# Investigation of Methylene Tetrahydrofolate Reductase Gene Polymorphisms in Coronary by-passed Patients Due to Coronary Atherosclerosis Etiology

## Koroner Ateroskleroz Etiyolojisi ile Koroner Bypass Olan Olgularda Metilen Tetrahidrofolat Redüktaz Gen Polimorfizmlerinin Araştırılması

Müzeyyen İZMİRLİ[a]
Davut ALPTEKİN, MD,[a]
M. Şah TOPÇUOĞLU, MD[b]
A. İrfan GÜZEL, MD[a]

[a]Department of Medical Biology,
[b]Thoracic and Cardiovascular Surgery,
Çukurova University Faculty of Medicine,
Adana

Geliş Tarihi/*Received:* 03.06.2009
Kabul Tarihi/*Accepted:* 28.09.2009

*This study was presented at "10th National Congress of Medical Biology and Genetics (6-9 July 2007, Belek, Antalya, Turkey).*

Yazışma Adresi/*Correspondence:*
Davut ALPTEKİN, MD
Çukurova University Faculty of Medicine,
Department of Medical Biology,
Adana,
TÜRKİYE/TURKEY
alptekin@cu.edu.tr

**ABSTRACT Objective:** Methylene tetrahydrofolate reductase (MTHFR) enzyme plays a role in the folate metabolism which is present in many bio-chemical pathways such as homocysteine methylation and nucleotide bio-synthesis. MTHFR also provides the balance between DNA synthesis and methylation reactions. Furthermore, this enzyme affects the plasma homocysteine level which is a risk factor for cardiovascular diseases. In this study, it was aimed to investigate the relationship between coronary atherosclerosis and 677 C→T and 1298 A→C polymorphisms in the MTHFR gene. **Material and Methods:** Study group consist of 60 coronary atherosclerosis patients (mean age= 55.4 ± 1.20; 45 male, 15 female) and 60 healthy control group (mean age= 44.5 ± 1.26; 34 male, 26 female). DNA isolation was performed from blood samples of the subjects and polymorphism regions were amplified by Polymerase Chain Reaction (PCR). The PCR products were cut by using Hinf I and Ita I restriction enzymes and evaluated after Poly Acrylamide Gel Electrophoresis (PAGE) for the determination of 677 (CC, C→T or TT) and the 1298 (AA, A→C or CC) genotypes. **Results:** The differences of MTHFR 677 C→T and 1298 A→C polymorphisms were p= 0.240 and p= 0.278 between patients and controls, p= 0.961 and p= 0.980 between gender and p= 0.181 and p= 0.747 for the number of obstructed vessels respectively and were not significant statistically. **Conclusion:** It was suggested that, MTHFR 677 C→T and 1298 A→C polymorphisms has no effect on the development of coronary artery disease and on the formation of clogged arteries.

**Key Words:** Atherosclerosis; coronary artery disease; homocysteine; 5,10-methylenetetrahydrofolate reductase (FADH2)

**ÖZET Amaç:** Metilen tetrahidrofolat redüktaz (MTHFR) enzimi; 5,10 metilen tetrahidrofolatın 5 metil tetrahidrofolata dönüşümünü katalize eden, homosisteinin metilasyonu ve nükleotid biyosentezi gibi pek çok biyokimyasal yolda rol alan bir enzimdir. MTHFR, DNA sentezi ve metilasyon reaksiyonları arasındaki dengeyi sağlar. Ayrıca bu enzim, kardiyovasküler hastalıklar için bir risk etmeni olan plazma homosistein seviyesini de etkilemektedir. Bu çalışmada, koroner ateroskleroz hastalığı ve MTHFR geni 677 C→T ve 1298 A→C polimorfizmleri arasındaki ilişkiyi araştırmak hedeflenmiştir. **Gereç ve Yöntemler:** Çalışma grubu 60 koroner ateroskleroz hastası (yaş ortalaması= 55.4 ± 1.20; 45 erkek, 15 kadın) ve 60 sağlıklı kontrol grubu (yaş ortalaması= 44.5 ± 1.26; 34 erkek, 26 kadın) içermektedir. Kişilerden alınan periferik kan örneklerinden DNA izolasyonu yapılarak polimorfizmlerin bulunduğu bölgeler Polimeraz Zincir Reaksiyonu (PCR) ile amplifiye edilmiştir. PCR ürünleri Hinf I ve Ita I restriksiyon enzimleri ile kesilmiş, ve 677 CC, C→T veya TT ve 1298 AA, A→C veya CC genotiplerinin belirlenmesi için Poli Akrilamid Jel Elektroforezinde (PAGE) yürütülerek değerlendirilmiştir. **Bulgular:** MTHFR geninin 677 C→T ve 1298 A→C polimorfizmleri sırasıyla hasta ve kontrol grupları arasında p= 0.240 ve p= 0.278, cinsiyetler arasında p= 0.961 ve p= 0.980, tıkalı damar sayısı açısından ise p= 0.181 ve p= 0.747 olarak belirlenmiş ve istatistiksel olarak anlamlı bulunmamıştır. **Sonuç:** MTHFR 677 C→T ve 1298 A→C polimorfizmlerinin koroner arter hastalıklarının gelişiminde ve tıkalı damar oluşumunda etkisinin olmadığı düşünülmektedir.

**Anahtar Kelimeler:** Ateroskleroz; koroner arter hastalığı; homosistein; 5,10 methilentetrahidrofolat redüktaz



**Turkiye Klinikleri J Cardiovasc Sci 2009;21(3):303-8**

Methylene tetrahydrofolate reductase (MTHFR) enzyme catalyzes the conversion of 5,10-methylene tetrahydrofolate (MTHF) into 5-methyl tetrahydrofolate (THF).[1] The MTHFR enzyme is a key enzyme that has a mission in the purin-pyrimidine synthesis, methylation reactions and in the folate metabolism where homocysteine converted to methionine.[2] Therefore, various metabolic problems may occur when the activity of the MTHFR enzyme tends to decrease. One of these problems is the intracellular accumulation of homocysteine which may lead to atherosclerosis.[3]

The MTHFR gene is localized on chromosome 1 p36.3 and consisted of 11 exons.[4,5] The 2.2 kilo base (kb) cDNA of the human MTHFR gene was isolated at 1994.[6] The product of this gene is a 77 kilo Dalton (kD) protein with a catalytic activity consisting of 656 amino acids.[2]

The 677 C→T polymorphism is located in exon 4 and causes Ala222Val substitution in the amino terminal catalytic zone of the enzyme. The binding zone of FAD which is a co-factor of MTHFR is the amino acid determined by the 677$^{th}$ base pair of the MTHFR gene. However, a problem may occur at the mentioned binding site as a result of a 677 C→T mutation. On the other hand, 1298 A→C polymorphism on the 7$^{th}$ exon causing Glu429Ala substitution in the carboxy terminal zone where is a critical point coding the S-adenosyl methionine regulatory region of the enzyme. Conformational alterations at the S-adenosyl methionine binding site inhibit the activity of the enzyme.[7]

According to the statistical study of Turkish Adult Risk Factor (TEKHARF), the prevalence of atherosclerotic cardiac diseases in adults is approximately 3.8% (4.1% in males, and 3.5% in females) in Turkey. It was determined that the moderately-graded plasma homocysteine levels is a risk factor in coronary cardiovascular disease, in myocardial infarction, ischemic stroke and in venous thrombosis.[8-11] Additionally, a few relationships were determined between MTHFR gene polymorphisms and breast and colon carcinoma, leukemia, some types of migraine, schizophrenia, vascular dementia, depression and Alzheimer disease.[2,12-15] Furthermore, the polymorphisms were shown as risk factors for pregnancy complications such as pre-eclampsia, placental rupture and spontaneous abortus and for neural tube defects.[16,17] Recent studies had been demonstrated that the mentioned polymorphisms have roles on the formation of cleft palates, Down syndrome and congenital cardiac defects.[18-20]

The objective of this study is to investigate the MTHFR gene 677 C→T and the 1298 A→C polymorphisms by means of PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) method on the target sites of DNA samples of patient and control groups.

## MATERIAL AND METHODS

### PATIENT AND CONTROL GROUPS

This study was performed on 60 patients (mean age= 55.4 ± 1.20; 45 male, 15 female) who did not received a blood transfusion within the recent six months and planned to undergo a coronary by-pass surgery in our institute and 60 healthy controls (mean age= 44.5 ± 1.26; 34 male, 26 female) who have no family history with coronary atherosclerotic disease. Venous blood samples obtained from the subjects were transferred to CBC tubes and stored at 4°C.

### GENOTYPE ASSESSMENT

DNA samples were extracted from whole blood using salting-out procedure.[21] For the 677 C→T polymorphism, 198 base pair (bp) PCR product of the MTHFR gene was obtained by using mixture containing 10 pmol from each primers (Forward: 5'TGA AGG AGA AGG TGT C→TG CGG GA3' and Reverse: 5'AGG A→CG GTG CGG TGA GAG TG3'), 2mM $MgCl_2$, 0.2 mM dNTPs, 2.5 U Taq polymerase and 100-500 ng of DNA sample for each reaction. After initial denaturation of the reaction mixture at 95°C for 3 min, amplification was achieved by 30 cycles of 95°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec, and final extension at 72°C for 10 minutes. For the 1298 A→C polymorphism of the MTHFR gene, 138 bp product was obtained by using mixture containing 10 pmol from each primers (Forward primer: 5'GGG AGG AGC TGA

CCA GTG CAG3', Reverse primer: 5'GGG GTC AGG CCA GGG GCA G3'), 2 mM $MgCl_2$, 0.2 mM dNTPs, 2.5 U Taq polymerase and 100-500 ng of DNA sample for each reaction. After initial denaturation of the reaction mixture at 95°C for 3 min, amplification was achieved by 30 cycles of 95°C for 30 sec, 66°C for 30 sec and 72°C for 30 sec, and final extension at 72°C for 10 minutes.

Restriction digestion of the PCR products were performed by using *Hinf* I which cuts at GANTC and produces two fragments (175 and 23 bp), and *Ita* I/*FnU4*HI which cuts at GCNGC and produces two fragments (119 and 19 bp), restriction endonucleases for the 677 C→T and 1298 A→C polymorphisms respectively.

Original PCR products and restriction digestions were loaded on 10% polyacrilamide gels and electrophoresed at 100 V for 90 min, stained in EtBr for 5 min, visualized by using UviTech Gel documentation system and then evaluated.

### STATISTICAL ANALYSIS

The analysis of data was performed by a SPSS (11.5 version) program. Pearson Chi-Square test was used to compare ratios and $p< 0.05$ was accepted as statistically significant.

## RESULTS

Frequencies of 677 C→T and 1298 A→C polymorphisms at MTHFR in patient (n= 60) and controls (n= 60) are shown in Table 1 and 2. We could not demonstrate any difference between the patients and controls for 677 C→T and 1298 A→C polymorphisms (for 677 C→T polymorphism p= 0.240 p> 0.05; for 1298 A→C polymorphism p= 0.278 p> 0.05).

**TABLE 1:** Distribution of 677 CT polymorphism between patient and control groups.

| 677 CT polymorphism | Patient | | Control | | |
|---|---|---|---|---|---|
| Genotype | n | % | n | % | p |
| CC | 32 | 53.3 | 39 | 65 | |
| CT | 24 | 40 | 20 | 33.3 | 0.240, |
| TT | 4 | 6.7 | 1 | 1.7 | p> 0.05 |
| Total | 60 | 100 | 60 | 100 | |

**TABLE 2:** Distribution of 1298 AC polymorphism between patient and control groups.

| 1298 AC polymorphism | Patient | | Control | | |
|---|---|---|---|---|---|
| Genotype | n | % | n | % | p |
| AA | 25 | 41.7 | 21 | 35 | |
| AC | 26 | 43.3 | 34 | 56.7 | 0.278, |
| CC | 9 | 15 | 5 | 8.3 | p> 0.05 |
| Total | 60 | 100 | 60 | 100 | |

**TABLE 3:** Distribution of 677 CT polymorphism between male and female.

| 677 CT polymorphism | Male | | Female | | |
|---|---|---|---|---|---|
| Genotype | n | % | n | % | p |
| CC | 47 | 59.5 | 24 | 58.5 | |
| CT | 29 | 36.7 | 15 | 36.6 | 0.961, |
| TT | 3 | 3.8 | 2 | 4.9 | p> 0.05 |
| Total | 79 | 100 | 41 | 100 | |

When patients and controls were assessed together, it was found that the CC genotype of the MTHFR gene 677 C→T polymorphism was usually present in both sexes, but that the mentioned fact was not related with the gender of the individuals (p= 0.961, p> 0.05) (Table 3).

When we assess together patients and the controls in 1298 A→C polymorphism, we determined that the number of A→C genotyped individuals (he-terozygote mutants) were higher in both sexes; there was no differences regarding to gender statistically (p= 0.980, p> 0.05) (Table 4).

The number of clogged arteries of patients with coronary artery disease may display individual differences. In our study, the patient group had 1-7 clogged arteries and the healthy control group who did not carry any symptoms for coronary artery disease. We analyzed the relationship between the number of clogged arteries and 677 C→T and 1298 A→C polymorphisms. Accordingly, we assume that the number of clogged arteries in C→T and TT genotypes mutant patients would be higher than CC genotypes patients regarding 677 C→T polymorphism. However, statistical evaluation demonstrated no meaningful relationship be-

**TABLE 4:** Distribution of 1298 AC polymorphism between male and female.

| 1298 AC polymorphism | | | | | |
|---|---|---|---|---|---|
| Genotype | Male | | Female | | |
| | n | % | n | % | p |
| AA | 30 | 38 | 16 | 39 | |
| AC | 40 | 50 | 20 | 48.8 | 0.980, |
| CC | 9 | 11.4 | 5 | 12.2 | p> 0.05 |
| Total | 79 | 100 | 41 | 100 | |

tween the number of clogged arteries and polymorphisms (p= 0.181, p> 0.05 for 677 C→T and p= 0.747, p> 0.05 for 1298 A→C).

The cigarette smoking and alcohol consumption status of the patient and control groups were also considered in the study. The number of cigarette smokers were 35 (58.3%) in the patient and 32 (53.3%) in the control groups (p= 0.713; p> 0.05). When the patient and control groups were assessed in terms of alcohol consumption, there were 16 persons (26.7%) in the patient and 21 persons (35%) in the control group (p= 0.429, p> 0.05).

## DISCUSSION

The MTHFR enzyme reduces 5,10 MTHF to MTHF, the major carbon donor in the remethylation of homocysteine to methionine.[5] Lots of miscellaneous nonsense and missense mutations had been demonstrated in the MTHFR gene. Some of these mutations are effects the activity of the enzyme at different forms.[4,5] The MTHFR gene 677 C→T polymorphism is effective on the enzyme activity and lead to a decrease at a ratio of 55-65% while 1298 A→C polymorphism lead to a decrease at a ratio of 40%. Hyperhomocysteinemia may occur due to the decrease in the enzyme activity and as a consequence various clinical problems may arise.[3] Coronary atherosclerosis that occurs as a result of hyperhomocysteinemia is known to be responsible of death events in Europe at a ratio of 46%. Therefore, numerous studies were performed on MTHFR gene codon 677 C→T and 1298 A→C polymorphisms. Hyperhomocysteinemia and many other factors such as folate deficiency, Vitamin $B_6$ inadequacy, advanced age, gender, cigarette smoking, cholesterol, sedentary life style, diabetes, hypertension and obesity may cause the disease.[22,23]

In the present study, we have determined the MTHFR gene 677 CC and 1298 A→C polymorphisms. For the 677 C→T polymorphism, percent ratio of CC, C→T and TT genotypes in the patients (53.3%, 40%, 6.7% respectively) and control group (65%, 33.3%, 1.7%; respectively) were not so different but C→T and TT genotype ratios were tended to be higher in the patient group when compared with the control group. Similar results were obtained in a recent study performed on 79 coronary artery patients and 93 control group.[24] This study also indicates that coronary artery disease is not related with the polymorphisms of the MTHFR gene alone. According to studies performed on different races and communities, the MTHFR gene 677 C→T polymorphism displayed no statistical differences between the patient and control groups and this result is compatible with our findings. The ratios of MTHFR gene 677 CC, C→T and TT genotype frequencies may differ in every country where as in patients with a coronary artery disease the TT genotype frequency may increase.

Regarding the MTHFR gene codon 677 C→T polymorphism, contrary to our findings, a relationship with coronary artery disease was found in Japan. In the study, the CC, C→T and TT genotypes (34.6%, 45.6%, 19.8%; respectively) were different from the control group (42.6%, 46.4%, 11%; respectively) and was also related with coronary cardiac disease. Consequently, it was proven that the MTHFR gene codon 677 C→T polymorphism was a risk factor for the Japanese population.[25] The reason of different genotype frequencies may originate from the differences of the selected patient and control groups. Nevertheless, according to other studies performed, it was suggested that the mentioned polymorphisms were not a risk factor alone for the Poland, Spanish, Dutch, Pakistani, American, Arabian and Turkish populations.[23,26-31]

In our study, if the MTHFR gene codon 677 C→T polymorphism of the patient and control groups are studied, we can notice that the reason of the undifferentiated frequencies of AA, A→C, CC

genotypes was not a risk factor alone for coronary artery diseases. In studies which were carried out using this type of polymorphisms, it was reported that the reason of finding no differences between patient and control groups, in different populations, where the mentioned ratio may differ in different ethnic groups.[31-33] The compatibility with our study, demonstrates that MTHFR gene 1298 A→C polymorphism was not related with coronary hearth diseases alone.

Cigarette smoking alone is the most important risk factor for coronary artery disease. In a study carried out in Japan, it was reported that 53.2% of ischemic cardiac patients and 24.5% of patients enrolled in the control group were cigarette smokers whereas cigarette smoking was one of the main factors that increased the risk of coronary artery disease,[34] this difference may be involved in the effect of mentioned gene polymorphism distinction in this population. The cigarette smoker ratio in our study was not different (58.3%) when compared to the controls (53.3%).

In our study, alcohol consumption ratio was found 26.7% in the patient group and 35% in the control group. In a recent study which assessed the Japanese population revealed a ratio of 55.8% alcohol consumption in the patient group and 50.2% in the control group.[34] Both in the present and the Japanese population studies, no meaningful relationship was found between alcohol consumption and coronary atherosclerosis. Recent studies reported that increased homocysteine levels may elevate the risk for coronary artery diseases. Clarke et al reported that when compared with controls, higher homocysteine levels were present in patients with a coronary artery disease.[35] Some studies reported that when compared with the C→T and CC genotype, TT genotype of 677 C→T polymorphism increased the total plasma homocysteine level.[36] However, in our study the mentioned issue that coronary artery disease may elevate homocysteine level was not considered as this fact was already recognized.

Consequently, a relationship between coronary atherosclerosis and MTHFR gene polymorphisms had been indicated in some studies but according to our findings the results were not statistically significant.

*Acknowledgements*

*This study was supported by Cukurova University Research Fund (TF.2003YL8).*

## REFERENCES

1. Schwahn B, Rozen R. Polymorphisms in the methylenetetrahydrofolate reductase gene: clinical consequences. Am J Pharmacogenomics 2001;1(3):189-201.
2. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57(6):1098-102.
3. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274(13):1049-57.
4. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994;7(2):195-200.
5. Tran P, Leclerc D, Chan M, Pai A, Hiou-Tim F, Wu Q, et al. Multiple transcription start sites and alternative splicing in the methylenetetrahydrofolate reductase gene result in two enzyme isoforms. Mamm Genome 2002;13(9): 483-92.
6. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome 1998;9(8): 652-6.
7. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci USA 2001;98(26):14853-8.
8. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 2000; 72(2):324-32.
9. Rothenbacher D, Fischer HG, Hoffmeister A, Hoffmann MM, März W, Bode G, et al. Homocysteine and methylenetetrahydrofolate reductase genotype: association with risk of coronary heart disease and relation to inflammatory, hemostatic, and lipid parameters. Atherosclerosis 2002;162(1):193-200.
10. Wu Y, Tomon M, Sumino K. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke: sex difference in Japanese. Kobe J Med Sci 2001;47(6):255-62.
11. Rigamonti A, Carriero MR, Boncoraglio G, Leone M, Bussone G. Cerebral vein thrombosis and mild hyperhomocysteinemia: three new cases. Neurol Sci 2002;23(5):225-7.
12. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. Am J Epidemiol 2003;157(7):571-82.
13. Kara I, Sazci A, Ergul E, Kaya G, Kilic G. Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk. Brain Res Mol Brain Res 2003;111(1-2):84-90.

14. Sazcı A, Ergul E, Guzelhan Y, Kaya G, Kara I. Methylenetetrahydrofolate reductase gene polymorphisms in patients with schizophrenia. Molecular Brain Research 2003;10:117(1): 104.
15. Rozen R. Methylenetetrahydrofolate reductase: a link between folate and riboflavin? Am J Clin Nutr 2002;76(2):301-2.
16. Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999;340(1):9-13.
17. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000;151(9):862-77.
18. Mills JL, Kirke PN, Molloy AM, Burke H, Conley MR, Lee YJ, et al. Methylenetetrahydrofolate reductase thermolabile variant and oral clefts. Am J Med Genet 1999;86(1):71-4.
19. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999;70(4):495-501.
20. Wenstrom KD, Johanning GL, Johnston KE, DuBard M. Association of the C677T methylenetetrahydrofolate reductase mutation and elevated homocysteine levels with congenital cardiac malformations. Am J Obstet Gynecol 2001;184(5):806-17.
21. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
22. McCully KS. Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr 2007;86(5):1563S-8S.
23. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97(18):1837-47.
24. Yilmaz H, Isbir S, Agachan B, Ergen A, Farsak B, Isbir T. C677T Mutation of methylenetetrahydrofolate reductase gene and serum homocysteine levels in Turkish patients with coronary artery disease. Cell Biochemistry Function 2006;24(1):87-90.
25. Wu Y, Tomon M, Sumino K. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke: sex difference in Japanese. Kobe J Med Sci 2001;47(6):255-62.
26. Goracy I, Goracy J, Suliga M, Ciechanowicz A. [C677T gene polymorphism of methylenetetrahydrofolate reductase (MTHFR) in patients with myocardial infarction]. Pol Arch Med Wewn 1999;102(4):849-54.
27. Guillén M, Corella D, Portolés O, González JI, Mulet F, Sáiz C. Prevalence of the methylenetetrahydrofolate reductase 677C > T mutation in the Mediterranean Spanish population. Association with cardiovascular risk factors. Eur J Epidemiol 2001;17(3):255-61.
28. Rassoul F, Richter V, Hentschel B, Geisel J, Herrmann W, Kuntze T. Plasma homocysteine levels & 677C-->T methylenetetrahydrofolate reductase gene polymorphism in patients with coronary artery disease of different severity. Indian J Med Res 2008;127(2):154-8.
29. Iqbal MP, Fatima T, Parveen S, Yousuf FA, Shafiq M, Mehboobali N, et al. Lack of association of methylenetetrahydrofolate reductase 677C>T mutation with coronary artery disease in a Pakistani population. J Mol Genet Med 2005;1(1):26-32.30.
30. Kluijtmans LA, Whitehead AS. Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease; evidence that all three MTHFR C677T genotypes confer different levels of risk. Eur Heart J 2001;22(4):294-9.31.
31. Abu-Amero KK,. Wyngaard CA, Dzimiri N. Prevalence and role of methylentetrahydrofolate reductase 677 CT and 1298 AC polymorphisms in coronary artery disease in Arabs. Arch Pathol Lab Med 2003;127(10): 1349-52.
32. Keku T, Millikan R, Worley K, Winkel S, Eaton A, Biscocho L, et al. 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. Cancer Epidemiol Biomarkers Prev 2002;11(12):1611-21.
33. Meisel C, Cascorbi I, Gerloff T, Stangl V, Laule M, Müller J, et al. Identification of six methylentetrahydrofolate reductase (MTHFR) genotypes resulting from common polymorphisms: impact on plasma homocysteine levels and development of coronary artery disease. Atherosclerosis 2001;154:651-8.
34. Ou T, Yamakawa-Kobayashi K, Arinami T, Amemiya H, Fujiwara H, Kawata K, et al. Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. Atherosclerosis 1998;137(1):23-8.35.
35. Clarke R, Daly L, Robinson K. Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 1991; 324(17):1149-55.
36. Brattström L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998;98(23): 2520-6.