Original Article

Common Polymorphism A1298C in Methylenetetrahydrofolate Reductase Gene Is not a Risk Factor for Coronary Artery Disease in Selected Iranian Patients

Mahboobeh Ghaedi, MSc[1], Ahmad Aleyasin, PhD[1*], Mohammad Ali Boroumand, MD[2], Seyed Hesameddin Abbasi, MD[2,3], Saeed Davoodi, MD[2], Mojgan Mirakhori, MSc[1]

[1]National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.
[2]Research Department, Tehran Heart Center, Medical Sciences/University of Tehran, Tehran, Iran.
[3]National Iranian Oil Company Health Organization, Central Hospital, Tehran, Iran.

Received 13 February 2007; Accepted 19 March 2007

Abstract

Background: *Coronary artery disease (CAD) is emerging as a major public health concern in most developing countries. During the past 10 years, the vast majority of over 100 case-control retrospective studies have shown that elevated plasma homocysteine level is a strong independent risk factor for coronary artery disease. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. A second polymorphism, A1298C, in MTHFR gene, is reported to be associated with decreased enzyme activity and may give rise to elevated blood homocysteine level and increased risk of coronary artery disease.*

Methods: *In the present study we used PCR-RFLP analysis to investigate the association between A1298C polymorphism and blood homocysteine level and the risk of CAD in 100 patients compared to 100 normal controls.*

Results: *The frequency of mutated allele and genotype distribution showed no significant difference between patient and control groups. Although the elevated level in blood homocysteine were observed in Iranian CAD cases compared to the normal control, the A1298C polymorphism was not associated with increased CAD risk in studied population as supported by a P value>0.05 and chi-square equal to 0.697.*

Conclusion: *An increased plasma homocysteine concentration confers an independent risk factor for CAD. Although A1298C polymorphism in MTHFR gene has effects on enzyme activity but our findings do not support a major role for this polymorphism in homocysteine metabolism and it can not be considered a major risk factor for coronary artery disease in a selected Iranian population*

J Teh Univ Heart Ctr 3 (2007) 161-166

Keywords: *MTHFR • Polymorphism • Homocysteine • Folate*

Introduction

Coronary artery disease (CAD) is the major cause of death in industrial nations. Despite advances in our understanding of cardiovascular disease, traditional risk factors such as hypertension, smoking, diabetes mellitus, and dislipidemia do not accurately predict cardiovascular events.[1-3] Homocysteine is an emerging new risk factor for CAD. Mild-to-moderate hyperhomocysteinemia is a well-established independent risk factor for coronary cerebral and peripheral atherosclerotic diseases and venous thrombosis.[4-6]

Numerous clinical studies have shown that total homocysteine is a risk factor for CAD and stroke in humans and predicts mortality independently of traditional risk

*Corresponding Author: ***Ahmad Aleyasin****, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran. P. O. Box: 14155-6343. Tel: +98-21-44580383. Fax: +98-21-44580395. E-mail: ahmad.aleyasin@nrcjeb.ac.ir.*

factors in patients with CAD.[7,8]

Until recently, the normal range for homocysteine was considered to be 5 to 15 μmol/L. It is now widely accepted that upper range of normal may be 10 to 12 μmol/L for middle-aged adults and that risk for cardiovascular disease occurs if plasma homocysteine exceed this value. Patients with CAD and other cardiovascular diseases usually have mild hyperhomocysteinemia (>12 to 25 μmol/L) with an incidence of 30 to 50 percent.[9]

Plasma homocysteine concentration is determined by both genetic and nutritional factors. Deficiencies of folate, vitamin B12, or B6 can lead to impaired homocysteine metabolism and hyperhomocysteinemia.[9-11] In addition, mutations in the genes coding for methylenetetrahydrofolate reductase (MTHFR), methionine synthase, and cysthathionine B synthase may also produce hyperhomocysteinemia. Smoking, excessive coffee consumption, and lack of exercise are associated with elevation in homocysteine as well.[12,13] Because homocysteine has a thiol , it can undergo auto-oxidation and oxidation with other thiols. The resulting reactive oxygen species, hydrogen peroxide and superoxide anion radical, generate oxidative stress and induce vascular dysfunction.[14,15] Recent evidence suggests that homocysteine may limit the bioavailability of nitric oxide, resulting in the impairment of folate-mediated vasodilation. The limited bioavailability of nitric oxide could be due to nitrosothiol formation with homocysteine. Homocysteine may also target specific proteins and impair their activity and function through disulfide bond formation.[16]

Homocysteine is a thiol compound derived from methionine that can be metabolized by two pathways; folate and cobalamine dependent remethylation to methionine or transsulfuration to cysteine with ultimate degradation.[5-7] In both pathways, major genetic defects or enzyme deficiencies are associated with high plasma homocysteine.[7,8,17] A less frequent form of mild hyperhomocysteinemia is genetic defect in methylenetetrahydrofolate reductase (MTHFR), a key enzyme regulating folate metabolism. MTHFR catalyses the reduction of 5,10 Methylenetetrahydrofolate to 5, Methylenetetrahydrofolate, which donates a methyl group to homocysteine to be transformed into methionine.18,19 The MTHFR gene is located on chromosome 1. There are two well-described and commonly occurring polymorphisms in the MTHFR gene: C677T and A1298C. The C677T polymorphism occurs in exon 4 and results in an alanine to valine substitution at codon 222.This polymorphism lies in the binding site of the MTHFR cofactor flavin adenine dinucleotide (FAD) and produces a thermolabile form of the enzyme.20 Individuals with this polymorphism have elevated plasma homocysteine in comparison with normal people.[8,21-23]

Recently another polymorphism in the MTHFR gene, A1298C, has been identified. This polymorphism occurs in exon 7 and results in a glutamate to alanine substitution at codon 429.[15-17] This polymorphism lies in the S-adenosylemethionine regulatory domain of the enzyme. The binding of the S-adenosylemethionine (SAM) results in conformational change within the MTHFR enzyme, which inhibits the enzyme's activity.[24,25]

The A1298C polymorphism is associated with decreased enzyme activity and altered distribution of intracellular folate metabolites toward an accumulation of the 5,10 Methylenetetrahydrofolate, the precursor for thymidilate and purine synthesis.[26-28] A decreased availability of 5,Methylenetetrahydrofolate in remethylation homocysteine to methionine affects blood homocysteine and folate concentration.[25]

Only a few studies have investigated the relationship between the MTHFR A1298C polymorphism and the alteration of the risk of CAD. Some study reports that although this polymorphism affects the MTHFR activity, neither the homozygous nor the heterozygous status for A1298C polymorphism is associated with higher homocysteine or a lower plasma folate concentration.[29,30] Other studies, however, have shown that A1298C mutation influences specific enzyme activity and homocysteine and folate concentration , but to a lesser extent than the C677T mutation.[25,30] It is noteworthy that the subjects in the said studies had different ethnic backgrounds; consequently, whether the A1298C polymorphism has any relevance in other ethnic populations including Iran is still unclear. We speculate that a possible link exists between the MTHFR polymorphism and the occurrence of CAD, but with a smaller relative risk than the C677T polymorphism. Therefore, this study was designed to examine the prevalence of the MTHFR A1298C mutation and to assess the association between this polymorphism and plasma homocysteine level and CAD in a selected Iranian population.

Methods

Our case group was comprised of 100 patients (72 males and 28 females) at a mean age of 56.9 years (range 32-89 years) with angiographically documented CAD in Tehran Heart Center. A physician as well as cardiologists consulted all the chosen cases. The normal control group consisted of 100 healthy volunteers (61 males and 39 females) at a mean age of 45 years (range 32-57 years) without any clinical disorders selected from the general population in Tehran. Informed consent was obtained from all the patients and healthy subjects according to the guidelines of our ethics committee.

Venous blood samples were taken from fasting individuals (cases and controls) into EDTA vials. Total homocysteine was measured in plasma because the use of anticoagulant allows immediate sample processing. The samples were immediately ice packed and centrifuged within 30 min to avoid false increases of homocysteine due to release from red blood cells. Plasma samples were then refrigerated and

stored at -70 °C until analysis was done. The total plasma homocysteine concentration of the patients and normal controls was determined by using a homocysteine measuring kit via the Elisa method

DNA was extracted from peripheral lymphocytes by standard salting out method as described by Miller et al.[31]

The MTHFR polymorphism genotype of A1298C was analyzed using polymerase chain reaction–based restriction fragment length polymorphism (PCR-RFLP). The A1298C mutation alters a glutamate into alanine residue and abolishes the MboII restriction site. For the detection of the A1298C transition in the MTHFR gene ~100 ng DNA was used for PCR amplification. The PCR was carried out in a total volume 50µ1, containing 50 ng of the forwardLprimerL5' CTTGGGGAGCTGAAGGACTACTAC3' and 50 ng of the reverse primerL5'CACTTTGTGACCATTCCGGTTTG3' 200 µM each dNTP, 10mM Tris-Hcl (PH=8.3), 50mM KCL, 3mM Mgcl2, and 1 unit Taq polymerase. PCR parameters for the detection of the A1298C transition were as follows: an initial denaturation step of 5 min at 95˚C, followed by 32 cycles of 95˚C/60s (denaturation), 63˚C/60s (annealing) and 72˚C/45s (extension) and final extension for 10 min at 72˚C to ensure a complete extension of all PCR products. The amplified PCR fragment of 163bp was digested with restriction enzyme MboII, followed by gel electrophoresis on 12% poly acrylamide. The mutated homozygous variant produced 4 fragments of 84, 31, 30, and 28bp, while 1298AC heterozygote produced 5 fragments of 84, 56, 31, 30, and 28bp and 1298AA wild-type produced 4 fragments of 56, 31, 30, and 28 bp (Figure 1).

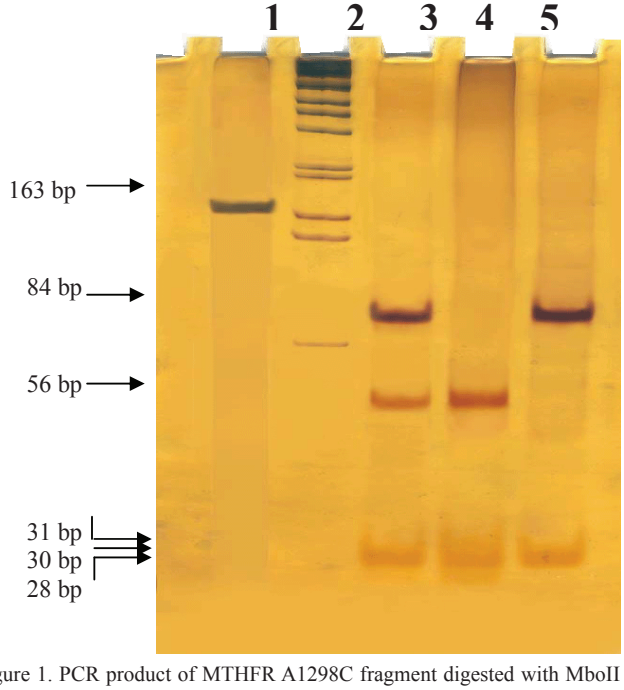

Figure 1. PCR product of MTHFR A1298C fragment digested with MboII restriction enzyme. From left to right line 1, PCR product 163bp; line 2, size marker ladder X; line 3, heterozygous A/C genotype; line 4, homozygous normal A/A genotype; line 5, homozygous mutant C/C genotype

Descriptive values were expressed as mean±SD. Allele frequencies were calculated for each genotype by allele counting. Comparison of allele frequencies between the case and control groups was made using the Chi-square test via SPSS for Windows version 10 (Chicago, Illinois software). Differences between the patient and control groups were assessed with the student's t-test for continuous variables (homocysteine). The Fisher exact test was used when the number of observations in any group was less than or equal to 5. All the tests were two-tailed, and $p<0.05$ was considered significant.

Results

One hundred CAD cases and 100 normal controls were genotyped for common mutation A1298C MTHFR. Their blood homocysteine level was measured to determine its linkage with CAD. The mean age of the cases and controls was 56.9 and 45 years respectively; 28% of cases and 39% of controls were woman. The frequencies of 1298AA, 1298AC, and 1298CC genotypes were 30%, 52%, and 18% in the CAD patients; and 35%, 50 %, and 15% in the controls. The frequencies of mutated allele (C) and normal allele (A) in patient and control groups were 44%, 56%, 40 %, and 60%, respectively. The difference between two groups was not significant ($\chi 2=0.697$, $P>0.05$). The prevalence of the A1298C polymorphism in the CAD patients and normal controls was compared with each other (Table 1).

Table 1. Distribution of A1298C genotype and allele's frequencies among cases and control group

	CC	AC	AA	[%]A	[%]C
Cases	18	52	30	56	44
Controls	15	50	35	60	40

Heterozygous A/C, Homozygous mutant C/C, Normal A/A

There was no significant difference in the incidence of A1298C MTHFR genotype between the males and females in either group (in patients $\chi 2=1.64$, $P>0.05$ and in controls $\chi 2=5.61$, $P= 0.07$). There was also no significant difference in the incidence of this polymorphism among different age groups ($P>0.05$). Plasma homocysteine concentration in both case and control groups was measured. The proportion of subjects with moderate hyperhomocysteinemia was substantially higher among the CAD patients compared to the controls. By comparison with the controls, the cases had higher plasma homocysteine concentration and the difference between the two groups was significant (15.56±6.77 versus 11.51±4.63, $P<0.05$).

The correlation between homocysteine levels with the MTHFR genotype was studied. The average of plasma homocysteine in CC, AC, and AA genotypes in the cases was 15.73±6.87, 15.08±5.51, and 14.68±6.81 and 12.81±5.30,

11.81±4.81 and 10.61±2.86 in the control group respectively (Table 2). There was no significant difference between the plasma homocysteine level in CC and AC genotypes in comparison with the AA genotype in both case and control groups.

Table 2. Distribution of plasma homocystiene concentration (μ/mol) among different A1298C MTHFR genotypes in case and control groups*

	MTHFR genotype	Homocysteine concentration
Cases (100)	CC	15.73±6.87
	AC	15.08±5.51
	AA	14.68±6.81
Controls (100)	CC	12.81±50.30
	AC	11.81±4.81
	AA	10.61±2.68

*Data are presented as mean±SD

Discussion

Over the past decade, the role of genetic polymorphisms of enzymes in folate metabolism has attracted much interest in epidemiological research on cardiovascular disease. Numerous studies have demonstrated a significant relationship between elevated plasma homocysteine concentration and CAD.[32,33] Of several enzyme defects and deficiencies that can lead to elevated plasma homocysteine is polymorphism in MTFHR gene.[34] Genetic aberrations in the methylenetetrahydrofolate reductase gene may account for reduced enzyme activity and elevated plasma homocysteine. As reported previously, the MTFHR C677T polymorphism has a significant effect on MTFHR activity and increased risk of CAD.[35-37] A second common polymorphism in this gene is A1298C. There have been fewer studies on this polymorphism than there have been on the C677T polymorphism, and most of these studies have demonstrated no association between the MTFHR A1298C polymorphism and CAD.[25,29]

We studied the association between MTHFR A1298C polymorphisms and CAD in 100 Iranian cases and 100 normal controls. We speculated that a possible link existed between this polymorphism and the occurrence of CAD in the selected Iranian population. We did not observe any association between the A1298C polymorphism and CAD. The genotype distribution and frequency of mutated allele showed no significant difference between the patient and control groups. The frequency of mutated C allele was similar in the patient and normal control groups (χ 2=0.697, P>0.05).

The correlation between homocysteine and A1298C MTFHR genotype was studied in 100 cases and 100 normal controls. In line with several previous studies, we found elevated plasma homocysteine concentration as a risk factor for CAD.[17,18,20] The mean plasma homocysteine concentration was higher in CAD patients than that in the control group, and there was a significant difference between the two groups supported by P<0.05 (15.56±6.77 versus 11.51±4.63). The correlation between homocysteine level and MTHFR genotype was also studied. The mean plasma homocysteine concentration in CC, AC, and AA genotypes in the cases was 15.73±6.87, 15.08±5.51, and 14.68±6.81, respectively. In the control group, the mean plasma homocysteine concentration in CC, AC, and AA genotypes was 12.81±5.30, 11.81±4.81, and 10.67±2.86, respectively. There was no significant difference between plasma homocysteine level in individual homozygous or heterozygous for this polymorphism in comparison to the normal one. Homozygous individuals for C/C genotype were not associated with higher levels of plasma homocysteine than were A/C and A/A genotypes. In summery, we concluded that although the A1298C polymorphism in the MTHFR gene is reflected in enzyme activity, it has no significant effect on plasma homocysteine level and cannot be considered a major factor for CAD. The present findings are limited in that they were obtained from a relatively small study population. It means that some of our insignificant findings may be due to weak statistical power, and a small difference, if any, may not be detected with this sample size. Therefore, further investigation into the association between this polymorphism and risk of CAD is warranted and should include a larger sample size and other polymorphisms in folate metabolism and address interactions with folate status.

Acknowledgment

This study was supported by project grants from National Research Center for Genetic Engineering and Biotechnology, Ministry of Sciences, Research and Technology, Tehran, Iran and from Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran.

References

1. Gupta M, Sharma P, Gitanjali G, Kaur K, Kaur Bedi G, Vij A. Plasma homocysteine: an independent or an interactive risk factor for coronary artery disease. Clinica Chimica Acta 2005;325:121-125.
2. Kerkeni M, Addad F, Chauffert M, Chuniaud L, Miled A, Trivin F, Maaroufi K. Hyperhomocysteinemia, paraoxnase activity and risk of coronary artery disease. Clinical Biochemistry 2006;39:821-825.
3. Meisel C, Cascorbi I, Gerloff T, Stangl V, Laule M, Müller JM, Wernecke KD, Baumann G, Roots I, Stangl K. Identification of six methylenetetrahydrofolate reductase (MTHFR) genotypes resulting from common polymorphisms: impact on plasma homocysteine levels and development of coronary artery disease.Atherosclerosis 2001;154:651-658.
4. Mallinow M R. Hyperhomocysteinemia: a common and easily reversible risk factor for occlusive atherosclerosis. Circulation

1990;81:2004-2006.
5. Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocysteine and risk factor of ischemic strock. Strock 1994;25:1924-1930.
6. Laraqui A, Allami A, Carrié A, Raisonnier A, Coiffard AS, Benkouka F, Bendriss A, Benjouad A, Bennouar N, El Kadiri N, Benomar A, Fellat S, Benomar M. Relation between plasma homocysteine, gene polymorphisms of homocysteine metabolism-related enzymes, and angiographically proven coronary artery disease. Eur J Intern Med 2007;18:474-483.
7. Falcon CR, Cattaneo M, Panzeri D Cattaneo M, Martinelli I, Mannucci PM. High prevalence of hyperhomocystienemia in patient with juvenile venous thrombosis. Arteroscler Thromb 1994;14:1080-1083.
8. Den Heijer M, Blom HJ, Gerrits WB, Rosendaal FR, Haak HL, Wijermans PW, Bos GM. Is hyperhomocystienemia a risk factor for recurrent venous thrombosis? Lancet 1995;345:882-885.
9. Panigrahi I, Chatterjee T, Biswas A, Behari M, Choudhry PV, Saxena R. Role of MTHFR C677T polymorphism in ischemic stroke. Neurol India 2006;54:48-50.
10. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325:1202– 1208.
11. Lee BJ, Huang MC, Chung LJ, Cheng CH, Link KL, Su KH, Huang YC. Folic acid and Vitamin B12 are more effective than Vitamin B6 in lowering fasting plasma homocysteine concentration in patients with coronary artery disease. Eur J Clin Nutr 2004;58:481–487.
12. Schneede J, Refsum H, Ueland PM. Biological and environmental determinant plasma homocysteine. Semin Thromb Hemost 2000;26:263-279.
13. Rozen R. Genetic modulation of homocysteinemia. Semin Thromb Hemost 2000;26:255-261.
14. Jacobsen DW. Hyperhomocysteinemia and oxidative stress. Time for reality check? Arterioscler Throm Vasc Biol 2000;20:1182-1184.
15. Jakubowski H. Molecular basis of homocysteine toxicity in humans. Cell Mol Life Sci 2004;61:470–487.
16. Jakubowski H, Zhang L, Bardeguez A, Aviv A. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. Circ Res 2000;87:45–51.
17. Kang SS , Zhou J , Wong PWK , Mallinow R. Hyperhomocysteinemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1997;12:279-298.
18. Mudd SH, Levy HL, Kraus JP. Disorders of transsulffuration. In: Scriver CR, Beaudentsly WS, Valle D, eds. The metabolic and molecular basis of inherited disease. 8th ed. New York: McGraw-Hill; 2001. p. 2007-2056.
19. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1997;78:523-526.
20. Hanson NQ, Aras O, Yang F, Tsai MY. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. Clin Chem 2001;47:661-666.
21. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase an inherited risk factor for coronary artery diseases. Am J Hum Genet 1991;48:536-545.
22. Friso S, Girelli D, Trabetti E, Stranieri C, Olivieri O, Tinazzi E, Martinelli N, Faccini G, Pignatti PF, Corrocher R. A1298C methylenetetrahydrofolate reductase mutation and coronary artery disease: relationships with C677T polymorphism and homocysteine/folate metabolism. Clin Exp Med 2002;2:7-12.
23. Bailey LB, Gregory JF 3rd. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: metabolic significance, risks and impact on folate requirement. J Nutr 1999;129:919-922.
24. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemi. Nat Struct Biol 1999;6:359-365.
25. Jenks DA, Mathews RG. Allosteric inhibition of methylenetetrahydrofolate reductase by adenosylmethionine. Effects of adenosylmethionine and NADPH on the equilibrium between active and inactive forms of the enzyme and on the kinetics of approach to equilibrium. J Biol Chem 1987;262:2485-2493.
26. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, Rozen R, Genest J. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 1994;7:195–200.
27. Szczeklik A, Sanak M, Jankowski M, Dropiński J, Czachór R, Musiał J, Axenti I, Twardowska M, Brzostek T, Tendera M. Mutation A1298C of methylenetetrahydrofolate reductase: risk for early coronary disease not associated with hyperhomocysteinemia. Am J Med Genet 2001;101:36-39.
28. Lievers KJ, Boers GH, Verhoef P, den Heijer M, Kluijtmans LA, van der Put NM, Trijbels FJ, Blom HJ. A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J Mol Med 2001;79:522-528.
29. Chango A, Boisson F, Barbé F, Quilliot D, Droesch S, Pfister M, Fillon-Emery N, Lambert D, Frémont S, Rosenblatt DS, Nicolas JP. The effect of 677C-->T and 1298A-->C mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects. Br J Nutr 2000;83:593-596.
30. Wiesberg 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.
31. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
32. 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.
33. 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;274:1049-1057.
34. Verhoef P, Kok FJ, Kluijtmant LA, Willett WC, Stampfer MJ. The C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Atherosclerosis 1997;132:105-113.
35. Gardemann A, Weidemann H, Philipp M, Katz N, Tillmanns H, Hehrlein FW, Haberbosch W. The TT genotype of the methylenetetrahydrofolate reductase C677T gene polymorphism is associated with the extent of coronary atherosclerosis in patients at high risk for coronary artery disease. Eur Heart J 1999;20:584-592.

36. Kluijtmans LA, Kastelein JJ, Lindemans J, Boers GH, Heil SG, Bruschke AV, Jukema JW, van den Heuvel LP, Trijbels FJ, Boerma GJ, Verheugt FW, Willems F, Blom HJ. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. Circulation 1997;96:2573-2577.
37. Christensen B, Frosst P, Lussier-Cacan S, Selhub J, Goyette P, Rosenblatt DS, Genest J Jr, Rozen R. Correlation of a common polymorphism in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. Arterioscler Thromb Vasc Biol 1997;17:569-573.