ASSOCIATION OF THE MTHFR 677C>T POLYMORPHISM WITH OBESITY AND BIOCHEMICAL VARIABLES IN A YOUNG POPULATION OF MEXICO

POVEZANOST MTHFR 677C>T POLIMORFIZMA SA GOJAZNOŠĆU I BIOHEMIJSKIM PROMENLJIVIMA U MLADOJ POPULACIJI MEKSIKA

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Summary

Background: Methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms have been associated with overweight people and obesity. The goal of this study was to investigate the relationship of the *MTHFR* 677C>T polymorphism with obesity and biochemical variables in young individuals of Mexico.
Methods: A total of 316 young individuals were included in the study, 172 with normal weight (NW) and 144 with overweight/obesity. Body mass index (BMI) was classified as NW, overweight, and obesity. Also, waist circumference was measured. Moreover, glucose, total cholesterol, and triglycerides were determined. Genotyping for *MTHFR* 677C>T polymorphism was performed by the PCR-RFLP method.
Results: There was no difference in the distribution of the *MTHFR* 677C>T polymorphism between individuals with NW and overweight/obesity; neither when they were divided by overweight vs NW, nor when we contrasted obese vs NW. However, an analysis stratified by gender showed a significant protector effect of the TT genotype against obesity in males and elevated waist circumference

Kratak sadržaj

Uvod: Polimorfizam genetske metilenetetrahidrofolat reduktaze (MTHFR) je povezan sa prekomernom težinom i gojaznošću. Cilj ove studije bio je da se istraži odnos *MHTFR* 677C>T polimorfizma sa gojaznošću i bio-hemijskim promenjivim kod mladih u Meksiku.
Metode: U studiju je uključeno ukupno 316 mladih osoba, 172 sa normalnom težinom (NV) i 144 sa prekomernom težinom/gojaznih. Indeks telesne mase (BMI) je klasifiko-van kao NT (normalna težina), prekomerna težina i gojaz-nost. Takođe, izmeren je obim struka. Štaviše, određena je i glukoza, ukupni holesterol i trigliceridi. Genotipizacija za *MTHFR* 677C>T polimorfizam je izvedena metodom PCR-RFLP.
Rezultati: Nije postojala razlika u distribuciji *MHTFR* 677C>T polimorfizma između pojedinaca sa NT i preko-mernom težinom/gojaznih; ni u slučaju kada su bili po-deljeni na one sa prekomernom težinom naspram NT, niti kada smo uporedili gojazne naspram onih normalne težine. Međutim, analiza stratifikovana po polu je pokazala zna-čajan zaštitni efekat TT genotipa protiv gojaznosti kod muškaraca i povećanog obima struka kod žena. Takođe,

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in females. Also, overweight/obese individuals with TT genotype had less risk of high cholesterol or triglycerides than overweight/obese subjects with the other genotypes.

Conclusions: These results suggest that the MTHFR 677T polymorphism might not be a risk factor for being overweight/obesity. Rather, on the basis of our results, this variant could be a protector effect. However, further large-scale population-based studies are still necessary to clarify the role of the MTHFR 677C>T polymorphism in overweight, obesity, and lipid profile level.

Keywords: MTHFR, 677C>T, obesity, overweight, biochemical variables

osobe sa preteranom težinom/gojazne osobe sa TT genotipom imale su manje rizika od visokog holesterola ili triglicerida nego osobe prekomerne težine/gojazne osobe sa drugim genotipovima.

Zaključak: Ovi rezultati ukazuju na to da polimorfizam MTHFR 677C>T možda nije faktor rizika za preveliku težinu/gojaznost. Potpuno suprotno, naši rezultati pokazuju da ova varijanta može imati zaštitni efekat. Ipak, potrebne su dodatne studije širokog opsega da bi se pojasnila uloga MHTFR 677C>T polimorfizma u prekomernoj težini, gojaznosti i nivou lipidnog profila.

Ključne reči: MTHFR 677C>T, gojaznost, prekomerna težina, biohemijske promenljive

Introduction

Overweight and obesity have become a serious public health problem worldwide since they increase the risk of chronic diseases and early death (1). Overweight and obesity are disorders of energy balance with excessive fat storage. According to the *World Health Organization* (WHO), obesity has increased more than twofold in the last three decades worldwide; in 2014, it was reported that more than 1,900 million people over the age of 18 years were overweight, and of those, over 600 million were obese (2). In Mexico, in the most recent report by the National Survey of Health and Nutrition, the prevalence of overweight and obesity in people over the age of 20, adolescents, and children was 72.5%, 36.3% and 33.2%, respectively (3). Obesity is a multifactorial disease that involves environmental and genetic factors (4). Candidate genes associated with obesity include the methylenetetrahydrofolate reductase gene (*MTHFR*), located in 1p36.3, of which the rs1801133 polymorphism (677C>T; A222V) is the most common and majorly studied (5). MTHFR is a pivotal enzyme in folate metabolism, since catalyze 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Moreover, it regulates the proportional usage of one-carbon units between methylation reactions and nucleic acid synthesis. The 677TT genotype is associated with a reduction in the availability of folate, high homocysteine levels, reduced enzyme activity, and thermolabile enzyme (6). Moreover, the 677T allele has been linked with overweight/obesity risk, dyslipidemias, and diabetes in several populations. However, so far, the studies have been controversial (4, 5, 7–12, 14–30). Here, we analyzed whether there is an association of the MTHFR 677C>T polymorphism with overweight and obesity, as well as its relationship with lipid profile, glucose level, and anthropometric measurements in a young Mexican population.

Materials and Methods

Three hundred and sixteen students, 232 females and 84 males, from the Multidisciplinary

Academic Unit Reynosa Aztlán of the Autonomous University of Tamaulipas, in Reynosa, Tamaulipas, Mexico were enrolled in this study. Individuals were od the average age of 19, without apparent comorbidities (hypertension, diabetes, or dyslipidemia). All of them had their weight (kilogram; kg), and height (meter; m) measured. They were weighted in a precision balance with very light clothes and no shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The BMI was classified as normal weight (NW) (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obesity (≥30.0 kg/m^2) (2). Moreover, waist circumference was measured in all participants; in females, 88 cm was considered normal, and ≥88 was considered elevated, in males, <102 cm (normal) and ≥102 (elevated). Glucose, cholesterol, and triglycerides were determined in an analyzer AutoKem II (KontroLab, Roma, Italy). Reference values (mmol/L were as follows: for glucose, normal (≤5.55) and high (>5.55); for cholesterol, normal (≤5.2) and high (>5.2); for triglycerides, normal (≤1.71) and high (>1.71). This study was approved by the ethics committee of each participating institute. Every individual was asked for informed consent to take part in this research.

Genotyping by PCR-RFLP

Genomic DNA was isolated from peripheral blood by CTAB–DTAB (denaturing cationic detergents, to precipitate DNA) and Miller (salting out the cellular proteins by dehydration and precipitation with a saturated NaCl solutions) methods (13). Genotyping was performed using PCR-RFLP with the following primers: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' 5'-AGGACGGTGCGGTGAGAGTG-3' a total of 100 ng genomic DNA was amplified in a 20 μL PCR reaction, PCR buffer containing 3 mmol/L $MgCl_2$, 0.25 mmol/L dNTP's (Bioline), 5 pmol of each primer, 1 U Taq polymerase. The PCR cycles were as follows: primary denaturation was carried at 94 °C for 4 min followed by 35 cycles of 94°C for 30 s, 61°C for 30 s, 72°C for 30 s. The final extension

was carried out at 72 °C for 7 min (14). Thus, a fragment of 198 base pairs (bp) was obtained; 10 µL of the PCR product was digested by 4 hours at 37 °C with the enzyme *Hinf* I (BioLabs, New England, USA); the digestion products were separated in poly-acrylamide gels. Homozygous individuals for the wild allele (CC) result in a fragment of 198 base pair (bp); heterozygous (CT) two fragments (198 bp and 175 bp) and homozygous (TT) a fragment of 175 bp.

Statistical analysis

Allelic and genotypic frequencies were established by counting and the distribution of the genotypes between both groups was compared by Chi-square test. Hardy-Weinberg equilibrium (HWE) was analyzed by Fisher exact test. Association was estimated by odds ratio (OR) and 95% confidence interval (CI) in SPSS v10.0 software (the C allele was taken as reference), $p \leq 0.05$ was considered significant. Likewise, in overweight and obese individuals, risk factors such as glucose, triglycerides, and cholesterol were assessed. Moreover, analysis of means of anthropometric measurements and

metabolic traits was evaluated according to the genotype by Kruskal-Wallis test (available at http://vassarstats.net/kw3.html)

Results

One hundred and seventy-two individuals were NW, 74 overweight, and 70 obese. Allelic and genotypic frequencies of the *MTHFR* 677C>T polymorphism are shown in the *Table I*. Allele frequency in NW was consistent with HWE (p=0.65). There was no difference in the distribution of this polymorphism between NW and overweight/obese individuals [OR=0.94 (0.69–1.28); p=0.69], nor when they were divided by NW vs overweight, or NW vs obese [OR=0.87 (0.59–1.3); p=0.47] and [OR=0.99 (0.67–1.47); p=0.96], respectively (*Table I*). However, when the sample was stratified by gender, significant differences between NW males vs obese males were observed (*Table II*), suggesting a protector effect for obesity of the T homozygotes. Regarding the analysis of waist circumference, stratified by gender, this showed that the females carrying the T allele had a minor risk of having

Table I Analysis of allelic and genotypic frequencies of the MTHFR 677C>T polymorphism between individuals with normal weight, overweight/obese, overweight, and obese.

Genotype/allele	NW BMI <25 n=172	Overweight /obesity BMI ≥25 n=144	OR (95% CI)	*p value
C/C	43	34	Reference	
C/T	90	84	1.18 (0.69–2.02)	0.55
T/T	39	26	0.84 (0.43–1.65)	0.62
C	176	152	Reference	
T	168	136	0.94 (0.69–1.28)	0.69
	NW BMI<25 n=172	Overweight (≥25BMI <30) n=74		
C/C	43	16	Reference	
C/T	90	49	1.46 (0.75–2.9)	0.27
T/T	39	9	0.62 (0.25–1.6)	0.31
C	176	81	Reference	
T	168	67	0.87 (0.59–1.3)	0.47
	NW BMI <25 n=172	Obesity BMI >30 n=70		
C/C	43	18	Reference	
C/T	90	36	0.96 (0.49–1.87)	0.89
T/T	39	16	0.98 (0.44–2.18)	0.96
C	176	72	Reference	
T	168	68	0.99 (0.67–1.47)	0.96

*Chi square test.

Table II Analysis of allelic and genotypic frequencies of the MTHFR 677C>T polymorphism between males with BMI <25 vs BMI ≥25 and females with waist circumference <88 cm vs ≥88 cm.

Genotype/allele	Male BMI <25 n=37	Male BMI ≥25 n= 47	OR (95% CI)	*p value
C/C	7	18	Reference	
C/T	21	23	0.43 (0.15–1.22)	0.10
T/T	9	6	0.26 (0.07–1.00)	<0.05
C	35	59	Reference	
T	39	35	0.53 (0.29–0.99)	<0.05
Genotype/allele	Female <88 cm n=155	Female ≥88 cm n=77	OR (95% CI)	*p value
C/C	36	43	Reference	
C/T	79	22	0.23 (0.12–0.45)	<0.001
T/T	40	12	0.25 (0.12–0.55)	<0.001
C	151	108	Reference	
T	159	46	0.40 (0.27–0.61)	<0.001

Table III Analysis of allelic and genotypic frequencies of the MTHFR 677C>T polymorphism between overweight/obese individuals depending on the level of triglycerides, cholesterol, and glucose.

Genotype/alleles	Overweight /obesity triglycerides ≤1.71 mmol/L n=109	Overweight /obesity triglycerides >1.71 mmol/L n=35	OR (95% CI)	*p value
C/C	25	12	Reference	
C/T	60	21	0.73 (0.31–1.70)	0.47
T/T	24	2	0.17 (0.04–0.86)	0.02
C	110	45	Reference	
T	108	25	0.57 (0.32–0.99)	0.04
	Overweight /obesity cholesterol ≤5.2 mmol/L n=126	Overweight /obesity cholesterol >5.2 mmol/L n=18		
C/C	30	6	Reference	
C/T	70	12	0.86 (0.29–2.50)	0.78
T/T	26	0	0.09 (0.01–1.65)	0.02
C	130	24	Reference	
T	122	12	0.53 (0.26–1.11)	0.09
	Overweight /obesity glucose ≤5.55 mmol/L n=111	Overweight /obesity glucose >5.55 mmol/L n=33		
C/C	28	9	Reference	
C/T	65	18	0.86 (0.35–2.15)	0.75
T/T	18	6	1.04 (0.32–3.41)	0.95
C	121	36	Reference	
T	101	30	0.99 (0.58–1.73)	0.99

*Chi square test.

Table IV Analysis of anthropometric measurements, lipid, and glycemic traits in 316 Mexican youths according the MTHFR 677C>T genotype.

Trait	CC	CT	TT	p value*
n	80	172	64	
Age (years)	19.5 ± 1.9	19.7 ± 1.8	20.0 ± 3.6	0.34
BMI (kg/m^2)	25.9 ± 6.4	25.8 ± 5.9	25.8 ± 7.8	0.63
Hip circumference (cm)	99.2 ± 13.0	99.7 ± 13.7	98.5 ± 12.8	0.68
Waist circumference (cm)	89.9 ± 22.9	90.1 ± 21.0	98.5 ± 17.4	0.03
**Waist hip ratio	0.84 ± 0.7	0.84 ± 0.7	0.83 ± 0.7	0.79
Systolic pressure (mm Hg)	108.6 ± 18.5	106.5 ± 13.5	104.3 ± 14.9	0.63
Diastolic pressure (mm Hg)	75.6 ± 10.0	74.8 ± 10.1	72.7 ± 9.6	0.33
Total cholesterol (mmol/L)	4.18 ± 1	4.04 ± 1.93	3.88 ± 0.75	0.22
Triglycerides (mmol/L)	1.19 ± 0.69	1.17 ± 0.4	1.04 ± 0.49	0.18
Glucose (mmol/L)	4.8 ± 0.79	4.93 ± 1.18	4.85 ± 0.82	0.88

Data are expressed as mean ± standard deviation. BMI: body mass index. *Kruskal-Wallis test (available at http://vassarstats.net/kw3.html). **Genotypes of waist hip ratio were 73, 148, and 61 for CC, CT, and TT, respectively.

elevated waist circumference, both CT genotype and TT homozygote; but this was not observed in males (*Table II*). Testing between overweight/obese individuals based on glucose, cholesterol, and triglycerides levels, showed that the TT genotype was more common in subjects with triglycerides ≤1.71 mmol/L and cholesterol ≤5.2 mmol/L (*Table III*). As for the analysis of quantitative variables according to genotype, only significant differences were detected for waist circumference, and individuals with TT genotype had a larger waist circumference than CC and TT subjects (p=0.03; *Table IV*).

Discussion

Besides known causes involved in overweight/ obesity development (poor eating habits, lack of physical activity, and psychological disorders), genetics plays a very important role, since it represents up to 70% of factors associated with overweight/obesity (4). The results of this study showed no statistical differences about the prevalence of the *MTHFR* 677C>T polymorphism between NW and overweight/obesity individuals, which agrees with the majority of researches (4, 8, 10–12, 14, 17, 18, 20, 22, 29). However, opposite findings have also been described; so Lambrinoudaki et al. (9) observed that healthy postmenopausal women carrying the 677CT or 677TT genotype had central adiposity and waist higher hip ratio compared with women carrying the 677CC genotype. Tabassum et al. (19) reported association of 677C>T polymorphism with overweight/obesity [1.24 (1.01–1.52) p=0.04] in Indian

population. Similarly, Di Renzo et al. (7) studied 56 obese individuals who underwent a diet and observed that before starting the regime there were more obese who carried the T (+) genotype (CT or TT) and less with T (-) genotype (CC carriers). They found that after twelve weeks the subjects who carried the T (-) genotype (CC homozygote) decreased more weight than the T allele carriers (CT or TT) (5). Others researchers suggest that overweight/obese individuals with *MTHFR* 677TT genotype are at increased risk for type 2 diabetes mellitus (30).

To our knowledge, the finding that carrier females of the *MTHFR* 677T allele have a minor risk of elevated waist circumference has not been reported in healthy people, but Wang et al. analyzed this parameter between patients with metabolic syndrome from China and they found a trend towards a protector effect against elevated waist circumference in the patients with the TT genotype [OR=0.60 (0.27–1.32); p=0.204] (31), which is in accordance with our observations. On the other hand, the analysis of means of anthropometric measurements showed that waist circumference was increased in individuals with TT genotype. Although this looks like a contradiction, it could be explained by an unequal distribution of the people with abdominal obesity and the size sample.

Regarding lipid profiles, the *MTHFR* 677T allele is associated in non-obese patients with unfavourable serum lipid profiles in several studies (15, 18, 21, 24, 26, 28). In this study, we detected low levels of triglycerides and cholesterol in overweight/obese individuals carrying the TT genotype. In contrast to

our observation, Zhi et al. (27) found that over-weight/obese females carrying the TT genotype had high triglycerides levels (p=0.0007), and in over-weight/obesity males, the CT carriers had significantly higher LDL-cholesterol levels than the TT carriers did (p=0.018) in Chinese population. Moreover, Di Renzo et al. (23) studying obese women with metabolic syndrome who underwent a diet, found that CC carriers had lower triglycerides and minor fasting glucose (p≤0.05).

On the other hand, it is known that the TT genotype is associated with high homocysteine levels (6). Moreover, the significant increment of cholesterol has been detected in diet-induced hyperhomo-cysteinemic rats (32). Accordingly, it has been suggested that elevation of homocysteine increases HMG-CoA reductase expression, which in turn activates three transcription factors: sterol regulatory element-binding protein 2 (SREBP-2), cyclic adeno-sine monophosphate response element-binding protein (CREB), and nuclear factor Y (NF-Y), and likely increases cholesterol biosynthesis, with subsequent hypercholesterolemia (32). In this study, we did not evaluate the plasma homocysteine levels. Contradictorily, our results suggest that individuals carrying the TT genotype have a minor risk to develop high levels of triglycerides, and cholesterol.

Discordances observed among studies may be occasioned by several factors such as differences in dietary, physical activity habits, genetic background, gender differences, sample size, statistical analysis criteria, and the participation of other genes that coding enzymes involved in the homocysteine pathway. We only investigated one polymorphism and did not have data on other environmental factors, such as physical activity, dietary habits, alcohol consumption and cigarette smoking. We consider that identification of polymorphisms associated with obesity and overweight in young individuals will allow the detection of people in risk at an early age, which could to permit to take preventive actions.

In conclusion, these results suggest that the *MTHFR* 677T polymorphism might not be a risk factor for being overweight/obese. Rather, on the basis of our results, this variant could be a protector effect. However, further large-scale population-based studies are still necessary to clarify the role of the *MTHFR* 677C>T polymorphism in overweight, obesity, and lipid profile level.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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