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ORIGINAL ARTICLE

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

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KEYWORDS

Childhood obesity;
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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

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glucose metabolism in obese subjects indicating a possible gene-environment interaction. POMC variant may be involved in the natural history of polygenic obesity, contributing to the link between type 2 diabetes and obesity.

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) > 95th 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:

- Weight: was measured on a digital scale in kilograms and to the nearest 0.1 kg with the subjects standing motionless without shoes and with minimal clothing. Weight for height SDS (standard deviation score) was calculated according to the norms of— [14].
- 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 Tanner et al. [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.

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

Parameters		Controls ($n = 50$)	Cases ($n = 50$)	t/X^2	p-value
Sex	Male	27 54%	27 54%	$X^2 = 0.000$	1.00
	Female	23 46%	23 46%		
Age	Mean ± SD	8.99 ± 3.21	8.63 ± 3.42	$t = -0.551$	0.58
	Range	3–16	3–15.61		
Weight SDS	Mean ± SD	−0.34 ± 0.85	+3.85 ± 1.06	$t = 21.779$	< 0.001*
	Range	−1.98 to 1.24	+2–8		
Height SDS	Mean ± SD	−0.30 ± 0.91	+0.86 ± 1.60	$t = 4.477$	< 0.001*
	Range	−1.8 to 1.88	−1.6 to 6.39		
BMI	Mean ± SD	16.62 ± 1.96	32.98 ± 4.82	$t = 22.224$	< 0.001*
	Range	13.67 to 21.8	24.9 to 43.59		
BMI SDS	Mean ± SD	-0.06 ± 0.95	+4.13 ± 0.83	$t = 23.477$	< 0.001*
	Range	−1.8 to 1.5	+2.5–6.2		
Waist circumference (cm)	Mean ± SD	57.80 ± 7.64	94.00 ± 15.32	$t = 14.956$	< 0.001*
	Range	44–77	63–129		
Waist SDS	Mean ± SD	-0.57 ± 0.71	+6.16 ± 2.17	$t = 20.817$	< 0.001*
	Range	−2.47 to 1.39	+1.33–12.38		
Hip circumference (cm)	Mean ± SD	67.40 ± 9.95	97.76 ± 14.62	$t = 12.136$	< 0.001*
	Range	48 to 92	61–131		
Hip SDS	Mean ± SD	-0.76 ± 0.76	+4.63 ± 1.79	$t = 19.589$	< 0.001*
	Range	−2.95 to 0.64	–1.14 to 8.39		
Waist/hip	Mean ± SD	0.86 ± 0.04	0.96 ± 0.05	$t = 10.901$	< 0.001*
	Range	0.74–0.95	0.84–1.1		
Waist/hip SDS	Mean ± SD	+0.10 ± 0.85	+2.30 ± 1.44	$t = 9.331$	< 0.001*
	Range	−1.3 to 2	+0.25–6.6		
Systolic blood pressure percentile	Mean ± SD	51.60 ± 7.92	72.64 ± 21.24	$t = 6.564$	< 0.001*
	Range	50–90	50–99		
Diastolic blood pressure percentile	Mean ± SD	55.60 ± 14.02	76.84 ± 20.48	$t = 6.051$	< 0.001*
	Range	50–90	50–99		

BMI: body mass index, SDS: standard deviation score.
* $p \leqslant 0.05$: significant.

(C) Laboratory investigations:

- Sampling was 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 −20 °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:* ALT (alanine transferase) 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 2 Laboratory investigations in cases and controls.

Laboratory investigations		Controls (n = 50)	Cases (n = 50)	t/z	p-value
ALT (IU/L)	Mean ± SD Range	9.74 ± 2.64 6–17	24.82 ± 11.00 9–53	t = 9.426	<0.001*
Fasting insulin (μu/ml)	Median (IQR) Range	4.2 (3.2–6.6) 0.91–11.7	10.05 (6.6–13.5) 2–49.6	z = -5.833	<0.001*
Fasting glucose (mg/dl)	Mean ± SD Range	80.44 ± 5.42 70–95	90.00 ± 10.36 70–120	t = 5.783	<0.001*
HOMA-IR	Median (IQR) Range	0.85 (0.63–1.27) 0.18–2	2.11 (1.46–3.06) 0.41–11.27	z = -6.057	<0.001*
Fasting cholesterol (mg/dl)	Mean ± SD Range	159.24 ± 19.63 123–209	181.70 ± 37.52 106–267	t = 3.751	<0.001*
Fasting LDL (mg/dl)	Mean ± SD Range	38.20 ± 6.15 28–62	50.62 ± 18.24 23–113	t = 4.561	0.17
Fasting HDL (mg/dl)	Mean ± SD Range	105.10 ± 19.20 69–149	114.36 ± 43.32 23–207	t = 1.381	<0.001*
Fasting triglycerides (mg/dl)	Mean ± SD Range	94.76 ± 18.51 48–144	112.40 ± 41.16 35–245	t = 2.764	0.007*

HOMA-IR: homeostasis model assessment for insulin resistance, HDL: high density lipoproteins, LDL: low density lipoproteins, ALT: alanine transferase.
* $p \leqslant 0.05$: 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 pro-opiomelanocortin 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 3 Frequency of NAFLD in cases and controls by abdominal ultrasound.

NAFLD grade	Controls (n = 50)		Cases (n = 50)		X^2	p-value
	No.	%	No.	%		
Grade 0	50	100	2	4	92.308	0.00*
Grade 1	0	0	7	14		
Grade 2	0	0	41	82		

NAFLD: non alcoholic fatty liver disease.
* $p \leqslant 0.05$: significant.

Table 4 Frequency of Binge Eating Disorder in cases and controls by the DSM-5.

Binge	Controls (n = 50)		Cases (n = 50)		X^2	p-value
	No.	%	No.	%		
Non	50	100	42	84	8.696	0.01*
Sub threshold BED	0	0	7	14		
BED	0	0	1	2		

BED: Binge Eating Disorder.
* $p \leqslant 0.05$: significant.

Table 5 Allele frequency of POMC gene in cases and controls.

POMC gene	Controls (n = 50)		Cases (n = 50)		X^2	p-value
	No.	%	No.	%		
Alleles					0.003	0.956
POMC 108 bp	65	65	66	66		
POMC 117 bp	35	35	34	34		

- 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

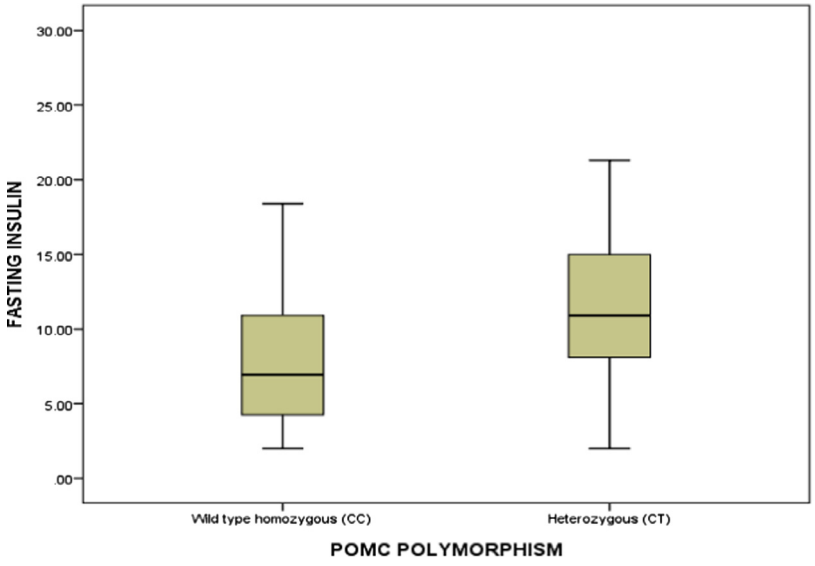
Fifty obese children and adolescents (27 males and 23 females) with simple obesity were included. Their mean age was 8.99 ± 3.21 years. The control group included 27 males and 23 females, their mean age was 8.63 ± 3.42 years, Table 1.

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



- **Wild type homozygous (N) =66, Heterozygous type (N) = 34**

Figure 1 Fasting insulin levels among the homozygous and heterozygous cases (p = 0.01).

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 insertional 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 9-bp insertion were comparable in patients (0.05) and in 300 lean controls of Mediterranean descent (0.04). Similar results were obtained by [26] 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 insertional 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.

Conflict 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

[1] Banjade B, Naik VA, Narasannavar A. Comparison of CDC, WHO and IOTF growth references in relation to overweight and obesity in college adolescents of North Karnataka, 2015.
[2] Fairley L, Santorelli G, Lawlor DA, Bryant M, Bhopal R, Petherick ES, et al. The relationship between early life modifiable risk factors for childhood obesity, ethnicity and body mass index at age 3 years: findings from the Born in Bradford birth cohort study. BMC Obesity 2015;2(1):9.
[3] El-Sayed Moustafa JS, Froguel P. From obesity genetics to the future of personalized obesity therapy. Nat Rev Endocrinol 2013. http://dx.doi.org/10.1038/nrendo.2013.57.
[4] Shawky RM, Sadik DI. Genetics of obesity. Egypt J Med Hum Genet 2012;13(1):11–7. http://dx.doi.org/10.1016/j.ejmhg.2011.08.005.
[5] Symonds ME, Budge H, Frazier-Wood AC. Epigenetics and obesity: a relationship waiting to be explained. Hum Hered 2013;75(2–4):90–7. http://dx.doi.org/10.1159/000352009, Epub 2013 Sep 27.
[6] Moleres A, Martinez JA, Marti A. Genet Obes 2013;2(1):23–31.
[7] Mercer AJ, Hentges ST, Meshul CK, Low MJ. Unraveling the central proopiomelanocortin neural circuits. Front Neurosci 2013;2013(7):19. http://dx.doi.org/10.3389/fnins.2013.00019, Epub 2013 Feb 22.
[8] Zhan C, Zhou J, Feng Q, Zhang JE, Lin S, Bao, et al. Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. J Neurosci 2013;33(8):3624–32. http://dx.doi.org/10.1523/JNEUROSCI.2742-12.2013.
[9] Mencarelli M, Zulian A, Cancello R, Alberti L, Gilardini L, Di Blasio AM, et al. A novel missense mutation in the signal peptide of the human POMC gene: a possible additional link between

early-onset type 2 diabetes and obesity. Eur J Hum Genet 2012;20 (12):1290–4. http://dx.doi.org/10.1038/ejhg.2012.103, Epub 2012 May 30.
[10] Hasnain S. Obesity, more than a 'cosmetic' problem. Current knowledge and future prospects of human obesity genetics. Biochem Genet 2015:1–28.
[11] Madonna ME. Melanocotins of the central nervous system: structural insights on pigmentation and appetite; 2012.
[12] Cole TJ. A chart to link child centiles of body mass index, weight and height. Eur J Clin Nutr 2002;56(12):1194–9.
[13] National High Blood Pressure Education Program (NHBPEP). The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(2 Suppl 4th Report):555–76.
[14] Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965. Arch Dis Child 1966;41(219):454–71.
[15] Schwandt P, Kelishadi R, Haas GM. First reference curves of waist circumference for German children in comparison to international values: the PEP Family Heart Study. World J Pediatr 2008;4(4):259–66.
[16] Mederico M, Paoli M, Zerpa Y, Briceño Y, Gómez-Pérez R, Martínez JL, et al. Reference values of waist circumference and waist/hip ratio in children and adolescents of Mérida, Venezuela: comparison with international references. Endocrinol Nutr 2013;60(5):235–42.
[17] Moreno LA, Fleta J, Mur L, Rodriguez G, Sarria A, Bueno M. Waist circumference values in Spanish children-Gender related differences. Eur J Clin Nutr 1999;53:429–33.
[18] Lauer RM, Barness LA, Clark R, Deckelbaum RJ, Finberg L, Kwiterovich Jr PO, et al. Highlights of the report of the expert panel on blood cholesterol levels in children and adolescents. National cholesterol education program expert panel on blood cholesterol levels in children and adolescents. Am Fam Physician 1992;45:2127–36.
[19] American Diabetes Association Standards of Care for Diabetes. Brief summary of screening and diagnosis of type 2 diabetes and its complications. Diabetes Care 2013;36(Suppl 1):S11–66.
[20] England K, Thorne C, Pembrey L, Tovo PA, Newell ML. Age- and sex-related reference ranges of alanine aminotransferase levels in children: European paediatric HCV network. J Pediatr Gastroenterol Nutr 2009;49(1):71–7.
[21] Monteiro PA, Mota J, Silveira LS, Cayres SU, Bárbara de Moura MA, Fernandes RA, et al. Morphological and metabolic determinants of nonalcoholic fatty liver disease in obese youth: a pilot study. BMC Res Notes 2013;6(1):89.
[22] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9.
[23] Atabek ME, Pirgon O. Assessment of insulin sensitivity from measurements in fasting state and during an oral glucose tolerance test in obese children. J Pediatr Endocrinol Metab 2007;20 (2):187–96.
[24] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
[25] Santoro N, Miraglia del Giudice Emanuele, Cirillo G, Raimondo P, Corsi I, Amato A, et al. An insertional polymorphism of the proopiomelanocortin gene is associated with fasting insulin levels in childhood obesity. J Clin Endocrinol Metabol 2004;89:104846–9. http://dx.doi.org/10.1210/jc.2004-0333, October 1.
[26] del Giudice E1 Miraglia, Cirillo G, Santoro N, D'Urso L, Carbone MT, Di Toro R, et al. Molecular screening of the proopiomelanocortin (POMC) gene in Italian obese children: report of three new mutations. Int J Obes Relat Metab Disord 2001 Jan;25(1):61–7.
[27] American Psychiatric Association; 2013.
[28] Shannon A, Alkhouri N, Carter-Kent C, Monti L, Devito R, Lopez R, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children with nonalcoholic fatty liver disease (NAFLD). J Pediatr Gastroenterol Nutr 2011;53(2):190–5.
[29] Bult MJ, van Dalen T, Muller AF. Surgical treatment of obesity. Eur J Endocrinol 2008;158(2):135–45.
[30] Fick LJ, Belsham DD. Nutrient sensing and insulin signaling in neuropeptide-expressing immortalized, hypothalamic neurons: a cellular model of insulin resistance. Cell Cycle 2010;2010 (9):3186–93.
[31] Önal ZE, Atasayan V, Gürbüz T, Hepkaya E, Nuhoğlu C. Association of glycosylated hemoglobin (HbA1c) levels with insulin resistance in obese children. Afr Health Sci 2014;14 (3):533–8.
[32] Yang HR, Yi DY, Choi HS. Comparison between a pediatric health promotion center and a pediatric obesity clinic in detecting metabolic syndrome and non-alcoholic fatty liver disease in children. J Korean Med Sci 2014;29(12):1672–7.
[33] Wunsch R, de Sousa G, Toschke AM, Reinehr T. Intima-media thickness in obese children before and after weight loss. Pediatrics 2006;118(6):2334–40.
[34] Morris JC, Savva D, Lowry PJ. Reduced expression of a naturally deleted form of human proopiomelanocortin complementary deoxyribonucleic acid after transfection into Chinese hamster ovary cells. Endocrinology 1995;136:195–201.
[35] Feng N, Adler Wailes D, Elberg J, Chin JY, Fallon E, Carr A, et al. Sequence variants of the POMC gene and their associations with body composition in children. Obes Res 2003;11(5):619–24.