Study of obesity associated proopiomelanocortin gene polymorphism: Relation to metabolic profile and eating habits in a sample of obese Egyptian children and adolescents

Farida El-Baz Mohamed, Rasha T. Hamza, Nermine H. Amr, Azza M. Youssef, Tarek M. Kamal*, Rana A. Mahmoud

Department of Pediatrics, Ain Shams University, Cairo, Egypt
Genetics Unit, Department of Pediatrics, Ain Shams University, Cairo, Egypt

Received 23 December 2015; accepted 21 February 2016
Available online 10 March 2016

Abstract  Background: Melanocortinergic system represents a known system involved in the central regulation of body weight with the central proopiomelanocortin (POMC) neurons forming a potent anorexigenic network. Polymorphisms in the POMC gene locus are associated with obesity phenotypes.
Aim: To assess the contribution of the POMC gene 9-bp insertional polymorphism in the susceptibility to obesity and its relation to body mass index (BMI) and adiposity-related co-morbidities in obese children and adolescents; as well as binge eating behavior.
Patients and methods: Fifty obese children and adolescents with simple obesity were screened for Binge Eating Disorder (BED) by The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), they were compared to 50 age, sex and pubertal stage-matched non obese controls. Anthropometric measurements, blood pressure, abdominal ultrasound for fatty liver, measurement of fasting lipid profile, fasting insulin, fasting blood glucose and assessment of POMC gene 9-bp insertional polymorphism were done.
Results: Obese patients had significantly higher anthropometric measurements, blood pressure percentiles, fasting glucose, fasting insulin, homeostasis model assessment for insulin resistance (HOMA-IR) and fasting lipid profiles, and higher frequency of occurrence of non alcoholic fatty liver disease and BED. Allelic frequencies of POMC gene 9 bp insertional polymorphism were comparable in patients and controls ($p = 0.956$). Fasting insulin levels were significantly higher in the heterozygous cases having the polymorphism than in wild homozygous cases; whereas no difference was observed among the controls.
Conclusion: This polymorphism was associated with higher fasting insulin levels in the obese patients only. These findings support the hypothesis that the melanocortin pathway may modulate...
1. Introduction

Childhood obesity is a worldwide health concern with a multifaceted etiology [1]; which includes modifiable and non-modifiable risk factors [2]. Genetic factors estimated to account for > 40% of the population variation in BMI, where clear differences exist in obesity susceptibility among individuals exposed to the same obesogenic environment, implicating genetic risk factors [3].

The genetic influences are likely to be particularly powerful in people with severe and early-onset obesity [4]. In the past few years, scientists have been trying to localize the genes responsible for obesity which code for hormones and neurotransmitters regulating satiety; where the alteration in these genes or their pathways are implicated in the pathology of obesity [5].

Monogenic obesity caused by single gene polymorphism accounts for about 5% of the cases of obesity, of which 11 genes are identified including leptin (LEP), LEP receptor, preproopiomelanocortin, and melanocortin-4 receptor (MCR4) [6].

The central proopiomelanocortin (POMC) neurons form a potent anorexigenic network [7] where it regulates feeding and energy homeostasis by integrating long-term adiposity signals from the hypothalamus and short-term satiety signals from the brainstem [8]. Polymorphisms in the POMC gene locus are associated with obesity phenotypes [9] which is a risk factor of several short term and long term complications including type 2 diabetes, hypertension, cardiovascular disease, orthopedic and psychological problems [10].

All this genetic evidence for POMC pathogenesis in obesity made us choose the POMC gene as a candidate to search for 9 bp insertional polymorphism that might correlate with childhood obesity [11].

2. Subjects and methods

This case-control study included fifty children and adolescents with simple obesity recruited from the Pediatric Obesity Clinic, Children’s hospital, Ain Shams University during the period from January 2014 till the end of August 2014. Obesity was defined as body mass index (BMI) > 95\textit{th} percentile [12]. Cases were compared to fifty age-, sex- and pubertal stage-matched healthy non obese children and adolescents as a control group. The work was carried out in accordance with "The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in Human". A written informed consent was taken from the legal guardians of cases and controls.

2.1. Methods

(A) Full medical history: age, sex, complications of obesity (diabetes mellitus, hypertension, heart disease, breathing problems, sleep disorders) and family history of obesity, hypertension, diabetes mellitus, liver disease or cardiac disease were recorded.

(B) Thorough physical examination laying stress on:

1. Blood pressure: measured by sphygmomanometer in the right arm of a relaxed, seated child with comparison of values to normal reference percentiles for age/height according to National High Blood Pressure Education Program [13].

2. Auxological measurements:

- Height: was measured to the nearest 0.1 cm on a wall mounted stadiometer without shoes. The participants were asked to stand with their back against the wall-mounted stadiometer with their back (scapulae), buttocks and both heels touching the wall-plate. The shoulders are relaxed and arms are relaxed and hanging loosely at the sides. The head should be in the “Frankfort Horizontal Plane” in which the lowest point on the inferior orbital margin and the upper margin of the external auditory meatus form a horizontal line and the participant was asked to look straight ahead. Height SDS was calculated according to the norms of—[14].

- Body mass index (BMI): was calculated as follows: weight (kg)/height (m)\(^2\) according to Cole [12]. BMI SDS was calculated from the age- and sex-specific reference values [12].

- Waist circumference: was measured midway between the lowest rib and the top of the iliac crest. Waist circumference SDS was calculated and compared to normal references for age and sex according to Schwandt et al. [15].

- Hip circumference: was measured in a horizontal plane at the extension of the buttocks. Hip circumference SDS was calculated and compared to normal references for age and sex according to Schwandt et al. [15] till the age of 11 years and according to Moreno et al. [17] above 11 years.

- Waist/hip ratio: was calculated and compared to normal age and sex reference range together with calculation of waist/hip ratio SDS according to Schwandt et al. [15] till age of 11 years and according to Mederico et al. [16] above 11 years.
Laboratory investigations:

- **Sampling**: performed after an overnight fast (12–14 h). From each participant in the study, 6 mL venous blood was withdrawn under complete aseptic conditions. Two hundred microliters were dispensed in an EDTA tube and stored at 0°C for the assay of POMC gene 9-bp insertional polymorphism. The remaining blood was dispensed in a plain vacutainer. After clotting, the tube was centrifuged at 3000 g for 30 min, the separated serum was dispensed into aliquots used for immediate assay of ALT, fasting lipid profile, fasting blood glucose on SYNCHRON CX-9 Autoanalyzer and fasting insulin was measured by an immunometric, chemiluminescent assay on IMMULITE Autoanalyzer (Siemens Medical Solution Diagnostics, Los Angeles, USA).

- **Fasting lipid profile**: the normal reference ranges of the pediatric age group were determined according to Lauer et al. [18] as follows:
  - TC (total cholesterol) <200 mg/dl
  - LDL (low density lipoprotein) <110 mg/dl
  - HDL (high low density lipoprotein) >35 mg/dl
  - TG (triglycerides) <105 mg/dl for children <10 years and <136 mg/dl for children >10 years.

- **Fasting blood sugar**: a value from 100 to 125 mg/dl was the cutoff to define impaired fasting glucose. While a value ≥126 mg/dl was defined as frank diabetes mellitus according to American Diabetes Association Standards of Care for Diabetes [19].

- **ALT**: levels above 40 IU/L in boys and above 35 IU/L in girls were considered pathologically high [20].

- **Fasting insulin**: the cutoff values for hyperinsulinemia were:
  - >15 mU/L in pre-pubertal
  - >30 mU/L in pubertal (stages 2–4 Tanner)
  - >20 mU/L in pospubertal (stage 5 Tanner) [21].

- **HOMA-IR**: is calculated using the formula HOMA-IR = fasting glucose in millimoles per liter × fasting insulin in milli-international units per liter/22.5 [22]. A value of >2.7 was the cutoff used as an index was calculated of insulin resistance in children and adolescents [23].

---

**Table 1**  Sex, age, anthropometric measurements and blood pressure percentiles of cases and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 50)</th>
<th>Cases (n = 50)</th>
<th>t/X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8.99 ± 3.21</td>
<td>8.63 ± 3.42</td>
<td>t = 0.551</td>
<td>0.58</td>
</tr>
<tr>
<td>Range</td>
<td>3–16</td>
<td>3–15.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.34 ± 0.85</td>
<td>+3.85 ± 1.06</td>
<td>t = 21.779</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-1.98 to 1.24</td>
<td>+2 to 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.30 ± 0.91</td>
<td>+0.86 ± 1.60</td>
<td>t = 4.477</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-1.8 to 1.88</td>
<td>-1.6 to 6.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>16.62 ± 1.96</td>
<td>32.98 ± 4.82</td>
<td>t = 22.224</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>13.67 to 21.8</td>
<td>24.9 to 43.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.06 ± 0.95</td>
<td>+4.13 ± 0.83</td>
<td>t = 23.477</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-1.8 to 1.5</td>
<td>+2.5 to 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>57.80 ± 7.64</td>
<td>94.00 ± 15.32</td>
<td>t = 14.956</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>44–77</td>
<td>63–129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.57 ± 0.71</td>
<td>+6.16 ± 2.17</td>
<td>t = 20.817</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-2.47 to 1.39</td>
<td>+1.33–12.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>67.40 ± 9.95</td>
<td>97.76 ± 14.62</td>
<td>t = 12.136</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>48 to 92</td>
<td>61–131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.76 ± 0.76</td>
<td>+4.63 ± 1.79</td>
<td>t = 19.589</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-2.95 to 0.64</td>
<td>-1.14 to 8.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist/hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.86 ± 0.04</td>
<td>0.96 ± 0.05</td>
<td>t = 10.901</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>0.74–0.95</td>
<td>0.84–1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist/hip SDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>+0.10 ± 0.85</td>
<td>+2.30 ± 1.44</td>
<td>t = 9.331</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>-1.3 to 2</td>
<td>+0.25–6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure percentile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>51.60 ± 7.92</td>
<td>72.64 ± 21.24</td>
<td>t = 6.564</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>50–90</td>
<td>50–99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure percentile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>55.60 ± 14.02</td>
<td>76.84 ± 20.48</td>
<td>t = 6.051</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Range</td>
<td>50–90</td>
<td>50–99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index, SDS: standard deviation score.

* p ≤ 0.05: significant.
Assessment of POMC gene 9-bp insertional polymorphism: DNA was extracted from peripheral blood samples (200 μL) on the average using “salting out” method [24] and purification by spin column method of GeneJET Genomic DNA purification kit (#K0722-0721; Thermo Scientific, Lithuania). Polymerase chain reaction (PCR) was performed using gradient thermal cycler (HYBAID Express; HYBAID Limited, UK). In vitro amplification of the extracted DNA was done to amplify the 9-bp insertional polymorphism, 94-AGC AGC GGC-100, between nucleotides 6979 and 6998 of the POMC gene according to the published techniques [25]. Analysis of the amplified PCR products and allele discrimination was done on high resolution agarose (Nusieve) stained with ethidium bromide for the wild allele of proopiomelanocortin gene (POMC 108 bp) as well as the mutant allele (POMC 117 bp). Hardy–Weinberg equilibrium (HWE) test was performed for the determination of the deviation of genotype distribution in all patients versus healthy controls.

(D) Psychometric study: Screening for Binge Eating Disorder by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) for eating disorders scale [27].

(E) Abdominal ultrasound examination to assess the presence of fatty liver: Multiple transverse and longitudinal gray scale was done, images of the abdomen were acquired using GE LOGIQ 9, USA ultrasound with a 2–5 MHz vector transducer. Cases and controls were scanned in the supine and left lateral decubitus position, utilizing subcostal and intercostal approaches. The time-gain compensation was set to adjust the tissue echogenicity as constant as possible regardless of depth and the following data were collected:
1. Liver size in centimeters (cm).
2. Liver echogenicity.
3. Echo penetration and visibility of diaphragm.
4. Clarity of liver blood vessel structure.
5. Echogenicity of the kidneys.
6. Fibrosis was recognized in the presence of steatosis by coarse echoes ("pin–head echoes") within the fine-echo pattern of steatosis.

From these data the grade of fatty liver was categorized according to Shannon et al. [28] as follows:

<table>
<thead>
<tr>
<th>NAFLD grade</th>
<th>Controls (n = 50)</th>
<th>Cases (n = 50)</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>50</td>
<td>100</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>82</td>
</tr>
</tbody>
</table>

NAFLD: non alcoholic fatty liver disease.

* p ≤ 0.05: significant.
Grade 0, normal echogenicity.

Grade I (mild): A slight diffuse increase in fine echoes in the hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders.

Grade II (moderate): A moderate diffuse increase in fine echoes with slightly impaired visualization of the intrahepatic vessels and diaphragm.

Grade III (marked): A marked increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm and posterior portion of the right lobe of the liver.

2.2. Statistical analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Statistical presentation and analysis was conducted, using the mean, standard deviation and Student’s t-test [Unpaired], Chi-square, Mann–Whitney test by SPSSV17. Unpaired Student’s T-test was used to compare between two groups in quantitative data. Chi-square test was used to study the association between 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error (p value) < 0.05 was considered statistically significant.

3. Results

Fifty obese children and adolescents (27 males and 23 females) with simple obesity were included. Their mean age was 8.99 ± 3.21 years. The control group included 27 males and 23 females, their mean age was 8.63 ± 3.42 years, Table 1.

Upon comparison of the two groups studied, there was no statistically significant difference between cases and controls regarding age or sex, (p = 0.58) (p = 1.00) respectively. On the other hand cases had significantly higher anthropometric measurements (p < 0.001*) as shown in Table 1. Cases also showed significantly higher values of blood pressure (p < 0.001*), ALT (p < 0.001*), fasting glucose (p < 0.001*), fasting insulin (p < 0.001*), HOMA-IR (p < 0.001*), fasting lipid profile (p < 0.001*) for the total cholesterol, HDL and (p = 0.007*) for the triglycerides as shown in Table 2. Cases also had a higher frequency of NAFLD by abdominal ultrasound alone (p < 0.001*) with 14% having grade 1% and 82% having grade 2 as shown in Table 3, and a higher frequency of BED (p = 0.013*) as seen in Table 4.

There was no statistically significant difference between cases and controls regarding POMC gene allelic frequency (p = 0.956) with 34% of cases and 35% of the controls having the POMC 117 bp allele as shown in Table 5. Fasting insulin levels were significantly higher in the heterozygous cases than in wild homozygous cases (p = 0.01), Fig. 1; yet no difference concerning fasting insulin levels between wild-type homozygous and heterozygous controls was detected (p = 0.335).

4. Discussion

Overweight and obesity are associated with insulin resistance, type 2 diabetes, dyslipidemia, hypertension and hepatic steatosis [29]. POMC polymorphisms show consistent evidence for association with obesity phenotypes. The 9-bp insertional polymorphism is the most common with the insertion of AGC AGC GGC between nucleotides 6979 and 6998 leading...
to the insertion of three amino acids (Ser-Ser-Gly) between codons 94 and 100 in the region of the 16-kDa fragment carboxy terminal to γ-MSH. [25]. It is well established that POMC-expressing neurons have a critical role in the control of homeostatic mechanisms such as food-intake, energy balance and glucose metabolism [30]. The disruption of central POMC secretion might represent an additional link between type 2 diabetes and obesity [9].

In the current study, fasting blood glucose, fasting insulin and HOMA-IR were significantly higher in obese cases than in controls (p < 0.001*). Similar results were obtained in a study done by Önal et al. [31] who investigated 70 obese and 60 non obese children (3–15 years) and concluded that both fasting insulin and HOMA-IR were significantly higher in obese than in non obese children.

In the present study, the levels of fasting cholesterol, HDL (p < 0.001*) and triglycerides (p = 0.007*) were significantly higher in cases than in controls. Our results are similar to a study by Yang et al. [32] who found that fasting cholesterol, LDL, HDL and triglycerides were significantly higher in obese participants than in controls.

In the present study, systolic and diastolic blood pressure percentiles were significantly higher in cases than in controls; with systolic blood pressure percentiles mean values 72.64 ± 21.24 and 51.60 ± 7.92 for the cases and controls respectively (p < 0.001*) and diastolic blood pressure percentiles mean values were 76.84 ± 20.48 and 55.60 ± 14.02 for the cases and controls respectively (p < 0.001*). Our results are similar to the results of a study done by [33] who studied 56 obese children and 10 normal weight children of mean age 9 years and found that blood pressure was significantly higher in obese children.

In our study, there was no statistically significant difference between cases and controls regarding POMC gene 9 bp insertion polymorphism with 68% of cases and 70% of the controls having the polymorphism in the heterozygous form (p = 0.828). Also no statistically significant difference was found between cases and controls regarding POMC gene allelic frequency with 34% of cases and 35% of the controls having the POMC 117 bp allele (p = 0.956). Our results are similar to those obtained by [25] Santoro et al. (2004) who found that in 380 Italian obese children and adolescents (185 girls), (mean age, 10.7 ± 3.1 years; range, 2–16 years), allelic frequencies of the POMC 117 bp allele were obtained by [25] who studied eighty-seven unrelated Italian obese children and adolescents suggesting that this polymorphism is not primarily related to early onset obesity. Additionally, the fasting insulin levels were significantly higher in the heterozygous cases than in wild-type homozygous cases (p = 0.01), yet there was no statistical difference regarding fasting blood glucose levels and HOMA-IR (p = 0.258) and (p = 0.36) respectively. Our results are comparable to those obtained by [25], where heterozygotes had 24% higher mean insulin levels than those homozygous for the wild-type allele, supporting the hypothesis for an involvement of the melanocortinergic system in the regulation of glucose metabolism in humans. This might be due to the affection of the translation and protein synthesis [9]. Also a direct action of the polymorphism cannot be excluded. [34]. On the other hand, Feng et al., 2003 [35] did not find a statistically significant difference concerning fasting insulin levels between wild-type homozygotes and obese subjects carrying the POMC variant. The discrepancy between our data and those of Feng et al., 2003 [35] is likely due to lower BMI in the patients of Feng et al. who were heterozygous for the 9-bp POMC variant compared to heterozygous patients reported in the present study (BMI SDS 4.13 ± 0.83 in our study versus 2.8 ± 3.4). Interestingly; no difference concerning fasting insulin levels between wild-type homozygous and heterozygous controls was detected (p = 0.335). This finding could be explained by the fact that the effect of POMC polymorphism on insulin levels may be noticed only in obese children, where insulin production increases in response to fat accumulation [35].

1. Study limitation: small sample size and larger studies are needed to determine POMC variant types, optimum multifactorial management plans involving nutrition, environmental control, medication, and behavioral therapies tailored to individual’s genetic make-up.

To conclude; POMC gene 9 bp insertion polymorphism was significantly related to the fasting insulin levels in obese cases compared to the control group having the polymorphism; indicating a gene-environment interaction and suggesting that this POMC variant may be involved in the natural history of polygenic obesity and the link between type 2 diabetes and obesity.

Conflicts of interest

The authors declare no conflict of interest. There are no financial or personal relationships with other people or organizations that could inappropriately influence the work.

References


