ANALYSIS OF THE METHYLENETETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM AND SERUM LIPID LEVELS IN YOUNG INDIVIDUALS OF NORTHEAST MEXICO

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SUMMARY

Dyslipidemia is a common alteration in lipid metabolism that is a main cardiovascular risk factor. There are multiple causes of dyslipidemia and environmental and genetic factors are involved. We analyze the methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism and its association with serum lipids in young individuals of northeast Mexico. A total of 139 voluntary participants were separated into two groups based on body mass index were evaluated. Lipid profile and genotype were determined in both groups. No statistically significant difference was found in either group and among genotypes. Its is concluded that the TT genotype of the MTHFR gene polymorphism is not associated with dyslipidemia in young individuals.

Introduction

Dyslipidemias are a group of plasma lipid disorders characterized by an increase in serum lipid levels (Bosomworth, 2013) related to the formation of atherogenic plaques in the vascular endothelium (Ahmed *et al*., 1998). Dyslipidemias are classified as secondary and primary. Secondary dyslipidemia has a multifactorial origin: drugs (retinoids, glucocorticoids, beta-blockers, antirretroviral therapy), diseases (systemic lupus erythematosus, diabetes mellitus, hypothyroidism), a high-fat diet, and a sedentary lifestyle (Yanes, 2008), while primary dyslipidemia has a genetic predisposition (Fernández-Pardo *et al*. 2009) with mutations in genes associated with lipid metabolism, such as upstream stimulatory factor-1 (USF1) (Naukkarinen *et al*., 2005), apolipoprotein E2 (APOE2; Sun *et al*., 2016), a carbohydrate-response element binding protein known as MLX interacting protein like (MLXIPL; Aguilar-Salinas *et al*., 2016), apolipoprotein B (APOB; Harper and Jacobson, 2010; Zhang *et al*., 2010) and methylenetetrahydrofolate reductase (MTHFR; Yilmaz *et al*., 2003).

The MTHFR gene is located in chromosome 1 (1p36.3) and encodes the enzyme related to folic acid, vitamins B6 and B12, and homocystein metabolism. MTHFR converts 5, 10-metylenetetrahydrofolate into 5-metyltetrahydrofolate, being a methyl donor that transforms homocystein into methionine and purines, and intervenes in S-adenosylmethionine biosynthesis (Neagos *et al*., 2012). The C677T single nucleotide polymorphism known as a thermolabile variant (Frosst *et al*., 1995) consisting of a cytosine to thymidine substitution at nucleotide position 677, causes the substitution of alanine for valine in the MTHFR enzyme, thus reducing enzyme activity (Bennouar *et al*., 2007) and increasing drug toxicity of methotrexate, metformin (Shaheen *et al*., 2006) and statins (León-Cachón *et al*., 2016). Many authors have reported that the homozygosity of the T allele is associated with multiple diseases such as epilepsy (Scher *et al*., 2011), schizophrenia (Moustafa *et al*., 2014), erectile dysfunction (Lombardo *et al*., 2010) and dyslipidemia (Rojas *et al*., 2008).

In Mexico, dyslipidemias are very common because they are strongly linked to the obesity epidemic (Navarro-Hernández *et al*., 2015). In this study, we investigated the association of the MTHFR C677T (rs1801133) gene polymorphism and serum lipid profiles in a sample of young students from northeast Mexico. We hypothesize that this polymorphism may influence lipids levels from a young age.

Material and Methods

Study population

Pharmacy students of the Universidad Autónoma de Nuevo León participated voluntarily during the period of January-June 2015. Participants were separated into two groups based on body mass index (BMI) with an established cutoff of 25kg/m^2. Information such as age, gender, weight, and height, and drug consumption, and the presence of chronic degenerative diseases as exclusion criteria, were obtained from each volunteer. The protocol for this study followed the Declaration of Helsinki and participants provided written informed consent. The Review Board of the Facultad de Ciencias Químicas UANL approved this

KEYWORDS / Dyslipidemia / Lipids / Lipoprotein / MTHFR C667T / Polymorphism /

Received: 05/12/2017. Modified: 04/25/2018. Accepted: 04/26/2018.

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ANÁLISIS DEL POLIMORFISMO C677T DEL GEN METILENTETRAHIDROFOLATO-REDUCTASA Y NIVELES SÉRICOS DE LÍPIDOS EN JÓVENES DEL NORESTE DE MÉXICO

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RESUMEN

Las dislipidemias son alteraciones del metabolismo de lípidos y son uno de los principales factores de riesgo cardiovascular. Las causas son múltiples y están involucrados factores genéticos y ambientales. Se analizó el polimorfismo C677T del gen metilentetrahidrofolato reductasa (MTHFR) y su asociación con los niveles de lípidos séricos en sujetos jóvenes del noreste de México. Participaron 139 voluntarios que fueron agrupados según su índice de masa corporal en dos grupos con un punto de corte de 25. Se determinó el genotipo y el perfil de lípidos en ambos grupos. No se observó diferencia significativa en los perfiles lipídicos entre los dos grupos ni entre los tres genotipos. Se concluye que el genotipo TT del polimorfismo MTHFR no está asociado con dislipidemias en individuos jóvenes.

ANÁLISE DO POLIMORFISMO C677T DO GENE METILENTETRAHIDROFOLATO-REDUCTASE E NÍVEIS SÉRICOS DE LIPÍDEOS EM JÓVENS DO NORDESTE DO MÉXICO

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RESUMO

As dislipidemias são alterações do metabolismo de lipídeos e um dos principais fatores de risco cardiovascular. São múltiplas as causas e estão envolvidos fatores genéticos e ambientais. Analisou-se o polimorfismo C677T do gene metilentetrahidrofolato reductase (MTHFR) e sua associação com os niveis de lipideos séricos em sujeitos jovens do nordeste do México. Participaram 139 voluntários que foram agrupados segundo seu índice de massa corporal em dois grupos com um ponto de corte de 25. Determinou-se o genótipo e o perfil de lipídeos em ambos os grupos. Não se observou diferença significativa nos perfis lipídicos entre os dois grupos nem entre os três genótipos. Conclui-se que o genótipo TT do polimorfismo MTHFR não está associado com dislipidemias em indivíduos jovens.

study with reference number 04-99604- FAR-11/193.

Blood sampling and DNA extraction

Two peripheral blood samples were obtained from each volunteer by venipuncture to carry out a biochemical analysis and DNA extraction using the phenol chloroform method (Ausubel *et al*. 1992). DNA samples were stored at −20ºC until further analysis.

Genotyping

The sequence of the primers for the MTHFR gene was 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' (forward) and 5'-AGG ACG GTG CGG TGA GAG TG-3' (reverse). Each PCR assay was performed in a total volume of 25µl, with 4µl genomic of DNA, 1 U Taq DNA polymerase (GenScript Corp., Piscataway, NJ, USA), 3mM $MgCl_2$, 0.25mM dNTPs and buffer 10X. The PCR cycles were performed in three steps; the first includes one cycle of 94ºC for 4min; the second includes 35 cycles, each at 94ºC for 30s, 61ºC for 30s and 72ºC for 30s; and the third cycle is one cycle of 72ºC during 7min. An amplicon of 198bp was obtained and was observed on 2% agarose gel (Raza *et al*., 2012), stained with ethidium bromide and photographed.

Restriction fragment length polymorphism (RFLP)

The amplicon was digested with 10 U HinfI (New England Biolabs Inc., Woburn, MA, USDA) and incubated at 37ºC overnight (Li *et al*. 2014). Digested products were a 198 bp wild type variant (CC), a 198 and 175 bp heterozygous variant (CT) and a 175 and 23 bp homozygous variant (TT). These were analyzed on 4% agarose gel (Amela *et al.* 2013) stained with ethidium bromide and photographed.

Statistical analysis

Anthropometric and biochemical data were analyzed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and expressed in terms of mean and standard deviation (SD). Genotype and allele frequencies were calculated by direct count. The Hardy-Weinberg equilibrium (HWE) was assessed with a chi-square test. Differences among genotypes and between alleles were determined with one-way ANOVA and Student´s t-test, respectively. Finally, to analyze the association between lipid profiles and MTHFR genotypes, the odds ratio (OR) and confidence interval (CI) were calculated with a logistic regression. P values <0.05 were considered statistically significant.

Results

A total of 139 volunteers participated in the study, 64.7% were women with a mean age of 19.7 years, a mean height of 1.64m and a mean weight of 65.7kg. This last value differed according to their BMI. Biochemical parameters were within normal range. There were significant differences ($P<0.05$) between individuals with a BMI<25kg/m^2 and individuals with a BMI>25kg/m^2 in the following parameters: HDL, 57.2 *vs* 52.0mg/dl; triglycerides, 65.3 *vs* 84.8mg/dl; VLDL, 13.3 *vs* 17.00mg/dl; with an atherogenic index of 1.64 *vs* 2.04 and a TC/HDL ratio of 2.9 *vs* 3.4 (Table I).

The frequency of the wild type CC genotype was 48.2%; of the heterozygote CT, 42.4%; and of the TT variant, 9.4%. The goodness of fit results showed that the population is in Hardy-Weinberg equilibrium ($P<0.01$). The frequency of allele T was 30.6%. According to genotype and allele, biochemical parameters were within normal range (Table II). Except for creatinine levels ($P=0.02$), the rest of the parameters did not show a significant difference among genotypes and between alleles.

Discussion

In this study, we investigated the association of MTHFR

TABLE I
ANTHROPOMETRIC AND BIOCHEMICAL CHARACTERISTICS*

Variable	Total mean (SD)	BMI		
		< 25 mean (SD)	> 25 mean (SD)	P value
Male	49	25 (30.12)	24 (42.85)	
Female	90	58 (69.87)	32 (57.14)	
Age (years)	19.70 (1.89)	19.76 (1.74)	19.71 (2.10)	0.90
Height (m)	1.64 (0.48)	1.63 (0.07)	1.65 (0.10)	0.18
Weight (kg)	65.64 (14.35)	56.97 (7.90)	78.48 (11.93)	0.00
Glucose	84.54 (7.92)	84.14 (6.63)	85.12 (9.55)	0.48
Urea	24.23 (7.17)	23.95 (6.74)	24.64 (7.81)	0.58
Creatinine	0.93 (0.17)	0.91 (0.16)	0.95 (0.19)	0.20
Total cholesterol	162.16 (31.82)	158.71 (30.00)	167.27 (34.04)	0.12
HDL	55.11 (14.33)	57.23 (13.28)	51.96 (15.35)	0.03
LDL	92.35 (29.65)	88.43 (27.34)	98.16 (32.16)	0.06
TG	73.14 (37.98)	65.25 (32.90)	84.82 (42.11)	0.00
VLDL	14.76 (7.65)	13.25 (6.65)	17.00 (8.47)	0.00
Aterogenic Index	1.79 (0.78)	1.64 (0.69)	2.04 (0.85)	0.00
TC/HDL	3.09 (0.92)	2.89 (0.82)	3.39 (0.99)	0.00

* Concentrations are expressed as mg/dl except atherogenic index and TC/HDL. BMI: body mass index.

TABLE II
BIOCHEMICAL PARAMETERS ACCORDING TO GENOTYPE AND ALLELE

Biochemical parameters*	Genotype				Allele		
	CC	CT	TT	P value	C	T	P value
Glucose	85.08	84.44	82.15	0.47	84.78	84.03	0.51
Urea	23.15	25.65	23.31	0.13	24.32	25.23	0.40
Creatinine	0.90	0.97	0.86	0.02	0.93	0.95	0.45
Total cholesterol	161.78	162.42	162.92	0.99	162.08	162.51	0.93
HDL	56.42	55.25	47.69	0.13	55.87	53.89	0.36
LDL	91.01	92.64	97.92	0.74	91.78	93.59	0.68
TG	71.88	71.61	86.54	0.41	71.75	74.31	0.65
VLDL	14.38	14.62	17.29	0.45	14.50	15.11	0.59
Atherogenic index	1.71	1.81	2.15	0.17	1.76	1.87	0.33
TC/HDL	2.99	3.11	3.56	0.12	3.04	3.19	0.29

* Concentration is expressed as mg/dl except atherogenic index and TC/HDL.

C677T gene polymorphism and serum lipids profiles in a sample of young students of northeast Mexico. No association was found between the MTHFR polymorphism and the frequency of obesity (BMI> 25kg/m^2) and dyslipidemia using codominant, recessive, dominant, over-dominant and paradominant models (Table III).

The prevalence of the TT genotype verify in our study population was 9.40%, which is less than a previous study in Mexican population (19.70%; Aguirre-Rodríguez *et al*., 2008) as well as others (Table IV), such as Circassian and Chechen population, where the prevalence is 8.30% and 2.50%, respectively (Dajani *et al*., 2013).

Our results do not show a significant difference in biochemical parameters among genotypes, except for creatinine. A possible explanation is that MTHFR enzyme activity in the T allele carrier is decreased and the concentration of homocysteine is increased (Petr *et al*., 2013). This is a sulfur-containing amino acid involved in two metabolic pathways: transulfuration and transmetilation (Chen *et al*., 2010), the latter being of importance because in the formation of creatine and creatinine (Figure 1, from Taes *et al*., 2003) precursor molecules are synthesized, such as S-adenosyl-L-methionine (donor of methyl groups), S-adenosyl-homocysteine (Stead *et al*., 2001) and guanidineacetate (Ostojic *et al*., 2018).

The results are similar to those reported in Russian and Brazilian populations where the MTHFR polymorphism was not associated with any biochemical parameter (Spiridonova *et al*., 2000, Massa *et al*., 2016). However, a study in a Turkish population found that diabetic patients with the CC genotype had elevated total cholesterol and LDL levels (Kucukhuseyin *et al*., 2013). Another study in Chinese patients with hypertension also found an association between the TT genotype and LDL levels (Jiang *et al*., 2014). In view of these last results, we speculate that this polymorphism had a major impact in individuals susceptible to hypertension and diabetes or other genes that could reinforce the potential effect of MTHFR on the lipid profile. In addition, it is possible that the effect of the polymorphism manifests itself in time, when people are at a mature age. A follow-up of TT homozygotes would be interesting research topic.

We did not find any association with obesity expressed as a BMI >25kg/m^2. This agrees with a study in the UK and Denmark (Lewis *et al*. 2008), and with a study in a Chinese population (Fan *et al*. 2015). All these findings suggest that

TABLE III
ASSOCIATION OF MTHFR POLYMORPHISM GEN WITH OBESITY AND DYSLIPIDEMIA

Model	Obesity OR (95% CI)	Dyslipidemia OR (95% CI)
Codominant		
CC	1	1
CT	0.58 (0.28-1.21)	1.34 (0.25-7.17)
TT	1.44 (0.44-4.74)	1.16 (0.41-3.30)
Recessive		
CC + CT	1	1
TT	1.83 (0.58-5.78)	1.25 (0.25-6.16)
Dominant		
CC	1	1
CT + TT	0.70 (0.35-1.38)	1.19 (0.44-3.22)
Paradominant		
No CT	1	1
CT	0.55 (0.27-1.11)	0.84 (0.39-1.81)
Over-dominant		
CC + TT	1	1
CT	1.81 (0.90-3.65)	1.19 (0.55-2.58)

OR: odds ratio, CI: confidence interval.

TABLE IV
GENOTYPE FREQUENCIES OF MTHFR (C677T) POLYMORPHISM IN DIFFERENT POPULATIONS AND ITS CLINICAL IMPLICATIONS

Authors	Population	Sample size (n)	C677T genotype (%)			Clinical implications
			CC	CT	TT	
Vázquez-Rodríguez *et al.* (2017) Mexico	Northeast of Mexico	139	48.20	42.40	9.40	This study
Aguirre-Rodríguez *et al.* (2008) Mexico	Nuevo Leon, Mexico	533	26.10	54.20	19.70	Prevalence study
Dajani *et al.* (2013) Jordan	Circassian	72	50	41.60	8.30	Prevalence study
	Chechen	120	72.50	25	2.50	
Spiridonova *et al.* (2000) Russia	Control	122	53	39	8	No association between studied populations and TT genotype
	CAD	94	47	43	10	
	Rural	117	48	38	15	
Massa *et al.* (2016) Brazil	Control	21	38.10	28.60	33.30	No association but T allele carriers of control group showed more responsive to watermelon extract
	Dyslipidemia	22	54.60	22.70	22.70	
Kucukhseyin *et al.* (2013) Turkey	Control	138	44.93	44.20	10.87	CC genotype increased LDL and TC plasma levels in DM(+)CHD
	DM(-)CHD	112	56.25	35.71	8.04	
	DM(+)CHD	82	48.78	34.14	17.08	
Jiang *et al.* (2014) China	Patients with hypertension	340	39.20	47.60	13.20	Association between the TT genotype and LDL plasma levels
Lewis *et al.* (2008) UK and Denmark	UK	3416	44.70	43.70	11.60	TT genotype (UK group) is associated with increased to risk of obesity
	Denmark	9173	48	42.80	9.20	
	Avon, UK	11621	44.23	44.29	9.72	
Fan *et al.* (2015) China	Control	741	21.60	50.60	27.80	MTHFR C677T gene polymorphism is not associated with overweight/obesity
	Cases	517	22.20	47.20	30.60	
Ruiz-Franco *et al.* (2016) Mexico	Control	100	28	60	12	TT genotype is associated with CCAD patients in Mexican mestizo population
	CCAD	100	20	52	28	
Perez-Razo *et al.* (2015) Mexico	CNH	209	17.58	54.27	28.14	TT genotype is associated with increase to risk of hypertension
	CH	209	17.08	49.24	33.66	
	ANH	391	23	51.20	25.70	
	AH	372	30	46.60	23.30	
Ramos-Silva *et al.* (2015) Mexico	Control	339	49	41	10	TT genotype increase to risk of breast cancer
	BC	497	43	36	21	
Estandia-Ortega *et al.* (2014) Mexico	Control	370	15	46	39	T allele is associated to low risk of NSCLP
	NSCL/P	132	29	41	30	
Ibarra-López *et al.* (2013) Mexico	Control	116	25.90	47.40	26.70	Absence of folic acid supplementation and T allele is associated to high risk of NSCLP
	NSCL/P	88	15.90	47.70	36.40	
Gallegos-Arreola *et al.* (2009) Mexico	Control	170	34.70	46.50	18.80	TT genotype increased risk of CRC
	CRC	369	34	34	32	
Canto *et al.* (2008) (Mexico)	Control	274	22.20	47.80	29.90	TT genotype decreased risk of PE in Mexican-Mayan Mestizo population
	PE	125	28.80	52.80	18.40	
Delgado-Enciso *et al.* (2006) Mexico	Control	89	23.59	53.93	22.47	TT genotype decreased risk of CaCU in woman with multipregnancies
	CaCU	70	25.71	48.57	25.71	
Torres-Sánchez *et al.* (2006) Mexico	Healthy non-pregnant women	130	21.50	52.30	26.20	Vitamin B12 supplements decrease to risk of spontaneous abortion in TT genotype carriers.
Esfahani *et al.* (2003) United States	MW	193	37.80	44	18.10	TT genotype carriers increase to risk of folate deficiency in MW.
	WW	139	49.60	43.20	7.20	
	AW	53	60.40	35.80	3.80	
	AAW	48	81.30	18.80	0.00	
Mutchinick *et al.* (1999) Mexico	Different regions of Mexico	250	17.60	47.60	34.80	TT genotype increase to risk of NTD in MW

CAD: coronary artery disease, DM(-)CHD: coronary heart disease in patients without diabetes, DM(+)CHD: coronary heart disease in patients with diabetes, UK: United Kingdom, IRPL: idiopathic recurrent pregnancy loss, CCAD: spontaneous cervico-cerebral artery dissection, CNH: children non-hypertensive, CH: children hypertensive, ANH: adult non-hypertensive, AH: adult hypertensive, BC: breast cancer, NSCL/P: non-syndromic cleft lip/palate, CRC: colorectal cancer, PE: preeclampsia, CaCU: cervical cancer, MW: Mexican women, WW: White women, AW: Asian women, AAW: African-American women, NTD: neural tube defects.

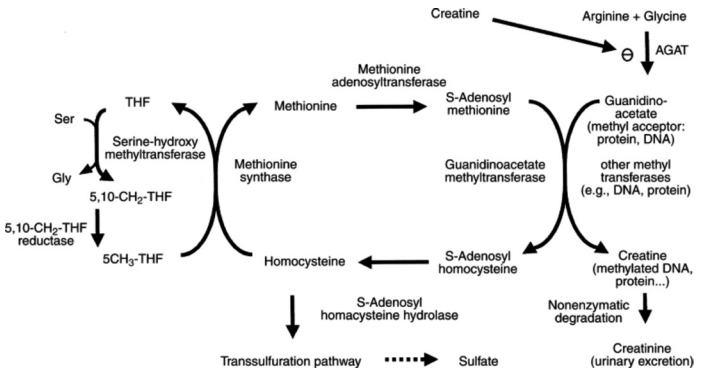

Figure 1. Metabolic pathways of homocysteine. From Taes *et al.* (2003).

the MTHFR polymorphism is not associated with obesity.

Finally, not including measurements of homocysteine, folates, vitamins B6 and B12 in plasma which are not routine biochemical tests in our laboratories, is a limitation of this study. We also did not consider the volunteers' diet, and it would be interesting to include this consideration in a later study.

Conclusions

The prevalence of the TT genotype and the T allele of MTHFR-C677T was 9.40% and 30.60%, respectively. No association was found between genotype and serum lipids and a BMI>25kg/m^2 in young Mexican individuals.

ACKNOWLEDGEMENTS

The authors acknowledge funding by the Scholl of Chemical Science, Universidad Autonoma de Nuevo Leon, Mexico.

REFERENCES

Aguilar-Salinas C, Melgarejo-Hernández M, Gómez-Velasco D, Muñoz-Hernández L, Guillén-Pineda L, Moreno-Villatoro C, Moreno-Macías H, Huerta-Chagoya A, Gómez-Diaz R, Mehta R, Arellano-Campos O, Cruz-Bautista I, Tusie-Luna M (2016) Genética de las dislipidemias en México. *Mensaje Bioquím. 4*: 125-142.

Aguirre-Rodríguez A, Martínez-Villarreal L, Velazco-Campos M, Sampallo-Hernández E, Esmer-Sánchez M (2008) Prevalencia del polimorfismo C677T del gen MTHFR en una muestra de la población de Nuevo León, México. *Salud Públ. Méx. 50*: 5-7.

Ahmed S, Clasen M, Donnelly J (1998) Management of dyslipidemia in adults. *Am. Fam. Physician 57*: 2192-2204.

Ausubel F, Brent R, Kingston R, Moore D, Seidman J, Smith J, Struhl K (2003) *Current Protocols in Molecular Biology.* Vol. 1. Wiley. New York, USA. pp. 180-184.

Amela K, Rifet T, Zoran J, Jaminska M (2013) The frequency of C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism in Southern East Bosnian population. *J. Biomet. Biostat. 4*(4): 1-3.

Bennouar N, Allami A, Azeddoug H, Bendris A, Laraqui A, El-Jaffali A, El-Kadiri N, Benzidia R, Benomar A, Fellat S, Benomar M (2007) Thermolabile Methylenetetrahydrofolate Reductase C677T Polymorphism and Homocysteine Are Risk Factors for Coronary Artery Disease in Moroccan Population. *J. Biomed. Biotechnol. 2007*: 1-9.

Bosomworth N (2013) Approach to identifying and managing atherogenic dyslipidemia. *Can. Fam. Physician 59*: 1169-1180.

Canto P, Canto-Cetina T, Juárez-Velázquez R, Rosas-Vargas H, Rangel-Villalobos H, Canizales-Quinteros S, Velázquez-Wong A, Villarreal-Molina M, Fernández G, Coral-Vázquez R (2008) Methylenetetrahydrofolate reductase C677T and glutathione S-transferase P1 A313G are associated with a reduced risk of preeclampsia in Maya-Mestizo women. *Hypertens. Res. 31*: 1015-1019.

Chen C, Gan Y (2010). The allele frequency of three polymorphisms in genes involved in homocysteine metabolism in a group of unrelated healthy Singaporeans. *Dis. Markers. 29*: 111-119.

Dajani R, Fathallah R, Arafat A, AbdulQader M, Hakooz N, Motassem Y, El-Khateeb (2013) Prevalence of MTHFR C677T single nucleotide polymorphism in genetically isolate populations in Jordan. *Biochem. Genet. 13*(9): 780-788.

Delgado-Enciso I, Martínez-Garza S, Rojas-Martínez A, Espinosa-Gómez F, Canseco-Ávila L, Vidal-Guerrero O, Garza-Leal J, Barboza-Quintana O, Flores-Gutiérrez J, Barrera-Saldaña H, Ortiz-López R (2006) The effect of MTHFR polymorphisms, pregnancy and first intercourse on cervical cancer in a population from the Northeastern Mexico. *Rev. Invest. Clin. 58*: 462-469.

Esfahani S, Cogger E, Caudill M (2003) Heterogeneity in the prevalence of methylentetrahydrofolatereductase gene polymorphisms in women of different ethnic groups. *J. Am. Diet. Assoc. 103*: 200-207.

Estandia-Ortega B, Velázquez-Aragón J, Alcántara-Ortigoza M, Reyna-Fabian M, Villagómez-Martínez S, González-Del Angel A (2014) 5,10-Methylenetetrahydrofolate reductase single nucleotide polymorphisms and gene-environment interaction analysis in non-syndromic cleft lip/palate. *Eur. J. Oral. Sci. 122*: 109-113.

Fan SJ, Yang BY, Zhi XY, He M, Wang D, Wang YX, Wang YN, Wei J, Zheng QM, Sun GF (2015) Are MTHFR C677T and MTRR A66G polymorphisms associated with overweight/obesity risk? From a case-control to a meta-analysis of 30,327 subjects. *Int. J. Mol. Sci 15*: 11849-11863.

Fernández-Pardo J, Royo-Villanueva C, Giménez-Bello M, Sánchez-Rojas C (2009) Dislipidemias aterogénicas. Concepto. Mecanismos etiopatogénicos. Manifestaciones clínicas. Historia Natural. Desarrollo de complicaciones. Criterios de sospecha, diagnóstico y pronósticos. *Medicine. 10*(40): 2678-2687.

Frosst P, Blom H, Milos R (1995) A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nat. Genet. 10*: 111-113.

Gallegos-Arreola M, Figuera L, Puebla-Pérez A, Morgan-Villela G, Zúñiga-González G (2009) Association of the C677T polymorphism in the MTHFR gene with colorrectal cancer in Mexico patients. *Cancer Genom. Proteom. 6*: 183-188.

Harper C, Jacobson T (2010) Using apolipoprotein B to manage dyslipidemic patients: Time for a change? *Mayo Clin. Proc. 85*: 440-445.

Ibarra-Lopez J, Duarte P, Antonio-Vejar V, Calderon-Aranda E, Huerta-Beristain G, Flores-Alfaro E, Moreno-Godinez M (2013) Maternal C677T MTHFR polymorphism and environmental factors are associated with cleft lip and palate in a Mexican population. *J. Investig. Med. 61*: 1030-1035.

Jiang S, Zhao R, Pan M., Venners S, Zhong G, Hsu Y (2014) Association of MTHFR and MTRR polymorphism with serum lipids levels in Chinese hypertensive patients. *Clin. Appl. Throm. Hemost. 20*: 400-410.

Kucukhuseyin O, Kurnaz O, Akadam-Teker A, Isbir T, Bugra Z, Ozturk O, Yilmaz-Aydogan H (2013) The association of MTHFR C677T gene variants and lipid profiles or body mass index in patients with diabetic and nondiabetic coronary heart disease. *J. Clin. Lab. Anal. 27*: 427-434.

León-Cachón R, Ascacio-Martínez J, Gamino-Peña M, Cerda-Flores R, Meester I, Gallardo-Blanco H, Gòmez-Silva M, Piñeyro-Garza E, Barrera-Saldaña H (2016) A pharmacogenetic pilot study reveals MTHFR, DRD3, and MDR1 polymorphisms as biomarker candidates for slow atorvastatin metabolizers. *BMC Cance. 16*(74): 1-13.

Lewis SJ, Lawlor DA, Nordestgaard BG, Tybjærg-Hansen A, Ebrahim S, Zacho J, Ness A, Leary S, Smith GD (2008) The methylenetetrahydrofolate reductase C677T genotype and the risk of obesity in three large population-based cohorts. *Eur. J. Endocrinol. 159*: 35-40.

Li S, Li J, Xiao Z., Ren A, Jin L (2014) Prospective study of MTHFR genetic polymorphism as a possible ethiology of male infertility. *Genet. Mol. Res. 13*: 6367-6374.

Lombardo F, Tsamatropoulos P, Culasso F, Jannini E, Dondero F, Lenzi A, Gandini L (2010) Treatment of erectile dysfunction due to C677T mutation of the MTHFR gene with vitamin B6 and folic acid in patients non-responders to PDE5i. *J. Sex. Med. 7*: 216-223.

Massa N, Silva A, De Oliveira C, Costa M, Persuhn D, Barbosa C, Gonçalves M (2016) Supplementation with watermelon extract reduces total cholesterol and LDL in adults with dislipidemia under the influence of the MTHFR C677T poly-

morphism. *J. Am. Coll. Nutr. 2:* 1-7.

Moustafa A, Hewedi D, Eissa A, Frydecka D, Misiak B (2014) Homocystein levels in schizophrenia and affective disorders-focus on cognition. *Front. Behav. Neurosci. 8*: 1-10.

Mutchinik O, López M, Luna M, Waxman J, Babinsky V (1999) High prevalence of the thermolabile methylenetetrahydrofolate-reductase variant in Mexico: a country with a high prevalence of neural tube defects. *Mol. Genet. Metab. 68*: 461-467.

Naukkarinen J, Gentile M, Soro-Paavonen A, Saarela J, Koistinen H, Pajukanta P, Taskinen R, Peltonen L (2005) USF1 and dyslipidemias: converging evidence for a functional intronic variant. *Hum. Mol. Genet. 14*: 2595-2605.

Navarro-Hernández R, Flores-Alvarado L, Madrigal-Ruiz P, Aguilar-Aldrete M, Ruiz-Mejía M, González-Romero E, Ramírez-García S (2015) Estudio de asociación entre dislipidemia, obesidad central, grasa subcutánea y síndrome metabólico en población mestiza del occidente de México. *Rev. Med. MD. 6*: 181-188.

Neagos D, Creńu R, Sfetea R, Mierla D, Bohiltea L (2012) Investigation of the relationship between the risk of spontaneous abortion and C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene. *Rev. Rom. Med. Lab. 20*: 335-343.

Ostrojic S, Vranes M, Loncar D, Zenic N, Sekulic D (2018) Guanidinoacetic acid and creatine are associated with cardiometabolic risk factors in healthy men and women: A cross-sectional study. *Nutrients 10*: 1-8.

Petr M, Steffl M, Kohlíková E (2013) Effect of the MTHFR C677T polymorphism on homocysteinemia in response to creatine supplementation: A case study. *Physiol. Res. 62*: 721-729.

Perez-Razo J, Cano-Martínez L, Vargas-Alarcón G, Canizales-Quinteros S, Martínez-Rodríguez N, Canto P, Roque-Ramírez B, Palma-Flores C, Esteban-Martínez R, López-Hernández L, Rojano-Mejía D, Coral-Vázquez R (2015) Functional polymorphism rs13306560 of the MTHFR gene is associated with essential hypertension in a Mexican-mestizo population. *Circ. Cardiovasc. Genet. 8*: 603-609.

Ramos-Silva A, Figuera L, Soto-Quintana O, Puebla-Pérez A, Ramírez-Patiño R, Gutiérrez-Hurtado I, Carrillo-Moreno D, Zúñiga-González G, Dávalos-Rodríguez I, Gallegos-Arreola M (2015) Association of the C677T polymorphism in the methylenetetrahydrofolate reductase gene with breast cancer in a Mexican population. *Genet. Mol. Res. 14*: 4015-4026.

Raza S, Abbas S, Ahmed F, Fátima J, Zaidi Z, Mahdi F (2012) Association of MTHFR and PPARγ2 gene polymorphism in relation to type 2 diabetes mellitus cases among north Indian population. *Gene 511*: 375-379.

Rojas J, Bermúdez V, Leal E, Aparicio D, Peña G, Acosta L, Finol F, Urdaneta A, Colmenares C, Almarza J, Linares S, Carrillo M, Bustamante M (2008) Origen étnico y enfermedad cardiovascular. *Arch. Venez. Farmacol. Terap. 27*: 40-57.

Ruiz-Franco A, Barboza M, Jara-Prado A, Canizales-Quinteros S, León-Mimila P, Arguelles-Morales N, Vargas-González J, Quiroz-Compean A, Arauz A (2016) TGFBR2 mutation and MTHFR-C677T polymorphism in a Mexican mestizo population with cervico-cerebral artery dissection. *J. Neurol. 263*: 1066-1073.

Scher A, Wu H, Tsao J, Blom H, Feit P, Nevin R, Schwab K (2011) MTHFR C677T genotype as a risk factor for epilepsy including post-traumatic epilepsy in a representative military cohort. *J. Neurotrauma 28*: 1739-1745.

Shaheen H, El-Gawhary S, Neazeye M, Makhlof H, El-Shafay S (2006) Epilepsy, Hyperhomocysteinemia and mutant methylenetetrahydrofolate reductase gene. *Egypt J. Neurol. Psychiat. Neurosurg. 43*: 495-505.

Spiridonova M, Stepanov V, Pyzyrev V, Karpov R (2000) Relationship between polymorphism C677T of the methylenetetrahydrofolate reductase gene with clinical symptoms of coronary atherosclerosis. *Genetika 36*: 1269-1273.

Stead L, Au K, Jacobs R, Brosnan M, Brosnan J (2001) Methylation demand and homocysteine metabolism: effects of dietary provision of creatine and guanidinoacetate. *Am. J. Physiol. Endocrinol. Metab. 281*: 1095-1100.

Sun Y, Wei R, Yan D, Xu F, Zhang X, Zhang B, Yimiti D, Li H, Sun H, Hu C, Luo L, Yao H. (2016) Association between APOE polymorphism and metabolic syndrome in Uyghur ethnic men. *BMJ Open. 6*(1): 1-6.

Taes Y, Delanghe J, De Vriese A, Rombaut R, Van Camp J, Lameire N (2003) Creatine supplementation decreases homocysteine in an animal model of uremia. *Kidney Int. 64*: 1331-1337.

Torres-Sánchez L, Chen J, Díaz-Sánchez Y, Palomeque C, Bottiglieri T, López-Cervantes M, López-Carrillo L (2006) Dietary and genetics determinants of homocysteine levels among Mexican women of reproductive age. *Eur. J. Clin. Nutr. 60*: 691-697.

Yilmaz H, Agachan B, Isbir T, Akoglu E (2003) Is there additional effect of MTHFR C677T mutation on lipid abnormalities in renal allograft recipients? *Transpl. Proc. 35*: 1390-1392.

Yanes J (2008) Hiperlipidemias secundarias. Clasificaciones. Factores etiopatogénicos. Complicaciones. Valoración diagnóstica. *Medicine 10*(19): 1242-1245.

Zhang L, Yin R, Liu W, Miao L, Wu D, Aung L, Xu H, Cao X, Wu J, Pan S (2010) Association of methylenetetrahydrofolate reductase C677T polymorphism and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. *Lip. Health Dis. 123*(9): 1-12.