12)†         | 42.4(13)             |
| Women (n=358)             | 44.0(11)          | 44.0(12)             |
| Current smokers (%)       | 12.3              | 15.0                 |
| Low physical activity (%) | 36.8              | 33.5                 |
| Education level (≥14 years) (%) | 13.2         | 16.3                 |
| Baseline BMI (Kg/m²)      | 26.1(4)           | 22.9(4) *            |
| Obesity (%)               | 38.6              | 3.6 *                |
| Baseline WC (cm)          | 86.2(11)          | 78.0(10) *           |
| Abdominal obesity (%)     | 74.6              | 5.1                |
| Baseline systolic BP (mmHg) | 114(12)       | 109(11) *           |
| Baseline diastolic BP (mmHg) | 75.0(8)          | 71.5(8) *           |
| Elevated BP (%)           | 43.8              | 2.2                |
| Baseline HDL-C (mg/dl)    | 39.3(10)          | 47.4(10) *           |
| Low HDL-C (%)             | 78.5              | 1.4                 |
| Baseline TG (mg/dl)       | 148(74)           | 101(44) *            |
| High TG (%)               | 72.0              | 0.5                 |
| Baseline FBG (mg/dl)      | 90.0(13)          | 86.5(10) *           |
| High FBG (%)              | 9.4               | 1.0                 |
| Energy intake (kcal/day)  | 2334(1070)        | 2314(979)            |
| Carbohydrate (% of energy)| 58.9(7)           | 58.8(6)              |
| total fat (% of energy)   | 29.9(6)           | 30.1(6)              |
| saturated fat (% of energy) | 9.8 (3)       | 10.6(10)            |
| monounsaturated fat (% of energy) | 10.2(3) | 10.1(2)            |
| Fiber intake (g/1000 kcal/d) | 17.9(7)      | 18.1(7)              |

* P<0.05; †values are mean (SD) unless otherwise listed. BMI: body mass index. WC: waist circumference. BP: blood pressure. HDL-C: high density lipoprotein cholesterol; TG: triglycerides. FBS: fasting blood glucose.

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Avcenna Journal of Medical Biotechnology, Vol. 9, No. 2, April-June 2017
Table 2. Genotype and allele frequency of APOA1 and APOC3 polymorphisms in subjects with metabolic syndrome (MetS) cases and controls. Tehran Lipid and Glucose Study

| Allele frequency rs670 | With MetS (n=414) | without MetS (n=414) | p-value |
|------------------------|-------------------|----------------------|---------|
| A                      | 186(45)           | 197(47)              | 0.20    |
| C                      | 132(32)           | 136(33)              | 0.65    |
| Genotype frequency rs670 |                   |                      |         |
| GG                     | 11(3)             | 7(2)                 | 0.12    |
| GA                     | 217(52)           | 210(51)              |         |
| AA                     | 261(62)           | 259(62)              |         |
| Allele frequency rs5069 |                   |                      |         |
| C                      | 648(78)           | 654(79)              |         |
| T                      | 180(22)           | 174(21)              |         |
| Genotype frequency rs5069 |                 |                      |         |
| CC                     | 258(62)           | 259(62)              |         |
| CT                     | 132(32)           | 136(33)              |         |
| TT                     | 24(6)             | 19(5)                |         |
| Allele frequency rs5128 |                   |                      |         |
| C                      | 645(81)           | 655(81)              | 0.20    |
| G                      | 155(19)           | 157(19)              |         |
| Genotype frequency rs5128 |                 |                      |         |
| CC                     | 261(65)           | 261(44)              |         |
| CG                     | 123(31)           | 133(33)              |         |
| GG                     | 16(4)             | 12(3)                | 0.76    |

* n(%) combined group was found; the ORs for the A and T allele carriers were (1, 2.80, 2.41, 4.0; P trend=0.20) in quartiles of fish group intake, relative to other combined genotypes (2.60, 3.91, 2.90, 1.82; P trend=0.88) (P interaction=0.03). The combination of APOA1 genotypes did not alter the decreasing trend of MetS risk in quartiles of other food group intakes (data not shown). The combined effect of genotypes of APOC3/APOA1 showed further decrease in MetS risk in higher quartiles of sugar group intake (OR: 1, 0.24, 0.26, 0.14, P trend=0.001) relative to other combinations (P interaction=0.008). The genotype carriers (GA+AA/CT+TT/CC) in the highest quartile of fish group intake had greater risk of MetS (OR: 6.27, CI: 1.86-21.2), compared to other genotypes. The SNPs×sweet beverage group intake was seen in relation to MetS risk. Carriers of GA+AA/CT+TT/CC genotypes and high intake of sweet beverage group showed further increase in MetS risk (OR: 1, 4.69, 7.62, 9.58, P trend<0.001) relative to other combinations (OR: 2.98, 3.93, 4.07, 4.41, P trend<0.009) (P interaction=0.03). Other food groups did not modulate the association of combined genotype carriers of APOA1 and APOA1/APOC3 in relation to MetS risk.

Discussion
The interaction between food group intakes and genetic variants of APOA1/APOC3 SNP (rs670, rs5069, rs5128) in relation with MetS risk was assessed in the present nested case-control study of Tehranian adults. Intake of sugar was associated with a lower risk of the MetS among the A allele carriers of rs670, T allele carriers of rs5069, the APOA1 combined GA+AA/CT+TT/CC genotypes. Moreover, subjects with GG genotypes, in the highest quartiles of nuts consumption, in comparison with minor allele carriers with low nuts intake had the highest MetS risk. Sugar-sweet beverages (SSB) intakes interacted with combined APOA1/APOC3 genotypes on MetS risk. Intakes of SSB and fish groups were associated with higher MetS risk among individuals with combined GA+AA/CT+TT/CC genotypes.

The association between changes in the SSB consumption and the incidence of the MetS and its components was assessed in a Spanish Mediterranean cohort of university graduates 30. Participants who increased their consumption of SSB (upper v. lower quintile) after 6 years of follow-up, had a significantly higher risk of developing the MetS (adjusted OR 2.2, 95% CI: 1.4, 3.5; P for trend=0.003); in contrast to this study, the results of another Iranian study are in agreement with those of the current study 31. Khosravi-Boroujeni et al found no significant association between SSB intake and MetS, a lack of significant associations that persisted even after controlling for potential confounders. In addition to this study, Lutsey et al in a well-designed prospective study also showed that consumption of SSBs was not associated with incident MetS among middle-aged adults 32. Another study analyzing data from two well-known cohorts about SSB and genetic risk of obesity, assessed 32 polymorphisms that are known to be associated with BMI and observed that the higher the SSB intakes, the greater the BMI increase per increment of 10 risk allele 33.

Other studies have shown a direct correlation between sugar intake and risk of metabolic syndrome 32,34,35. One of the reasons that might explain why MetS risk was lower in the A allele carrier across the quartiles of sugar intake in the current study, is this fact that jam consumption has been considered in the definition of sugar group intake. Jams are made from different fruits rich in several antioxidants and polyphenols. Polyphenolic compounds are ubiquitous dietary components, mainly flavonoids and tannins. Specific polyphenols, effective in scavenging reactive oxygen and reactive nitrogen species, are able to modulate genes associated with metabolism, stress defense, drug metabolizing enzymes, and detoxification and transporter proteins. Their overall effect is protective in overcoming damaging effects of chronic diseases such as MetS 36. As mentioned above, another finding of the present study showed that consumption of nuts is related to MetS risk. Nuts as a whole are healthy foods because of their good fatty acid profile (low in saturated fats and high in mono- and polyunsaturated fats (MUFA and PUFA, respectively)) and low available carbohy-
Table 3. Adjusted ORs (95% CI) for metabolic syndrome according to quartile classification of food group intakes by dominant model of APOA1/APOC3 genotypes

Table 3. Adjusted ORs (95% CI) for metabolic syndrome according to quartile classification of food group intakes by dominant model of APOA1/APOC3 genotypes

| APOA1 genotypes (rs670) | Q1 | Q2 | Q3 | Q4 | P for trend | P for interaction |
|--------------------------|----|----|----|----|------------|-----------------|
| Fast foods               |    |    |    |    | 0.08       |                 |
| GG (rs670) (n=383)       | 1  | 0.7 (0.35-1.56) | 1.20 (0.57-2.44) | 0.6 (0.27-1.22) | 0.32       |
| GA+AA (rs670) (n=445)    | 0.7 (0.31-1.32) | 1.10 (0.55-2.21) | 1.20 (0.57-2.41) | 1.30 (0.60-2.85) | 0.04       |
| Salty snacks             |    |    |    |    | 0.06       |                 |
| GG (rs670) (n=383)       | 1  | 0.41 (0.19-0.87) | 1.40 (0.64-2.85) | 1.04 (0.50-2.16) | 0.51       |
| GA+AA (rs670) (n=445)    | 0.69 (0.33-1.43) | 1.07 (0.52-2.20) | 1.23 (0.60-2.54) | 1.34 (0.66-2.71) | 0.006      |
| Sugar                    |    |    |    |    | 0.02       |                 |
| GG (rs670) (n=383)       | 1  | 1.70 (0.87-3.44) | 1.31 (0.64-2.77) | 2.20 (1.02-4.48) | 0.46       |
| GA+AA (rs670) (n=445)    | 3.0 (1.44-6.22) | 1.60 (0.87-3.53) | 1.52 (0.76-3.09) | 1.30 (0.66-2.67) | <0.01      |
| Nuts                     |    |    |    |    | 0.0006     |                 |
| GG (rs670) (n=383)       | 1  | 0.87 (0.38-1.64) | 1.31 (0.65-2.63) | 2.40 (1.13-4.91) | 0.01       |
| GA+AA (rs670) (n=445)    | 1.80 (0.86-3.60) | 1.82 (0.93-3.66) | 0.80 (0.30-1.61) | 1.92 (0.96-3.78) | 0.31       |
| APOA1 genotypes (rs5069) |    |    |    |    |            |                 |
| Fish                     |    |    |    |    | 0.04       |                 |
| CC (rs5069) (n=517)      | 1  | 1.34 (0.70-2.57) | 1.07 (0.58-1.96) | 0.63 (0.34-1.18) | 0.75       |
| CT+TT (rs5069) (n=311)   | 0.48 (0.23-0.98) | 1.14 (0.58-2.28) | 0.96 (0.46-1.98) | 1.30 (0.64-2.77) | 0.20       |
| Tea-coffee               |    |    |    |    | 0.007      |                 |
| CC (rs5069) (n=517)      | 1  | 0.78 (0.41-1.47) | 0.32 (0.16-0.61) | 0.67 (0.36-1.27) | 0.09       |
| CT+TT (rs5069) (n=311)   | 0.69 (0.34-1.43) | 0.44 (0.21-0.92) | 0.92 (0.42-1.96) | 0.48 (0.24-0.98) | 0.70       |
| Sugar                    |    |    |    |    | 0.04       |                 |
| CC (rs5069) (n=517)      | 1  | 1.39 (0.76-2.55) | 1.04 (0.54-2.01) | 1.53 (0.84-2.81) | 0.66       |
| CT+TT (rs5069) (n=311)   | 1.97 (0.94-4.13) | 1.21 (0.59-2.49) | 1.11 (0.55-2.23) | 0.73 (0.36-1.47) | 0.003      |
| APOC3 genotype (rs5128)  |    |    |    |    |            |                 |
| Fish                     |    |    |    |    | 0.15       |                 |
| CC (rs5128) (n=522)      | 1  | 0.78 (0.41-1.49) | 1.17 (0.62-2.18) | 1.73 (0.92-3.24) | 0.02       |
| GG+GG (rs5128) (n=284)   | 0.75 (0.36-1.55) | 0.66 (0.32-1.39) | 1.73 (0.82-3.61) | 0.76 (0.34-1.69) | 0.26       |

OR: Odds Ratio, Q: Quartile of food group intakes. APOA1: rs670 (-75G>A), rs5069 (+83C>T), APOC3: rs5128 (3238C>G)

1 ORs (95% CI) were calculated by using conditional logistic regression model, adjusted for baseline BMI and energy intake. Participants were joint classified (8 groups) according to quartiles (Q) of food group intakes and dominant model of rs5128, rs670 and rs5069 genotypes. The lowest quartile of food group intakes and homozygote genotype of major allele were used as the reference group.

Interactions of APOA1 alleles with fast food consumption and genotype of APOC3 polymorphism. Fast foods have a high amount of saturated fat and trans-fatty acid, energy density, glycemic index, sodium, refined carbohydrate and therefore poor diet quality. Besides these negative points, the low dietary phytochemical index and total antioxidant capacity of mentioned food, fast foods have become one of the most important risk factors of MetS.

Some studies showed that carriers of the A allele were more responsive to changes in dietary fat than GG subjects. Results of one study examining the effect of fast food intake on the risk of MetS were consistent with those of the present study, and showed that after adjustment for all potential confounding variables, the risk of MetS, in the highest quartile of fast foods compared with the lowest, was 1.85 (95% CI: 1.17-2.95). In the Qi et al’s study, interaction of fried food consumption with genetic risk in relation to BMI was assessed on 9623 women from the Nurses’ Health Study and 6379 men from the Health Professionals Follow-up Study. Interaction between fried food consumption and genetic risk score was observed based on 32 BMI-associated variants in both the Nurses’ Health Study and Health Professionals Follow-up Study (p≤0.001 for interaction).
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Table 4. Adjusted OR (95% CI)\(^1\) for metabolic syndrome and combined APOA1/APOC3 genotypes according to quartile classification of food group intakes

| APOA1 (rs670/rs5069) | Q1 | Q2 | Q3 | Q4 | P for trend | P for interaction |
|----------------------|----|----|----|----|-------------|------------------|
| Sugar                |    |    |    |    | 0.02        |                  |
| GA+AA/CT+TT (n=229)  | 1  | 0.44 (0.17-1.16) | 0.36 (0.13-0.99) | 0.23 (0.08-0.64) | 0.001         |                  |
| Others (n=599)       | 0.40 (0.17-0.92) | 0.53 (0.23-1.24) | 0.44 (0.19-1.03) | 0.58 (0.25-1.33) | 0.64         |                  |
| Fish                 |    |    |    |    | 0.03        |                  |
| GA+AA/CT+TT (n=229)  | 1  | 2.80 (1.10-6.94) | 2.41 (0.87-6.77) | 4.0 (1.45-10.88) | 0.20         |                  |
| Others (n=599)       | 2.60 (1.15-5.77) | 3.91 (1.64-8.94) | 2.90 (1.26-6.44) | 1.82 (0.78-4.08) | 0.88         |                  |

APOA1 (rs670/rs5069)/APOC3 (rs5128)

| Sugar                |    |    |    |    | 0.008       |                  |
|----------------------|----|----|----|----|-------------|------------------|
| GA+AA/CT+TT/CC (n=147)| 1  | 0.24 (0.07-0.87) | 0.26 (0.06-1.02) | 0.14 (0.04-0.52) | 0.001        |                  |
| Others (n=659)       | 0.24 (0.07-0.70) | 0.32 (0.11-0.97) | 0.22 (0.07-0.69) | 0.32 (0.10-0.95) | 0.55         |                  |
| Fish                 |    |    |    |    | 0.01        |                  |
| GA+AA/CT+TT/CC (n=147)| 1  | 3.29 (1.09-9.92) | 2.13 (0.61-7.33) | 6.27 (1.86-21.2) | 0.09         |                  |
| Others (n=659)       | 2.24 (0.87-5.79) | 3.32 (1.28-8.63) | 3.00 (1.16-7.77) | 1.78 (0.68-4.61) | 0.98         |                  |
| Sweet beverages      |    |    |    |    | 0.03        |                  |
| GA+AA/CT+TT/CC (n=147)| 1  | 4.69 (1.46-15.08) | 7.62 (2.19-26.53) | 9.58 (2.70-33.94) | 0.001        |                  |
| Others (n=659)       | 2.98 (1.17-7.58) | 3.93 (1.51-10.23) | 4.07 (1.62-10.25) | 4.41 (1.72-11.31) | 0.009       |                  |

Regarding the interaction of salty snack consumption and APOA1 genotype on MetS risk, it should be said that salty snack because of the high levels of salt can increase blood pressure over time. Although it is not clear how the mutant allele affects MetS risk, future studies are recommended to clarify the molecular mechanisms underlying these gene-nutrients interactions.

Participants with higher consumption of fish had a high risk of MetS, if they were the carriers of the T allele. Unlike many studies which show protective role of fish consumption on MetS risk, some studies demonstrated no association between fish consumption and risk of MetS. Fish preparation methods (e.g., frying) and certain contaminants in fish may attenuate the potential and beneficial effects of fish consumption. Since most Iranian people eat deep-fried fish, this may limit the protective effect of fish consumption on MetS, not seen in this population. In subjects with the major homozygote of APOA1 rs5069, higher quartiles of fish preparation methods were associated with a higher risk of MetS, if they were the carriers of the T allele. The main strength of the present study was its longitudinal design and the use of appropriate number of cases and matched controls from all demographic strata, and our results also emphasized the importance of taking into account the gene-diet interaction effect in association studies. Excluding subjects who created changes in dietary pattern because of having MetS was the strength of this study. Evaluation of dietary intake at just one time point was one of the limitations. However, assessing dietary intake by FFQ in about one year before developing MetS can cover this limitation. The second limitation was the lack of measurement of insulin sensitivity as a sensitive marker of MetS. For this reason, the present study did not detect any interaction between food groups and genotypes in relation to it. Another limitation of this study was considering the limited number of polymorphisms in the analysis of the interaction between dietary patterns and polymorphism in association with MetS that most polymorphisms in the interaction between dietary patterns and polymorphism in association with MetS do not cover. The small effect size of polymorphisms reduced the probability of observing a significant correlation. Future
studies should investigate the genetic basis of individuals namely the cumulative effect of polymorphisms involved in the desired outcome and calculate their genetic risk score. Due to the limited sample size, there was no possibility of gender segregation in the analyses. Although lifestyle factors such as smoking, physical activity, education levels were considered in this study, inevitably some other confounding factors were not measured.

**Conclusion**

In conclusion, this study provides evidence that some food groups modulate the effect of APOA1 and APOC3 SNPs in relation to MetS risk; being A and T allele carriers of APOA1 (rs670, rs5069) and C allele carriers of APOC3 (rs5128) further increased MetS risk in individuals with higher consumption of sweet beverages and fish groups. Also, A and T alleles of APOA1 appear to be protective for MetS risk in participants with higher scores of the sugar group intake.

**Acknowledgement**

The authors acknowledged Ms. Nilufar Shiva for critical editing of English grammar and syntax of this manuscript. The present study was conducted as a PhD thesis (Grant No. 407) and supported by the Research Institute for Endocrine Sciences, SBMU, Tehran, Iran.

**Conflict of Interest**

None of the authors has any conflict of interest to declare.

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