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Association between the apelin rs2235306 gene polymorphism and metabolic syndrome

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Aim: The rs2235306 of apelin polymorphism has been shown to be associated with fasting plasma glucose levels and hypertension. The present study aimed to investigate the impact of the apelin rs2235306 gene polymorphism on the risk of metabolic syndrome (MeS) in a sample of the Iranian population.

Materials and methods: This population-based cross-sectional study was performed on 151 subjects with MeS and 149 without MeS, as defined by ATPIII criteria. Apelin rs2235306 polymorphism detection was done using the tetra amplification refractory mutation system–polymerase chain reaction. Because the apelin gene is located on the X chromosome, statistical analyses were conducted in a sex-specific manner.

Results: Our findings proposed that the apelin rs2235306 polymorphism was not associated with MeS susceptibility in the codominant, dominant, and recessive inheritance models tested (OR = 0.93, 95% CI = 0.51–1.71 for TC vs. TT; OR = 2.39, 95% CI = 0.70–8.16 CC vs. TT; OR = 1.09, 95% CI = 0.62–1.93 for TC+CC vs. TT; and OR = 2.45, 95% CI = 0.73–8.21 for CC vs. TT+TC). We found that the apelin TC+CC genotypes were associated with lower HDL-cholesterol in women without MeS.

Conclusion: Our findings indicated no association between the apelin rs2235306 polymorphism and MeS. However, the results suggest that healthy females carrying apelin TC+CC genotypes have lower HDL-cholesterol in comparison with those carrying TT, which remains to be confirmed.

Key words: Metabolic syndrome, apelin, polymorphism

1. Introduction

Metabolic syndrome (MeS; also known as the insulin resistance syndrome or syndrome X) has become one of the main public health challenges worldwide. It is characterized by abdominal obesity, increased blood pressure, triglycerides levels, and fasting blood glucose levels and by lower HDL cholesterol levels (1,2). MeS is prevalent worldwide (1,3–6), and it independently predicts the development of type 2 diabetes mellitus and coronary heart disease (7). It has been proposed that lifestyle, environmental, and genetic factors may have effects on the prevalence of MeS. Several candidate gene polymorphisms are involved in MeS (8–14).

Apelin, a peptide that, in humans, is encoded by the *APLN* gene, is the endogenous ligand for the human G-protein coupled receptor APJ (15). The apelin gene is located in the X chromosome (Xq25-26.1) and contains 3 exons, with the coding region spanning exons 1 and 2 (Figure 1). The *APLN* gene encodes a 77-amino acid prepropeptide that is then cleaved to shorter mature peptides, including apelin-36, apelin-17, and apelin-13, which activate the APJ receptor (16). The apelin–APJ system has a wide representation in the central nervous system and in a variety of peripheral tissues, such as the lungs, kidneys, heart, and vasculature, and is emerging as an important regulator of cardiovascular homeostasis (16,17). It stimulates contractility without inducing ventricular hypertrophy, has mild diuretic effects, antagonizes the release of vasopressin centrally, and functions as both an arterial and venous dilator (16,17). Cekmez et al. (18) found that the large-for-gestational-age (LGA) infants with diabetic mothers had significantly higher apelin, HOMA-

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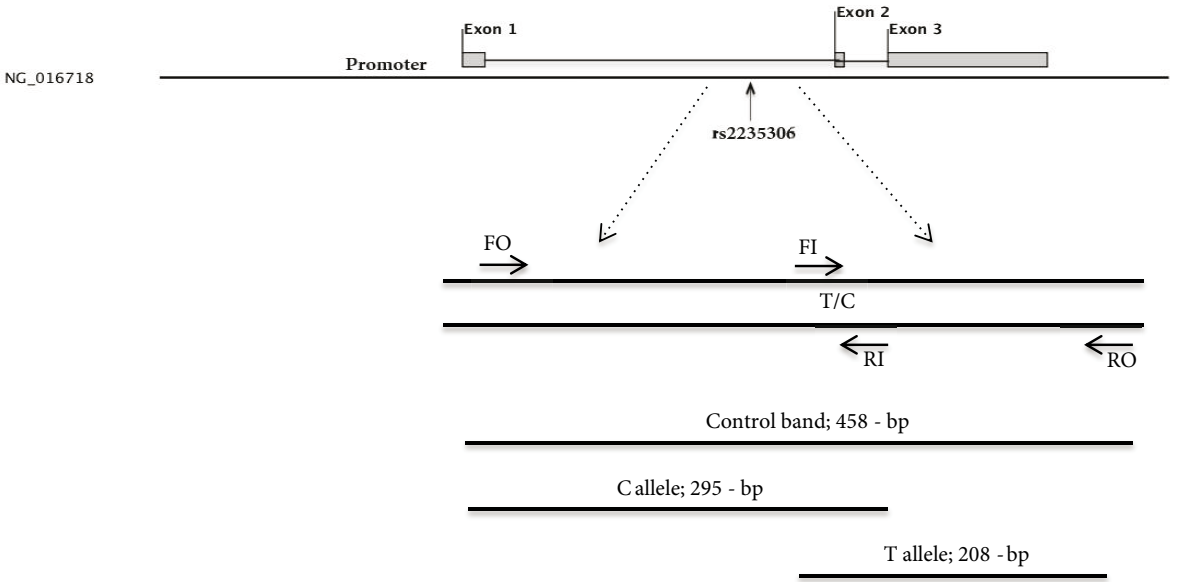

Figure 1. Map of the human apelin gene. Exons 1–3 are numbered. Position of the SNP rs2235306 is shown.

IR values, and fasting insulin levels. The findings suggest that apelin can be used as a good predictor for MeS. In another study, it was reported that apelin polymorphisms are associated with risks of obesity phenotypes (19). There is some evidence that the apelin–APJ system plays a role in blood pressure (20,21). The apelin rs2235306 polymorphism was significantly associated with diastolic blood pressure response to potassium supplements in women (22). To the best of our knowledge, there are no data regarding the association between the apelin gene polymorphism and MeS. On the other hand, it has been proposed that the rs2235306 variant of apelin may be associated with a component of MeS (23,24). Thus, the present study was aimed at finding the possible association between apelin rs2235306 gene polymorphism and MeS in a sample of the Iranian population.

2. Material and methods

This case-control study was performed on 151 individuals with MeS (101 females, 50 males) and 149 without MeS (104 females, 45 males). MeS was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) (25), which establishes the diagnosis by the presence of 3 or more of the following components: abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, high blood pressure, and high fasting plasma glucose levels (1). The project was approved by local ethics committee of Zahedan University of Medical Sciences, and informed consent was obtained from all individuals. Whole-blood DNA was extracted using the salting-out method as described previously (26).

2.1. Genotyping

The apelin rs2235306 polymorphism was determined by tetra amplification refractory mutation system–polymerase chain reaction (T-ARMS-PCR). This is a simple, rapid, and sensitive method for the detection of single nucleotide polymorphisms (SNPs) (27,28).

The apelin genomic sequences (NG_016718.1) were obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), and the primers for T-ARMS-PCR were designed (Table 1).

PCR was performed using a commercially available PCR premix (AccuPower PCR PreMix; BIONEER, Daejeon, Korea), prepared according to the manufacturer's

Table 1. The primers used for the detection of SNP of apelin rs2235306.

Primers	Sequence (5' → 3')
Forward inner (T allele)	CCCCCTGCACACCATCTGCTT
Reverse inner (C allele)	GGGACAGGGATCTAGATGCAGGAAG
Forward outer	AAGTGGTGCAGGGTATCCTTGGGT
Reverse outer	AAGGAGCCAAGGAAGGAACAGAGC

instructions. Briefly, 1 μL of template DNA (~100 ng/μL), 1 μL of each primer (10 pmol/μL), and 15 μL of DNase-free water were added to the AccuPower PCR PreMix.

Amplification was done with an initial denaturation step at 95 °C *for 5 min, followed by 30 cycles of 30 s at 95 °C,* 20 s at 63 °C, and 30 s at 72 °C, with a final step at 72 °C for 10 min (Corbett Research, Australia). PCR products were verified on a 2.0% agarose gel containing 0.5 µg/mL ethidium bromide and photographs were taken (Figure 2).

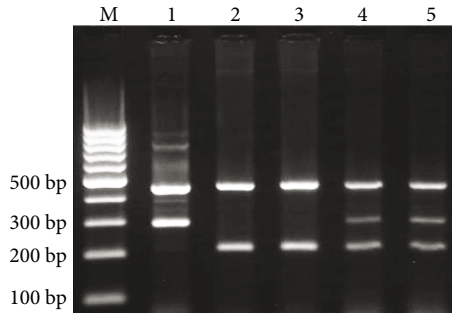

Figure 2. Electrophoresis pattern of T-ARMS-PCR for detection of apelin rs2235306 polymorphism. In the T-ARMS-PCR method, 2 external primers (control band) and 2 inner primers (allele specific primers) are used. The product sizes were 208 bp for T allele, 295 bp for C allele, and 458 bp for control band. M: DNA Marker; Lane 1: rs2235306 CC; Lanes 2, 3: TT; Lanes 4, 5: CT.

Product sizes were 208 bp for the T allele, 295 bp for the C allele, and 458 bp for the 2 outer primers (control band). In order to verify the genotyping quality, we also regenotyped randomly selected samples.

2.2. Statistical analysis

Statistical analyses of the results were done using SPSS 18.0. Since the apelin gene is located on the X chromosome, statistical analyses were conducted in a sex-specific manner. Genotype and allelic frequencies were compared between the groups using the chi-square test. Logistic regression analyses were applied to estimate the odds ratio (OR) and 95% confidence interval (CI) of genetic risks in MeS. A P-value of less than 0.05 was considered statistically significant.

3. Results

In women, the apelin rs2235306 polymorphism was not associated with MeS susceptibility in any of the tested genetic inheritance models (OR = 0.93, 95% CI = 0.51–1.71 for TC vs. TT; OR = 2.39, 95% CI = 0.70–8.16 CC vs. TT; OR = 1.09, 95% CI = 0.62–1.93 for TC+CC vs. TT; and OR = 2.45, 95% CI = 0.73–8.21 for CC vs. TT+TC) (Table 2). The allele frequency of apelin rs2235306 polymorphism was not associated with MeS in women (OR = 0.81, 95% CI = 0.51–1.34 for C vs. T).

Table 2. Analysis of the association of the apelin rs2235306 polymorphism with MeS in females.

Genotypes	MeS (Yes)	MeS (No)	OR (95% CI)	P
Codominant				
TT	63 (62.4)	67 (64.4)	1.00	-
TC	29 (28.7)	33 (31.7)	0.93 (0.51–1.71)	0.877
CC	9 (8.9)	4 (3.8)	2.39 (0.70–8.16)	0.244
Dominant				
TT	63 (62.4)	67 (64.4)	1.00	-
TC+CC	38 (37.6)	37 (35.6)	1.09 (0.62–1.93)	0.773
Recessive				
TT+TC	92 (91.1)	100 (96.2)	1.00	-
CC	9 (8.9)	4 (3.8)	2.45 (0.73–8.21)	0.160
Alleles				
T	155 (76.7)	167 (80.3)	1.00	-
C	47 (23.3)	41 (19.7)	0.81 (0.51–1.34)	0.401

In men, MAF (C allele) was found to be 0.14 and 0.089 in the case and control groups, respectively. The apelin rs2235306 polymorphism was not associated with MeS in males (OR = 2.23, 95% CI = 0.54–9.19 for C vs. T) (Table 3).

In women, the apelin genotype was in HWE in subjects with and without MeS (χ^2 = 2.43, P = 0.118, and χ^2 = 0.15, P = 0.691, respectively).

We evaluated the effect of MeS and apelin gene polymorphism on anthropometric, biochemical, and clinical parameters in women (Table 4). No significant differences between the genotypes TT and TC+CC were found regarding waist circumference (WC), body mass index (BMI), total cholesterol (TC), triglyceride (TG), LDL-cholesterol, fasting blood glucose (FBG), or systolic and diastolic blood pressure in women with or without MeS. However, in subjects without MeS, the levels of HDL-cholesterol were significantly higher in the TT genotype than the TC+CC genotype (P = 0.045) (Table 4).

Regarding menopausal status, 133 women were premenopausal and 72 were postmenopausal. No significant differences were found between the TT and TC+CC genotypes concerning WC, BMI, TC, TG, LDL-cholesterol, FBG, or systolic and diastolic blood pressure in women of premenopausal or postmenopausal status (data not shown).

4. Discussion

Genetic polymorphisms, including FTO (12), paraoxonase (8), TNF-alpha (11), cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA) (29), and angiotensin-1-converting enzyme (ACE) (13), have been shown to be associated with MeS. Recently, we showed an association between genetic variants in vaspin rs2236242 and chemerin rs17173608 gene polymorphisms and the risk of MeS. While the vaspin rs2236242 polymorphism plays a protective role in susceptibility to MeS, the chemerin rs17173608 polymorphism increased the risk of MeS in our population (30).

The current study is the first investigation regarding the possible association between apelin rs2235306 gene polymorphism and the risk of MeS. Our findings showed no significant association between the apelin rs2235306 variant and MeS in a sample of the Iranian population.

Table 3. Analysis of the association of the apelin rs2235306 polymorphism with MeS in males.

Alleles	MeS (Yes)	MeS (No)	OR (95% CI)	P
T	43 (86.0)	41 (91.1)	1.00	-
C	7 (14.0)	4 (8.9)	2.23 (0.54–9.19)	0.321

Table 4. Effect of MeS and apelin rs2235306 gene polymorphism on anthropometric, clinical, and biochemical parameters in women.

Parameters	MeS (Yes) (n = 101)			MeS (No) (n = 104)		
	TT (n = 63)	TC+CC (n = 38)	P	TT (n = 67)	TC+CC (n = 37)	P
WC (cm)	93.48 ± 11.82	92.11 ± 16.09	0.624	89.68 ± 12.86	91.32 ± 12.88	0.536
BMI (kg/m^2)	27.38 ± 4.90	27.67 ± 5.90	0.785	25.92 ± 4.38	27.10 ± 5.27	0.230
TG (mg/dL)	163.51 ± 121.94	159.87 ± 80.32	0.870	142.52 ± 56.60	142.84 ± 69.33	0.980
TC (mg/dL)	198.81 ± 43.07	203.68 ± 45.92	0.592	193.52 ± 40.75	200.68 ± 36.16	0.375
HDL-C (mg/dL)	44.52 ± 8.47	44.11 ± 8.82	0.813	47.52 ± 8.48	44.27 ± 6.51	0.046
LDL-C (mg/dL)	121.58 ± 44.83	127.60 ± 41.93	0.503	117.49 ± 35.77	127.83 ± 33.04	0.158
FBG (mg/dL)	92.79 ± 23.02	97.63 ± 22.84	0.307	94.70 ± 30.77	98.84 ± 39.93	0.557
Systolic blood pressure (mmHg)	121.83 ± 18.62	121.58 ± 19.80	0.956	119.85 ± 22.64	116.89 ± 21.64	0.514
Diastolic blood pressure (mmHg)	76.35 ± 12.61	75.53 ± 16.06	0.775	76.82 ± 14.37	74.14 ± 8.68	0.303

In obese patients, both plasma apelin and insulin levels were significantly higher, suggesting that the regulation of apelin by insulin could influence blood concentrations of apelin (19). Li et al. found a significant association between hypertension and 2 SNPs, rs3761581 and T-1860C, within the apelin gene (21). It was reported that the apelin rs3115757 polymorphism was significantly associated with BMI and WC in women (19). No association was found between this SNP and BMI or WC in men. Zhao et al. (31) found that apelin rs2235306 gene polymorphism was significantly associated with diastolic blood pressure response to potassium supplements among women. In the present study, we found no association between apelin rs2235306 polymorphism and anthropometric, biochemical, and clinical parameters in women. However, the levels of HDL-cholesterol were significantly higher in the TT genotype than the TC+CC genotype in patients without MeS. One limitation of this study was the relatively small sample size. Therefore, subgroup analysis was not possible.

In conclusion, our findings showed, for the first time to the best of our knowledge, that rs2235306 polymorphism in the apelin gene was not associated with susceptibility to MeS in a sample of the Iranian population. The HDL-cholesterol levels were significantly lower in women without MeS who had TC+CC genotypes. Independent studies are required to validate our findings in a larger sample, as well as in patients of different ethnic origins.

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