Genes & Diseases (2018) 5, 150–157

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

FULL LENGTH ARTICLE

Obesity phenotype in relation to gene polymorphism among samples of Egyptian children and their mothers

Nayera E. Hassan [a], Sahar A. El-Masry [a,*], Waheba Zarouk [b], Rokia A. El Banna [a], Rehab M. Mosaad [b], Muhammad Al-Tohamy [a], Abeer Ramadan Salamah [b]

[a] *Biological Anthropology Department, Medical Research Division, National Research Centre, Giza, Egypt*
[b] *Molecular Genetics and Enzymology Department, Human Genetics and Genomic Research Division, National Research Centre, Giza, Egypt*

Received 15 August 2017; accepted 6 December 2017
Available online 18 December 2017

KEYWORDS
Egyptian;
Gene polymorphism;
Insulin receptor (INSR);
Leptin receptor (LEPR);
Obesity;
Uncoupling protein 2 (UCP2);
Phenotype

Abstract Obesity is complex heterogeneous disease controlled by genes, environmental factors, and their interaction. Genetic factors account for 40–90% of the body mass index variations. Body mass index (BMI) of children correlates more closely with maternal than paternal BMI. So, this studu was aimed to investigate the role of leptin receptor LEPR Gln223Arg, the uncoupling protein 2 (UCP2 G 866 A) and insulin receptor gene (INSR exon 17) polymorphisms in the pathogenesis of obesity. A cross-sectional study executed on 130 children and their obese mothers; classified into 2 groups according to their BMI. The 2 groups were evaluated regarding the anthropometry. Restriction fragment length analysis for LEPR Gln223Arg, UCP2 -866 G/A and INSR exon 17 polymorphisms were applied. It was reported that increased risk of obesity was found in LEPR AG + AA genotype and the A allele. Significant statistical difference was detected only in female children. Concerning UCP2, the AG followed by the GG genotype was the most frequent in all groups and the G allele was the mostly present in obese mothers and obese male children but with no statistical significance. There was difference in the INSR genotype and alleles between groups, but this difference was not statistically significant. This study concluded that the LEPR Gln223Arg, UCP2 G 866 A and INSR exon 17 polymorphisms are related to obesity in Egyptian population. Further researches on larger population are recommended to ascertain the implications of LEPR, UCP2 and INSR polymorphisms in obesity.

* Corresponding author. Biological Anthropology Dept., National Research Centre, El-Bohooth St., Dokki, Giza, Cairo, 12622, Egypt.
E-mail address: masrysa@yahoo.com (S.A. El-Masry).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2017.12.004

Introduction

Obesity prevalence has increased all over the world as a pandemic.[1] Obesity is a multifactorial disease, controlled by genetic and environmental factors as well as the complex interactions among them. Approximately 118 candidate genes are associated with obesity.[2] Some of them are genes encoding leptin (LEP), leptin receptor (LEPR), uncoupling proteins (UCP) and insulin receptor (INSR) gene.

Body weight regulation and stability depends upon an axis with three interrelated components: food intake, energy expenditure and adiposeness.[3] The most important factor leptin; an endogenous hormone; decreases appetite and increases energetic consumption and insulin, with its peripheral regulating role.[4] Leptin controls lipid homeostasis effect by binding to leptin receptor (LEPR), belongs to class I cytokine receptor family. The long isoform LEPRb plays a key role in body weight regulation expressed in the hypothalamus.[5] It was reported that allele frequencies in the Gln223Arg variant of the LEPR gene are characterized by a significant population of origin effect.[6] Hence, LEPR Gln223Arg polymorphism may be expected to have more impact on risk of obesity in the developing countries with a much higher percentage of obese women as in Egypt.

Uncoupling proteins (UCP) is associated with energy and maintain fatty acid homeostasis. Uncoupling protein UCP2 is considered as candidate genes for obesity. This is due to reduce energy expenditure by increasing coupling of oxidative phosphorylation, thereby contributing to the development of obesity.[7] The most interesting polymorphisms in UCP2 gene is 866G/A (rs659366) in the promoter region. Development and progression of diabetes and obesity phenotypes are related by UCP2 $-866\ G > A$.[8]

Insulin resistance and obesity are interrelated.[9] Moreover, insulin resistance may occur secondary to resistance at the insulin receptor. Insulin receptors expressed in the brain were found to reduce food intake. The most important polymorphism for INSR gene is at exon 17 which is necessary for insulin signal transduction as it has been shown mutation in exon 17 of the INSR causes severe insulin resistance and hyperinsulinemia.[10]

The aim of the present study was to explore the role of leptin receptor LEPR Gln223Arg, uncoupling proteins UCP2 866G/A and insulin receptor INSR axon 17 polymorphisms; at genetic level; in the pathogenesis of obesity. Since a large proportion of adult obesity starts during childhood, the differences in genotype and allele frequencies in obese mothers and in juvenile obesity were examined.

Subjects and methods

Subjects

This study was derived from a cross-sectional survey through a project funded by National Research Centre (NRC) Egypt: entitled "Familiar Overweight and Obesity in Children and Adolescents: Diagnostic Clinical, Behavioral, Genetic and Biochemical Markers and Intervention" (10th Research Plan of the NRC); after taking approval from Ethical Committee of NRC (Registration Number is 13/168). It was carried in the "Medical Excellence Research Center (MERC)" through the period 2013—2016.

It included 130 children of both sexes (74 males and 56 females) and their mothers. All the mothers were obese; their BMI above 30 kg/m^2. While the children were classified into 2 groups according to their BMI: 32 obese children with BMI above 95th percentile (12 males and 20 females) and 98 normal weight children with BMI ranged between 15th and 85th percentiles (62 males and 36 females) according to the Egyptian Standard Growth Curves[11] for corresponding age and sex. According to the child BMI, the mothers were reclassified into 2 groups: group I included obese mothers and their children are obese (32 mothers), and group II included obese mothers and their children were of normal healthy weight (98 mothers).

The mothers were chosen randomly from all categories of the employee (at the National Research Centre (NRC)) and their relatives and neighbors. They participated in the study after signing a written informed consent form of the Medical Ethical Committee of NRC. The age range of the children was 5—18 years with a mean age $10.83 + 3.82$. All participants were informed about the purpose of the study and their permission in the form of written consent was obtained.

Methods

Anthropometric measurements including weight, height, and body mass index (BMI) of all the children and their mothers were conducted; in addition to the genetic analysis.

Anthropometric measurements

Weight was measured using a commercial scale (Seca Scale, Germany) with accuracy up to nearest 100 g. The subjects were asked to remove their footwear and wear minimal clothes before weighing them. Standing body height was measured, to the nearest 0.1 cm by using Holtain Stadiometer with the shoulder in a relaxed position and arms hanging freely and without shoes. The scales were recalibrated after each measurement following the recommendations of the International Biological Program.[12] Body Mass Index (BMI) was calculated as body weight in kilograms/height in meter^2. Children BMI percentile was calculated according to their age and sex based on the Egyptian Growth Reference Charts.[11] A child with BMI below 85th percentile was considered healthy weight, with BMI between 85th and 95th percentile overweight and those with BMI $\geq$95th percentile obese. While mothers with BMI below 25 kg/m^2 were considered healthy weight, with $25 \leq$ BMI ≤ 29.9 kg/m^2 overweight and with BMI $\geq$30 kg/m^2 were considered obese.

Genetic analysis

DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood by using DNA extraction and purification kit (Qiagen) according to the manufacturer's protocol. The concentration of genomic DNA was determined by the quantitative method based on the

optical density measurement. The purity was determined by calculation the ratio of absorbance at 260 nm to absorbance at 280 nm (A260/A280). Genotyping of polymorphism was carried out by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). Specific primers upstream and downstream to amplify target sequences gene was selected according to the GENBANK databank. The PCR was carried out using thermocycler (Icycler 5, BioRad, USA) according to the optimized conditions. In a total volume of 30 μl reaction, PCR components comprised of 100 ng DNA, 1X Taq buffer, 2 mM $MgCl_2$, 0.2 mmol/l each of dNTPs, 0.5 U Taq DNA polymerase and 10 pmol each of the SNP detection primers. Thermal cycling was performed as follows for Leptin receptor polymorphism (Gln223Arg); initial denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 90 s, for 30 cycles. The cycling profile for UCP2 −866G/A (rs659366) polymorphism in the promoter region consisted of; denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, with initial denaturation at 94 °C for 4 min and final extension at 72 °C for 10 min. For INSR at the 3′ end of exon 17 the cycling parameters were; denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, with initial denaturation at 94 °C for 4 min and final extension at 72 °C for 10 min. Amplified PCR products were digested with the corresponding restriction enzyme to check polymorphism by RFLP assay; *Msp* I (LEPR), *mlu1* (UCP2) and *Pml*I (INSR). The digested PCR fragments along with DNA ladder were resolved by electrophoresis using 3% agarose gel. The gel was run at 120 V for 1 h. After that, the gel was placed in a Gel Documentation System (GDS) to visualize the digested PCR fragments under UV light (Molecular Imager Gel DocTM XR + Systems with Image LabTM 2.0 Software, BioRad, USA).[13,14]

Statistical analysis

Data analysis was carried out using the statistical package for social science (SPSS) software version 16 (Chicago, IL, USA). Evaluation of the statistical normal distribution of the variables was done using Kolmogorov−Smirnov Goodness of Fit Test. The variables have asymmetric distribution ($P < 0.05$). Frequency distribution of the genotypes and alleles was presented as percentage. Chi-square test was used in comparing qualitative data. Statistical significance was set at $P < 0.05$.

Results

Anthropometric assessment

Mothers with obese children (group 1) had significantly higher weight than those with normal weight children (group 2); however, there were insignificant differences in height and BMI between the 2 groups of mothers. Obese males and females (group 1) had significantly higher weight and BMI than normal weight ones (group 2); inspite of the insignificant differences in height in either sex (Table 1)

Genotype comparisons

a) Mothers

Table 2 shows the distribution of genotypes for; Leptin receptor polymorphism (Gln223Arg), UCP2 (866) A/G gene polymorphism and INSR at the 3′ end of exon 17, in the group (I) and (II) of obese mothers. Among the obese mothers with obese children group (group I), regarding leptin genotype, homozygous (AA) predominate as it was 56.2% followed by the heterozygous (AG) 43.8% and homozygous (GG) was undetected. While in the group of obese mothers with normal children (group II); the heterozygous genotype (AG) was the most prevalent, 53.1%, then the homozygous (AA), 44.9%, and (GG), 2%. The UCP2 (866) A/G gene polymorphism we found that, in both group of mothers the heterozygous genotype (AG) was the most frequent 56.2% and 63.3%, followed by the homozygous (GG) 37.5% and 26.5% then (AA), 6.2% and 10.2%; in group I and II respectively. Analysis of INSR at the 3′ end of exon 17; showed that, the heterozygous genotype (CT) represented the highest frequency in the two groups of obese mothers, 43.8% and 59.4% followed by the homozygote genotype (TT)

Table 1 Comparison between the anthropometric measurements of the sample under study.

		Group I			Group II			P
		N	Mean	+SD	N	Mean	+SD	
Mothers	Weight (Kg)	32	90.18	16.06	98	81.48	11.81	0.007**
	Height (cm)	32	159.63	7.47	98	156.98	6.03	0.076
	BMI (Kg/cm^2)	32	35.35	5.72	98	33.16	5.36	0.052
Children								
Males	Weight (Kg)	12	70.77	32.08	62	39.400	21.7249	0.000**
	Height (cm)	12	151.00	20.21	62	140.90	18.26	0.107
	BMI (Kg/cm^2)	12	36.18	6.18	62	17.82	4.89	0.000**
Females	Weight (Kg)	20	50.86	22.66	36	40.06	19.06	0.045*
	Height (cm)	20	139.60	16.01	36	140.64	20.44	0.839
	BMI (Kg/cm^2)	20	25.61	4.05	36	17.56	3.74	0.000**

*$P > 0.05$ = significant differences.
**$P < 0.01$ = highly significant differences.

Table 2 Comparison between the genotypes of the mothers in the 2 groups.

	Genotype	Obese mothers				Chi-square P
		Group I (N = 32)		Group II (N = 98)		
		N	%	N	%	
LEPR	AA	18	56.2	44	44.9	0.336
	AG	14	43.8	52	53.1	
	GG	0.0	0.0	2	2.0	
UCP2	AA	2	6.2	10	10.2	0.454
	AG	18	56.2	62	63.3	
	GG	12	37.5	26	26.5	
INSR	TT	12	37.5	26	26.5	0.312
	CT	14	43.8	58	59.2	
	CC	6	18.8	14	14.3	

Table 3 Comparison between the genotypes of the male children in the 2 groups.

	Genotype	Male children				Chi-square P
		Group I (N = 12)		Group II (N = 62)		
		N	%	N	%	
LEPR	AA	6	50.0	20	32.3	0.197
	AG	6	50.0	42	67.7	
	GG	0.0	0.0	0.0	0.0	
UCB2	AA	2	16.7	2	3.2	0.121
	AG	8	66.7	36	58.1	
	GG	2	16.7	24	38.7	
INSR	TT	0.0	0.0	6	9.7	0.050
	CT	6	50.0	44	71.0	
	CC	6	50.0	12	19.4	

37.5% and 26.5%; while the homozygote (CC) genotype was 18.8% and 14.3%; in group (I) and (II) respectively. Statistical analysis revealed insignificant differences in frequencies of genotype distribution between mothers of either obese or non-obese children.

b) Siblings

Table 3 shows comparison between obese and non-obese male children in their genotype. Leptin receptor polymorphism results demonstrated increased frequency of (AA) genotype in obese than non obese male children (50% and 32.3% respectively). In contrast, there was an increased frequency of the heterozygous (AG) genotype in non obese male children than in obese (67.7% and 50% respectively). The homozygous GG wasn't present in the two groups. The UCP2 (866) A/G gene polymorphism in the male children revealed the existence of the three genotype in obese (group I) and non obese (group II) children as follow: (AA) genotype 16.7% vs 3.2%, (AG) genotype 66.7% vs 58.1%, and (GG) genotype 16.7% vs 38.1%. While INSR genotype at the 3′ end of exon 17 was detected in the obese males as 0.0% homozygous (TT), 50% heterozygous (CT) and 50% the homozygous (CC) compared to the non obese males, the results were 9.7% homozygous (TT), 71% heterozygous (CT) and 19.4% homozygous (CC). These differences in genotype distribution didn't attain any statistical significance.

Table 4 demonstrated that; comparing the genotype distribution in obese (group I) and non obese (group II) female children. Concerning Leptin receptor, results showed that; in obese, the homozygous (AA) and the heterozygous (AG) genotype were equally presented (50%); while in non obese female children, the heterozygous (AG) was more frequent than the homozygous (AA) (77.8% and 22.2% respectively). The homozygous GG was not present in female children studied. When comparing the female children group I and II, for UCP2; results revealed that, the frequency of the homozygous (AA) was 0.0% and 66.7%, respectively, the heterozygous (AG), 60% and 33.3% respectively and the homozygous (GG) 40% and 0.0% respectively. While analysis of INSR at the 3′ end of exon 17; in female children; the obese group genotyping were; undetected homozygous (TT), 70% heterozygous (CT) and 30% homozygous (CC). While in the non-obese group 11.1% homozygous (TT), 61.1% heterozygous (CT) and the (CC) homozygous genotype was 27.8%. Statistical significant differences in frequency of genotype were detected in case of leptin receptor only.

Frequency of different alleles

a) Mothers

The presence of alleles between mothers of obese children (group I) and non-obese children (group II) for Leptin receptor (Gln223Arg) were as follow: the A allele 100% vs 98% (P = 0.41), the G allele, 43.8% vs 55.1% (P = 0.26) in group I and II respectively. The alleles of the UCP2 (866G/A) were presented as; A allele 62.5% vs 73.5% (P = 0.23) and G allele 93.8% vs 89.8% (P = 0.50) in group I and II respectively. The distribution of alleles of INSR at the 3′ end of exon 17 was; C allele 62.5% vs 73.5% (P = 0.23) and T allele 81.2% vs 85.7% (P = 0.54) in group I and II respectively (Table 5).

Table 4 Comparison between the genotypes of the female children in the 2 groups.

	Genotype	Female children				Chi-square P
		Group I (N = 20)		Group II (N = 36)		
		N	%	N	%	
LEPR	AA	10	50.0	8	22.2	0.033
	AG	10	50.0	28	77.8	
	GG	0.0	0.0	0.0	0.0	
UCP2	AA	0.0	0.0	24	66.7	0.619
	AG	12	60.0	12	33.3	
	GG	8	40.0	0.0	0.0	
INSR	TT	0.0	0.0	4	11.1	0.156
	CT	14	70.0	22	61.1	
	CC	6	30.0	10	27.8	

Table 5 Comparison between the presences of alleles (present/absent)of the Mothers in the 2 groups.

	Alleles	Mothers				Chi-square P
		Group I (N = 32)		Group II (N = 98)		
		N	%	N	%	
LEPR	A	32	100	96	98.0	0.415
	G	14	43.8	54	55.1	0.264
UCP2	A	20	62.5	72	73.5	0.236
	G	30	93.8	88	89.8	0.502
INSR	C	20	62.5	72	73.5	0.236
	T	26	81.2	84	85.7	0.543

b) Siblings

Table 6, shows the results of alleles distribution among obese (group I) and non obese (group II) male children. Regarding Leptin; the frequency of the A allele was high in both groups (100%) than the G allele as it is presented in 50% of group I and 67.7% group II (P = 0.23). In case of UCP2; the frequency of the A allele was 83.3% vs 61.3% (P = 0.14) whereas the frequency of the G allele was 83.3% vs 96.8% (P = 0.059) in group I and II respectively. Concerning INSR, the frequency of the C allele was 100% vs 90.3% (P = 0.26); and T alleles frequency was 50% vs 80.6% (P = 0.024) among group I and II respectively.

Table 7, shows presence of alleles of the 3 studied genes among obese and non-obese females. For GLN223ARG leptin receptor; the frequency of the A allele is the same in group I as in group II (100%). G alleles was highly expressed in group II (77.8%) than in group I (50%) and this difference is statistically significant (P = 0.033). The alleles of the UCP2 (866) A/G gene polymorphism were presented in obese (group I) and non obese (group II) female children as follow; A allele 60% vs 66.7% (P = 0.61), while the G allele was highly present as it was 100% in both groups. Presence of the INSR alleles in group I and II respectively was; C 100% vs 88.9% (P = 0.12), T allele was less found, 70% and 72.2% (P = 0.86).

Discussion

Childhood obesity increases the risk of adult obesity, this fact leading to the increased importance of determining the causes of childhood obesity and preventing it. As Personalized genomics is the future of medicine; this includes the identification of genetic factors that confer risk of susceptibility to obesity. In children, the phenotypic presentation caused by gene polymorphisms may be more convenient; because they are less affected by life style intervention due to environmental factors. Inheritance studies show that; body mass index (BMI) of children correlates more closely with maternal than paternal BMI and that genetic factors account for 40–90% of the BMI variations.[3]

This study reported an association of the LEPR Gln223Arg polymorphism in the LEPR with obesity risk, as it was found that; the highest risk of developing obesity was owned by the obese mothers and their children with AG + AA genotype and carrying the A allele of the LEPR 223 gene. There were insignificant statistical differences, but only in case of female children Genotype and allele frequencies observed in the present study were similar to those found in other populations.[15–17] In contrast to current findings; LEPR 223 G allele (polymorphic), was observed had a higher probability of becoming obese in different Caucasian populations including Tunisian,[18] Mexican Mestizo[6] and Romanian.[19] A meta-analysis study by Bender et al[20] on the Q223R polymorphism indicated the presence of variant G allele as being associated with high risk of obesity in 8 studies, whereas in 5 studies a protective effect was found. Another studies done by Komşu-Ornek et al,[21] Zandoná et al[22] and Gajewska et al;[23] found no association. Despite, several lines of evidence suggest that this polymorphism may play a role in the pathogenesis of obesity; however, such mutations are extremely rare and are not likely to be responsible for the obesity, because there are many factors that contribute to the appearance of obesity.[24] Other authors observed that the wild-type variant of Gln223Arg correlated with impaired glucose metabolism and dyslipidemia; therefore this variant of LEPR polymorphism may carry a risk of insulin resistance.[23]

UCP2 expression was found to be increased after birth and decreased later on in the life.[25] In various ethnicities, the UCP2 G (−866)A SNP has also been associated with

Table 6 Comparison between the presences of alleles of the male children in the 2 groups.

	Alleles	Males				Chi-square P
		Group I (N = 12)		Group II (N = 62)		
		N	%	N	%	
LEPR	A	12	100.0	62	100.0	–
	G	6	50.0	42	67.7	0.239
UCP2	A	10	83.3	38	61.3	0.143
	G	10	83.3	60	96.8	0.059
INSR	C	12	100.0	56	90.3	0.261
	T	6	50.0	50	80.6	0.024*

*P > 0.05 = significant differences.

Table 7 Comparison between the presences of alleles of the female children in the 2 groups.

	Alleles	Females				Chi-square P
		Group I (N = 20)		Group II (N = 36)		
		N	%	N	%	
LEPR	A	20	100.0	36	100.0	–
	G	10	50.0	28	77.8	0.033*
UCP2	A	12	60.0	24	66.7	0.618
	G	20	100.0	36	100.0	–
INSR	C	20	100.0	32	88.9	0.122
	T	14	70.0	26	72.2	0.860

*P > 0.05 = significant differences.

central obesity and with several phenotypes related to obesity.[26,27] The present study revealed that; the heterozygous AG genotype was the most frequent in all groups in the study followed by the homozygous GG genotype and the G allele was the mostly present in obese mothers and obese male children but with no statistical significance. Present finding coincide with that of Esterbauer et al;[28] which showed that the GG genotype was associated with an increased risk of obesity among 596 and 791 white Europeans. Dhamrait and his colleagues[29] also found that, a haplotype containing the −866G-allele showed association with childhood obesity. A Case control study and meta-analysis studies showed that A allele of −866G/A polymorphism had a protective effect on overweight and obesity, especially for European populations.[30,31] A possible mechanism for the observed association between the −866G-allele and both insulin resistance and obesity is that, the obesity-induced increase of UCP2 mRNA could be lower for the −866G-allele compared with the A allele, The lower expression at the transcription level may result in a decreased production of UCP2, resulting in increased reactive oxygen species generation, decreased energy expenditure; hence, increased accumulation of body fat and insulin resistance in G-allele carriers.[8] In contrast; in the Austrian population,[28] in the northern Indian,[26] in Hungarian children[32] and In the Balinese population studied,[33] they observed that subjects with A/A genotype and A allele carriers of the SNP G (−866)A has higher BMI. Lack of association was detected; in French Caucasians,[34] in young German subjects[35] and in an Italian Caucasian population that studied morbidly obese patients.[36]

Insulin receptor (INSR), being an integral part of insulin signaling could be a potential candidate gene in obesity.[9] Results of the present study conferred that; the TT genotype and the T allele were the less frequent among children, while the CC genotype and the C allele were less found among their obese mothers; these differences in distribution didn't reach statistical significant. It is interesting to note that, lean PCOS women with polymorphic genotype had significantly higher waist circumference, which is a measure of central obesity compared with wild type genotype. Since this SNP is a silent one, can't exert a major effect on the development of insulin resistance; rather it might be in linkage disequilibrium with others genetic variants.[37] Feng et al[38] detected insignificant difference in the BMI between (CC) and CT + TT genotypes in PCOS. Insulin or its receptors potentially influence fatty acid and triacylglycerol metabolism which can lead to obesity. However, the underlying mechanisms of the association between INS gene and INSR gene with obesity are still unknown.[9]

Recent studies have confirmed the association between the genes encoding; uncoupling proteins (UCP2), Insulin receptor gene (INSR) and leptin receptor (*LEPR*) with body mass index, as they are involved in energy expenditure and lipid and glucose metabolism and long-term weight regulation. Thus, they can be regarded as candidate genes of obesity.[39,40]

Despite these contradictory findings, it is now well known that obesity is a complex disorder involving a multitude of genetic, behavioral, and environmental factors. Polymorphisms or mutations may not be the cause for obesity, and many genetic and life style factors act cumulatively to affect energy metabolism. Also the contradiction in results may be due to differential fat distribution in populations.

Previous analyses of the association between the GLN223ARG polymorphism and obesity or BMI are controversial among different ethnic population and geographic area. More investigations are needed to further clarify this association.

We recommend further studies with larger sample sizes and linkage disequilibrium with other genetic variants must be considered as well as collective meta-analyses are needed in order to have a better understanding on the effect of these SNPs in obesity.

Conclusion

The LEPR Gln223Arg, UCP2 G 866 A and INSR exon 17 polymorphisms are related to obesity in Egyptian population. Further researches on larger population are necessary to ascertain the implications of LEPR, UCP2 and INSR polymorphisms in obesity.

Statement of authorship

Nayera E. Hassan: Conception and design of the study. She is the PI of the project from which this data was derived. Sahar A. El-Masry: Analysis and interpretation of the data. She is the Co-PI of the project from which this data was derived. Wahba Zarouk, and Rehab M.Mosaad: Responsible for genetic analysis. Rokia A El Banna and Muhamed Al-Tohamy: Share in data collection and drafting the article. All authors share in data collection, drafting the article and final approval of the version to be submitted.

Conflict of interest

The authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) the present work.

Acknowledgments

We would like to acknowledge our institute ''National Research Centre; Egypt"; without its support; this study could not have been done. We would also like to acknowledge everybody who participated in this study; the employers of our institute who were the participants of this study and their children, the technicians who helped in the laboratory analysis and the doctors who participated in collection of the data. Without their help, this study could not have been completed.

References

1. WHO. *Updates Fact Sheet on Overweight and Obesity*; 27 June 2016. http://www.who.int/mediacentre/factsheets/fs311/en/.
2. Jiao H, Kaaman M, Dungner E, Kere J, Arner P, Dahlman I. Association analysis of positional obesity candidate genes

based on integrated data from transcriptomics and linkage analysis. *Int J Obes (Lond)*. 2008;32:816—825.
3. Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. *Trends Genet*. 2010;26:266—274.
4. Perry B, Wang Y. Appetite regulation and weight control: the role of gut hormones. *Nutr Diabetes*. 2012;2:e26. https://doi.org/10.1038/nutd.2011.21.PMID:23154682.
5. Frühbeck G. Intracellular signaling pathways activated by leptin. *Biochem J*. 2006;393:7—20.
6. Chavarria-Avila E, Vázquez-Del Mercado M, Gomez-Bañuelos E, et al. The impact of LEP G-2548A and LEPR Gln223Arg polymorphisms on adiposity, leptin, and leptin-receptor serum levels in a Mexican Mestizo population. *Biomed Res Int*. 2015; 2015:539408.
7. Vozza A, Parisi G, De Leonardis F, et al. UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. *Proc Natl Acad Sci U S A*. 2014;111(3): 960—965. https://doi.org/10.1073/pnas.1317400111.PMID: 24395786.
8. Andersen G, Dalgaard LT, Justesen JM, et al. The frequent UCP2 -866G>A polymorphism protects against insulin resistance and is associated with obesity: a study of obesity and related metabolic traits among 17 636 Danes. *Int J Obes (Lond)*. 2013;37(2):175—181. https://doi.org/10.1038/ijo.2012.22.
9. Pey C, Pei Nizam N, Nee C, Jamal A, Bee Koon P, Ngah W. Association between polymorphisms of insulin and insulin receptor gene with childhood obesity in Malay population. *Jurnal Sains Kesihatan Malays*. 2016;14(1):5—9. ISSN 1675-8161.
10. Mutib M, Hamdan F, Al-Salihi A. INSR gene variation is associated with decreased insulin sensitivity in Iraqi women with PCOs. *Iran J Reprod Med*. 2014;12(7):499—506.
11. Ghalli I, Salah N, Hussien F, et al. In: Sartorio A, Buckler JMH, Crescere nel mondo Marazzi N, eds. *Egyptian Growth Curves 2002 for Infants, Children and Adolescents*. Ferring Publisher; 2008, 2008.
12. Hiernaux J, Tanner JM. Growth and physical studies. In: Weiner JS, Lourie SA, eds. *Human Biology: A Guide to Field Methods*. 1969:315—340. IBP. London, Blackwell Scientific Publications. Oxford. UK.
13. Sesti G, Cardellini M, Mi Marin, et al. A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects. *Diabetes*. 2003;52: 1280—1283.
14. Dhahi MA. Molecular screening for single nucleotide polymorphism in insulin receptor gene in Iraqi women with polycystic ovarian syndrome. *Int J Mod Biol Med*. 2013;3(1):17—26.
15. Wauters M, Mertens I, Chagnon M, et al. Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women. *Int J Obes*. 2001;25(5):714—720.
16. Takahashi-Yasuno A, Masuzaki H, Miyawaki T, et al. Leptin receptor polymorphism is associated with serum lipid levels and impairment of cholesterol lowering effect by simvastatin in Japanese men. *Diabetes Res Clin Pract*. 2003;62:169—175.
17. Fan SH, Say YH. Leptin and leptin receptor gene polymorphisms and their association with plasma leptin levels and obesity in a multi-ethnic Malaysian suburban population. *J Physiol Anthropol*. 2014;33(1):15.
18. Boumaiza I, Omezzine A, Rejeb J, et al. Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in tunisian volunteers. *Genet Test Mol Biomark*. 2012;16(7):726—733.
19. Mărginean C, Mărginean C, Voidăzan S, et al. Correlations between leptin gene polymorphisms 223 A/G, 1019 G/A, 492 G/C, 976 C/A, and Anthropometrical and biochemical parameters in children with obesity. *Medicine (Baltimore)*. 2016;95(12):e3115.
20. Bender N, Allemann N, Marek D, et al. Association between variants of the leptin receptor gene (LEPR) and overweight: a systematic review and an analysis of the CoLaus study. *PLoS One*. 2011;6, e26157. https://doi.org/10.1371/journal.pone.0026157.
21. Komşu-Ornek Z, Demirel F, Dursun A, Ermiş B, Pişkin E, Bideci A. Leptin receptor gene Gln223Arg polymorphism is not associated with obesity and metabolic syndrome in Turkish children. *Turk J Pediatr*. 2012;54:20—24.
22. Zandoná MR, Rodrigues RO, Albiero G, et al. Polymorphisms in LEPR, PPARG and APM1 genes: associations with energy intake and metabolic traits in young children. *Arq Bras Endocrinol Metabol*. 2013;57:603—611.
23. Gajewska J, Kuryłowicz A, Ambroszkiewicz J, et al. ADIPOQ -11377C > G polymorphism increases the risk of adipokine abnormalities and child obesity regardless of dietary intake. *J Pediatr Gastroenterol Nutr*. 2016;62:122—129.
24. Ben Ali S, Kallel A, Sediri Y, et al. LEPR p.Q223R polymorphism influences plasma leptin levels and body mass index in tunisian obese patients. *Arch Med Res*. 2009;40:186—190.
25. Saleh MC, Wheeler MB, Chan CB. Endogenous islet uncoupling protein-2 expression and loss of glucose homeostasis in ob/ob mice. *J Endocrinol*. 2006;190:659—667.
26. Srivastava N, Prakash J, Lakhan R, Agarwal CG, Pant DC, Mittal B. A common polymorphism in the promoter of UCP2 is associated with obesity and hyperinsulenemia in northern Indians. *Mol Cell Biochem*. 2010;337:293—298.
27. Martinez-Hervas S, Mansego ML, de Marco G, et al. Polymorphisms of the UCP2 gene are associated with body fat distribution and risk of abdominal obesity in Spanish population. *Eur J Clin Investig*. 2012;42:171—178.
28. Esterbauer H, Schneitler C, Oberkofler H, et al. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat Genet*. 2001;28:178—183.
29. Dhamrait SS, Stephens JW, Cooper JA, et al. Cardiovascular risk in healthy men and markers of oxidative stress in diabetic men are associated with common variation in the gene for uncoupling protein 2. *Eur Heart J*. 2004;25:468—475.
30. Brondani LA, Assmann TS, de Souza BM, Bouças AP, Canani LH, Crispim D. Meta-analysis reveals the association of common variants in the uncoupling protein (UCP) 1-3 genes with body mass index variability. *PLoS One*. 2014;9(5), e96411.
31. Zhang M, Wang M, Zhao ZT. Uncoupling protein 2 gene polymorphisms in association with overweight and obesity susceptibility: A meta-analysis. *Meta Gene*. 2014 Jan 31;2:143—159. https://doi.org/10.1016/j.mgene.2013.10.009.
32. Csernus K, Pauler G, Erhardt E, Lanyi E, Molnar D. Uncoupling protein-2 gene polymorphisms are associated with obesity in Hungarian children. *Acta Paediatr*. 2013;102(5):e200—e204.
33. Oktavianthi S, Trimarsanto H, Febinia CA, et al. Uncoupling protein 2 gene polymorphisms are associated with obesity. *Cardiovasc Diabetol*. 2012 Apr 25;11:41. https://doi.org/10.1186/1475-2840-11-41.
34. Otabe S, Clement K, Rich N, et al. Mutation screening of the human UCP 2 gene in normoglycemic and NIDDM morbidly obese patients: lack of association between new UCP 2 polymorphisms and obesity in French Caucasians. *Diabetes*. 1998; 47(5):840—842.
35. Schäuble N, Geller F, Siegfried W, et al. No evidence for involvement of the promoter polymorphism -866 G/A of the UCP2 gene in childhood-onset obesity in humans. *Exp Clin Endocrinol Diabetes*. 2003;111(2):73—76.
36. Maestrini S, Podestà F, Di Blasio AM, et al. Lack of association between UCP2 gene polymorphisms and obesity phenotype in Italian Caucasians. *J Endocrinol Investig*. 2003;26(10): 985—990.
37. Mukherjee S, Shaikh N, Khavale S, et al. Genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with polycystic ovary syndrome. *Eur J Endocrinol*. 2009;160(5):855—862.

38. Feng C, Lv PP, Yu TT, et al. The association between polymorphism of INSR and polycystic ovary syndrome: a meta-analysis. *Int J Mol Sci*. 2015;16(2):2403—2425. https://doi.org/10.3390/ijms16022403.PMID:25622255.
39. Wang Y, Yang W, Gui L, Wang H, Zan L. Association and expression analyses of the UCP2 and UCP3 gene polymorphisms with body measurement and meat quality traits in Qinchuan cattle. *J Genet*. 2016 Dec;95(4):939—946.
40. El-Hussiny M, Atwa M, Rashad W, Shaheen D, Elkady N. Leptin receptor Q223R polymorphism in Egyptian female patients with breast cancer. *Contemp Oncol (Pozn)*. 2017;21(1):42—47.