Pakistan J. Zool., pp 1-8, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230830151236

Association of MTHFR C677T with Obesity in Human Female Population

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ABSTRACT

The hypothalamo-pituitary-thyroid axis is responsible for the maintenance of the metabolic processes in the human body. The hypothalamus releases thyroid releasing hormone (TRH) which stimulates the pituitary gland to release thyroid stimulating hormone (TSH) which in turn leads to stimulation of the thyroid gland and release of thyroxine (T4) and its active derivative triiodothyroxine (T3). Malfunctioning of any component at any level of the hierarchy of the axis may lead to disorders of metabolism including obesity. The thyrotropin releasing hormone (TRH) is a hypothalamic peptide hormone that possesses a broad spectrum of effects mainly determined by its stimulatory effects on energy metabolism together with iodine containing hormones of the thyroid. The objective of this study was to determine association of a specific methylene tetrahydrofolate reductase (MTHFR) single nucleotide polymorphism (SNP) with obesity (BMI) in human females at early age. This research included the obese and normal females visiting the hospitals of Islamabad and Rawalpindi. Blood samples were used for isolation of DNA. *MTHFR* gene was amplified. A particular restriction enzyme was used identify polymorphisms. The DNA fragments of the wild-type and mutant varieties were obtained on the gel. The allele frequency of the C to T polymorphism was determined by counting alleles through electrophoresis gel analysis. Chi-square analysis was used to determine the Hardy-Weinberg equilibrium of the alleles in the population. By adjusting the effects of confounding factors such age and socioeconomic characteristics, logistic regression analysis was used to establish the correlation between the polymorphism and BMI. Statistical significance was set at a p value of <0.05. MTHFR C677T was not linked to obesity in women in this study.

Article Information
Received 30 August 2023
Revised 05 May 2024
Accepted 12 May 2024
Available online 09 July 2025
(early access)

Authors' Contribution
RR: Collected samples, performed the experiments and analyzed the data and wrote the original draft of manuscript. MI: Conceived idea and planned the experiments, provided resources and supervised the study. All authors read and approved the manuscript.

Key words
Obesity, SNP, *MTHFR* gene, Allelic frequency, Homocysteine, Body mass index

INTRODUCTION

Methylene tetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate. This reduction reaction creates methyl donor that plays a key role for the conversion of homocysteine (Hcy) to methionine (Yamada *et al*., 2001). Methionine synthase catalyze this conversion along with vitamin B12 as a cofactor and this catalyst always coexist with vitamin B12 in all mammalian tissues. Methionine is incorporated in dietary protein, and act as a predecessor of S-adenosylmethionine. When methionine is converted to Hcy, S-adenosylmethionine acts as a methyl donor. Similarly, in transsulfuration process the Hcy can also be converted to cysteine through vitamin B6 dependent pathway (Selhub, 1999).

The chromosome 1 at 1p36.3 possesses the gene that codes for 5,10-methylenetetrahydrofolate reductase (MTHFR). This gene consists of 2.2 kb long complementary DNA sequence and 11 exons (Goyette *et al*., 1998). 70-77 kDa subunits of dimeric proteins are encoded by this cDNA. The cDNA of human has the most catalytic activity and binding sites as compared to catalytic activity of porcine and bacterial enzymes (Daubner and Matthews, 1982; Goyette *et al*., 1994).

Goyette *et al*. (1994, 1996, 1998) have reported 15 mutations of *MTHFR* gene that are associated with enzymatic deficiency, out of which 14 are infrequent and cause severe enzymatic deficiency and one is common that causes slight enzymatic shortage. A point mutation at C677T in the *MTHFR* gene replaces alanine to valine in the enzyme (Rosenberg *et al*., 2002). Kang *et al*. (1988) and Rozen (1997) reported that thermostability of MTHFR enzyme was reduced due to this mutation and the enzyme showed decreased activity at 37 ºC or higher temperature. As compared to normal subjects the activity of MTHFR enzyme in homozygous subjects is reduced to 50-60% at 37 ºC and 65% at 46 ºC. The plasma Hcy level rise in the homozygous mutated subjects due to incapability of the

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0030-9923/2024/0001-0001 $ 9.00/0

MTHFR enzyme to convert 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. The Hcy level is higher in homozygous mutated subjects and slightly elevated in heterozygous mutated subjects as compared to the normal subjects (Rozen, 1997).

Methionine is only obtained by using the 5-MTHF as donor group. The use of Hcy in the biochemical cycle is maintained by 5-MTHF, because rise in plasma Hcy level is associated with vascular injury that can start or speed up atherogenic and thrombotic reactions. Loscalzo (1996) reported that hydrogen peroxide and superoxide free radicals are formed quickly by oxidation of free Hcy in plasma which causes injury to the biological cellular membrane by oxidation, or initiates the per-oxidation reactions of lipoprotein particles in plasma.

In obese individuals the levels of circular oxidative stress markers are high because of processes involving pro-oxidant reactions for example inflammatory adipokine synthesis similar to leptin, TNF- α (tumor necrosis-factor-alpha) and IL-β (interleukine-1β) by macrophages and adipocytes. Obese individuals have enhanced respiratory action and less utilization of anti-oxidant molecules and vitamins (Vincent and Taylor, 2006). It appears that in obese individuals little utilization of vitamins B12 and B6 and folic acid is linked with the existence of a greater prevalence of C677T of MTHFR that can be a factor responsible for low availability of cofactors and substrate necessary for the production of 5-MTHF.

Mojtabai (2008) established the relationship of low levels of folate with elevated body mass index. It is also assumed that obesity is influenced by folate levels through epigenetic control of the genes which regulate the body fat storage (Smith *et al*., 2008). The methylation of DNA cytosine and histone amino acid residues is done with methyl groups provided by folate that may change the epigenetic gene expression (Fuks, 2005). The methylation of dinucleotides of gene involved in food intake, fat storage and cellular physiology or body weight will be affected by any defect in the genes that are involved in methyl group metabolism. Therefore, in the present study we hypothesized that the genetic polymorphisms C677T of *MTHFR* gene may compromise the potency of MTHFR enzyme resulting in decreased levels of folate which is associated with obesity. The objectives of the present study was to determine association of *MTHFR* C677T polymorphism with obesity in humans.

MATERIALS AND METHODS

Subjects

The present study included human normal and obese female subjects visiting various hospitals in Rawalpindi and Islamabad. All the subjects were from the same socioeconomic status and eating habits. The overweight subjects with minor illness like temperature, flu, and cough without any concurrent diseases and having obesity were recruited in the study after a written consent. The controls were normal weight female subjects taken from the same population.

Data of socio-demographic, physical health and lifestyle aspects was collected in the form of a predefined questionnaire.

Measurement of body mass index (BMI)

For determination of BMI, Harpender Standiometer was used to measure the standing height close to 0.1 cm. Digital weight scale was used to determine the weight with a precision of 0.1 Kg. Following formula was used to calculate the body mass index.

$$\text{BMI} = \frac{\text{Weight in kilogram}}{(\text{Height in meter})^2}$$

The subjects with BMI of 18-25 normal weight and above 30 were considered obese.

PCR amplification of MTHERC677T

Standard phenol-chloroform method was used to extract DNA from blood samples. *Hinf*I restriction endonuclease was used to digest the PCR amplified products to determine polymorphisms (Irfan et al., 2016). The genotype and allelic frequency of the genetic polymorphism was obtained by directly counting bands on 3% agarose gel.

Biotechnology Information sequences database (http://www.ncbi.nlm.nih.gov) was used to construct primers and Primer 3 programme was used to design primers 5'-ACC CAC AGA AAA TAC CCA G-3' (forward) and 5'-TGC CCC ATT ATT TA-3' (reverse) (Irfan et al., 2016) (http://www.patch.com.ac.uk/cgi.bin/primer3.cgi). The specificity, dimmer and multiple priming sites were determined by using PCR simulation programme amplify 1.2.

Statistical analysis

The Mean±S.E. of the quantitative variables was calculated. Chi-square analysis was used to determine the Hardy-Weinberg equilibrium of the alleles in the population. The association of the polymorphism with overweight condition and BMI was determined by logistic regression analysis adjusting the effects of confounding factors i.e., age, socioeconomic factors and lifestyle. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Association of lifestyle and medical factors with obesity

The mean age of obese (37.18±1.232) were

significantly ($p<0.05$) higher as compared to normal (31.50±1.011) subjects. However, the multiple logistic regression analysis shows no significant ($p<0.05$) association between age and obesity (Table I). Our data show that the female consuming extra meal (4th meal) are at a higher risk of obesity. Obsessive eating also increases the odds of obesity. A routine daily walk and increasing sleep time have protective role against obesity (Table I).

We found an association between obesity and liver disease. Digestive disease significantly ($p<0.0$) increases the odds ratio of obesity. There is also a significant ($p<0.05$) association of diabetes with obesity (Table I).

Though, previous studies reported an association between age and obesity (Cynthia *et al*., 2012; Amira *et al*., 2012) we did not find a significant association, which may be due to our age matched subjects. However, our results of association of obesity with an extra meal (4th meal) consumption and obsessive eating (a meal after every hour) and lack of physical activity are consistent with previous reports (Swinburn *et al*., 2004). It is well established previously that the obsessive and frequent eating of carbohydrates and fat rich foods may increase the fats deposition and central obesity especially in elderly as anabolic lipolytic hormones such as growth hormone (GH) and sex steroids decline gradually with age.

We have observed an inverse association between obesity and sleep time that is also reported previously. The values of BMI, body fat, waist and hip circumferences and fat mass index were higher for subjects with short sleeping time, especially for females (Garaulet *et al*., 2011; Taheri, 2006; Sun *et al*., 2009; Shaikh *et al*., 2009). The association between reduced sleep duration and obesity can be explained in different ways such as shorter sleep time leads to an excess of energy intake and lower energy expenditure as the awaking person tends to consume more and frequent food. On the other hand, insufficient sleep is associated with low anabolic lipolytic hormones (GH and sex steroids) and an anorexic (eating and fat deposition inhibiting) hormone, *i.e.,* leptin. Appetite and hunger might be affected by this association, which leads to overeating and obesity. Hence in this way, the subjects with short sleep duration become obese because they have much time to eat and decreased energy expenditure due to fatigue and changed thermoregulation (Cauter and Knutson, 2008).

As in the present study, the association between obesity and digestive disease was also established similar to previous study (Giovannucci and Michaud, 2007). The poor digestion, reflux and digestive ulcers may lead to central obesity. The liver diseases result in disorders of fat metabolism and lead to deposition of fats. The previous studies also reported the association between obesity and thyroid or goiter (Zheng *et al*., 2015; Tamer *et al*., 2011). The hypothyroidism based goiter is associated with an increase in body weight and obesity (Scheen and Luyckx, 2002). It is also observed that obesity and insulin has a relation, but the status of cause and effect is dubious (Hussain *et al*., 2010).

Table I. Association of non-genetic factors with obesity.

Factors	Control n (%)	Obese n (%)	OR (95% CI)	p Value	AOR (95% CI)	p Value
Age	-	-	1.047 (1.019-1.075)	0.001	1.025 (0.962-1.092)	0.449
Diabetes	3 (3.0)	25 (25.5)	11.073 (3.219-38.091)	0.000	20.944 (3.063-143.217)	0.002
Extra meal (4th meal)	4 (4.0)	31 (31.6)	11.104 (3.745-32.930)	0.000	25.061 (4.330-145.062)	0.000
Digestive disease	1 (1.0)	59 (60.2)	149.769 (20.049-1.119E3)	0.000	305.196 (30.864-3.018E3	0.000
Goiter	3 (3.0)	9 (9.2)	3.270 (0.858-12.461)	0.083	NC	-
Frequent fast food	23 (23.0)	30 (30.6)	1.477 (0.784-2.783)	0.228	NC	-
Liver disease	3 (3.0)	39 (39.8)	21.373 (6.322-72.254)	0.000	18.483 (2.893-118.087)	0.002
Obsessive eating	2 (2.0)	23 (23.5)	15.027 (3.435-65.739)	0.000	18.341 (1.640-205.137	0.018
Daily walk	87 (87.0)	62 (63.3)	0.257 (0.126-0.525)	0.000	0.064 (0.016-0.262)	0.000
Sleeping time	-	-	0.652 (0.496-0.857)	0.002	0.874 (0.472-1.617)	0.667
Family history of thyroid disorders	3 (3.0)	6 (6.1)	2.109 (0.512-8.680)	0.301	NC	-

Logistic regressions; significant if $p\leq0.05$; NC, not calculated. OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval

Association of MTHFR C677T *with obesity*

Genotype distribution of the *MTHFR 677C*>T polymorphism of the 198 subjects was analyzed: 117 (59. 09%) subjects were homozygous for the C allele (CC), 77 (38.89%) subjects were heterozygous (CT), and 4 (2.02%) subjects were homozygous for the T allele (TT). The minor allele (T) frequency of the *MTHFR 677C*>T polymorphism was 0.205, and the genotype distributions did not deviate from the Hardy-Weinberg equilibrium (p >0.05) (Table II).

The alleles and genotype distribution were compared between obese (98) and control (100) subjects. Out of 98 obese subjects 57 (58.2%) were homozygous for C allele (CC), 38 (38.8%) were heterozygous (CT) and 3 (3.1%) were homozygous for T allele (TT). Out of 100 control subjects 60 (60.0%) were homozygous for C allele (CC), 39 (39.9%) were heterozygous (CT) and 1 (1.0%) was homozygous for T allele (TT).

Furthermore, C allele was present in 152 (77.6%) obese and 159 (79.5%) control subjects. The frequency of T allele in obese and control was 44 (22.4%) and 41 (20.5%), respectively.

Allelic frequencies

According to the Table II the allelic frequencies were statistically similar (P>0.05) in the both groups of subjects suggesting lack of association between minor allele (T) and obesity.

Genotype frequencies

The genotypic frequencies were not significantly (p>0.05) different in obese and normal females. Therefore, no significant (p>0.05) association between *MTHFR C677T* and obesity was observed. Both the heterozygous (CT) and mutated homozygous (TT) increase the odds of obesity statistically non-significantly (p>0.05) (Table II). However, the heterozygous and mutated homozygous (CT+TT) collectively showed a statistically significant (p<0.05) association (OR:1.776, CI: 0.287-2.954) with obesity, after adjusting odds ratios for age, diabetes, extra meal, digestive diseases, goiter, fast food frequent consumption, liver disease, obsessive eating, physical activity, sleeping time and family history of thyroid disorders.

The present study found no association between *MTHFR C677T* and obesity in human female obese population. However, it is observed that other factors such as lifestyle habits and medical condition are responsible to cause obesity.

A number of studies have been conducted to investigate the association of *MTHFR C677T* polymorphism with obesity in various populations (Tables III and IV). Although, the results of most of these studies are similar but they did not find significant relationship between C677T and obesity (Fan *et al*., 2015; Gara *et al*., 2011; Hernandez-Guerrero *et al*., 2013; Lewis et al., 2008; Settin *et al*., 2009; Thawnashom *et al*., 2005; Yin *et al*., 2012; Bazzaz et al., 2010). There are however two studies, *i.e.,* Terruzzi *et al*. (2007) and Yang *et al*. (2014), based on a Caucasian and an Asian, respectively, that have shown a significant association between obesity and the polymorphism. Therefore, the differences in the results could be attributed to other factors such as variations in recruitment of subjects, sample size, ethnicity and geographic factors (Table IV). The results are also dependent on the general health, medical conditions (diabetes, liver diseases and digestive diseases, etc.) and lifestyle (frequency and type of food, sleeping and resting time and daily physical activity etc.), which were not addressed while selecting subjects in many of the previous studies.

Therefore, the present study was conducted to address the role of non-genetic factors along with *MTHFR C677T* polymorphism in obesity. The results reports no association between the polymorphism and obesity in spite of adjusting non-genetic factors significantly. However, there are few limitations in the present study that we could not measure folate and Hcy levels of the subjects. A mutation in the *MTHFR* such as C677T may reduce conversion of Hcy

Table II. Genotype and allelic frequencies of the *MTHFR* C677T mutation in the studied subjects.

Genotype	Obese n (%)	Control n (%)	OR (95% CI)	p Value	AOR (95 % CI)	p Value
CC	57 (58.2)	60 (60.0)	1		1	
CT	38 (38.8)	39 (39.9)	1.026 (0.577-1.823)	0.931	0.739 (.217-2.518)	0.628
TT	3 (3.1)	1 (1.0)	3.158 (0.319-31.247)	0.325	20.002 (0.871-459.331)	0.061
CT+TT	41 (41.8)	40 (40.0)	1.079(0.612-1.902)	0.793	1.776 (0.287-2.954)	0.000
C	152 (77.6)	159 (79.5)	1		1	
T	44 (22.4)	41 (20.5)	1.123(0.695-1.814)	0.637	1.182 (0.469-2.981)	0.723

Logistic regression adjusted for age, diabetes, extra meal, digestive diseases, goiter, fast food, liver disease, obsessive eating, daily walk, sleeping time and family history of thyroid disorders. Significant at p<0.05

Table III. Previous studies based on the relationship of MTHFR C677T and obesity.

Study name	Country	Cases						Controls						
		Total	CC	CT	TT	CT+ TT	C	T	Total	CC	CT	TT	CT +TT	C	T
Asian															
Thawnashom *et al.* (2005)	Thailand	37/112*	67		23				23/90*	34		16			
Bazzaz *et al.* (2010)	Iran	74	44	21	9	30	109	39	207	113	80	14	94	306	108
Fan *et al.* (2015)	China	517	115	244	158	402			741	160	375	206	581		
Yin *et al.* (2012)	China	378/373*	354	341	56	397	1049	453	490/488*	471	441	66	507	1383	573
Yang *et al.* (2014)	China	692	129	335	228	563	593	791	878	202	431	245	676	835	921
Caucasian															
Terruzzi *et al.* (2007)	Italy	82	18	54	12	66	90	78	54	14	33	5	38	61	43
Settin *et al.* (2009)	Saudi Arabia	66/64*	89	34	5	39	212	44	57/54*	69	36	5	41	174	46
Gara *et al.* (2011)	Tunisia	31	15	14	2	16	44	18	22	9	12	1	13	30	14
Guerrero *et al.* (2013)	Mexico	75	18	38	19	57	74	76	53	15	28	10	38	58	48
Lewis *et al.* (2008)	United Kingdom	882	360	410	112	522	1130	634	2534	1165	1086	283	1369	1130	634
Lewis *et al.* (2008)	United Kingdom	356	163	155	38	193	481	231	6135	2707	2713	715	3428	8127	4143
Lewis *et al.* (2008)	United Kingdom	233	115	93	25	118	323	143	4897	2155	2190	552	2742	6500	3294
Lewis *et al.* (2008)	United Kingdom	1269	588	574	107	681	1750	788	7904	3812	3356	736	4092	10980	4828

*Male/female.

Table IV. The characteristics of the previous studies.

Study [Refs.]	Association	Sample size (n) obese/control	Age	BMI	Folate level	Hcy level	Serum vitamin B12	Body fat	Waist circumference	Skin fold thickness	Blood pressure	Triglyceride
Thawnashom *et al.* (2005)	No	149/113	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Bazzaz *et al.* (2010)	No	74/207	Yes	Yes	No	Yes	No	No	No	No	No	No
Guerrero *et al.* (2013)	No	75/53	Yes	Yes	Yes	No	Yes	No	No	No	No	No
Lewis *et al.* (2008)	No	1858/18936	Yes	Yes	No	No	No	No	Yes	No	No	No
Fan *et al.* (2015)	No	517/741	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes
Terruzzi *et al.* (2007)	Yes	82/54	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Settin *et al.* (2009)	No	130/111	Yes	Yes	No	No	No	No	No	No	No	No
Yin *et al.* (2012)	No	751/978	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes
Gara *et al.* (2011)	No	31/22	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
Yang *et al.* (2014)	Yes	692/878	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes

into methionine may lead to hyper-homocysteinemia that is found to be responsible for various health conditions such as cardiovascular diseases, infertility and obesity. Similarly, lower levels of folate were also found to be responsible for the obesity (Rassoul *et al*., 2008; Klerk *et al*., 2002; Thawnashom *et al*., 2005; Vincent and Taylor, 2006).

CONCLUSION

We concluded that there is no association of *MTHFR C677T* with obesity in females. But, the extra meal (4th meal), obsessive eating, diabetes, digestive and liver diseases are among the major causes of obesity. However, a daily walk and increase in sleeping time have protective

role. There are few limitations in the present study that we did not measure folate and homocysteine levels of the subjects. Therefore, further study is needed to measure the folate and Hcy levels in the obese patients along with *MTHFR C677T* polymorphism.

DECLARATIONS

Funding

This study was financially supported by Higher Education Commission of Pakistan via Access to scientific instrumentation (Grant number HEC/R and D/ ASIP/2016/000063).

IRB approval

The study was approved by the Institutional Review Board at Shaheed Zulfiqar Ali Bhutto Medical University, PIMS Islamabad vide reference No. F. I-1/2015/ERB/ SZABMU/ Dated: 26-1-2016.

Ethical statement

The research was approved by the ethical committee at Pakistan Institute of Medical Sciences (PIMS) Hospital, Islamabad and all experiments were performed in accordance with relevant guidelines and regulations. Informed consent of participants was taken in the form of a questionnaire.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Amira, C.O., Sokunbi, D.O.B. and Sokunbi, A., 2012. The prevalence of obesity and its relationship with hypertension in an urban community: Data from world kidney day screening programme. *Int. J. med. biomed. Res*., **1**: 104-110. https://doi.org/10.14194/ijmbr.124

Bazzaz, J.T., Shojapoor, M., Nazem, H., Amiri, P., Fakhrzadeh, H., Heshmat, R., Parvizi, M., Ranjbar, S.H. and Amoli, M.M., 2010. Methylenetetrahydrofolate reductase gene polymorphism in diabetes and obesity. *Mol. Biol. Rep.,* **37**: 105–109. https://doi.org/10.1007/s11033-009-9545-z

Bokor, S., Meirhaeghe, A., Ruiz, J.R., Zaccaria, M., Kurt, W., Gonzalez-Gross, M., Amouyel, P., Moreno, L.A., Molnar, D. and Dallongeville, J., 2011. Common polymorphisms in six genes of the methyl group metabolism pathway and obesity in European adolescents. *Int. J. Pediat. Obes*., **6**: 1-2. e336–e344. https://doi.org/10.3109/17477166.2010.500386.

Cardona, H., Cardona-Maya, W., Gomez, J.G., Castaneda, S., Gomez, J.M., Bedoya, G., Alvarez, L., Torres, J.D., Tobon, L.I. and Cadavid, Y.A., 2008. Relacion entre los polimorfismos de la metilen-tetrahidrofotato-reductasa y los niveles de homocisteína en mujeres con perdidagestacionalrecurrente: perspective desde la nutrigenetica. *Nutr. Hosp*., **23**: 277-282.

Cauter, V.E. and Knutson, K.L., 2008. Sleep and the epidemic of obesity in children and adults. *Eur. J. Endocrinol*., **159**: S59-S66. https://doi.org/10.1530/EJE-08-0298

Cynthia, L.O., Carroll, M.D., Kit, B.K. and Flegal, K.M., 2012. Prevalence of obesity in the United States, 2009-2010. *Natl. Center Hlth. Stat. Data Brief*, **82**: 1-5.

Daubner, S. and Matthews, R., 1982. Purification and properties of methylenetetrahydrofolate reductase from pig liver. *J. biol. Chem*., **257**: 140-145. https://doi.org/10.1016/S0021-9258(19)68337-7

Di Renzo, L., Marsella, L.T., Sarlo, F., Soldati, L., Gratteri, S., Abenavoli, L. and Lorenzo, A.D., 2014. C677T gene polymorphism of MTHFR and metabolic syndrome: response to dietary intervention. *J. transl. Med*., **12**: 329. https://doi.org/10.1186/s12967-014-0329-4

Fan, S.J., Yang, B.Y., Zhi, X.Y., He, M., Wang, D., Wang, Y.X., Wang, Y.N., Wei, J., Zheng, Q.M. and Sun, G.F., 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*., **16**: 11849-11863. https://doi.org/10.3390/ijms160611849

Fuks, F., 2005. DNA methylation and histone modifications: Teaming up to silence genes. *Curr. Opin. Genet. Dev. Sci. Direct*, **15**: 490-495. https://doi.org/10.1016/j.gde.2005.08.002

Gallistl, S., Sudi, K., Mangge, H., Erwa, W. and Borkenstein, M., 2000. Insulin is an independent correlate of plasma homocysteine levels in obese children and adolescents. *Diabetes Care*, **23**. https://doi.org/10.2337/diacare.23.9.1348

Gara, S., Ochi, H., Chango, A., Najjar, L., Feki, M., Chir, F.B., Kaabachi, N., Becher, S.B., Boukthir, S.S. and Abdennebi, M., 2011. C677T polymorphism of MTHFR and G80A polymorphism of *RFC* genes and their relation with homocysteine levels in obese Tunisian children. *La Tunisie Med*., **89**: 565–568.

Garaulet, M., Ortega, F.B., Ruiz, J.R., Rey-Lopez, J.P., Beghin, L., Manios, Y., Cuenca- Garcıa, M.,

Plada, M., Diethelm, K., Kafatos, A., Molnar, D., Al-Tahan, J. and Moreno, L.A., 2011. Short sleep duration is associated with increased obesity markers in European adolescents: Effect of physical activity and dietary habits. The Helena study. *Int. J. Obes*., **35**: 1308–1317. https://doi.org/10.1038/ijo.2011.149

Giovannucci, E. and Michaud, D., 2007. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology*, **132**: 2208-2225. https://doi.org/10.1053/j.gastro.2007.03.050

Goyette, P., Pai, A., Milos, R., Frosst, P., Tran, P., Chen, Z., Chan, M. and Rozen, R., 1998. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). *Mammal. Genome*, **9**: 652-656. https://doi.org/10.1007/s003359900838

Goyette, P., Christensen, B., Rosenblatt, D. and Rozen, R., 1996. Severe and mild mutations in cis for the methylenetetrahydrofolate reductase (MTHFR) gene, and description of five novel mutations in MTHFR. *Am. J. Hum. Genet*., **59**: 1268-1275.

Goyette, P., Sumner, J.S., Milos, R., Duncan, A.M., Rosenblatt, D.S., Matthews, R. and Rozen, R., 1994. Human methylenetetrahydrofolate reductase: Isolation of cDNA, mapping, and mutation identification. *Nat. Genet*., **7**: 195-200. https://doi.org/10.1038/ng0694-195

Hernandez-Guerrero, C., Romo-Palafox, I., Diaz-Gutierrez, M.C., Iturbe-Garcia, M., Texcahua-Salazar, A. and Perez-Lizaur, A.B., 2013. Prevalence of metilentetrahidrofolate reductase C677T polymorphism, consumption of vitamins B6, B9, B12 and determination of lipidic hydroperoxides in obese and normal weight Mexican population. *Nutr. Hosp*., **28**: 2142-2150.

Hussain, A., Hydrie, M.Z.I., Claussen, B. and Asghar, S., 2010. Type 2 diabetes and obesity: A review. *J. Diabetol*., **2**: 1.

Irfan, M., Ismail, M., Azhar Beg, M., Shabbir, A., Rashid Kayani, A. and Kaukab R.G., 2016. Association of the MTHFR C677T (rs1801133) polymorphism with idiopathic male infertility in a local Pakistani population. *Balkan J. med. Genet.,* **19**: 51–62. https://doi.org/10.1515/bjmg-2016-0007

Kang, S., Zhou, J., Wong, P., Kowalisyn, J. and Strokosch, G., 1988. Intermediate homocysteinaemia: A thermolabile variant of methylenetrahydrofolatereductase. *Am. J. Hum. Genet*., **43**: 414-421.

Klerk, M., Verhoef, P., Clarke, R., Blom, H.J., Kok, F.J. and Schouten, E.G., 2002. MTHFR studies collaboration group. MTHFR 677C to T polymorphism and risk of coronary heart disease: A meta-analysis. *J. Am. med. Assoc*., **288**: 2023-2031. https://doi.org/10.1001/jama.288.16.2023

Lewis, S.J., Lawler, D.A., Nordestgaard, B.G., Tybjærg-Hansen, A., Ebrahim, S., Zacho, J., Ness, A., Leary, S. and Smith, G.D., 2008. The methylenetetrahydrofolate reductase c677t genotype and the risk of obesity in three large population-based cohorts. *Eur. J. Endocrinol*., **159**: 35–40. https://doi.org/10.1530/EJE-08-0056

Loscalzo, J., 1996. The oxidant stress of hyperhomocystein (e) inemia. *J. clin. Invest*., **98**: 5-7. https://doi.org/10.1172/JCI118776

Mojtabai, R., 2008. Body mass index and serum folate in childbearing age women. *Eur. J. Epidemiol*., **19**: 1029-1036. https://doi.org/10.1007/s10654-004-2253-z

Norris, J.M., Langefeld, C.D., Scherzinger, A.L., Rich, S.S., Bookman, E., Beck, S.R., Saad, M.F., Haffner, S.M., Bergman, R.N., Bowden, D.W. and Wagenknecht, L.E., 2005. Quantitative trait loci for abdominal fat and BMI in Hispanic-Americans and African-Americans: The IRAS family study. *Int. J. Obesity*, **29**: 67-77. https://doi.org/10.1038/sj.ijo.0802793

Rassoul, F., Richter, V., Hentschel, B., Geisel, J., Herrmann, W. and Kuntze, T., 2008. Plasma homocysteine levels and 677C to T methylenetetrahydrofolate reductase gene polymorphism in patients with coronary artery disease of different severity. *Indian J. med. Res*., **127**: 154-158.

Rosenberg, N., Murata, M., Ikeda, Y., Opare-Sem, O., Zivelin, A., Geffen, E. and Seligsohn, U., 2002. The frequent 5, 10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in Whites, Japanese and Africans. *Am. J. Hum. Genet*., **70**: 758-762. https://doi.org/10.1086/338932

Rozen, R., 1997. Genetic predisposition to hyperhomocysteinaemia: Deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thromb. Haemost*., **78**: 523-526. https://doi.org/10.1055/s-0038-1657581

Scheen, A.J. and Luyckx, F.H., 2002. Obesity and liver disease. *Clin. Endocrinol. Metab*., **16**: 703-716. https://doi.org/10.1053/beem.2002.0225

Scorsatto, M., Luiz, R.R., de-Oliveira, G.M.M., Santos-Reboucas, C.B., Pimente, M.M.G. and Rosa, G., 2015. Association between homocysteine and polymorphisms in MTHFR in Brazilian obese

women. *Int. J. Cardiovasc. Sci*., **28**: 16-24. https://doi.org/10.5935/2359-4802.20150004

Selhub, J., 1999. Homocysteine metabolism. *Annls Rev. Nutr*., **19**: 217-246. https://doi.org/10.1146/annurev.nutr.19.1.217

Settin, A.A., Algashamb, A., Dowaidara, M. and Ismail, H., 2009. Methylenetetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms related to overweight/obesity among Saudi subjects from Qassim Region. *Dis. Mark*., **27**: 97–102. https://doi.org/10.1155/2009/384718

Shaikh, W.A., Patel, M. and Singh, S., 2009. Sleep deprivation predisposes Guajarati Indian adolescents to obesity. *Indian J. Commun. Med*., **34**: 192–194. https://doi.org/10.4103/0970-0218.55282

Smith, A.D., Kim, Y.I. and Refsum, H., 2008. Is folic acid good for everyone? *Am. J. clin. Nutr*., **87**: 517-533. https://doi.org/10.1093/ajcn/87.3.517

Sun, Y., Sekine, M. and Kagamimori, S., 2009. Lifestyle and overweight among Japanese adolescents: The Toyama birth cohort study. *J. Epidemiol*., **19**: 303-310. https://doi.org/10.2188/jea.JE20080095

Swinburn, B.A., Caterson, I., Seidell, J.C. and James, W.P.T., 2004. Diet, nutrition and the prevention of excess weight gain and obesity. *Publ. Hlth. Nutr*., **7**: 123-146. https://doi.org/10.1079/PHN2003585

Taheri, S., 2006. The link between short sleep duration and obesity: We should recommend more sleep to prevent obesity. *Arch. Dis. Child*., **91**: 881–884. https://doi.org/10.1136/adc.2005.093013

Tamer, G., Mert, M., Tamer, I., Mesci, B., Kilic, D. and Arik, S., 2011. Effects of thyroid autoimmunity on abdominal obesity and hyperlipidaemia. *Endokrynol. Polska*, **62**: 421–428.

Terruzzi, I., Senesi, P., Fermo, I., Lattuada, G. and Luzi, L., 2007. Are genetic variants of the methyl group metabolism enzymes risk factors predisposing to obesity? *J. Endocrinol. Invest*., **30**: 747-753. https://doi.org/10.1007/BF03350812

Thawnashom, K., Tungtrongchitr, R., Petmitr, S., Pongpaew, P., Phonrat, B., Tungtrongchitr, A. and Schelp, F.P., 2005. Methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) in relation to homocysteine concentration in overweight and obese Thai. *Southeast Asian J. Trop. Med. Pub. Hlth.,* **36**: 459-66.

Tremblay, A., Perusse, L. and Bouchard, C., 2004. Energy balance and body-weight stability: Impact of gene-environment interactions. *Br. J. Nutr*., **92(Suppl. 1)**: S63–S66. https://doi.org/10.1079/BJN20041144

Vincent, H.K. and A.G. Taylor. 2006. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int. J. Obesity*, **30**: 400-418. https://doi.org/10.1038/sj.ijo.0803177

Yamada, K., Chen, Z., Rozen, R. and Maththews, R.G., 2001. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc. natl. Acad. Sci. U.S.A*., **98**: 14853-14858. https://doi.org/10.1073/pnas.261469998

Yang, B., Fan, S., Zhi, X., Wang, D., Li, Y., Wang, Y., Wang, Y., Wei, J., Zheng, Q. and Sun, G., 2014. Associations of *MTHFR* C677T and *MTRR* A66G gene polymorphisms with metabolic syndrome: A case-control study in northern China. *Int. J. mol. Sci*., **15**: 21687-21702. https://doi.org/10.3390/ijms151221687

Yin, R.X., Wu, D.F., Miao, L., Aung, L.H.H., Cao, X.L., Yan, T.T., Long, X.J., Liu, W.Y., Zhang, L. and Li, M., 2012. Several genetic polymorphisms interact with overweight/obesity to influence serum lipid levels. *Cardiovasc. Diabetol*., **11**: 123. https://doi.org/10.1186/1475-2840-11-123

Zheng, L., Yan, W., Kong, Y., Liang, P. and Mu, Y., 2015. An epidemiological study of risk factors of thyroid nodule and goiter in Chinese women. *Int. J. environ. Res. Publ. Hlth*., **12**: 14114. https://doi.org/10.3390/ijerph120911608