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Original Article

Association of Methylenetetrahydrofolate Reductase gene Polymorphism (C677T) with Metabolic Syndrome in an Iranian Population: Tehran Homocysteine Survey

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Abstract

Background: The association of MTHFR and metabolic syndrome (MS) has been shown in special groups of diabetic and schizophrenic subjects, but no single study has investigated this relation in metabolic syndrome subjects. Our aim was to examine the association of MTHFR gene polymorphism with metabolic syndrome, type II diabetes mellitus and hypertension in an Iranian population.
Methods: As a cross-sectional study, the relevance of metabolic syndrome, hypertension and type II diabetes was investigated. Subjects were recruited from Tehran Homocysteine survey. Fasting serum levels of blood sugar, triglyceride (TG), total cholesterol (TC), HDL-Cholesterol (HDL-C) and LDL-Cholesterol (LDL-C), homocysteine, folic acid, and B12 were measured. MTHFR polymorphism was determined using PCR-RFLP.
Results: Of participants, 150, 160, 191 subjects met the criteria for metabolic syndrome, hypertension and diabetes, respectively. Compared to control group, frequency of CC, CT, and TT genotypes were not significantly different. In control and hypertensive groups, serum homocysteine levels were significantly higher in TT than CC and CT genotypes ($P<0.05$), serum folic acid was significantly lower in TT than CC genotype in hypertensive group ($P<0.001$). In diabetic subjects, serum homocysteine levels were significantly lower in CC than TT genotype ($P<0.01$), and reverse was true for serum folic acid ($P<0.05$). In hypertensive and diabetic subjects, serum folic acid levels and difference between C and T alleles were significant ($P< 0.001$ for both), whereas in MS group only homocysteine levels differed significantly between C and T alleles ($P< 0.001$).
Conclusion: We found no significant association between MTHFR polymorphism and metabolic syndrome, hypertension, and diabetes in this Iranian population. Results of present the study should be confirmed in larger population-based studies.

Keywords: MTHFR, Metabolic syndrome, Population

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Introduction

Methylenetetrahydrofolate reductase (MTHFR) enzyme donates methyl group for homocysteine remethylation (1). A missense mutation in which cytosine is replaced by thymine at position 677 of the MTHFR gene translates into substitution of alanine by valine and results in thermolability and decreased specific activity of the enzyme (2). This would results in higher homocysteine concentrations, endothelial dysfunction and accelerated lipoprotein oxidation (3). Elevated homocysteine concentrations have been shown in diabetes mellitus (DM) (4, 5) and hypertension (6, 7). Also, it has been shown that hyperhomocysteinemia is an integral component of metabolic syndrome (MS) in rats (8). Several studies have found a relationship between hyperhomocysteinemia and insulin resistance (9-12). MTHFR polymorphism may influence these findings due to the association of C677T polymorphism with hypertension (13), diabetes (14) and diabetic nephropathy (15). The association between MTHFR and metabolic syndrome has been investigated in type II diabetic (16) and schizophrenic patients (17). However, to our knowledge there is not any population –based study on this issue in the literature. Regarding high prevalence of metabolic syndrome (27%) in Iranian population (18) and possible role of genetic factors in susceptibility to MS (19), it may be prudent to determine the association between MTHFR polymorphism and metabolic syndrome. This would lead to earlier interventions for genetically susceptible individuals. On the other hand, the coexistence of hypertension and glucose intolerance as metabolic syndrome components, makes it difficult to interpret the relation between MTHFR gene polymorphism and MS. Thus, we aimed to investigate the association between MTHFR gene polymorphism and metabolic syndrome, type II diabetes and hypertension in an Iranian population.

Methods

Study design

A subgroup of subjects was extracted randomly from the data base of a population based study, *Tehran Homocysteine survey*. Details of this study are described elsewhere (20). Briefly, this study was conducted to assess atherosclerosis risk factors in adult population of downtown Tehran according to the methodology of WHO (World Health Organization) / MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) project.

Study population

Five hundred seventy seven participants with diagnoses of diabetes, hypertension, Metabolic syndrome and healthy controls were recruited from the total of 1500 Participants of *Tehran Homocysteine Survey*. Subjects with secondary hypertension, previous history of stroke, coronary artery disease, myocardial infarction, peripheral Vascular disease, renal hypertension, and diabetic nephropathy were excluded from The study. Hypertensive and diabetic groups had less than 3 NCEP-ATP III criteria of Metabolic syndrome diagnosis. Hypertensive group were not diabetic and vice versa.

Weight and Height were measured using standard scale with light clothing and barefoot. BMI (body mass index) was calculated as weight (kg) by height squared (m^2).

Blood pressure was measured by a trained team according to World Health Organization (WHO) criteria. After 10 minutes rest, BP was measured in sitting Position at right arm. The first and fifth korotkoff sounds were considered as systolic And diastolic blood pressures, respectively. The average of two measurements was recorded.

Waist circumference (WC) was measured at the part of the trunk located midway

between the lower costal margin and the iliac crest while the person was standing.
The study protocol was approved by Endocrinology and Metabolism Research center (EMRC) ethics committee and conformed to the Declaration of Helsinki. All subjects gave written informed consent.

Biochemical analyses

Whole blood samples of 8 milliliters were drawn and transformed immediately to clinical laboratory within 1 hour where they were centrifuged (10 min, RT, at 2000 RPM) in room temperature. Serum aliquots were divided into micro tubes and stored at -70°C until analyses. Serum homocysteine was determined by HPLC method. Blood samples collected in tubes were allowed to clot, centrifuged and serum were divided into micro tubes and stored at –70ºC for serum folic acid and vitamin B_{12} measurement by RIA assay (Simul TRAC, ICN Pharmaceutical). Fasting blood sugar, TG, TC, HDL-C and LDL-C was measured by enzymatic method (Pars Azmun, Iran).

Diagnosis

According to modified ATP (Adult Treatment Panel) III of NCEP (National Cholesterol Education Program), metabolic syndrome is identified by the presence of at least three of the following components: increased waist circumference (>102cm for men, > 88 cm for women), blood pressure elevation (≥ 130/85 mmHg), low HDL cholesterol (<40 mg/dl in men, < 50 mg/dl in women), high triglycerides (≥ 150 mg/dl), hyperglycemia (fasting glucose ≥ 100 mg/dl) (21). Hypertension diagnosis was based on WHO criteria, as SBP ≥ 160 mm Hg and/or DBP ≥ 95 mm Hg or if they were taking antihypertensive drugs (22). Type II diabetes diagnosis was based on the current American Diabetes Association guidelines, that is blood glucose ≥ 126 mg/dl (23).

Genetic analyses

Genomic DNA was isolated from anti-coagulated whole blood using salting- out protocol.

Genotyping was performed using PCR-RFLP with the following primers:
5'-TGAAGGAGAAGGTGTCTGCGGGA-3'
5'-AGGACGGTGCGGTGAGAGTG-3'
A total of 100ng genomic DNA was amplified in a 20 µl PCR reaction, PCR buffer containing 3 mM MgCl2, 0.25 mM dNTP's (Bioline), 5 pmol of each primer, and 1 U Taq polymerase. The PCR cycles were as follows: primary denaturation was carried at 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 30 seconds. The final extension was carried at 72°C for 7 minutes. The presence of product (198 base pairs) was verified on a 2% agarose gel stained with ethidium bromide. PCR products were digested with *Hinf I* in a 15 µl final volume. This contained 10µl of PCR product, 1x NE Buffer, 10U *Hinf I*. The digest was incubated overnight at 37°C and the products of the digest were then visualized on a 4% agarose gel stained with ethidium bromide. *Hinf I* restriction endonuclease digested the PCR product yielding DNA fragments of 176, 22 base pairs when T allele was present and leaving fragment of 198 base pairs undigested when allele C was present.

Statistical analysis

Statistical analysis was performed using SPSS software, release 15.0 (SPSS Inc Chicago). Values less than 0.05 were considered significant. Chi-square test was used to compare differences in genotype distribution from those expected for Hardy-Weinberg equilibrium. Fisher's exact test was performed to examine any differences in allele frequency. Multiple logistic regression analysis was performed to identify the relevancy of (age, sex, BMI, and MTHFR) to metabolic syndrome, hypertension, and type II diabetes and interaction of these outcomes with relevant factors.

Results

Table 1 provides baseline characteristics of participants. One hundred fifty, 160, 191 of persons met the criteria for MS, hypertension and DM, respectively. Age was found to be significantly higher in all study groups compared to control (P< 0.001). Mean systolic and diastolic blood pressures were significantly higher in hypertensive, diabetic and MS subjects compared to control group (P<0.001). The same result was true for serum TG (P< 0.01). Serum B12 was significantly higher in diabetic patients than control group (P<0.01).

Genotype distributions and allele frequencies of MTHFR polymorphism are presented in Table 2. The allele frequencies of all participants within the groups were in concordance with Hardy-Weinberg equilibrium (HWE). There were not significantly difference in frequencies of CC, CT, and TT genotypes among MS, hypertensive and diabetic compared to control groups. Frequency of T allele was significantly higher in control group compared to either case groups (P<0.05).

Homocysteine, folic acid, and B12 serum concentrations within different MTHFR genotypes are revealed in Table 3.

Table 1- General Characteristics of study participants

	MS n=150	End HTN n=160	Diabetes n=191	Control n=76
Gender (M/F)	35/115	50/110	63/128	26/50
Age(years)	51±10*	50±11*	51±11*	34±8
Total cholesterol (mg/dl)	213.05±49.12	211.87±46.84	208.47±46.20	194.74±39.00
HDL-cholesterol (mg/dl)	63.68±15.85	63.94±17.62	63.84±18.00	60.12±16.21
LDL-cholesterol (mg/dl)	103.95±32.88	105.78±31.74	103.63±31.00	108.80±18.66
Triglyceride (mg/dl)	274.03±205.59*	242.27±193.05*	255.33±201.18*	148.66±100.66
Systolic blood pressure (mmHg)	144.01±21.38*	146.28±21.16*	139.17±24.00*	111.17±9.99
Diastolic blood pressure (mmHg)	92.03±12.70*	93.40±11.31*	87.09±13.96*	72.82±8.47
BMI (kg/m^2)	31.6±5	30.8 ±5.4	29.9±5.5	27.5±5.6
Homocysteine (µmol/L)	15.48±9.15	16.96±11.67	17.19 ±10.58	15.75±8.92
Folic acid (ng/ml)	4.77±2.29	4.37±2.03	4.78±2.44	3.90±1.94
B12 (pg/ml)	312.09±223.58	290.40±194.46	338.49±225.32*	269.13±151.07

Data are means ± SD, HTN: hypertension, MS: Metabolic Syndrome, BMI: Body Mass Index , *: P<0.05 compared with control

Table 2- Genotype distributions and allele frequencies

	MS n=150	End HTN n=160	Diabetes n=191	Control n=76
Genotype				
CC	102 (68 %)	99 (61.87%)	122 (63.8%)	36 (47.4%)
CT	38 (23.3 %)	44 (27.5%)	53 (27.7 %)	31 (40.7%)
TT	10 (6.6 %)	17 (10.6%)	16(8.3 %)	9 (11.8%)
Alleles				
C	79.65%	75.62%	77.74%	67.75 %
T	18.25%	24.35%	22.49%	32.15%

Data are *n* (%), HTN: hypertension, MS: Metabolic Syndrome, BMI: Body Mass Index

Table 3- Comparison of serum B12, folic acid, and homocysteine levels according to MTHFR polymorphism

	Hcy (µmol/L)				Folic acid (ng/ml)				B12 (pg/ml)			
	MS	**HTN**	**Diabetes**	**Control**	**MS**	**HTN**	**Diabetes**	**Control**	**MS**	**HTN**	**Diabetes**	**Control**
Genotype												
CC	14.68 ± 9.27	15.05 ± 9.25	15.60 ± 10.11	15.11 ± 6.27	5.05 ± 2.35	4.64 ± 2.08	5.10 ± 2.49	4.05 ± 2.05	355.51 ± 244.95	295.05 ± 194.62	363.89 ± 224.40	262.30 ± 139.77
CT	17.27 ± 7.98	17.67 ± 7.48	17.90 ± 8.23	14.08 ± 7.67	4.34 ± 2.24	4.12 ± 2.01	4.63 ± 2.47	4.01 ± 1.95	296.86 ± 230.46	284.09 ± 266.94	322.40 ± 241.96	277.90 ± 160.37
TT	14.82 ± 4.31	31.77 ± 27.33	26.17 ± 15.63	15.10 ± 16.68	3.67 ± 1.14	3.26 ± 1.10	3.41 ± 1.54	2.93 ± 1.15	243.70 ± 185.72	248.31 ± 151.97	235.94 ± 155.99	267 ± 178.85
Alleles												
C	15.51 ± 9.32	15.85 ± 8.81	16.30 ± 9.59	14.36 ± 6.92	4.86 ± 2.33	4.48 ± 2.07	4.96 ± 2.49	4.36 ± 1.99	317.68 ± 225.95	294.45 ± 198.98	351.05 ± 229.65	269.39 ± 148.51
T	16.93 ± 7.58	20.44 ± 14.57	19.92 ± 10.92	16.34 ± 10.86	4.20 ± 2.05	3.92 ± 1.86	4.27 ± 2.29	3.76 ± 1.84	285.30 ± 220.68	275.55 ± 194.75	296.61 ± 222.48	274.43 ± 162.36

Data are means ± SD, HTN: hypertension, MS: Metabolic Syndrome, Hcy: Homocysteine

Homocysteine was significantly higher and folic acid was significantly lower in TT than CC genotypes both in diabetic and hypertensive patients [(P<0.05 and P<0.001, respectively for hypertensive and (P<0.01 P<0.05, respectively for diabetics)]. The difference of serum folic acid level between participants with C and T alleles was significant in all three groups including hypertension, MS, and diabetes (P< 0.001); whereas the difference of serum homocysteine level between subjects with C and T alleles was significant only in hypertensive and diabetic patients (P< 0.001).

As shown in Table 4, multiple logistic regression analysis was used to test the relevancy of outcome variables with MTHFR, homocysteine, serum B12, and folic acid. MTHFR was found to be significantly associated with MS (P<0.01); however, after including confounding factors such as BMI, age, and cigarette smoking in the regression model, no significant association observed (Table 5).

Table 4- Logistic regression determinants of MS, HTN and Diabetes

	MS		Diabetes		HTN	
Parameters	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
MTHFR	0.58*	(0.38-0.9)	0.95	(0.63-1.44)	0.70	(0.46-1.06)
B12	0.99	(0.99-1.00)	1.00*	(1.00-1.005)	0.99*	(0.99- 1.00)
Folic acid	1.2*	(1.01-1.42)	1.1	(1.00-1.004)	1.13	(0.96-1.34)
Hcy	0.99	(0.96-1.02)	1.01	(0.99-1.04)	1.01	(0.98-1.03)

* P- Value was significant (P<0.05), HTN: hypertension, MS: Metabolic Syndrome

Table 5- Logistic regression determinants of MS, HTN and Diabetes after considering confounding factors

Parameters	MS		Diabetes		HTN	
	OR	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
MTHFR	0.69	(0.39-1.21)	1.07	(0.62-1.85)	0.75	(0.44-1.26)
B12	1.0	(0.99-1.00)	1.00†	(1.00-1.005)	0.99	(0.99- 1.001)
Folic acid	1.1	(0.88-1.38)	1.03	(0.82-1.28)	1.07	(0.86-1.33)
Hcy	0.99	(0.96-1.02)	0.99	(0.955-1.02)	0.99	(0.95-1.02)
BMI category*	2.08†	(1.50-2.90)	0.99	(0.73-1.33)	1.54†	(1.15-2.08)
Age	1.09†	(1.06-1.13)	1.10†	(1.07-1.13)	1.08†	(1.05-1.11)
No of cigarette**	1.29	(0.46-3.65)	0.377	(0.12-1.12)	0.51	(0.192-1.36)

*BMI: BMI category (underweight<18.5 kg/m^2, normal 18.6-24.9 kg/m^2 , overweight 25- 29.9 kg/m^2, obese 30-40 kg/m^2, morbid obese >40 kg/m^2), ** Number of cigarette smoking: < 10, 10-20, >20 per day, HTN: hypertension, MS: Metabolic Syndrome, Hcy: Homocysteine , † P- Value was significant (P<0.05)

Discussion

This is the first study to assess the association between C677T variant of MTHFR polymorphism and metabolic syndrome, hypertension, and diabetes in an Iranian population. No significant association between outcome variables and MTHFR polymorphism was observed. The results of the present study are in accordance with findings in Czech population which reported no significant association between MTHFR polymorphism and hypertension (14). A recent study in hypertensive adolescents

also showed that systolic blood pressure was not significantly higher in TT compared to CC genotypes (24). On the contrary, CT genotype was associated with increased risk of hypertension in an Indian population (25). In Spanish population and Japanese women also, hypertension was associated with TT genotype (26, 27). Likewise, in Caucasians, a modest significant association was found between MTHFR C677T mutation and essential hypertension (28). The discrepancy among the findings of these studies may not merely due to the difference in T allele frequency, as in the Indian population with 18% prevalence of TT variant; there was a significant association between hypertension and CT genotype (25). Another explanation for these inconsistent results may be the effect of complex heterozygosity for C677T and A1298C polymorphisms resulting in the reduced MTHFR enzyme activity (29, 30) as has been demonstrated by Markan et al. (25).

There was a gender-specific significant association between MTHFR polymorphism and type II diabetes in the Czech population which was not observed in the present study (14). This inconsistency may be related to different sample size as well as different logistic models in our study. Aforementioned study recruited 49 DM subjects while we evaluated 160 DM individuals in our study. In addition, they entered MTHFR, age, gender, and BMI as covariates in multiple logistic regression, while we also added Hcy, serum B12, folic acid, and cigarette smoking in the model.

Regarding the association of MTHFR C677T gene polymorphism and metabolic syndrome, our results are consistent with those of Russo et al. and Yamada et al. (16, 31). Russo et al. recently found no association between MTHFR polymorphism and metabolic syndrome in a group of type II diabetic subjects with mild hyperhomocysteinemia (16); also, Yamada et al. observed no association between MTHFR polymorphism and metabolic syndrome. On the other hand, Ellingrod et al. (17) observed MTHFR C/T variant, predisposes schizophrenic patients who also take atypical antipsychotics to the metabolic syndrome. This discrepancy may be explained by the effect of epigenetic mechanisms.

Epigenetic mechanisms affect gene expression via DNA methylation, various modifications of histone proteins and autoregulation of transcription factors (32). There is a hypothesis that in addition to "thrifty genotype" inheritance, epigenetic mechanisms during fetal and postnatal development affect components of metabolic syndrome, including insulin resistance, regional adiposity, dyslipidemia, and hypertension (33). These traits may also be inherited to the next generations. Due to the existence of mitosis in adulthood, epigenetic pathways continue to affect gene expression throughout the life. MTHFR enzyme provides methyl group for homocysteine remethylation, and consequently methionine. Methionine donates methyl group for DNA methylation, almost in CpG pairs. These pairs of specific loci are served as promoters for associated genes (34). It is possible that environmental/nutritional interactions may affect the association of MTHFR and metabolic syndrome through these epigenetic mechanisms (35).

In conclusion, we found no significant association between MTHFR C677T polymorphism and metabolic syndrome, hypertension, and diabetes in this Iranian population. To our knowledge, no single study has investigated the relation between MTHFR polymorphism and metabolic syndrome in a general population. Findings of the present study should be confirmed in further population-based studies with larger sample size.

Partly, life style and nutritional modifications may influence gene expression via epigenetic mechanisms. A widely available modification may be food fortification with folic acid. It is

hypothesized that folic acid fortification could overcome metabolic block caused by thermolabile MTHFR mutation, and consequently affects DNA methylation and gene expression. It is also noteworthy to assess the effects of folic acid fortification/supplementation on gene expression, especially those related to chronic diseases such as hypertension, diabetes, and metabolic syndrome.

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