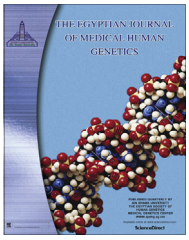
The Egyptian Journal of Medical Human Genetics (2015) 16, 35–40

HOSTED BY

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Apelin rs2235306 polymorphism is not related to metabolic syndrome in Egyptian women

Eman T. Mehanna [a,*], Dina M. Abo-Elmatty [a], Maivel H. Ghattas [b], Noha M. Mesbah [a], Samy M. Saleh [a]

[a] *Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, 41522 Ismailia, Egypt*
[b] *Department of Medical Biochemistry, Faculty of Medicine, Port Said University, 42526 Port Said, Egypt*

Received 27 October 2014; accepted 11 November 2014
Available online 27 November 2014

KEYWORDS

Apelin;
Rs2235306;
Metabolic syndrome;
Polymorphism;
Tetra amplification refractory mutation system.

Abstract *Background:* Apelin is an adipokine that was identified to play a role in the control of glucose homeostasis. Apelin rs2235306 gene polymorphism was linked to insulin resistance and poor glycemic control.

Aim of the study: To assess the relation of apelin rs2235306 polymorphism with metabolic syndrome and its component traits in Egyptian women from Suez Canal area.

Subjects and methods: The study included 100 metabolic syndrome patients and 100 healthy female subjects. The component traits of metabolic syndrome were determined and the genotypes of the polymorphisms were assessed using tetra amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) technique.

Results: There was no significant difference in the allele frequencies between the metabolic syndrome and control groups ($P = 0.841$). There was also no association of the different genotypes of this polymorphism with any of the component traits of metabolic syndrome.

Conclusion: Apelin rs2235306 polymorphism is not associated with the incidence of metabolic syndrome in the studied population.

1. Introduction

Apelin is derived from a 77 amino-acid prepropeptide that produces four active isoforms (apelin-12, 13, 17 and 36), each showing different receptor-binding affinities, from which (Pyr1)-apelin-13 has the highest abundance and activity [1,2]. Apelin exerts its paracrine function by binding and activating the apelin receptor (Aplnr or APJ) [3]. Increasing evidence suggests that apelin regulates multiple physiological functions, including fluid homeostasis, food intake, cell proliferation, blood pressure regulation, angiogenesis and glucose utilization

Abbreviations: T-ARMS-PCR, tetra amplification refractory mutation system polymerase chain reaction; T2DM, type 2 diabetes mellitus; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; SNP, single nucleotide polymorphism; FBG, fasting blood glucose; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; ANOVA, analysis of variance; SD, standard deviation.

* Corresponding author. Tel.: +20 10 26671785; fax: +20 643230741.
E-mail address: emanmehanna22@yahoo.com (E.T. Mehanna).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2014.11.004

[4,5] and therefore is capable of interfering with diabetes, obesity, hypertension or cardiovascular diseases [6,7].

Apelin system is widely expressed in various tissues, including adipose tissue, brain, heart, lungs and kidneys [7]. Apelin production in adipose tissue is regulated by factors, such as fasting and feeding, insulin level [5], hypoxia [8], growth hormone and tumor necrosis factor α levels [9]. Whereas insulin stimulates adipose tissue apelin expression, apelin inhibits insulin secretion [5,9].

Interestingly, apelin-13 has been found to have beneficial effects on high-fat-diet-induced obese mice, improving glucose tolerance and increasing glucose utilization in normal and insulin-resistant mice [10]. Furthermore, intraperitoneal administration of apelin in normal and obese mice for 14 days reduced body adiposity without altering food intake or insulin, leptin and triglyceride levels whereas it increased adiponectin levels [11]. In a well established mice model of type 1 diabetes, chronic apelin-13 treatment significantly ameliorated pancreatic islet mass and insulin production [12].

Considering these physiological actions in the control of glucose homeostasis, it is tempting to propose a link between apelin and obesity-associated insulin resistance. Accordingly, apelin overproduction in the obese might represent a protective mechanism before the emergence of type 2 diabetes mellitus (T2DM) or cardiovascular disease. Thus, apelin becomes a potential therapeutic target in diabetes and obesity [13].

The human apelin is localized on chromosome Xq25-q26.1 and contains 3 exons, with the coding region spanning exons 1 and 2 [14]. The rs2235306 variant of apelin showed association with insulin secretion [15], and fasting glucose level [16].

The metabolic syndrome is an aggregation of several metabolic risk factors, including elevated plasma triglyceride (TG), reduced high density lipoprotein-cholesterol (HDL-C), elevated blood pressure, and raised plasma glucose. This aggregation is recognized as a multiplex risk factor for atherosclerotic cardiovascular disease and T2DM [17]. Metabolic risk factors are more common in obese individuals. For this reason, obesity is commonly included in the clinical definition of metabolic syndrome [18].

This study aimed to analyze the relation of apelin rs2235306 single nucleotide polymorphism (SNP) with metabolic syndrome and its component traits in Egyptian women from Suez Canal area.

2. Subjects and methods

2.1. Study population

A cross-sectional study was conducted on 200 female subjects.They were divided into 100 healthy subjects and 100 metabolic syndrome patients. All subjects were Egyptians of the same ethnic group. Patients were selected from the Outpatient Clinic of the Ismailia General Hospital. Diagnosis of metabolic syndrome was done according to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATPIII). Metabolic syndrome is diagnosed by the presence of any three or more of the following factors: waist circumference $\geqslant$88 cm in women; fasting blood glucose (FBG) $\geqslant$100 mg/dL or known diabetes; serum TG $\geqslant$150 mg/dL; HDL-C $<$50 mg/dL in women; and blood pressure $\geqslant$130/85 mmHg or treated hypertension [17].

The study groups included non smokers. Subjects suffering from heart failure, myocardial infarction, diabetes mellitus type I, any type of cancer, renal failure or chronic liver disease were excluded. Pregnant or lactating women and those receiving hormone replacement therapy or contraceptive medications were also excluded. All participants had regular menstruation.

The present study was conducted according to the principles of the Declaration of Helsinki, and all the participants provided written informed consent following a protocol approved by the Faculty of Pharmacy, Suez Canal University Research Ethics Committee. Body mass index (BMI), waist circumference, and systolic and diastolic blood pressure were determined for all the subjects.

2.2. Laboratory measurements

Peripheral blood was drawn after a 12 h fast and a portion was collected with EDTA and used for DNA extraction. The serum was separated from the remaining portion of the blood samples and used for assessment of the following parameters:

Glucose homeostasis traits: FBG was measured by the enzymatic colorimetric method (Biodiagnostic, Egypt) and fasting serum insulin was measured by enzyme linked immune sorbent assay (Monobind). The homeostasis model assessment of insulin resistance (HOMA-IR) [19] and the quantitative insulin sensitivity check index (QUICKI) [20] were calculated.

Lipid profile: TG, total cholesterol (TC) and HDL-C were measured by enzymatic colorimetric methods (Biodiagnostic, Egypt). Low density lipoprotein-cholesterol (LDL-C) was calculated [21].

2.3. Genomic DNA extraction and genotyping

Genomic DNA was isolated from 300 μL of whole blood collected in EDTA anticoagulated tubes using the Wizard genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer's instructions. The apelin rs2235306 SNP was detected using tetra amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR). PCR was performed by using four primers: forward outer primer [5′-AAGTGGTGCAGGGTATCCTTGGGT-3′], reverse outer primer [5′-AAGGAGCCAAGGAAGGAACAGAGC-3′], forward inner primer (for T allele) [5′-CCCCCTGCACACCATCTGCTT-3′], and reverse inner primer (for C allele) [5′-GGGACAGGGATCTAGATGCAGGAAG-3′] [22].

PCR amplifications were performed in a total volume of 25 μL containing 1 μL genomic DNA (~100 ng/μL), 1 μL of each primer (10 pmol/μL), 12.5 μL Go Taq® Green Master Mix (2×) (Promega, Madison, USA) and 7.5 μL DNase-free water.

Thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 60 s; and ending with a final extension at 72 °C for 10 min. Thermal cycling was performed in an Eppendorf Mastercycler® machine (Eppendorf, Hamburg, Germany). PCR products were verified on a 2.0% agarose gel. The product sizes of apelin rs2235306 polymorphism were 458 bp for two outer primers (control band), 295 bp for C allele and 208 bp for T allele (Fig. 1).

2.4. Statistical analysis

Differences in baseline characteristics between metabolic syndrome patients and the control group were assessed through the Student *t*-test. Student *t*-test was also used to determine the relationship between different genotypes and the clinical parameters in the study population. Genotype and allele frequencies as well as other qualitative variables were analyzed with the chi-square test. Additionally, the Chi-square test was used to determine whether the genotype distribution was consistent with Hardy–Weinberg equilibrium expectations. Associations of genotypes with the metabolic syndrome component traits were analyzed by one way analysis of variance (ANOVA) test followed by Tukey's test for multiple comparisons. Analysis was performed using SPSS, version 17.0. All data are presented as means ± standard deviations (SD) except where otherwise stated. A value of $p < 0.05$ was considered statistically significant.

3. Results

The demographic, clinical and biochemical parameters of the metabolic syndrome patients and their age-matched controls are summarized in Table 1. In comparison with the control group, patients showed a significant increase in serum TG, TC, LDL-C, fasting blood glucose and fasting serum insulin levels. Patients had a significantly higher insulin resistance than controls indicated by a significant increase of HOMA-IR and a significant decrease of QUICKI. On the other hand, serum HDL-C levels were significantly decreased in the metabolic syndrome patients as compared to the control subjects. Additionally, BMI, waist circumference, systolic and diastolic blood pressure were higher in patients than in controls.

The genotype frequencies of apelin rs2235306 polymorphism were in accordance with Hardy–Weinberg equilibrium in the whole study sample ($\chi^2 = 2.47$, $p = 0.116$), as well as in subjects with metabolic syndrome ($\chi^2 = 0.53$, $p = 0.467$), and control subjects ($\chi^2 = 2.23$, $p = 0.135$). As shown in Table 2; apelin rs2235306 polymorphism did not appear to be associated with the incidence of metabolic syndrome in the studied groups.

We also investigated the relation of apelin rs2235306 genotypes with different clinical parameters in the studied population. The different genotypes of the polymorphism showed no association with any of the components of metabolic syndrome. These results are summarized in Table 3.

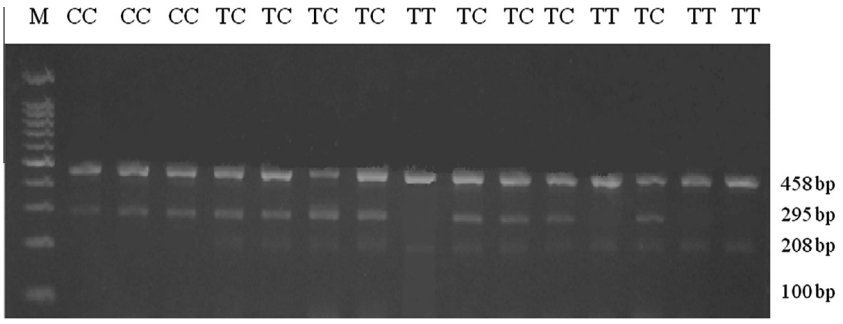


Figure 1 Detection of apelin rs2235306 polymorphism genotypes by agarose gel electrophoresis. Product sizes are 458 bp for two outer primers (control band), 295 bp for C allele, and 208 bp for T allele.

Table 1 General and biochemical characteristics of the study population.

Variables	Control ($n = 100$)	Metabolic syndrome patients ($n = 100$)	*P*
Age (years)	39.98 ± 12.13	38.76 ± 9.72	0.433
BMI (kg/m^2)	23.10 ± 1.68	32.31 ± 5.55*	0.001
Waist circumference (cm)	76.77 ± 8.50	107.12 ± 10.99*	0.001
Systolic blood pressure (mmHg)	109.15 ± 9.02	139.85 ± 16.85*	0.001
Diastolic blood pressure (mmHg)	72.40 ± 6.98	89.75 ± 8.36*	0.001
Glucose homeostasis traits			
FBG (mg/dL)	84.26 ± 10.67	157.02 ± 55.10*	0.001
Fasting serum insulin (μIU/ml)	8.50 ± 1.11	15.80 ± 5.58*	0.001
HOMA-IR	1.80 ± 0.45	6.91 ± 4.89*	0.001
QUICKI	0.351 ± 0.016	0.301 ± 0.026*	0.001
Lipid profile			
TG (mg/dL)	117.54 ± 22.65	202.00 ± 62.41*	0.001
TC (mg/dL)	166.85 ± 27.93	229.55 ± 37.22*	0.001
HDL-C (mg/dL)	59.06 ± 10.56	42.73 ± 5.66*	0.001
LDL-C (mg/dL)	84.37 ± 29.24	142.06 ± 35.11*	0.001

Data are presented as mean ± SD. Comparisons were performed by unpaired Student *t*-test.
* Significantly different from normal control at $p \leqslant 0.05$.

Table 2 Allele frequencies and genotype distribution of apelin rs2235306 polymorphism in the control group and metabolic syndrome patients.

	Control $n = 100$	Metabolic syndrome $n = 100$	P	OR (95%CI)
T allele	109	111		
C allele	91	89	0.841[a]	1.041 (0.702–1.544)
Genotype				
TT	26	29		
TC	57	53	0.584[b]	1.200 (0.627–2.294)
CC	17	18	0.920[c]	1.053 (0.451–2.461)

Comparisons were performed with the Chi-square test; (CI) = confidence interval; OR = odds ratio.
[a] T vs. C.
[b] TT vs. TC.
[c] TT vs. CC.

Table 3 The relationship between apelin rs2235306 polymorphism genotypes and different clinical parameters in the study population.

Variables	Carriers of TT ($n = 55$)	Carriers of TC ($n = 110$)	P (TC vs. TT)	Carriers of CC ($n = 35$)	P (CC vs. TT)
BMI (kg/m^2)	28.45 ± 5.99	27.17 ± 5.75	0.420	28.23 ± 7.57	0.985
Waist circumference (cm)	92.95 ± 17.02	91.24 ± 19.42	0.836	92.60 ± 15.64	0.996
Systolic blood pressure (mmHg)	123.27 ± 19.77	124.82 ± 20.69	0.892	125.43 ± 21.30	0.878
Diastolic blood pressure (mmHg)	81.27 ± 11.39	80.55 ± 11.26	0.924	82.43 ± 13.14	0.890
Glucose homeostasis traits					
FBG (mg/dL)	121.78 ± 55.35	119.12 ± 53.20	0.952	123.63 ± 54.78	0.986
Fasting serum insulin (μIU/mL)	12.25 ± 5.55	12.01 ± 5.42	0.961	12.43 ± 5.42	0.988
HOMA-IR	4.43 ± 4.50	4.27 ± 4.27	0.972	4.51 ± 4.23	0.996
QUICKI	0.325 ± 0.033	0.327 ± 0.033	0.971	0.324 ± 0.033	0.978
Lipid profile					
TG (mg/dL)	165.24 ± 70.19	157.55 ± 59.21	0.743	158.17 ± 64.67	0.864
TC (mg/dL)	203.44 ± 40.13	194.10 ± 46.73	0.429	202.86 ± 49.08	0.998
HDL-C (mg/dL)	49.91 ± 11.87	51.92 ± 12.17	0.556	49.23 ± 10.18	0.961
LDL-C (mg/dL)	118.40 ± 37.98	107.99 ± 44.46	0.312	121.49 ± 46.30	0.941

Data are presented as mean ± SD. Comparisons were performed by one way ANOVA test followed by the Tukey's test for multiple comparison.

4. Discussion

Metabolic syndrome is a risk factor for coronary artery disease as well as diabetes, fatty liver and several cancers. The prevalence of metabolic syndrome in women appears to be increasing, particularly in those of childbearing age [23]. Metabolic syndrome is thought to be attributable to genetic predisposing factors in combination with environmental factors [24]. Several candidate gene polymorphisms were found to show association with metabolic syndrome, such as estrogen receptor alpha [25], tumor necrosis factor alpha [26], angiotensin converting enzyme [27], fat mass and obesity-associated protein, transcription factor 7-like 2, apolipoprotein A5, apolipoprotein C3, interleukin 6 and cholesteryl ester transfer protein [28].

In humans, numerous studies have reported changes in plasma apelin concentrations and variations of apelin and its receptor expression in different tissues in physiological and pathological situations [29]. In obese and hyperinsulinemic subjects, plasma apelin levels as well as adipose tissue expression are increased [5]. Plasma apelin levels are also raised in morbidly obese [30] and T2DM subjects [31]. Non-obese patients with impaired glucose tolerance or with T2DM also exhibited higher concentrations of apelin when compared with control subjects [32]. It was shown that increased plasma apelin concentration in obese and T2DM subjects is positively correlated with insulinemia, glycemia and the percentage of glycated hemoglobin [33]. A decline in apelin levels after diet-induced weight loss [34] or bariatric surgery [31] in obese individuals has also been described, showing that the apelin upregulation can be reversed [35].

The results of this study show that apelin rs2235306 SNP had no association with increased risk of metabolic syndrome. We also found no association of the polymorphism genotypes with any of the components of metabolic syndrome in the studied population. These results are in accordance with the findings of Hashemi et al. [22] in Iranian population who indicated no association between the apelin rs2235306 polymorphism and metabolic syndrome. However, the mentioned article suggested that healthy females carrying apelin TC + CC genotypes have lower HDL-cholesterol in comparison with those carrying TT, which is not the case in our study.

The findings of the current research also agree with the results reported by Zhang et al. [36] who found lack of association of this polymorphism with the prevalence of hypertension

in Chinese population. However, they counteract with the findings of another study carried out in Egypt in which rs2235306 showed association with post prandial insulin in both normal and diabetic females [15]. Apelin rs2235306 showed also association with fasting plasma glucose levels in Chinese male subjects with normal glucose regulation [16].

In conclusion, our results showed no significant association between apelin rs2235306 polymorphism and the frequency of metabolic syndrome or any of its component traits in the study population. Our findings are limited by the relatively small sample size. Further studies on larger scales and from different ethnicities may be required.

Conflict of interest

The authors declare no conflict of interest.

Disclosure statement

None of the authors have any conflicts of interest.

Author contributions

All authors contributed to the design of the study, data collection and analysis, data interpretation and manuscript writing.

Funding

This study was funded by the authors.

References

[1] Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998;251:471–6.
[2] Maguire JJ, Kleinz MJ, Pitkin SL, Davenport AP. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. Hypertension 2009;54:598–604.
[3] Pitkin SL, Maguire JJ, Bonner TI, Davenport AP. International union of basic and clinical pharmacology. LXXIV. Apelin receptor nomenclature, distribution, pharmacology, and function. Pharmacol Rev 2010;62:331–42.
[4] Ishida J, Hashimoto T, Hashimoto Y, Nishiwaki S, Iguchi T, Harada S, et al. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. J Biol Chem 2004;279:26274–9.
[5] Boucher J, Masri B, Daviaud D, Gesta S, Guigné C, Mazzucotelli A, et al. Apelin, a newly identified adipokine upregulated by insulin and obesity. Endocrinology 2005;146:1764–71.
[6] Sorli SC, van den Berghe L, Masri B, Knibiehler B, Audigier Y. Therapeutic potential of interfering with apelin signaling. Drug Discov Today 2006;11:1100–6.
[7] Falcão-Pires I, Ladeiras-Lopes R, Leite-Moreira AF. The apelinergic system: a promising therapeutic target. Expert Opin Ther Targets 2010;14:633–45.
[8] Kunduzova O, Alet N, Delesque-Touchard N, Millet L, Castan-Laurell I, Muller C, et al. Apelin/APJ signaling system: a potential link between adipose tissue and endothelial angiogenic processes. FASEB J 2008;22:4146–53.
[9] Rayalam S, Della-Fera MA, Krieg PA, Cox CM, Robins A, Baile CA. A putative role for apelin in the etiology of obesity. Biochem Biophys Res Commun 2008;368:815–9.
[10] Dray C, Knauf C, Daviaud D, Waget A, Boucher J, Buléon M, et al. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metab 2008;8:437–45.
[11] Higuchi K, Masaki T, Gotoh K, Chiba S, Katsuragi I, Tanaka K, et al. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. Endocrinology 2007;148:2690–7.
[12] Chen H, Zheng C, Zhang X, Li J, Li J, Zheng L, et al. Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice. Peptides 2011;32:1634–9.
[13] Falcão-Pires I, Castro-Chaves P, Miranda-Silva D, Lourenço AP, Leite-Moreira AF. Physiological, pathological and potential therapeutic roles of adipokines. Drug Discov Today 2012;17:880–9.
[14] Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, et al. Characterization of apelin, the ligand for the APJ receptor. J Neurochem 2000;74:34–41.
[15] Ayoub DF, Elghroury EA, Saad NE, Rafaat M, Adel D. Evaluation of apelin genetic variant rs 2235306 in prediabetic and type 2 diabetic individuals. Aust J Basic Appl Sci 2012;6:592–5.
[16] Zhang R, Hu C, Wang CR, Ma XJ, Bao YQ, Xu J, et al. Association of apelin genetic variants with type 2 diabetes and related clinical features in Chinese Hans. Chin Med J 2009;122:1273–6.
[17] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. 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 2009;120:1640–5.
[18] Grundy SM, Neeland IJ, Turer AT, Vega GL. Ethnic and gender susceptibility to metabolic risk. Metab Syndr Relat Disord 2014;12:110–6.
[19] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[20] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402–10.
[21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[22] Hashemi M, Rezaei H, Eskandari-Nasab E, Kaykhaei MA, Taheri M. Association between the apelin rs2235306 gene polymorphism and metabolic syndrome. Turk J Med Sci 2014;44:775–80.
[23] Ramos RG, Olden K. The prevalence of metabolic syndrome among US women of childbearing age. Am J Public Health 2008;98:1122–7.
[24] Joy T, Lahiry P, Pollex RL, Hegele RA. Genetics of metabolic syndrome. Curr Diab Rep 2008;8:141–8.
[25] Ghattas MH, Mehanna ET, Mesbah NM, Abo-Elmatty DM. Association of estrogen receptor alpha gene polymorphisms with metabolic syndrome in Egyptian women. Metabolism 2013;62:1437–42.
[26] Gupta V, Gupta A, Jafar T, Gupta V, Agrawal S, Srivastava N, et al. Association of TNF-alpha promoter gene G-308A polymorphism with metabolic syndrome, insulin resistance, serum

TNF-alpha and leptin levels in Indian adult women. Cytokine 2012;57:32–6.
[27] Xi B, Ruiter R, Chen J, Pan H, Wang Y, Mi J. The ACE insertion/deletion polymorphism and its association with metabolic syndrome. Metabolism 2012;61:891–7.
[28] Povel CM, Boer JM, Reiling E, Feskens EJ. Genetic variants and the metabolic syndrome: a systematic review. Obes Rev 2011;12:952–67.
[29] Castan-Laurell I, Dray C, Attané C, Duparc T, Knauf C, Valet P. Apelin, diabetes, and obesity. Endocrine 2011;40:1–9.
[30] Heinonen MV, Purhonen AK, Miettinen P, Pääkkönen M, Pirinen E, Alhava E, et al. Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. Regul Pept 2005;130:7–13.
[31] Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, Garcia-Almeida JM, Garcia-Arnes J, Tinahones FJ, et al. Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. Obes Surg 2009;19:1574–80.
[32] Li L, Yang G, Li Q, Tang Y, Yang M, Yang H, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diab 2006;114:544–8.
[33] Dray C, Debard C, Jager J, Disse E, Daviaud D, Martin P, et al. Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. Am J Physiol Endocrinol Metab 2010;298:E1161–9.
[34] Castan-Laurell I, Vítkova M, Daviaud D, Dray C, Kováciková M, Kovacova Z, et al. Effect of hypocaloric diet-induced weight loss in obese women on plasma apelin and adipose tissue expression of apelin and APJ. Eur J Endocrinol 2008;158:905–10.
[35] Castan-Laurell I, Dray C, Knauf C, Kunduzova O, Valet P. Apelin, a promising target for type 2 diabetes treatment? Trends Endocrinol Metab 2012;23:234–41.
[36] Zhang R, Lu J, Hu C, Wang C, Yu W, Jiang F, et al. Associations of common variants at APLN and hypertension in Chinese subjects with and without diabetes. Exp Diab Res 2012;2012 [Article ID 917496].