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RESEARCH ARTICLE

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MTHFR gene C677T and A1298C variants are associated with FMF risk in a Turkish cohort

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Background: Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in homocysteine (Hcy) metabolism. We aimed to evaluate a possible relationship between MTHFR gene C677T (rs 1801133), A1298C (rs 1801131) variants and susceptibility to FMF in a Turkish cohort.

Material-Methods: This case-control study included 198 Turkish FMF patients and 100 healthy subjects as controls. MTHFR C677T and A1298C were analyzed by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) methods.

Results: The genotype distribution and allele frequency of the MTHFR C677T were statistically different between the patients and the control group (P=.006, P=.001, respectively). The frequency of the TT genotype and T allele of MTHFR C677T was significantly higher in the patients than in the controls. The genotype distribution of MTHFR A1298C variant did not show any statistically significant difference between the patients and the controls (P›.05). The patients had statistically different frequencies in allele C of MTHFR A1298C variant compared with the control (P=.032). We also examined the risk associated with inheriting the combined genotypes for the two MTHFR variants. According to these results, individuals who were CC homozygous at C677T locus and AA homozygous at A1298C locus have a lower risk of developing FMF (P=.002). Individuals who were TT homozygous at C677T locus and AC heterozygous at A1298C locus have higher risk of developing FMF (P=.033).

Conclusion: Our findings clearly showed there was an association the MTHFR C677T/A1298C variants and susceptibility to FMF in the Turkish sample.

KEYWORDS

FMF, homocystein, methylenetetrahydrofolate reductase, variant

1 | INTRODUCTION

Familial Mediterranean fever (FMF, OMIM 249100) is an autosomal recessive inherited, autoinflammatory disease.[1] The major clinical manifestations of FMF are self-limited attacks of acute fever accompanied by abdominal pain, arthritis, polyserositis including peritonitis, pleuritis and synovitis 1. FMF frequently affects Mediterranean populations including Jews, Armenians, Turks, and Arabs. MEFV gene, the gene associated with FMF, encodes a protein of 781 amino acids termed pyrin or marenostrin, which is primarily expressed in mature granulocytes. Pyrin has been shown to be a component of inflammasome complex (particularly the NLRP3 inflammasome), an intracellular organelle, which is implied for the production of interleukin-1β.[2] Acute FMF attacks are manifested by a nonspecific elevation in inflammatory mediators, including serum amyloid A (SAA), fibrinogen, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[3,4] However, a

significant subclinical inflammation can be also seen during the interval between attacks.[3]

TheMethylenetetrahydrofolatereductase(MTHFR)enzyme,encoded by MTHFR gene, irreversibly converts 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF).[5] This reaction is essential for the sequential biochemical mechanism that transforms the amino acid homocysteine (Hcy) to amino acid methionine.[6] The human MTHFR gene is composed of 11 exons and 10 introns and located on the short arm of chromosome 1 (1p36.3).[7] MTHFR gene have two common allelic variants, C677T (rs1801133) and A1298C (rs1801131), which lead to the amino acid substitutions Ala222Val and Glu429Ala, respectively.[8] The C to T change at nucleotide 677 leads to a more thermolabile MTHFR, decreases its enzyme activity, and is related with higher plasma homocysteine levels.[9] Similarly, the A1298C variant may also have an impact on MTHFR activity and plasma Hcy values.[10] Several in vitro studies reported that Hcy was associated with a proinflammatory response in animal models and in humans.[11] Therefore, we aimed to examine the possible association between MTHFR C677T and A1298C variants and FMF susceptibility in Turkish population.

2 | MATERIALS AND METHODS

2.1 | Patients

In total, 198 FMF patients (The mean age 20.36±13.441 years; male/female:87/111) and 100 healthy controls (The mean age 25.35±8.948; male/female:42/58) participated in the study. All patients and controls were examined in the clinics of Internal Medicine at Gaziosmanpasa University in Tokat, Turkey. The diagnosis of FMF was made according to the established criteria described previously by Livneh et al.[12] An accurate and detailed family history was obtained for each patient. Both the study group and the control group were of Turkish origin, from the inner Central Black Sea region of Turkey. Informed written consent was obtained from all patients and subjects before enrollment to the study, according to the ethical guidelines of the 2008 Declaration of Helsinki and the investigation was approved by the Intuitional Ethical Committee.

2.2 | Genotyping

Genomic DNA was extracted from EDTA anticoagulated peripheral blood leukocytes using a commercial DNA isolation kit (Sigma-Aldrich, Taufkirchen, Germany). The C677T and A1298C variants in MTHFR gene were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphisms (RFLP).[7] The MTHFR C677T variant was analyzed by using forward (F) 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and reverse (R) 5′-AGG ACG GTG CGG TGA GAG TG-3′ primers. The PCR protocol was consisted of an initial melting step of 5 minutes at 94°C; followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 61°C, and 30 seconds at 72°C; and a final elongation step of 5 minutes at 72°C. After amplification, the 198 bp of PCR product was digested with Hinf I in a 15 μL reaction solution containing 10 μL of PCR product, 1.5 μL of 10× buffer, and two units of Hinf I at 37°C overnight. The digestion products were separated on 3% agarose gels, and fragments stained with the ethidium bromide were photographed on an ultraviolet transilluminator. Wild type (CC) individuals were identified by only a 198 bp fragment, heterozygotes (CT) by both the 175/23 bp and 198 bp, and homozygote variants (TT) by the 175/23 bp.

MTHFR A1298C variant amplification was performed using primers forward (F) 5′-CTT TGG GGA GGT GAA GGA CTA CTA C-3′ and reverse (R) 5′-CAC TTT GTG AGC ATT CCG GTT TG-3′ and the protocol described previously by El-Baz et al.[13] Restriction digestion of PCR product with MboII endonuclease was performed. Wild type (AA) was produced 4 fragments (176, 30, 28, and 22 bp), whereas heterozygous (AC) yielded 5 fragments (204, 176, 30, 28, and 22 bp) and homozygous mutant (CC) variant produced 3 fragments (204, 30, and 22 bp). Second PCR was performed to confirm samples whose results were not clear.

2.3 | Statistical analysis

All statistical analyses were performed using computer SPSS Statistical Program Version 22.0 and OpenEpi info 2.2 software package program. Continuous data were given as mean±SD (standard deviation) and (min/max). Chi square test was used to significance of differences in the allele frequency and genotype distribution between the two study groups. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. *P* value $P<.05$ was considered statistically significant.

3 | RESULTS

A total number of 298 subjects from a Turkish population were recruited (100 controls, 198 patients with FMF) in this study. In the study group, there were 87 males (43.9%) and 111 (56.1%) females. There were 42 (42%) male controls and 58 (58%) female controls. The demographical characteristics of the two groups and clinical characteristics of patients are presented in Table 1. Age of first symptoms, age of diagnosis, the frequency of attacks, colchicine use, family history, fever, abdominal pain, thoracic pain, joint involvement, appendicitis, amyloidosis, and erythema were analyzed.

Table 2 shows the genotype distribution and allele frequency of the MTHFR C677T and A1298C variants in FMF patients and controls. The frequency of the CC, CT, and TT genotypes of the MTHFR C677T variant in the patients was 48%, 42.4%, and 9.6% respectively, and in the controls the frequency was 66%, 31%, and 3%, respectively. The genotype distribution of the MTHFR C677T variant was statistically different between the FMF patients and the control group (*P*=.006). There was a significant difference for allele frequency of the MTHFR C677T. MTHFR C677T TT genotype and T allele were more prevalent in patient group. High differences were also observed when the patients and the controls were compared according to CC vs CT+TT (*P*=.003, OR: 2.10 95CI%: 1.28-3.48) and TT vs CC+CT (*P*=.035, OR: 3.42 95CI%: 1.07-14.83).

TABLE 1 The demographical and clinical characteristics of groups and clinical characteristics of FMF patients

Demographical and clinical characteristics	FMF patients n=198	Healthy controls n=100
Age, mean±SD (years)	20.36±13.441	25.35±8.948
Gender, n (%)		
Male/Female	87/111 (43.9/56.1)	42/58 (42.0/58.0)
Age of first symptoms, mean±SD (years)	11.07±8.159	
Age of diagnosis, mean±SD (years)	17.03±10.579	
The frequency of attacks, mean±SD (day)	25.59±15.035	
Colchicine use, n (%)		
Yes/No	128/70 (64.6/35.4)	
Family history of FMF, n (%)		
Yes/No	87/111 (43.9/56.1)	
Fever, n (%)		
Yes/No	163/35 (82.3/17.7)	
Abdomianl pain, n (%)		
Yes/No	179/19 (90.4/9.6)	
Thoracic pain, n (%)		
Yes/No	54/144 (27.3/72.7)	
Joint involvement, n(%)		
Yes/No	137/61 (69.2/30.8)	
Appendicitis, n(%)		
Yes/No	24/174 (12.1/87.9)	
Amyloidosis, n(%)		
Yes/No	12/186 (6.1/93.9)	
Erythema, n(%)		
Yes/No	35/163 (17.7/82.3)	

FMF, Familial Mediterranean Fever; SD, standard deviation.

For the A1298C variants, the genotype distribution for FMF and control groups were as follows: 40.4% were AA, 51% had the heterozygote AC genotype, and 8.6% were homozygote for CC in the patients group compared with 55% who were AA, 39% with the AC genotype, and 6% who had CC in the control group. The genotype distribution of MTHFR A1298C variant did not show any statistically difference between patients and controls (*P*=.057). Moreover, a significant association was observed when the patients were compared with the controls according to AA vs AC+CC genotypes (*P*=.018, OR: 1.80 95CI%: 1.11-2.94).

We found that there was a statistically significant difference between the groups with respect to MTHFR A1298C variant allele frequencies (*P*=.032, OR:1.51, 95%CI: 1.04-2.22). The patients had statistically different frequencies in allele C of MTHFR A1298C variant compared with the control (Table 2).

We also examined the risk associated with inheriting the combined genotypes for the two MTHFR variants (Table 3). According to these results, individuals who were CC homozygous at C677T locus and AA homozygous at A1298C locus have a lower risk of developing FMF (*P*=.002). Also, individuals who were TT homozygous at C677T locus and AC heterozygous at A1298C locus have higher risk of developing FMF (*P*=.033).

4 | DISCUSSION

FMF is a systemic autoinflammatory disorder characterized by ostensibly unprovoked activation of distinct inflammatory pathways.[1,14] Although FMF is a single gene disease, the real etiopathogenesis of FMF is not well understood. An abnormal form of pyrin which is encoded by the MEFV gene results in inflammation in FMF. These inflammatory attacks are then manifested by an intense accumulation of neutrophils into the serous cavities and are associated with an increase in the levels of acute-phase proteins and cytokines.[2] Most of the patients experience symptom-free intervals which are characterized by subclinical inflammation with excess production of SAA, an acute phase reactant synthesized in response to pro-inflammatory cytokines; this finding may be considered as a marker of subclinical systemic inflammation.[4]

Hcy, an endogenous sulfur-containing amino acid, is produced by the methionine metabolism. Hcy has a potent oxidant effect and its oxidation end-products are toxic.[15] Hyperhomocysteinaemia (HCA), the accumulation of homocystein in the circulation, is acts as an independent risk factor for atherosclerotic vascular disease by inducing platelet activation, promoting smooth muscle cell proliferation, displaying cytotoxicity, leading to endothelial dysfunction, and stimulating the low-density lipoprotein (LDL) oxidation.[16] It is reported that HCA was associated with enhanced carotid artery permeability, which could affect LDL intravasation and accumulation in the arterial wall in rats.[17] Rohde et al. reported that HCA is associated with an elevation of CRP, acute phase reactant.[18]

Recently it has been shown that inflammation induces every aspect of atherosclerotic process. Chronic HCA and inflammatory cytokines could contribute to the occurrence of endothelial dysfunction. Disorders with ongoing subclinical inflammation, such as systemic lupus erythematosus, rheumatoid arthritis, and diabetes mellitus, are believed to have an increased risk of early structural vascular change and atherosclerosis.[19,20] Due to nature of the disease, patients with FMF should be considered to face an increased risk of early vascular change and atherosclerosis. Basar et al. reported that MEFV gene mutations had significantly increased in patients with young coronary heart disease.[21] Also, it was shown that atherogenic index values were significantly higher in patients with FMF than control group.[22] Besides, studies demonstrated that common carotide artery intima-media thickness, a non-invasive predictor of early arterial wall alteration, increased in FMF patients.[23,24]

MTHFR C677T variant in the catalytic domain of the protein, the binding site for the MTHFR co-factor flavin adenine dinucleotide, is associated with increased thermolability of the enzyme, reduction in the activity and elevated plasma Hcy concentrations, along with lowered plasma folate.[7] Homozygous TT have about 30% of

TABLE 2 Genotype distribution and allele frequencies of MTHFR gene C677T ans A1298C variants in patients and controls

MTHFR	FMF patients n=198 (%)	Healthy controls n=100 (%)	*P*	OR (CI 95%)
C677T				
Genotypes				
CC	95 (48.0)	66 (66.0)	**.006**	
CT	84 (42.4)	31 (31.0)		
TT	19 (9.6)	3 (3.0)		
CC: CT+TT	95 (48.0): 103 (62.0)	66 (66.0): 34 (34.0)	**.003**	**2.10 (1.28-3.48)**
CC+CT: TT	179 (90.4): 19 (9.6)	97 (97.0): 3 (3.0)	**.035**	**3.42 (1.07-14.83)**
Alleles				
C	274 (69.2)	163 (81.5)	**.001**	**1.96 (1.30-3.00)**
T	122 (30.8)	37 (18.5)		
A1298C				
Genotypes				
AA	80 (40.4)	55 (55.0)	.057	
AC	101 (51.0)	39 (39.0)		
CC	17 (8.6)	6 (6.0)		
AA: AC+CC	80 (40.4): 118 (59.6)	55 (55.0): 45 (45.0)	**.018**	**1.80 (1.11-2.94)**
AA+AC: CCI	181 (91.4): 17 (8.6)	94 (94.0): 6 (6.0)	.447	1.47 (0.57-4.19)
Alleles				
A	261 (65.9)	149 (74.5)	**.032**	**1.51 (1.04-2.22)**
C	135 (34.1)	51 (25.5)		

Data were analyzed by $\chi 2$ or fisher's exact test. FMF: Familial Mediterranean Fever MTHFR: Methylene tetrahydrofolate reductase. The results that are statistically significant are typed in bold.

TABLE 3 Comparative analysis of combined genotypes of MTHFR gene variants between FMF patients and controls

	FMF Patients (n=198)		Controls (n=100)		
Genotypes	n	%	n	%	*P*
C677T-A1298C					
CC-AA	42	21.2	38	38.0	**.002**
CC-AC	43	21.7	24	24.0	.654
CC-CC	10	5.1	4	4.0	.933
CT-AA	33	16.7	16	16.0	.894
CT-AC	45	22.7	14	14.0	.073
CT-CC	6	3.0	1	1.0	.512
TT-AA	4	2.0	1	1.0	.911
TT-AC	14	7.1	1	1.0	**.033**
TT-CC	1	0.5	1	1.0	-

Data were analyzed by $\chi 2$ or fisher's exact test. FMF: Familial Mediterranean Fever. *MTHFR*: Methylene tetrahydrofolate reductase. The results that are statistically significant are typed in bold.

normal enzyme activity, while heterozygous CT have approximately 60% of the normal enzyme activity, therefore they incline to collect 5,10-methylene-THF displacing the reaction toward the DNA production despite diminishing the pool of methyl donors.[25] MTHFR A1298T variant is located in the C-terminal end of the enzyme, the S-adenosymethionine-regulatory domain, and may lead to a reduction in 40% in enzyme activity of the variant genotype.[26] The prevalence of the two variants differs in certain geographical regions and ethnic groups.[27] Several studies have been performed to evaluate the effects of MTHFR C677T and A1298T variants on the risk of different conditions including cancer,[28] congenital heart disease,[29] leukemia,[30] recurrent pregnancy loss,[31] obesity,[32] and hypertension.[33]

In this study, we aimed to investigate possible association of the C677T and A1298C variants in the MTHFR gene and susceptibility to FMF in a cohort of Turkish patients. To the best of our knowledge, this is the first study to elucidate the potential association MTHFR variants with FMF risk in Turkish patients. The genotype distribution and allele frequency of the MTHFR C677T variant showed statistically significant difference between the patients and the controls (*P*=.006 and *P*=.001, respectively; Table 2). We found that MTHFR C677T TT genotype and T allele were higher in patients group. In vivo and in vitro studies reported that individuals homozygous for the T allele have significantly higher Hcy levels.[7] Also previously, Karatay et al.[34] reported that serum Hcy levels are often increased in FMF patients during attack-free periods. Our results were consistent with both data. Furthermore, they would help to explain the atherosclerosis in FMF. In addition, a significant association was observed when the patients were compared with the controls according to CC genotype vs CT+TT genotypes and TT genotype vs CC+CT genotype.

Although genotype distribution of MTHFR A1298C variant did not show statistically significant differences between patients and controls

(*P*=.057), a significant association was observed when the patients were compared with the controls according to MTHFR A1298C AA vs AC+CC genotypes (OR:1.80, 95% CI:1.11-2.94, *P*=.018). There was a statistically significant difference in the allele frequency for MTHFR A1298C variant (OR: 1.51, 95% CI: 1.04-2.22, *P*=.032) (Table 2). C allele was significantly higher in the patients group than the control group. It was previously reported that the C allele of A1298C variant generates a reduction in MTHFR enzyme activity,[35] and can also lead to an elevation of plasma Hcy.[36] These data were also consistent with MTHFR C677T variant results.

In addition, we evaluated genotype combinations for the two MTHFR loci. For the two genotype combinations, two of them were found to be significantly different FMF patients and control subjects. The CC-AA genotype was found to be significantly decreased in the FMF patients, while the TT-AC genotype was found to be significantly more frequent in the control subjects (*P*=.02, *P*=.033, respectively) (Table 3).

In conclusion, we report the first association to our knowledge between MTHFR gene variants and FMF in Turkish population. The present study revealed that MTHFR C677T and A1298C variants are strongly associated with a susceptibility to FMF. Our results suggest that the MTHFR C677T and A1298C variants may be a useful tool to predict the susceptibility of FMF and its complications. Further investigations with larger populations are needed to confirm the association between MTHFR gene variants and FMF. We believe that with the help of newly designed studies, the association first emphasized here will better elucidate this interaction and clarify the pathogenesis of FMF.

REFERENCES

1. Onen F. Familial Mediterranean fever. *Rheumatol Int*. 2006;26:489-496.
2. Ben-Zvi I, Livneh A. Chronic inflammation in FMF: markers, risk factors, outcomes and therapy. *Nat Rev Rheumatol*. 2011;7:105-112.
3. Korkmaz C, Ozdogan H, Kasapçopur O, Yazici H. Acute phase response in familial Mediterranean fever. *Ann Rheum Dis*. 2002;61:79-81.
4. Lachmann HJ, Sengul B, Yavuzşen TU, et al. Clinical and sub-clinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. *Rheumatology (Oxford)*. 2006;45:746-750.
5. Fekih Mrissa N, Mrad M, Klai S, et al. Association of methylenetetrahydrofolate reductase A1298C polymorphism but not of C677T with multiple sclerosis in Tunisian patients. *Clin Neurol Neurosurg* 2013;115:1657-1660.
6. Alatab S, Hossein-nezhad A, Mirzaei K, Mokhtari F, Shariati G, Najmafshar A. Inflammatory profile, age of onset, and the MTHFR polymorphism in patients with multiple sclerosis. *J Mol Neurosci*. 2011;44:6-11.
7. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10:111-113.
8. de Cássia Carvalho Barbosa R, da Costa DM, Cordeiro DE, Vieira AP, Rabenhorst SH. Interaction of MTHFR C677T and A1298C, and MTR A2756G gene polymorphisms in breast cancer risk in a population in Northeast Brazil. *Anticancer Res* 2012;32:4805-4811.
9. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*. 1998;64:169-172.
10. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet*. 1998;62:1044-1051.
11. Durga J, van Tits LJH, Schouten Evert G, Kok Frans J, Verhoef P. Effect of Lowering of Homocysteine Levels on Inflammatory MarkersA Randomized Controlled Trial. *Arch Intern Med* 2005;165:1388-1394.
12. Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum*. 1997;40:1879-1885.
13. El-Baz R, Settin A, Ismaeel A, et al. MTHFR C677T, A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. *J Renin Angiotensin Aldosterone Syst*. 2012;13:472-477.
14. Yuksel S, Ayvazyan L, Gasparyan AY. Familial mediterranean Fever as an emerging clinical model of atherogenesis associated with low-grade inflammation. *Open Cardiovasc Med J*. 2010;4:51-56.
15. McCully KS. Homocysteine and vascular disease. *Nat Med*. 1996;2:386-389.
16. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: current evidence and future prospects. *Am J Med*. 2002;112:556-565.
17. Symons JD, Mullick AE, Ensunsa JL, Ma AA, Rutledge JC. Hyperhomocysteinemia evoked by folate depletion: effects on coronary and carotid arterial function. *Arterioscler Thromb Vasc Biol*. 2002;22:772-780.
18. Rohde LE, Hennekens CH, Ridker PM. Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am J Cardiol*. 1999;84:1018-1022.
19. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation*. 2003;108:2957-2963.
20. de Leeuw K, Freire B, Smit AJ, Bootsma H, Kallenberg CG, Bijl M. Traditional and non-traditional risk factors contribute to the development of accelerated atherosclerosis in patients with systemic lupus erythematosus. *Lupus*. 2006;15:675-682.
21. Basar N, Kisacik B, Ercan S, et al. Familial Mediterranean fever gene mutations as a risk factor for early coronary artery disease. *Int J Rheum Dis*. 2014;7:1-5.
22. Acay A, Ulu MS, Ahsen A, et al. Atherogenic index as a predictor of atherosclerosis in subjects with familial Mediterranean fever. *Medicina (Kaunas)*. 2014;50:329-333.
23. Akdogan A, Calguneri M, Yavuz B, et al. Are familial Mediterranean fever (FMF) patients at increased risk for atherosclerosis? Impaired endothelial function and increased intima media thickness are found in FMF. *J Am Coll Cardiol*. 2006;48:2351-2353.
24. Bilginer Y, Ozaltin F, Basaran C, et al. Evaluation of intima media thickness of the common and internal carotid arteries with inflammatory markers in familial Mediterranean fever as possible predictors for atherosclerosis. *Rheumatol Int*. 2008;28:1211-1216.
25. Balley LB, Gregory JF. Polymorphism of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risk and impact on folate requirement. *J Nutr*. 1999;129:919-922.
26. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A → C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. *Atherosclerosis*. 2002;156:40.
27. Yang B, Liu Y, Li Y, et al. Geographical Distribution of MTHFR C677T, A1298C and MTRR A66G Gene Polymorphisms in China: Findings from 15357 Adults of Han Nationality. *PLoS ONE*. 2013;8:e57917.
28. Al-Motassem Y, Shomaf M, Said I, et al. Allele and Genotype Frequencies of the Polymorphic Methylenetetrahydrofolate Reductase and Lung Cancer in ther Jordanian Population: a Case Control Study. *Asian Pac J Cancer Prev*. 2015;16:3101-3109.
29. Li D, Yu K, Ma Y, Liu Y, Ji L. Correlationship between congenital heart disease and polymorphism of MTHFR gene. *Wei Sheng Yan Jiu*. 2015;44:933-938.

30. Roy Moulik N, Parveen F, Kumar A, Awasthi S, Agrawal S. MTHFR gene polymorphism in acute lymphoblastic leukemia among North Indian children: a case-control study and meta-analysis updated from 2011. *J Hum Genet* 2014;59:397-404.
31. Settin A, Elshazli R, Salama A, ElBaz R. Methylenetetrahydrofolate reductase gene polymorphisms in Egyptian women with unexplained recurrent pregnancy loss. *Genet Test Mol Biomarkers*. 2011;15:887-892.
32. Settin AA, Algasham A, Dowaidar M, Ismail H. Methylene tetrahydrofolate reductase and angiotensin converting enzyme gene polymorphisms related to overweight/obesity among Saudi subjects from Qassim Region. *Dis Markers*. 2009;27:97-102.
33. Markan S, Sachdeva M, Sehrawat BS, Kumari S, Jain S, Khullar M. MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians. *Mol Cell Biochem*. 2007;302:125-131.
34. Karatay S, Yildirim K, Uyanik A, et al. Increased serum concentrations of homocysteine and lipoprotein (a) in familial Mediterranean fever. *Ann Clin Lab Sci*. 2010;40:10-14.
35. Pereira TV, Rudnicki M, Pereira AC, Pombo-de-Oliveira MS, Franco RF. 5,10-Methylenetetrahydrofolate reductase polymorphisms and acute lymphoblastic leukemia risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2006;15:1956-1963.
36. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, Sengupta S. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. *J Hum Genet*. 2005;50:655-663.

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