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

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.

© 2014 Production and hosting by Elsevier B.V. on behalf of Ain Shams University.

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.
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 ≥ 88 cm in women; fasting blood glucose (FBG) ≥ 100 mg/dL or known diabetes; serum TG ≥ 150 mg/dL; HDL-C < 50 mg/dL in women; and blood pressure ≥ 130/85 mmHg or treated hypertension [17].

The study groups included non smokers. Subjects suffering from heart failure, myocardial infarction, diabetes mellitus type 1, 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′-AGTTGGTGCAGGGTATCCTTGGGT-3′], reverse outer primer [5′-AAGGAACAAAGGAAGGACAGAAGC-3′], forward inner primer (for T allele) [5′-CCCCCTGCCACACCATCTGCTT-3′], and reverse inner primer (for C allele) [5′-GGGACAGGGATCTAGATGCAGGAGG-3′] [22].

PCR amplifications were performed in a total volume of 25 μL containing 1 μL genomic DNA (~100 ng), 1 μL of each primer (10 pmol/μL), 12.5 μL Go Taq® Green Master Mix (2x) (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 (*χ² = 2.47, *p* = 0.116), as well as in subjects with metabolic syndrome (*χ² = 0.53, *p* = 0.467), and control subjects (*χ² = 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](https://example.com/image.png)

**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>
<thead>
<tr>
<th>Variables</th>
<th>Control (<em>n</em> = 100)</th>
<th>Metabolic syndrome patients (<em>n</em> = 100)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.98 ± 12.13</td>
<td>38.76 ± 9.72</td>
<td>0.433</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.10 ± 1.68</td>
<td>32.31 ± 5.55</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>76.77 ± 8.50</td>
<td>107.12 ± 10.99</td>
<td>0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>109.15 ± 9.02</td>
<td>139.85 ± 16.85</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.40 ± 6.98</td>
<td>89.75 ± 8.36</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Glucose homeostasis traits</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>84.26 ± 10.67</td>
<td>157.02 ± 55.10</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting serum insulin (µIU/ml)</td>
<td>8.50 ± 1.11</td>
<td>15.80 ± 5.58</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.80 ± 0.45</td>
<td>6.91 ± 4.89</td>
<td>0.001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.351 ± 0.016</td>
<td>0.301 ± 0.026</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>117.54 ± 22.65</td>
<td>202.00 ± 62.41</td>
<td>0.001</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>166.85 ± 27.93</td>
<td>229.55 ± 37.22</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>59.06 ± 10.56</td>
<td>42.73 ± 5.66</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>84.37 ± 29.24</td>
<td>142.06 ± 35.11</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Comparisons were performed by unpaired Student *t*-test.

* Significantly different from normal control at *p* < 0.05.
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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Carriers of TT (n = 55)</th>
<th>Carriers of TC (n = 110)</th>
<th>P (TC vs. TT)</th>
<th>Carriers of CC (n = 35)</th>
<th>P (CC vs. TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>28.45 ± 5.99</td>
<td>27.17 ± 5.75</td>
<td>0.420</td>
<td>28.23 ± 7.57</td>
<td>0.985</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.95 ± 17.02</td>
<td>91.24 ± 19.42</td>
<td>0.836</td>
<td>92.60 ± 15.64</td>
<td>0.996</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.27 ± 19.77</td>
<td>124.82 ± 20.69</td>
<td>0.892</td>
<td>125.43 ± 21.30</td>
<td>0.878</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81.27 ± 11.39</td>
<td>80.55 ± 11.26</td>
<td>0.924</td>
<td>82.43 ± 13.14</td>
<td>0.890</td>
</tr>
<tr>
<td><strong>Glucose homeostasis traits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>121.78 ± 55.35</td>
<td>119.12 ± 53.20</td>
<td>0.952</td>
<td>123.63 ± 54.78</td>
<td>0.986</td>
</tr>
<tr>
<td>Fasting serum insulin (µIU/mL)</td>
<td>12.25 ± 5.55</td>
<td>12.01 ± 5.42</td>
<td>0.961</td>
<td>12.43 ± 5.42</td>
<td>0.988</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.43 ± 4.50</td>
<td>4.27 ± 4.27</td>
<td>0.972</td>
<td>4.51 ± 4.23</td>
<td>0.996</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.325 ± 0.033</td>
<td>0.327 ± 0.033</td>
<td>0.971</td>
<td>0.324 ± 0.033</td>
<td>0.978</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>165.24 ± 70.19</td>
<td>157.55 ± 59.21</td>
<td>0.743</td>
<td>158.17 ± 64.67</td>
<td>0.864</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>203.44 ± 40.13</td>
<td>194.10 ± 46.73</td>
<td>0.429</td>
<td>202.86 ± 49.08</td>
<td>0.998</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.91 ± 11.87</td>
<td>51.92 ± 12.17</td>
<td>0.556</td>
<td>49.23 ± 10.18</td>
<td>0.961</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>118.40 ± 37.98</td>
<td>107.99 ± 44.46</td>
<td>0.312</td>
<td>121.49 ± 46.30</td>
<td>0.941</td>
</tr>
</tbody>
</table>

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


