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Methylenetetrahydrofolate Reductase and Peroxisome Proliferator-Activated Receptor-γ Gene Polymorphisms and Risk of Obesity in Saudi Females

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- Polymorphism
- PPAR-γ
- MTHFR

Abstract

Background: Obesity is a systemic and chronic disease with genetic susceptibility, characterized by an increase in storage and irregular distribution of body fat. **Aim:** The aim of this study is to evaluate the association of polymorphisms of methylenetetrahydrofolate reductase (MTHFR) and peroxisome proliferator-activated receptor (PPAR) genes with obesity among adult females in Al-Madina, Saudi Arabia. **Method:** The participants in this study were 114 healthy females aged 18–60 years. Body mass index (BMI) and body composition parameters were assessed. Triglycerides, total cholesterol, HDL, and LDL were measured. Real-time PCR was used to detect the selected genetic polymorphisms of PPAR-γ rs1801282 and MTHFR rs1801131. **Results:** Weight, BMI, fat-percentage, FFM, truncal fat mass, and LDL were significantly higher in the overweight and obese group than in the normal BMI group. PPAR-γ genotype and allele polymorphism frequencies in the overweight and obesity group showed no statistically significant differences compared to those of the normal weight group. However, a significant difference was found between the frequency of both T and G alleles of MTHFR in the normal BMI and overweight and obese group ($p = 0.03$) and a significant correlation with the fat-percentage in both groups ($p = 0.01$). A significant correlation between the MTHFR genotypes and the fat-percentage in both groups was also found ($p = 0.04$). **Conclusion:** In this population of Saudi females, we found that MTHFR GG genotype and G allele are significantly correlated with the deposition of fat in the body; women with this allele and genotype should be advised to control their weight and fat intake, as this is also associated with LDL levels that may affect their cardiovascular system. PPAR-γ did not seem to play a role in obesity of the recruited subjects.

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INTRODUCTION

Obesity is a systemic and chronic disease with genetic susceptibility, characterized by an increase in fat storage and its irregular distribution in the body. Metabolic changes usually accompany obesity, that predispose other health presentations and, in most cases, are associated with cardiovascular, orthopedic, and endocrine disorders relating to diverse biological, psychological, and sociocultural factors (Blakemore and Froguel 2008, Das 2010).

Obesity has two main characteristics: increase in body mass index (BMI) and disproportion of body composition, leading to a rise of fat mass (Mutch and Clement 2006, Dixon 2010). This increase, represented by expansion and irregular distribution of white adipose tissue (WAT), is the most consistent pathological process in obesity.

Peroxisome proliferator-activated receptor-gamma (PPAR-γ), as a transcription factor, plays a major role in the activation of adipocyte differentiation and is a key modulator of gene expression regulating the transcription of a number of target genes in several specialized cell types, including adipocytes. PPAR-γ signaling pathways affect lipid metabolism and have shown associations with obesity, cardiovascular disease, and diabetes (Walczak and Tontonoz 2002).

Since PPAR-γ 2 is expressed only in adipose tissue, the Pro12Ala polymorphism was originally studied for its association with obesity. The PPAR-γ polymorphism (Pro12Ala Allele) influences several biological factors, serving as the master regulator of fat-cell formation, and influences insulin resistance (Jo, Yang et al. 2006). PPARs exert a measure of transcriptional control regulating lipid metabolism, glucose transport and insulin sensitivity, oxidative stress, and inflammation (Lehrke and Lazar 2006). Mutations affecting the function of PPAR-γ can cause hypertension, insulin resistant, and dyslipidemia syndrome characteristic of cardiovascular dysmetabolic syndrome (Libby 2001).

Several studies have shown that high body mass index (BMI) is associated with low folate levels (Mojtabai 2004) and may have important health implications. Lower serum folate levels were found to have a strong correlation with increased BMI in women of childbearing age, in two waves of the National Health and Nutrition Examination Survey. An association was found between the MTHFR (C677T) TT genotype and reduced folate availability (Frosst, Blom et al. 1995), and a similar correlation was observed with obesity in the British Women's Heart and Health Study (BWHHS) (Lewis, Lawlor et al. 2006). These results confirm that this genotype could not have been affected by adult BMI, and is not confounded by lifestyle factors (Smith and Ebrahim 2003, Smith and Ebrahim 2004). Thus, their MTHFR genotype–obesity findings suggested that folate levels may causally correlate with greater BMI and obesity (Lewis, Lawlor et al. 2008).

The lack of such studies among women in Saudi Arabia prompted us to conduct this study with the objective of investigating the association of MTHFR and PPAR-γ polymorphisms with obesity in Saudi females from the Al-Madina region.

Participants and Methods

A cross-sectional analytical observational study was conducted from January to April 2016 at Medicine and Nursing Colleges, Taibah

University, AL-Madina Al-Monawara, Saudi Arabia. The participants in this study were 114 healthy females, aged 18–60 years. Participation was voluntary, and signed informed consent was obtained from each subject. The study was reviewed and approved by Taibah University College of Dentistry Research Ethics Committee (TUCDREC), approval No. TUCDREC/20151011/Youssef.

Each participant fulfilled the following procedures:

Anthropometric measurements: Height was measured to the nearest 0.1 cm using a standard stadiometer. Body composition was measured by a body composition analyzer, type BC-418 MA TANITA (Japan). Participants were asked not to eat or drink within 4 hours prior to the test, to void (empty the bladder) within 30 minutes prior, and to wipe off the soles of their feet before stepping onto the platform, as unclean foot pads interfere with the device's conductivity. The participant stood on a metal footpad scale and gripped the handles. After inputting the participant's sex, age, and height, the device calculates body mass index, fat mass, fat-free mass, truncal fat mass, and truncal fat percentage, and prints out a report immediately.

Body mass index was used to assess overweight and obesity. Calculation of BMI (kg/m^2) is body weight in kilograms (kg) ÷ height in square meters (m^2), and is classified according to the National Institutes of Health (NIH) standard. Adults were classified based on their BMI as underweight (BMI < 18.5), normal (BMI = 18.5–24.9), overweight (BMI = 25–29.9) or obese (BMI ≥ 30). Obesity was further subdivided into three grades: Grade 1 (BMI = 30–34.9), Grade 2 (BMI = 35–39.9), and Grade 3, or extreme obesity, (BMI ≥ 40) (Chang et al. 2003). Participants were classified as having low body-fat (BF < 21%), normal (BF = 21–33%), high (BF ≥ 35%) (Ko, Tang et al. 2001).

Biochemical values: Fasting blood samples were obtained for measurement of triglycerides, as determined by an enzymatic colorimetric method, according to Bucolo and David (Bucolo and David 1973), and total cholesterol and HDL-cholesterol were estimated by an enzymatic colorimetric method, according to Allain et al. (Allain, Poon et al. 1974) and Friedewald et al. (Friedewald, Levy et al. 1972). LDL-cholesterol was calculated according to (Friedewald, Levy et al. 1972), as follows: LDL-C = total cholesterol – (triglyceride/5 + HDL-C).

DNA extraction: Peripheral blood samples were collected in EDTA-vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). All DNA samples were extracted using the QIAamp DNA blood mini-kit (Qiagen, Germany) according to the manufacturer's protocol. The purity of the DNA was determined by calculating the ratio of absorbance at 260/280 nm, and all DNA samples had a ratio of 1.8–1.9. The purified genomic DNA concentration was measured by nanodrop and stored at –80°C till testing. Real-time polymerase chain reaction (PCR) was used to detect the selected genetic polymorphisms.

SNPs genotype assay: qPCR was used to detect the SNPs of interest in a patient's DNA, the SNP rs1801282 (PPAR-γ) and rs1801131 (MTHFR). TaqMan SNP genotyping Universal Master Mix and genotyping assays were purchased from Thermo Fisher (USA). Sample testing was carried out on a 96-well optical reaction plate. Each reaction contained 5 μL TaqMan® 2 × Universal PCR Master Mix No AmpErase® UNG (Thermo

Fisher), 0.5 μL TaqMan® SNP Genotyping Assays, 1.5 μL distilled water, and 1 μL purified DNA, with a final reaction volume of 10 μL for every SNP. The plate also contained at least two negative water controls without DNA.

Statistical analysis

Data were analyzed using the SPSS version 23.0 (SPSS, Inc. Chicago, IL). A frequency table was used to present the distribution of nominal variables. Results were expressed as means ± standard deviation. Qualitative variables were expressed in number and percentage. Parametric variables were analyzed using students' t-test and ANOVA, while the analyzed non-parametric variables were tested using Chi-squared tests. Pearson's correlation coefficients were used to test association and correlations. P values below 0.05 were considered statistically significant.

Results

A total of 114 female participants were recruited in this study with ages ranging from 18–60 years. Comparisons of anthropometric parameters between the two groups of the study (normal BMI and BMI ≥ 25) are presented in Table 1. The table shows that weight ($p < 0.001$), BMI ($p < 0.001$), fat-percentage ($p < 0.001$), FFM ($p < 0.001$), truncal fat mass ($p < 0.001$) were significantly more in the overweight and obese group than in the normal BMI group. Among the lipid profile parameters, only LDL had a significant difference between normal and high BMI subjects ($p = 0.001$) while HDL, total cholesterol, and triglycerides showed no significant difference. It is worth mentioning that HDL was lower in the overweight and obese group, but the difference was statistically insignificant. Table 1 also shows that there was no significant difference in height between the two groups.

Table 2 shows the correlation between the investigated parameters and the BMI. The table shows strong correlations between BMI and fat-percentage ($p = 0.002$), FFM ($p < 0.001$), truncal fat ($p = 0.016$), and LDL ($p < 0.001$).

The CG genotype of PPAR-γ was found in about 8% of our cohort, while the CC genotype was found in about 92% of the studied cohort. The frequency of TT, TG, and GG genotypes of MTHFR gene were 45%, 43.2%, and 11.8%, respectively, as illustrated in Table 3.

Table 4 shows significant positive correlations between MTHFR and the levels of fat-percentage in the recruited subjects. The frequency of TT was 2, 27, 19; TG was 3, 14, 27; and GG was 1, 1, 9, in low, normal, and high fat-percentage, respectively ($p = 0.031$). Figure 1 shows the relation between MTHFR and fat-percentage. Table 5 shows no statistically significant differences between either the normal BMI or the high BMI groups as to frequencies of the studied PPAR-γ and MTHFR genotypes ($p > 0.05$).

Table 6 shows the relation between alleles and genotypes of MTHFR and PPAR-γ genes single-nucleotide polymorphisms to body mass index, fat-percentage, and truncal fat mass in the two groups of recruited subjects. The table shows a significant difference between the frequency of both T and G alleles of MTHFR in the normal BMI and the overweight and obese group ($p = 0.03$), and a significant correlation with the fat-percentage in both groups ($p = 0.01$). Table 6 also shows that there is a significant correlation between the MTHFR genotypes and the fat-percentage in both groups ($p = 0.04$). Truncal fat

mass did not show a similar correlation for either the MTHFR genotypes (p = 0.3) or alleles (p = 0.15).

Table 1. Anthropometric parameters among different body mass index (BMI) groups.

Parameters	Normal BMI Mean ± SD (n = 73)	Overweight and obese (BMI ≥ 25) Mean ± SD (n = 41)	P[1]
Height	157.0 ± 6.44	154.8 ± 20.56	0.372
Weight	52.1 ± 9.71	76.9 ± 17.91	0.000
BMI	20.8 ± 3.79	34.7 ± 21.25	0.000
Fat-percentage	27.3 ± 7.14	40.2 ± 6.35	0.000
FFM	37.1 ± 4.51	45.5 ± 5.78	0.000
Truncal fat mass	7.5 ± 5.53	16.4 ± 5.02	0.000
Total cholesterol	185.7 ± 47.97	207.4 ± 67.97	0.12
Triglycerides (TG)	89.9 ± 27.23	109.0 ± 52.47	0.053
HDL	65.3 ± 17.06	60.5 ± 24.88	0.386
LDL	107.7 ± 42.70	163.7 ± 74.4	0.001

[1] Significance at $p < 0.05$

Table 2. The correlation coefficient between BMI and certain parameters.

BMI (n = 114)	R	P
Fat-percentage	0.287	0.002
FFM	0.372	0.000
Truncal fat	0.441	0.016
LDL	0.314	0.000

Table 3. Frequencies of PPAR-γ binary CC, CG, GG; and MTHFR binary TT, GT, GG.

PPAR-γ		MTHFR	
CC	106 (92.2%)	TT	50 (45%)
CG	8 (7.8%)	GT	48 (43.2%)
C	220 (96.49%)	GG	13 (11.8%)
G	8 (3.51%)	T	148 (66.7%)
		G	74 (33.3%)

Table 4. Correlation between MTHFR and fat percentage.

MTHFR	Fat Percentage			P =
	Low	Normal	High	
TT	2	27	19	0.03
GT	3	14	27	
GG	1	1	9	

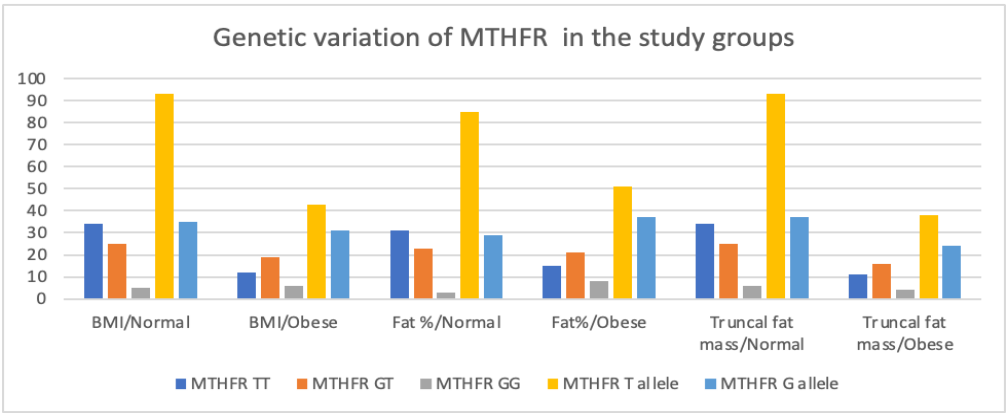
Table 5. Genotypic and allelic frequencies between the normal body mass index and the overweight/obesity groups (n (%)).

SNP	Genotype (Allele)	Normal BMI	Overweight and obese	P =
MTHFR rs1801131	TT	34 (53.1)	12 (32.4)	0.103
	GT	25 (39.1)	19 (51.4)	
	GG	6 (7.8)	5 (16.2)	
PPAR-γ rs1801282	CC	64 (91.4)	35 (94.6)	0.554
	CG	6 (8.6)	2 (5.4)	

Table 6. Analysis of MTHFR and PPAR-γ genes single nucleotide polymorphisms to body mass index, fat percentage, and truncal fat percentage in the different groups

Allele	BMI			Fat percentage			Truncal fat mass		
	Normal BMI	Overweight & obese group	P value	Normal BMI	Overweight & obese group	P value	Normal BMI	Overweight & obese groups	P value
MTHFR									
TT	34 (53.125%)	12 (32.43%)	0.10	31 (54.39%)	15 (34.09%)	0.04	34 (52.31%)	11 (35.48%)	
GT	25 (39.06%)	19 (51.35%)		23 (40.35%)	21 (47.73%)		25 (38.46%)	16 (51.61%)	
GG	5 (7.81%)	6 (16.22%)		3 (5.263%)	8 (18.18%)		6 (9.23%)	4 (12.90%)	0.30
T allele	93 (72.65%)	43 (58.11%)	0.03	85 (74.56%)	51 (57.95%)	0.01	93 (71.54%)	38 (61.29%)	
G allele	35 (27.34%)	31 (41.89%)		29 (25.44%)	37 (42.05%)		37 (28.46%)	24 (38.71%)	0.15
PPAR-γ									
CC	64 (91.42%)	35 (94.59%)	0.28	58 (92.06%)	41 (93.18%)	0.83	66 (91.67%)	28 (93.33%)	
CG	6 (8.57%)	2 (5.41%)		5 (7.94%)	3 (6.81%)		6 (8.33%)	2 (6.67%)	0.78
C allele	134 (95.71%)	72 (97.23%)	0.74	121 (96.03%)	85 (96.59%)	0.84	138 (95.83%)	58 (96.67%)	
G allele	6 (4.28%)	2 (2.70%)		5 (3.97%)	3 (3.41%)		6 (4.17%)	2 (3.33%)	0.78

(A)



(B)

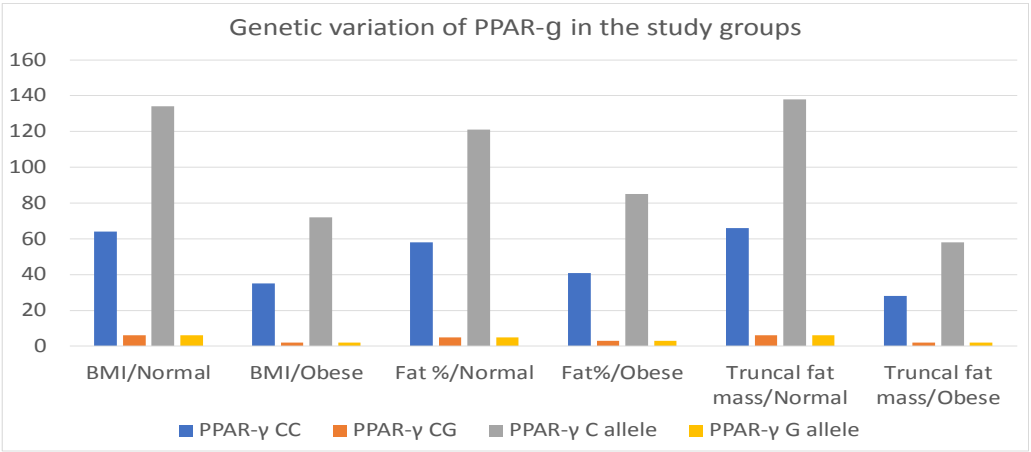


Figure 1: Variations in the frequency of genetic variants in MTHFR (A) and PPAR-γ (B) of the recruited subjects.

Discussion

Obesity is now considered a serious public health problem worldwide, increasing the risk of several diseases, such as diabetes mellitus, cardiovascular diseases, and some cancers (Van Gaal and Maggioni 2014). In 2016, WHO estimated that more than 1.9 billion adults were overweight, including over 650 million who were obese (WHO 2019). The development of obesity is influenced by several factors, including genetic factors, making the identification of these genetic factors essential for understanding the pathogenesis of obesity and to help provide effective preventive and therapeutic options. The C677T polymorphism in the MTHFR gene was investigated as potentially playing a role in obesity (Bell, Walley et al. 2005, Thawnashom, Tungtrongchitr et al. 2005, Terruzzi, Senesi et al. 2007, Lewis, Lawlor et al. 2008, Settin, Algasham et al. 2009, Tavakkoly Bazzaz, Shojapoor et al. 2010, Chauhan, Kaur et al. 2011, Gara, Ochi et al. 2011, Tabassum, Jaiswal et al. 2012). The mechanisms by which these mutations affect development of obesity/overweight are still unclear, but studies have hypothesized that elevated homocysteine levels may lead to the development of overweight or obesity via epigenetic control of gene expression in the regulation of fat storage in the body (Fuks 2005, Terruzzi, Senesi et al. 2007, Williams and Schalinske 2007, Lewis, Lawlor et al. 2008). PPAR-γ is a member of a receptor family responsible for regulating fat burning/storing. When activated, it in turn activates certain genes whose expression products cause the fat cell to store fat rather than to release it into the body. This increased fat storage also changes the levels of certain hormones released by the fat cell (Walczak and Tontonoz 2002).

The objective of this study was to investigate the possible association between PPAR-γ and MTHFR polymorphisms and obesity or body composition.

The present study demonstrates that weight, BMI, fat-percentage, FFM, and truncal fat mass were significantly higher in the overweight and obese group than in the normal BMI group. Total cholesterol, TG, and LDL were higher in the overweight and obese group, but the difference was statistically significant only in LDL (Tables 1 and 2). HDL was lower in the overweight and obese group, though statistically insignificant. These results are in line with a previous study on male adults in Saudi Arabia (Al-Ajlan 2011) that found that the mean HDL, LDL, and TG did not differ significantly among different weight groups at a 5% level of significance.

The present study showed that the frequency of G allele of MTHFR was 33.3%. This is higher than the global average frequency of 25%, according to the 1000 Genome Project. MTHFR GG genotype and G allele frequencies showed significant associations with fat percentage in the overweight and obese group compared to the normal BMI group, indicating that the G allele of the MTHFR gene has a positive correlation with BMI and fat accumulation in the body in our study population (Table 6, Figure 1).

Previous studies have shown conflicting results for the association of MTHFR C677T polymorphisms with being overweight or obese. Those studies investigated different ethnic populations (Terruzzi, Senesi et al. 2007, Lewis, Lawlor et al. 2008, Settin, Algasham et al. 2009,

Tavakkoly Bazzaz, Shojapoor et al. 2010, Gara, Ochi et al. 2011, Chauhan, Kaur et al. 2012, Tabassum, Jaiswal et al. 2012). No significant association of the MTHFR C677T polymorphism with being overweight/obese was observed in Danish/British (Lewis, Lawlor et al. 2008), Tunisian (Gara, Ochi et al. 2011), Iranian (Tavakkoly Bazzaz, Shojapoor et al. 2010), Saudi (Settin, Algasham et al. 2009), Italian (Terruzzi, Senesi et al. 2007) and Thai (Thawnashom, Tungtrongchitr et al. 2005) populations. A genome-wide association study of obesity-related traits did not observe significant associations of nine polymorphisms in the MTHFR gene with BMI, weight, or hip circumference (Scuteri, Sanna et al. 2007).

The Saudi study (Settin, Algasham et al. 2009) performed in 2009 showed no association of the MTHFR genotype and BMI/obesity, which is in disagreement with the results found in this study. A possible explanation may be that the 2009 study included both males and females as opposed to the all-female subjects recruited in this study. Another possibility for the difference is that the 2009 study included only subjects belonging to the Qassim Region ethnically, compared to subjects recruited from the Al-Madina region in this study. This difference in the role of MTHFR in obesity indicates the strong association of genotype and allele frequency with gender and ethnic origin of the recruited subjects.

In disagreement with results from this study are two studies carried out in England and India; those studies showed that the 677T allele or 677TT genotype was involved in significantly increased risk of obesity (Lewis, Lawlor et al. 2008, Tabassum, Jaiswal et al. 2012), which could be due to different ethnic backgrounds of participants in those two studies compared to ours.

The frequency of the G allele of PPAR-γ was found to be 8% in our cohort, which is close to the average global frequency of 7%, according to the 1000 Genome Project. The frequency of CC and CG of PPAR-γ gene was 92.2% and 7.8%, respectively. Frequencies of PPAR-γ genotype and allele polymorphism in the overweight and obese group showed no significant difference statistically compared to those of controls.

The present study demonstrated that the G allele in the MTHFR rs1801131 SNP was associated with a risk for increased BMI and fat percentage. However, the PPAR-γ rs1801282 SNP had no association with BMI or fat percentage. This result is inconsistent with numerous prior reports that did not observe direct associations between PPAR-γ Pro12Ala genotype and BMI (Ringel, Engeli et al. 1999, Oh, Min et al. 2000, Garaulet, Smith et al. 2011).

Conclusion

In this population of Saudi women, we found that MTHFR GG genotype and G allele are statistically correlated with the deposition of fat in the body; women with this allele and genotype should be urged to control their weight and fat intake, as it is also associated with LDL levels that may affect their cardiovascular system. PPAR-γ did not seem to play a role in obesity or the fat percentage of the recruited subjects. BMI values do not differentiate between fat and muscle tissue, so its use is not indicative of obesity.

Limitation

Limitations of this study may be the small sample size, the inclusion of only females, and not recruiting a wider range of ages to investigate the effects of aging.

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