**Peroxisome-Proliferator-Activated Receptor-Gamma
Coactivator-1 α Variation: A Closer Look at Obesity Onset
Age and its Related Metabolic Status and Body Composition**

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Peroxisome-Proliferator-Activated Receptor-Gamma Coactivator-1 α Variation: A Closer Look at Obesity Onset Age and its Related Metabolic Status and Body Composition

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ABSTRACT

Background/Aims: There is a lack of knowledge regarding the effect of polymorphisms of the peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α) gene on the age of obesity onset. Hence, three polymorphisms of the PGC-1 α gene (PGC-1 α rs17574213, rs8192678 and rs3755863) were examined in association with obesity onset age. Also, obesity onset age-related metabolic status and body composition were evaluated.

Methods: This cross-sectional study was conducted with a total of 321 obese participants. Anthropometric and biochemical information, body composition, DNA extraction and sequencing of the PGC-1 α gene variables, were analyzed.

Results: The rs17574213 polymorphism was associated with obesity onset in children aged <1-year-old and ages 10-18 years old. The rs8192678 polymorphism was associated with obesity in adulthood. BMI, body fat percentage and trunk fat in groups whose obesity began at aged <1 year old, and from ages 10-18 years old, were higher than other groups. Serum levels of HDL, LDL and total cholesterol were at the lowest level in the group who began to be obese between the ages of 10 and 18 years. Visceral fat and FBS was highest in those whose obesity began in adulthood.

Conclusions: Two polymorphisms of the PGC-1 α gene were associated with obesity onset age.

Keywords

PGC-1 α variation, obesity, biochemical factors, body composition

44

45 **INTRODUCTION**

46 Obesity is a condition that is associated with complications such as dyslipidemia, insulin
47 resistance, type 2 diabetes, hypertension and inflammation (Head 2015). Unfortunately, in recent
48 years, the prevalence of obesity among different age groups (from early childhood to adulthood)
49 has increased (Biro and Wien 2010, Lakshman et al. 2012, Nguyen and El-Serag 2010). There is
50 evidence that adulthood obesity may have its origins in obesity at earlier ages (Simmonds et al.
51 2016, Whitaker et al. 1997). It seems that a previous history of obesity is correlated with further
52 reductions of resting metabolic rate (RMR) and, therefore, risks of weight gain in future (Astrup
53 et al. 1999). Furthermore, early obesity at a younger age increases the burden of adulthood co-
54 morbidities, as well as premature death. For instance, one longitudinal study showed that women
55 who were overweight during childhood had an increased risk of death from various causes,
56 including breast cancer. In contrast, men who were overweight before puberty displayed an
57 increased risk of death from ischemic heart disease (Must et al. 2012). For these reasons, it is
58 imperative to identify and control the causes of early-onset obesity. In this regard, it is generally
59 agreed that sedentary lifestyles and energy-dense diets can be related to the incidence of
60 childhood and adolescent obesity (Saker et al. 2011, Teo et al. 2014). However, body mass index
61 (BMI) variations may be related to genetic factors (Silventoinen et al. 2009). Among these
62 factors, peroxisome proliferator-activated receptor gamma coactivator-1- α (PGC-1 α) may play a
63 crucial role in weight control by regulating the expression of oxytocin (Blechman et al. 2011),
64 mitochondrial biogenesis, cellular respiration and adaptive thermogenesis (Scarpulla 2011,
65 Wu et al. 1999). PGC-1 α is a co-activator of several transcription factors, by which it is able to
66 perform a variety of functions (Liang and Ward 2006). It has been well documented that some

variation in the PGC-1 gene (Gly482Ser) can disrupt the activity of this co-activator (Choi et al. 2006) and lead to obesity in inactive elderly men but not in females (Ridderstrale et al. 2006). The Thr394Thr polymorphism of the PGC-1 α gene has also been reported to show association with the accumulation of body fats in different areas (Vimalaswaran et al. 2006). However, other variants of this gene have been studied less in relation to obesity. Likewise, no study has been carried out on the relationship between PGC-1 α gene polymorphisms and the age of obesity onset.

Considering the importance of early onset obesity on public health, the main objective of this study was to understand how variations of PGC-1 could affect the obesity onset age in obese people. In addition, the age of onset of obesity-related metabolic status and body composition were also evaluated

METHODS

Study Population: This cross-sectional study was conducted from November 2013 through to December 2014, on 321 obese ($\text{BMI} \geq 30$) male ($n=66$) and female ($n=255$) participants, who had been referred to the nutrition clinic of Shariati Hospital (Tehran, Iran) and met all the inclusion criteria (including: aged between 23-62; absence of any acute or chronic diseases; no alcohol or drug abuse; and not being pregnant). Prior to the research, written informed consent was signed by all participants. Likewise, participants were asked to maintain their usual diet and avoid strenuous activities two days before the start of the study. Self-reported obesity onset age, family history of obesity, and experiencing the yo-yo effect (regaining weight after dieting) were recorded via face to face interview. The validity of self-reported obesity onset age was determined using participant's previous paper medical records. Appropriate standards for human experimentation have been followed and ethical approval was obtained from the local ethics committee of Tehran University of Medical Sciences (Ethics number: 02-27-18041-69439).

Anthropometric Measurements: A Seca 803 digital scale (100 gram accuracy) and a Seca 213 portable stadiometer (0.1 cm accuracy) were used to measure weight and height, respectively. Afterwards, BMI (body mass index) was determined by the following formula: $\text{weight (kg)} / \text{height}^2 \text{ (m)}$.

Biochemical Analysis: Fasting blood samples were collected between 8:00 and 10:00 am. Afterward, the blood samples were incubated at 37°C and then centrifuged for 15 minutes at a speed of 3000 rpm. Finally, the serum was separated and stored at -80°C for future analysis. The levels of serum glucose and triglyceride were measured using the Glucose oxidase-Peroxidase

(GOD/POD) and glycerol phosphate oxidase enzyme methods. Total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) values were determined by means of enzymatic endpoint analysis, enzymatic clearance assay, and direct clearance method respectively. All of the above measurements were carried out using the Randox Laboratories kit (Randox Laboratories LTD, Ardmore, United Kingdom). Serum insulin was measured by radioimmunoassay, using a commercial kit (Biosource, Denmark). In addition, hypersensitivity C-reactive protein (hs-CRP) determination was carried out by an immunoturbidimetric assay (high sensitivity assay, Hitachi 902).

Body Composition Analysis: A body composition analyzer BC-418MA-Tanita (UK) was used to estimate both male and female participants' fat percentage, fat mass, visceral fat, trunk fat and fat-free mass. This procedure has been described previously (Mirzaei et al. 2012).

DNA Extraction and Gene Sequencing: All stages of DNA extraction and sequencing of the PGC-1 α gene have already been published previously (Mirzaei et al. 2012).

Statistical Analysis:

The minimum sample size of 170 was calculated with the following equation (Charan and Biswas 2013):

$$\text{Sample size} = \frac{Z_{1-\alpha/2}^2 p (1-p)}{d^2}$$

Two sided $\alpha=0.05$, $P=0.46$ for the frequency of major allele (CC) of the genotype of SNP rs8192678 in the previous study (Mirzaei et al. 2012); and $d=0.075$ as an absolute precision which was decided by researchers. To increase the power of the study, we included almost twice as many participants who wished to cooperate ($n=321$). The Hardy–Weinberg equilibrium and the

comparison of categorical variables were assessed by χ^2 test. The comparison of means among several groups was evaluated using a one-way analysis of variance (ANOVA) test. Likewise, a multi-nominal logistic regression was used to investigate the relationship between various polymorphisms and obesity onset age. In all analyses, $p < 0.05$ was considered as statistically significant. SPSS statistical software (Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used for statistical analysis of the data.

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RESULTS

Study Population: The study population (n=321) included both men (n=66) and women (n=255) who were willing to participate. Face-to-face interviews showed that 264 participants (82.2%) had a family history of obesity, and 216 of them (67.3%) had experienced the yo-yo effect (regaining weight after dieting). The characteristics of the participants, including anthropometric measurements, laboratory results, body composition and RMR data are shown in Table 1.

Characteristics of the Study Population by Obesity Onset Age: Based on the obesity onset age, participants were divided into 4 groups: Less than one year of age (<1 year), 1-9 years old, 10-18 years and over 18 years (> 18 years).

The characteristics of obese people by age of onset of obesity are shown in Table 2.

PGC1 α Gene Sequencing and Target Single-Nucleotide Polymorphism (SNP) Frequency: Positions 23815227 through 23815706 on the PGC1 α gene were sequenced. Out of 6 SNPs located in the position mentioned, the SNPs (rs17574213), Gly 482 Ser (rs8192678) and (rs3755863) were studied. The frequencies of the genotypes in all three SNPs in our population were in Hardy- Weinberg equilibrium. The genotype of each polymorphism, its frequency and obesity- related risk alleles are summarized in Table 3.

Single-Nucleotide Polymorphisms (SNPs) and Obesity Onset Age: Multi-nominal logistic regression was used to understand the relationship between SNPs and the age of onset of obesity

(less than one year of age, 1-9 years old, 10-18 years and over 18 years) . In this model, the age group of over 18 was selected as a reference category. The findings are summarized in Table 4. After adjustment for current body mass index and physical activity, due to Beta coefficients and significance levels, having the SNP (rs8192678) was associated with a lower risk of obesity at less than one year of age (<1 year- old), compared to ages over 18 years. On the contrary, the risk allele of SNP (rs17574213) was related to a higher risk of obesity at earlier ages (<1 year- old and ages 10-18 years old) than adults aged over 18 years old. SNP (rs3755863) had no significant effect on the age of onset of obesity.

Genotype frequencies of polymorphisms by age of onset of obesity

Genotype frequencies of rs17574213, rs8192678 and rs3755863 by age of onset of obesity are shown in Table 3.

DISCUSSION

The present study showed that two SNPs in the PGC1 α gene (rs17574213 and rs8192678) were associated with obesity onset age. Interestingly, carriers of the rs17574213 polymorphism were at greater risk of obesity at earlier ages (<1-year-old and 10-18 years old). It seems that reducing the amount of RMR/kg (Kcal/24h/kg of body weight) or RMR/LBM (Kcal/24h/kg of lean body mass) in the holders of SNP (rs17574213) risk allele (Mirzaei et al. 2012) can justify the underlying mechanism on how the above-mentioned polymorphism in the PGC1 α gene contributes to the increased risk of obesity. Another possibility is the reduction of metabolic activity of brown adipose tissue (BAT), whose activity and amount is significant during infancy and puberty (Gilsanz et al. 2013). Cypess and Kahn (2010) reported that childhood obesity may be due to the impaired function of a protein called uncoupling protein-1 (UCP-1), which is largely found in BAT mitochondria, and which plays an important role in the thermogenesis of this tissue (Cypess and Kahn 2010). Intriguingly, UCP-1 expression is induced by PGC1 α (Lo and Sun 2013), and it is obvious that if SNP (rs17574213) in the PGC1 α gene disrupts the expression and activity of this protein, it could lead to obesity in children by reducing UCP-1 levels. Undoubtedly, further studies are needed to examine the association of this SNP and UCP-1 expression.

One finding that highlighted the association between SNP (rs17574213) and obesity in <1 year or 10-18 year-olds was that the highest levels of BMI, fat percent and trunk fat were observed in

adult participants whose obesity onset age fell in these two age ranges. In this context, the results of various published studies are contradictory: for example, Nader and colleagues (2006) found that being overweight during the preschool and elementary school period was associated with being overweight at age 12 years (Nader et al. 2006). Additionally, Taveras et al. (2011) suggested that weight gain during the first two years of life (especially 6 months of age) is associated with an increased risk of obesity in childhood (Taveras et al. 2011). However, Demerath et al. (2009) showed that rapid weight gain during the first two years of life was associated with increased risk of obesity and body fat accumulation in middle adulthood (Demerath et al. 2009). Therefore, it seems that more research is needed to determine the exact relationship. Another finding of this study which highlights the importance of early-life or adolescence obesity was that the level of hs-CRP was highest in participants whose age of onset of obesity was under 1 year. hs-CRP is a known marker of inflammation which is defined as a predictor of cardiovascular disease (CVD), and can be classified at three levels: A) <1 mg/l as a low risk of CVD; B) 1-3 mg/l as a moderate risk of CVD and C) >3 mg/l as a high risk of CVD (Kamath et al. 2015). Considering the serum levels of hs-CRP (Table 2), most groups, especially those who were obese at under one year old, are more likely to develop cardiovascular disease. In addition, the serum levels of HDL, LDL and total cholesterol were at the lowest level in the group who were obese between the ages of 10 and 18 years. In this regard, it is anticipated that obese participants with lower HDL, who are at greater risk of CVD (Ali et al. 2012), are protected by the significant lower level of LDL and total cholesterol. The association between SNP (rs17574213) and younger age obesity, body composition and biochemical parameters is summarized in Figure 1a. The results of this study also revealed that having the risk allele of SNP rs8192678 (Gly 482 Ser) could be a serious concern for adulthood obesity (aged over 18

years). There is evidence that Gly 482 Ser SNP is partly responsible for reducing age-related skeletal muscle PGC1 α (Ling et al. 2004). Since PGC1 α plays a vital role in energy homeostasis (Liu and Lin 2011), it is not surprising that its reduction at higher ages in carriers with Gly 482 Ser SNP could be related to weight gain. The predictive value of Gly 482 Ser SNP on adulthood obesity became apparent when the highest levels of visceral fat and fasting blood glucose were observed in obese participants whose obesity began after 18 years of age. It has been demonstrated that visceral fat accumulation (acquired or inherited) leads to metabolic syndrome and subsequent cardiovascular diseases by decreasing adiponectin and increasing inflammatory biomarkers such as TNF α , IL-6 (Di Chiara et al. 2012). Hence, having this polymorphism may be a warning sign of future cardiovascular disease. Despite higher visceral fat levels in this group, the body mass index, total fat percentage and trunk fat were less than in the other three groups (Figure 1b). The pattern of changes in body mass index and body fat percentage were also similar in other groups. This finding was consistent with prior literatures which showed a positive correlation between BMI and body fat percentage (Akindele et al. 2016, Ranasinghe et al. 2013). However as the data shows, the distribution of body fat varies among people. Finally, the result we have shown here, first proved the importance of PGC1 α variation in determining the age of onset of obesity and its consequences. However, the current study had some limitations. Firstly, the number of women and men was not equal (255 women versus 66 men). In fact, due to the lower number of men, it was not possible to perform sub-group analysis separately by sex. Secondly, the samples were selected from those who had been referred to the nutrition clinic, which might reduce generalizability. However, because obese people were referred from different parts of Iran, the generalizability problem may have been somewhat resolved. Thirdly, the non-obese control group was not included in the study.

CONCLUSION

PGC1 α variation might be one of the genetic roots of obesity in different periods of human life. Further studies are certainly recommended in this area.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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417 Figure Caption

418 Figure1. The association between SNP (rs17574213) or SNP (rs8192678) and obesity, body
419 composition and biochemical parameters in figure 1 a and 1b, respectively

420 Figure Legend

421 (Figure1a) the rs17574213 polymorphism was associated with obesity onset in children aged
422 <1-year-old and ages 10-18 years old, probably due to reduction of resting metabolic rate
423 (Mirzaei 2012) or UCP-1 expression (Cypess and Kahn 2010). BMI, body fat percentage and
424 trunk fat in the groups mentioned above were higher than other groups (1-9 years old and > 18
425 years). Serum levels of HDL, LDL and total cholesterol were at the lowest level in the group
426 who began to be obese between the ages of 10 and 18 years rather than other groups. The level of
427 hs-CRP was highest in participants whose obesity onset age was under 1 year. (Figure1b) the
428 rs8192678 polymorphism was associated with obesity in adulthood (>18 years old), probably
429 due to reduction of age-related skeletal muscle PGC1 α (Ling 2004). BMI, body fat percentage

430 and trunk fat in this group was the lowest compared to three other age groups. Although, visceral
431 fat and fasting blood sugar was the highest in those whose obesity began after 18 years old. hs-
432 CRP: high-sensitivity C-reactive protein, HDL: High-density lipoprotein, *LDL*: Low-density
433 lipoprotein, T-chol: Total cholesterol, BMI: Body Mass Index, FBS: Fasting Blood Sugar, RMR:
434 Resting Metabolic Rate, LBM: Lean Body Mass, UCP-1: uncoupling protein 1, PGC-1 α :
435 peroxisome proliferator-activated receptor gamma coactivator-1- α , : the highest level
436 compared to other age groups, : the lowest level compared to other age groups

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Table 1. Characteristics of Participants (n=321)

Characteristics	Mean (SD)
Anthropometric	
Age (years)	39.52 (11.73)
Height (cm)	162.70 (8.64)
Weight (kg)	94.66 (15.61)
BMI (kg/m ²)	35.47 (3.99)
Laboratory	
FBS (mg/dl)	107.62 (46.21)
TG (mg/dl)	136.45 (63.97)
T-chol (mg/dl)	192.00 (33.06)
HDL (mg/dl)	51 (13.03)
LDL(mg/dl)	103.64(21.46)
Insulin (μIU/ml)	15.00 (6.78)
hs-CRP (mg/L)	3.77 (3.68)
Body Composition	
Fat percent (%)	40.12 (6.65)
Fat mass (kg)	37.86 (8.43)
Fat-free mass (kg)	56.81 (12.46)
Visceral fat (kg)	11.09 (3.47)
Trunk fat (kg)	19.18 (4.49)

*BMI: Body mass index, FBS: Fasting blood sugar, TG: Triglyceride, T-chol: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, hs-CRP: high-sensitivity C-reactive protein.

Table 2. Characteristics of Participants by Obesity Onset Age

Characteristics	Age of onset of obesity (years)	Mean (SD)				P-value
		<1	1-9	10-18	>18	
		n=66	n=84	n=69	n=102	
Age (years)		36.72 ^a (7.88)	36.42 ^b (11.88)	33.6 ^c (8.65)	47.88 ^{abc} (11.28)	<0.0001
Height (cm)		160.06 ^a (8.31)	163.3 (8.38)	165.43 ^a (8.09)	162.48 (9.36)	0.004
Weight (kg)		92.85 ^a (13.7)	96.25 (16.32)	99.91 ^a (16.38)	90.97 (15.12)	0.0016
BMI (kg/m ²)		36.23 ^a (5.07)	35.82 (3.86)	35.96 ^b (3.65)	34.35 ^{ab} (3.45)	0.007
FBS (mg/dl)		115.79 ^a (64.80)	88.86 ^{ab} (12.7)	100.9 ^c (15.06)	119.95 ^{bc} (54.42)	<0.0001
TG (mg/dl)		140.14 (51.48)	137.47 (71.51)	137.30 (99.79)	132.47 (45.37)	0.9
T-chol (mg/dl)		194.07 ^a (37.91)	200.2 ^b (34.04)	175.6 ^{ab} (33.38)	192.63 (27.48)	<0.0001
HDL (mg/dl)		49.07 (9.27)	53.33 ^a (15.63)	45.1 ^{ab} (9.6)	53.68 ^b (14.31)	<0.0001
LDL (mg/dl)		107.21 ^a (25.19)	107.6 ^b (19.96)	93.2 ^{abc} (18.81)	103.37 ^c (20.73)	0.0001
Insulin (μIU/ml)		14.18 (5.99)	14.33 (6.44)	16.87 (9.02)	15.16 (6.63)	0.09
hs-CRP (mg/L)		4.67 ^a (4.31)	2.78 ^a (2.8)	3.68 (4.63)	3.92 (3.36)	0.0215
Fat percent (%)		42.59 ^a (5.58)	40.08 (8.29)	40.30 (6.24)	38.43 ^a (5.74)	0.0013
Fat mass (kg)		39.87 ^a (9.86)	38.29 (9.20)	40.06 ^b (7.86)	34.72 ^{ab} (6.29)	<0.0001
Fat-free mass (kg)		52.99 ^a (7.20)	57.98 (15.07)	59.86 ^a (12.95)	56.25 (12.27)	0.0107
Visceral fat (kg)		10.59 (2.88)	10.28 ^a (2.90)	11.26 (3.49)	11.97 ^a (4.1)	0.0055
Trunk fat (kg)		19.6 ^a (4.32)	19.37 ^b (5.43)	20.76 ^c (3.68)	17.66 ^{abc} (3.91)	<0.0001

* BMI: Body mass index, FBS: Fasting blood sugar, TG: Triglyceride, T-chol: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, hs-CRP: high-sensitivity C-reactive protein. P-values were determined using one-way ANOVA. Similar superscript letters mean statistically significant differences (p<0.05) using Tukey's post hoc test. P-values less than 0.05 were considered significant.

Table 3. Genotype of Selected SNPs and Their Frequencies

SNPs	Genotype	Frequency % (n)	Frequency % (n) for each age group			
Age groups (years)			<1	1-9	10-18	>18
rs17574213	GG	88.79 (285)	71.3 (47)	100 (66)	50 (33)	100 (66)
	GA	11.21 (36)	28.8 (19)	0 (0)	50.8 (35)	0 (0)
rs8192678	CC	33.02 (106)	84.9 (56)	37.9 (25)	0 (0)	0 (0)
	CT	58.88 (189)	15.2 (10)	62 (52)	100 (69)	80.4 (82)
	TT	8.1 (26)	0 (0)	0 (0)	0 (0)	19.7 (20)
rs3755863	CC	25.86 (83)	57.7 (38)	37 (31)	0 (0)	0 (0)
	CT	55.45 (178)	42.5 (28)	50 (42)	50.8(35)	70.6 (72)
	TT	18.69 (60)	0 (0)	13.1 (11)	53.7 (37)	31.4 (32)

Table 4. Relationship between Single-Nucleotide Polymorphisms (SNPs) and Obesity Onset**Age**

SNPs	Age (year)	β	SE	Wald χ^2	CI of β	P-value
rs17574213	<1	9.99	0.82	147.91	8.34 to 11.63	<0.00001
	1-9	-0.22	0.47	0.22	-1.16 to 0.71	0.63
	10-18	10.45	1.04	147.85	8.35 to 12.54	<0.00001
rs8192678	<1	-2.29	0.85	7.185	- 3.99 to -0.5	0.007
	1-9	-0.97	0.70	1.92	-2.28 to 0.42	0.16
	10-18	13.72	0.78	3.84	12.16 to 15.28	0.05
rs3755863	<1	-1.32	0.69	3.627	-2.7 to 0.06	0.056
	1-9	-0.97	0.66	2.13	-2.3 to 0.35	0.14
	10-18	-0.42	1.07	0.15	- 2.56 to 1.71	0. 69

* SNPs: Single-nucleotide polymorphism. P-values were determined using multi nominal logistic regression. Age group over 18 years was selected as a reference category. P-values less than 0.05 were considered significant.

39x17mm (300 x 300 DPI)