ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 03, pp. 27309-27315, March, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ASSOCIATION OF MTHFR GENE POLYMORPHISMS C677T AND A1298C WITH CORONARY ARTERY DISEASE

[1,*]Prasanna, P. V. V. S. L., [2]Dr. Lakshmi, V., [3]Devi, N. and [4]Dr. Padhy, K.

[1, 2]Department of Human Genetics, Andhra University, Visakhapatnam, AP, India
[3]Department of Biochemistry, MKCG Medical College, Berahmpur, Odisha
[4]CARE Hospitals, Institute of Medical Sciences, Visakhapatnam

ARTICLE INFO

Article History:
Received 15th December, 2015
Received in revised form
27th January, 2016
Accepted 20th February, 2016
Published online 16th March, 2016

Key words:

MTHFR,
Homocysteine,
Gene polymorphisms,
Coronary artery disease.

ABSTRACT

Gene environment interaction is an important aspect in the development of coronary artery disease (CAD). Elevated plasma Homocysteine has been identified as a risk factor for coronary atherosclerosis. Elevated Homocysteine may result from deficient Methylenetetrahydrofolate reductase (MTHFR) activity. MTHFR is involved in one carbon metabolism which is essential for DNA biosynthesis and methylation. The 5, 10-methyltetrahydrofolate reductase locus is mapped at the end of the short arm of chromosome 1(1p36.6). Though there are several singe nucleotide polymorphisms (SNPs) in this gene, two SNPs are most studied in association with diseases. A case control study was designed to assess the role of the two most commonly studied SNPs of MTHFR gene C677T and A1298C in the development of CAD. A total of 300 samples were recruited in the study which includes 150 CAD cases from CARE hospitals, Visakhapatnam and 150 controls. The MTHFR genotyping was performed based on polymerase chain reaction followed by restriction fragment length polymorphism. There was no significant difference either in the distribution of MTHFR C677T genotypes (CC vs CT: OR=1.05, 95%CI=0.58-1.91, P=0.858; CC vs TT: OR=2.03, 95%CI=0.18-22.7, P=0.564) or alleles (T vs C: OR=0.893, 95%CI=0.522-1.530, P=0.681) in patients and controls. Even in case of the SNP A1298C no significant difference was observed in the distribution of alleles though significant difference was observed in genotype distribution (AA vs AC: OR=0.90,95%CI=0.48-1.68, p=0.74; AA vs CC: OR=1.796, 95%CI=1.06-3.02, p=0.028).

Citation: Prasanna, PVVSL, Dr. Lakshmi V, Devi N and Dr. Padhy K., 2016. "Association of MTHFR Gene Polymorphisms C677T and A1298C with Coronary Artery Disease", *International Journal of Current Research,* 8, (03), 27309-27315.

INTRODUCTION

Coronary artery disease is one of the leading factors of mortality and morbidity accounting for approximately 30% of the deaths in the world (Matam *et al.,* 2014). It is a multifactorial disease which involves the contribution of many different genes and environmental factors. At genetic level, functional allelic variations or polymorphism in humans may play a role in an individual's susceptibility to the manifestation of the disease (Andreassi *et al.,* 2003). A major current challenge is therefore to explain the genetic components that contribute to the pathogenesis of complex disease (Fauci *et al.,* 2008). A high plasma level of homocysteine known to be associated with increased thrombotic tendency has been considered as a risk factor for atherosclerosis and CAD (Cattaneo *et al.,* 1999). The homocysteine levels are affected by genetic, environmental and physiological factors.

Corresponding author: Prasanna, PVVSL,
Department of Human Genetics, Andhra University, Visakhapatnam, AP, India.

Hyperhomocyst einemia may arise from genetic factors like mutation in MTHFR gene or due to environmental factors like vitamin B12 or folic acid deficiency (Eftychiou *et al.,* 2012). Genetic epidemiologic studies suggest that certain genetic variants including polymorphisms in the MTHFR gene have a strong association with CAD (Matam *et al.,* 2014). MTHFR is a key regulatory gene of the remethylation pathway and a vitamin B12 dependent enzyme. The MTHFR gene is located at 1p36.3. It is involved in folate metabolism catalysing the reduction of 5′, 10′methylenetetrahydrofolate reductase to 5′-methyltetrahydrofolate reductase, the major circulating form of folate and carbon donor for remethylation which is a multistep process that converts homocysteine to methionine (Saffari *et al.,* 2013) which is used to make proteins and other important compounds by the body (Wang *et al.,* 2013). Decrease in the function or amount of MTHFR leads to increased blood levels of homocysteine. Elevated homocysteine levels show an increased risk for hardening of the arteries (atherosclerosis), which may eventually result in a heart attack and venous thrombosis (Elizabeth *et al.,* 2005).

There are two common polymorphisms - C677T and A1298C in the gene that codes for MTHFR enzyme. The C677T polymorphism (rs1801133), in exon 4 of the gene, lies within the NH2-terminal catalytic domain, whereas the A1298C polymorphism is in the COOH-terminal regulatory region in exon 7 (Pandey *et al.,* 2011). The C677T genetic variant consists of a C to T transition in exon 4 at base 677 of the gene which encodes alanine in place of valine at amino acid 222 (Frosst *et al.,* 1995). The most striking characteristic of this mutant is its in vitro thermolability at 46^0C, which gives a clear distinction between this mutant and the normal enzyme found in the majority of the individuals (Kang *et al.,* 1988a, 1988b). The second prevalent polymorphism A1298C (rs1801131) is associated with decreased enzyme activity in vitro (Vanderput *et al.,* 1998; Weisberg *et al.,* 1998; Naomi *et al.,* 2001). This genetic variant consists of an A to C transversion at nucleotide 1298, in exon 7 which encodes glutamate in place of alanine at amino acid 429 (Jakubowski *et al.,* 2000; 2004). The prevalence of these two polymorphisms varies greatly in different ethnic groups. While several studies have been carried out to find out the association between C677T and CAD only few studies were carried out with respect to A1298C. Due to the lack of information in the Andhra Pradesh population, the present study has been attempted for the first time to find out the association between these two SNPs in MTHFR gene and their relation to CAD.

MATERIALS AND METHODS

This study was carried out on 150 angiographically documented CAD patients with at least 50% coronary artery stenosis or a history of coronary angioplasty or surgical revascularization in CARE Hospitals, Visakhapatnam during the period from December 2011-June 2012. The control group consists of 150 randomly chosen volunteers without a history of CAD. Informed consent was obtained from all the participants and the study was approved by Institutional Ethical Committee of Andhra University, Visakhapatnam. Five mL of blood sample was obtained from both the cases and the controls. Genomic DNA was extracted using standard salting out method described by Miller *et al.,* (1988). The MTHFR polymorphisms C677T and A1298C were analyzed using polymerase chain reaction– restriction fragment length polymorphism (PCR-RFLP).

For the detection of C677T polymorphism**,** amplification was carried out using the following primer set

F-5' TGA AGG AGA AGG TGT CTG CGG GA-3'
R-5' AGG ACG GTG CGG TGA GAG TG -3'

and the 198-bp product obtained was digested with HinfI restriction enzyme. Mutation creates a HinfI restriction site causing cleavage of the 198 bp fragment into 175 bp and 23 bp.

A1298C polymorphism was detected using the following primer set

F-5' CAA GGA GGA GCT GCT GCT GAA GA 3'
R-5' CCA CTC CAG CAT CAT CAC TCA CT 3'

and the resulted 128 -bp PCR product was digested with MboII restriction enzyme. After resolving in 3% agarose wild type allele shows two fragments of size 72 bp and 28 bp whereas the mutant allele shows 100 bp and 28 bp fragments.

Statistical analysis

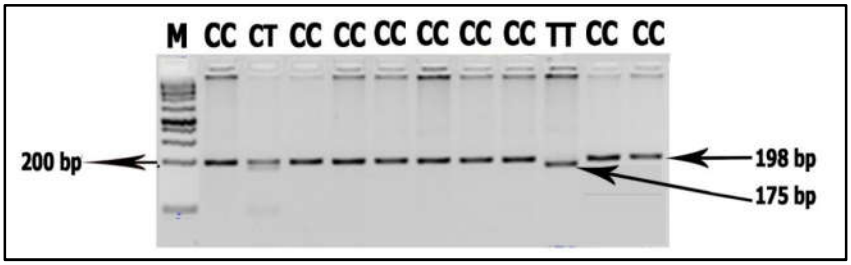
Chi-square test was performed to determine if the samples were in Hardy- Weinberg equilibrium. Binary logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for allele and genotype frequencies. Statistical analysis was carried out using SPSS Version 20.0 for windows software (SPSS, Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The genotype and the allele distribution of MTHFR C677T and A1298C variants are summarized in the Tables 1 and 2 respectively. Both the cases and the controls were in Hardy-Weinberg equilibrium. With reference to rs1801133 polymorphism, no significant difference was observed either in the genotype distribution or in the allele distribution between the patients and the controls. The wild genotype homozygous MTHFR 677CC was prevalent in both the groups (80.6% in cases and 81.3% in controls). The polymorphic allele MTHFR 677T presented a frequency of 5.1% in the CAD group and 4.6% in the control group. There was a statistically significant difference between the cases and the controls ($p = 0.028$) in the distribution of genotypes with regards to rs1801131 polymorphism. Though the CC genotype frequency is relatively higher in controls the heterozygous genotype MTHFR 1298AC frequency was higher in cases (52% in cases and 36.7% in controls). However no significant difference was observed between the cases and the controls in the distribution of alleles. The polymorphic allele MTHFR 1298C presented equal frequency of 28% in the CAD group and the control group.

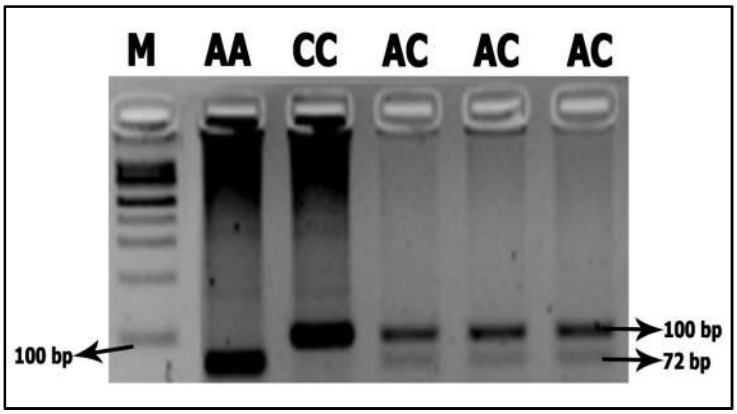
DISCUSSION

Genetic differences between individuals are caused by natural variations known as SNPs. For fine mapping of alleles, association studies at the population level are helpful (Pandey *et al.,* 2011). Polymorphisms in MTHFR gene is one of the several enzyme deficiencies and defects that can lead to elevated plasma homocysteine (Verhoef *et al.,* 1997). MTHFR C677T mutation was found to cause mild to moderate homocysteinemia due to the loss of FAD- binding capacity of the enzyme and dissociation of active dimer into monomers which leads to hypomethylation (Yamanda *et al.,* 2001). Several meta-analyses have shown a correlation between hyperhomocysteinemia and vascular disease (Boushey *et al.,* 1995, Wald *et al.,* 2002, 2012). As MTHFR is one of the candidate gene responsible for increasing homocysteine in serum, the present study was conducted to appraise the distribution of MTHFR polymorphisms C677T and A1298C in CAD cases and controls and to explore whether they confer susceptibility to CAD.



M = 100 bp DNA molecular ladder

Figure 1. Agarose gel picture with the three genotypes of MTHFR C677 polymorphism



M = 100 bp DNA molecular ladder

Figure 2. Agarose gel picture with the three genotypes of MTHFR A1298C polymorphism

Table 1. Distribution of genotype and allele frequencies of MTHFR C677T polymorphism in cases and controls

Genotype/allele	Cases (n=150)	Controls (n=150)	($\chi^{2)}$	Odds ratio	95% CI	p value
CC	121(80.6%)	122(81.3%)				
CT	27(18.0%)	27(18.0%)		1.05	0.583-1.9	0.858
TT	2(1.4%)	1(0.7%)	0.369	2.03	0.182-22.7	0.564
C	0.90	0.91				
T	0.10	0.09	0.169	0.893	0.522-1.530	0.681

Table 2. Distribution of genotype and allele frequencies of MTHFR A1298C polymorphism in cases and controls

Genotype/allele	Cases (n=150)	Controls (n=150)	($\chi^{2)}$	Odds ratio	95% CI	p value
AA	27(18%)	38(25.3%)				
AC	78(52%)	55(36.7%)		0.900	0.480-1.689	0.743
CC	45(30%)	57(38%)	7.251	1.796	1.066-3.026	0.028
A	0.44	0.44				
C	0.56	0.56	0.007	1.014	0.734-1.399	0.934

The present study failed to show any association between C677T polymorphism and CAD. This is consistent with other earlier findings (Biselli *et al*.,2009, Botto *et al.,* 2003, Chambers *et al.,* 2000, Eftychiou *et al.,* 2012, Folsom *et al.,* 1998 Girelli *et al.,* 1998, Gonzalez-perez *et al.,* 2002, Goracy *et al.,* 1999, Gueant Rodriguez *et al.,* 2005, Gupta *et al.,* 2010, Hanson *et al.,* 2001, Hsu *et al.,* 2001, Ibraheim *et al*.,2009, Iqbal *et al.,* 2005, Kolling *et al*.,2004, Lin PT *et al.,* 2008, Liu *et al.,* 2014, Meisel *et al*. (2001), Pandey *et al.,* 2011, Pezzini *et al.,* 2002, Rahimi *et al.,* 2009, Reinhardt *et al.,* 1998, Saffari *et al.,* 2013, Soriente *et al.,* 1998, Van Bockxmeer *et al.,* 1997,Vasisht *et al.,* 2002, Zee *et al.,* 2007, Zheng *et al.,* 2000). On the other hand some studies reported positive association between MTHFR C677T polymorphism and CAD (Alam *et al.,* 2008, Aleyasin *et al.,* 2006, Bennour *et al.,* 2007, Christensen *et al.,* 1997, Dhar *et al.,* 2010, Frosst *et al.,* 1995, Gardemann *et al.,* 1999, Gulec *et al.,* 2000, Kang *et al.,* 1988a,1991, Kluijtmans *et al.,* 1996, Lakshmi *et al.,* 2010, Matam *et al.,* 2014, Morita *et al.,* 1997, Nasiri *et al.,* 2014, Rassoul F *et al*.,2000, Tripathi *et al.,* 2010). There are only a few studies with regards to MTHFR A1298C polymorphism and the presence of CAD. While a lack of association was reported by several studies (Biselli *et al.,* 2009, Botto *et al.,* 2003, Chango *et al.,* 2000, Eftychiou *et al.,* 2012, Ghaedi *et al.,* 2007, Gueant Rodriguez *et al.,* 2005, Hanson *et al.,* 2001, Jenks *et al.,* 1987, Meisel *et al*. (2001), Nasiri *et al.,* 2014) a single study (Szczeklik *et al.,* 2001) reported significant difference in the allele frequency. Though the present study reveals a significant difference in the genotype distribution with high frequency of heterozygotes in the cases and CC

homozygotes in the controls it is contradicting the study of Szczeklik *et al.* (2001) by demonstrating no difference in the distribution of allele frequency. While no meta-analysis studies were available for MTHFR A1298C polymorphism, meta-analyses observations for MTHFR C677T SNP reflected the inconsistency in the findings reported by several individual studies. Meta-analyses by Kluijtmans and Whitehead (2001) and Klerk *et al.,* (2002) reported an increased risk of CAD in patients with TT genotype. A recent meta-analysis by Clarke *et al.* (2012) observed an increase risk of CAD in TT homozygotes when compared to CC homozygotes in both published and unpublished data with 15% in the former data and 2% in the latter. Interestingly another meta-analysis had given an estimate of 14% greater risk of CAD associated with MTHFR CC genotype (Lewis *et al.,* 2005). While in a meta-analysis carried out by Cronin *et al.* (2005) found no strong evidence to support the association of this polymorphism with CAD in Europe, North America or Australia but in Asia. Small size of the population is one of the limitations of the present study. Hence large and heterogeneous study populations are necessary to quantify the small effects of common mutations and their association. Further polymorphisms in MTHFR gene need to be evaluated together with other genetic markers involved in the homocysteine pathway for predisposition of the disease.

REFERENCES

Alam MA, Husain SA, Narang R, Chauhan SS, Kabra M and Vasisht S, 2008. Association of polymorphism in the thermolabile 5, 10-methylenetetrahydrofolate reductase gene and hyperhomocysteinemia with coronary artery disease. *Mol Cell Biochem* 310:111-117.

Aleyasin A, Ghaedi M, Davoodi S, Abbasi SH, Madani M.2006. Methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism is associated with coronary atherosclerosis disease in a sample of Iranian patients. *J Tehran Heart Cent*; 1(2):77-81.

Andreassi MG, Botto N, Cocci F, Battaglia D, Antonioli E, Masetti S, Manfredi S, Colombo MG, Biagini A and Clerico A., 2003. Methylenetetrahydrofolate reductase gene C677T polymorphism, homocysteine, vitamin B12, and DNA damage in coronary artery disease. *Hum Genet* 112:171-177.

Bennouar, N., A. Allami, H. Azeddoug, A. bendris and A. Laraqui *et al*., 2007. Thermolabile methylenetetrah ydrofolate reductase C677T polymorphism and homocysteine are risk factors for coronary artery disease in Moroccan population. *J. Biomed. Biotechnol.,* Vol. 2007.

Biselli, P.M., Guerzoni A.R., Goloni-Bertollo, E.M., Moacir Fernandes de Godoy ; Juliana Aparecida Barcelos Abou-CHAHLA ; Erika Cristina Bertelli Pavarino 2009. MTHFR genetic variability on coronary artery disease development. *Rev. Assoc. Med. Bras. vol.*55 no.3 São Paulo.

Botto,N., Andreassi,M.g., Manfredi,S., Masetti,S., Cocci,F., Colombo, M.G., Storti,S., Rizza,A., and Biagini,A. 2003. Genetic polymorphisms in folate and homocysteine metabolism as risk factors for DNA damage. *European Journal of Human Genetics* 11, 671–678.

Boushey, C.J., S.A.A. Beresford, G. Omenn and A.G. Motulsky, 1995. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: Probable benefits of raising folic acid intakes. *J. Am. Med. Assco*., 274: 1049-1057.

Cattaneo M, Tsai MY, Bucciarelli P, Taioli E, Zighetti ML, Bignell M, *et al*. 1997. A common mutation in the methylenetetrahydrofolate reductase gene (677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (Factor V:Q506). *Arterioscler Thromb Vasc Biol;*17:1662–6.

Chamber JC, Ireland H, Thompson E *et al*. 2000. Methylenetetrahydrofolate reductase 677 C_T mutation and coronary heart disease risk in UK Indian Asians. *Arterioscler Thromb Vasc Biol*, 20, 2448-2452.

Chango,A. F. Boisson, F. BarbeÂ, D. Quilliot, S. Droesch, M. Pfister, N. Fillon-Emery, D. Lambert, S. FreÂmont, D. S. Rosenblatt and J. P. Nicolas. 2000. The effect of 677CT and 1298AC mutations on plasma homocysteine and 5,10methylenetetrahydrofolate

Christensen B, Frosst P, Lussier-Cacan S, Selhub J, Goyette P, Rosenblatt DS, Genest J Jr, Rozen R. 1997. Correlation of a common polymorphism in the methylenetetrahydrofolate reductase with plasma homocysteine in patients with premature coronary artery disease. *Arterioscler Thromb Vasc Biol*;17:569-573.

Clarke RDA, Parish S, Verhoef P, Dötsch-Klerk M, Lathrop M, Xu P.2012. Homocysteine and coronary heart disease: Meta-analysis of MTHFR case-control studies, avoiding publication bias. *Plos Med*; 9(2):e1001177. PMID: 22363213.

Cronin S, Furie KL, Kelly PJ.2005. Dose-related association of MTH-FR 677T allele with risk of ischemic stroke: Evidence from a cumulative meta-analysis. *Stroke;* 36: 1581–1587.

Dhar S., S. Chatterjee, S. Ray, A. Dutta, B. Sengupta and S. Chakrabarti, 2010. Polymorphisms of methylenetetrahy drofolate reductase gene as the genetic predisposition of coronary artery disease in eastern India. *J. Cardiovasc. Di. Res*., 1: 152-157.

Eftychiou C, Antoniades L, Makri L, Koumas L, Costeas PA, Kyriakou E, *et al*. 2012. Homocysteine Levels and MTHFR Polymorphisms in Young Patients with Acute MyocardialInfarction: A Case Control Study. *HellenicJ Cardiol*. 53:189-194.

Elizabeth A. Varga, MS; Amy C. Sturm, MS; Caron P. Misita, PharmD; Stephan Moll, MD.2005. Homocysteine and MTHFR Mutations Relation to Thrombosis and Coronary Artery Disease. Circulation;111:e289-e293.

Fauci AS, Braundwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL and Loscalzo J.,2008. The pathogenesis of atherosclerosis. In: Harrison's Principle of Internal Medicine. 17th edition. Mc GrawHill, New York, pp 1425-1433.

Folsom AR, Nieto FJ, McGovern PG *et al*. 1998. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: The Atherosclerosis Risk in Communities (ARIC) study. *Circulation*; 98: 204–210.

Frosst, P., *et al.,* 1995. A candidate genetic risk factor for vascular disease: A common mutation in

methylenetetrahydrofolate reductase. *Nature Genet*.,10, 111–113.

Gardemann A, Weidemann H, Philipp M, Katz N, Tillmanns H, Hehrlein FW, Haberbosch

Ghaedi,M., Aleyasin, A., Boroumand,M.A., Abbasi,S.H., Davoodi, S., Mirakhori, M.., 2007. Common Polymorphism A1298C in Methylenetetrahydrofolate Reductase Gene Is not a Risk Factor for Coronary Artery Disease in Selected Iranian Patients. *J Teh Univ Heart Ctr* 3 161-166.

Girelli, D., S.Friso, E. Trabetti, O. Olivirevi and C. Russo *et al.,* 1998. Methylenetetrahydrofolate reductase C677t mutation, plasma homocysteine and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: Evidence for an important genetic-environmental interaction. *Blood*, 91: 4158-4163.

Gonzalez-Perez, E., M. Via, A. Lopez-Alomar, E. Eteban and N. Valveny *et al.,* 2002. Lack of association between methylenetetrahydrofolate reductase (MTHFR) C677T and

Goracy I, Goracy J, Suliga M, *et al*.1999. C677T gene polymorphism of methylenetetrahydrofolate reductase (MTHFR) in patients with myocardiat infarction. *Pol Arch Med Wewn*;102:849-54.

Guéant-Rodriguez RM, Juilliére Y, Candito M, Adjalla CE, Gibelin P, Herbeth B, *et al*. 2005. Association of MTRRA66G polymorphism (but not of MTHFR C677T and A1298C,MTRA2756G, TCN C776G) with homocysteine and coronary artery disease in the French population. *Thromb Haemost*; *94* : 510-5.

Gulec S. Aras O, Akar E, *et al*. 2001. Methylenetetrahydrofolate reductase gene polymorphism and risk of premature myocardial infarction. *Clin Cardiol*; 24:281-4.

Gupta S, Kotwal J, Kotwal A, Dhall A, Garg S. 2012. Role of homocysteine & MTHFR C677T gene polymorphism as risk factors for coroary artery disease in young Indians. *Indian J Med Res*; 135:506-512. PMID: 22664498.

Hanson, N. Q. *et al.,* 2001.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.*, 47, 661–666.

Hsu LA, Ko YL, Wang SM, Chang CJ, Hsu TS, Chiang CW, *et al*. 2001. The C677T mutation of the methylene tetrahydrofolate reductase gene is not associated with the risk of coronary artery disease or venous thrombosis among Chinese in Taiwan. *Hum Hered*; *51* : 41-5.

Ibrahiem MM, Gaber O, Mohammed SH, Salem EM. Methylenetetrahydrofolate reductase C677T polymorphism and relationship with coronary artery disease. *Egypt. J. Chem*. 2009; 27(1):177-194.

Iqbal,M.P., Tasneem Fatima, Siddiqa Parveen, Farzana A Yousuf, Majid Shafiq, Naseema Mehboobali1, Abrar H Khan, Iqbal Azam and Philippe M Frossard, 2005.

ischaemic heart disease (IHD): family-based association study in a Spanish population. *Clin. Genet*., 62: 235-239.

Jakubowski H, Zhang L, Bardeguez A, Aviv A.2000. Homocysteine thiolactone and protein homocysteinylation in human endothelial cells: implications for atherosclerosis. *Circ Res*; 87:45–51.

Jakubowski H. 2004. Molecular basis of homocysteine toxicity in humans. *Cell Mol Life Sci*; 61:470–487.

Jenks DA, Mathews RG. 1987. Allosteric inhibition of methylenetetrahydrofolate reductase by adenosylmethi onine. 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;*262:2485-2493.

Kang S-S, Wong PWK, Zhou J, Sora J, Lessick M, Ruggie N, Grcevich G (1988a) Thermolabile methylenetetrahy drofolate reductase in patients with coronary artery disease. Metabolism 37:611-613.

Kang S-S, Zhou J, Wong PWK, Kowalisyn J, Strokosch G (1988b) Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet* 43:414-421.

Kang, S-S, Wong PWK, Susmano A, Sora H, Norusis M, Ruggie N. 1991. Thermolabile methylenetetrahydrofolate reductase: An inherited risk factor for coronary artery disease. *Am J Hum Genet* 48:536.

Klerk M, Verhoef P, Clarke P, Blom HJ, Kok FJ, Schouten EG *et al*.2002. MTHFR 677C → T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA*. 288:2023-31.

Kluijtmans L, Whitehead A. 2001. Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease. *Eu Heart J;* 22:294-299. PMID: 11161947.

Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, den Heijer M, Trijbels FJ, Rozen R and Blom HJ., 1996. Molecular genetic analysis in mild hyperhomocysteinemia: A common mutation in the methylenetetrahydrofolate gene is as a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 58:35-41.

Kölling K, Ndrepepa G, Koch W, Braun S, Mehilli J, Schömig A, *et al* 2004. Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms, plasma homocysteine, folate, and vitamin B12 levels and the extent of coronary artery disease. *Am J Cardiol*; *93* : 1201-6.

Lack of association of methylenetetrahydrofolate reductase 677C>T mutation with coronary artery disease in a Pakistani population Journal of Molecular and Genetic Medicine, 1(1), 26-32.

Lewis SJ, Ebrahim S, Davey Smith G.2005. Meta-analysis of MTHFR 677C-T polymorphism and coronary heart disease: Does totality of evidence support causal role for homocysteine and preventive potential of folate? BMJ; 331: 1053–1058.

Lin PT, Huang MC, Lee BJ, Chang CH, Tsai TP, Huang YC, *et al*. 2008. High plasma homocysteine is associated with the risk of coronary artery disease independent of methylene tetrahydrofolate reductase 677 C→T genes. *Asia Pac J Clin Nutr* ; *17* : 3308.

Liu,R., Hanning Liu, Haiyong Gu, Xiao Teng, Yu Nie, Zhou Zhou, Yan Zhao, Shengshou Hu, and Zhe Zheng. 2014. A Polymorphism in Hepatocyte Nuclear Factor 1 Alpha, rs7310409, Is Associated with Left Main Coronary Artery Disease. Biochemistry Research International Volume, Article ID 924105, 7.

Matam, K., Imran Ali Khan, Qurratulain Hasan, and Pragna Rao , 2014. Coronary artery disease and the frequencies of MTHFR and PON1 gene polymorphism studies in a varied

population of Hyderabad, Telangana region in south India. *Journal of King Saud University*.

Meisel C, Cascorbi I, Gertoff T, *et al*.2001. Identification of six methylenetetrahydrofolate reductase (MTHFR) genotypes resulting from common polymorphisms: impact on plasma homocysteine levels and development of coronary artery disease. *Atherosclerosis* ;1 54:651-8.

Miller SA, Dykes DD, Polesky HF, 1998. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res; 11:1215.

Morita H, Taguchi J, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, Maemura K, Shindo T, Minamino T, Ohno M, Yamaoki K, Ogasawara K, Aizawa T, Suzuki S,Yazaki.Y.1997. Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease.*Circulation*.;95:2032–2036.

Naomi Q. Hanson, O¨ mer Aras, Feng Yang, and Michael Y. Tsai. 2001. 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. *Clinical Chemistry* 47:4 661–666.

Nasiri, M., Roostaei, A., Ehsanian, Z., 2014. Association of Methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C polymorphism with Myocardial Infarction from north of Fars province. Res Mol Med. 2(3): 37.

Pandey U, Kumari R, Nath B, Ganesh S, Banerjee I, Hasan O. 2011.Association of angiotensin- converting enzyme, Methylenetetrahydrofolate reductase and

paraoxonase gene polymorphism and coronary artery disease in an Indian population. *Cardiol J;* 18(4):385-394. PMID: 21769819.

Pezzini, A., E. Del Zotto, s. Archetti, R. Negrini and p. Bani *et al.,* 2002. Plasma homocysteine concentration, C677T MTHFR genotype, and 844ins68bp CBS genotype in young adults with spontaneous cervical artery dissection and atherothrombotic troke. Stroke, 33: 664-669.

Rahimi, Z., H. Nomani, H. Mozafari, A. Vaisi-Raygani, H.madani, S. Malek-Khosravi and A. Parsian, 2009. Factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate reductase polymorphism C677T are not associated with coronary artery disease and type 2 diabetes mellitus in western Iran. *Blood Coagul. Fibrinolysis*, 20:252-256.

Rassoul F., V. Richter, C. Janke, K. Purschwitz, B. Klotzer, J. Geisel and W.Herrmann, 2000. Plasma homocysteine and lipoprotein profile in patients with peripheral arterial occlusive disease. *Angiology*, 51: 189-196.

reductase activity in healthy subjects. British Journal of Nutrition (2000), 83, 593±596.

Reinhardt, D., Sigusch, H. H., Vogt, S.F., Farker, K., Muller, S. And Hoffman, A. 1998. Absence of methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. Eur. J. Clin. Invest. 28, 20-23.

Saffari, B., Senemar, S., karimi, M., Bahari, M., Jooyan, N., and Yavarian, M. 2013. An MTHFR variant, Plasma homocysteine levels and late-onset coronary artery disease in subjects from southern Iran. Pak. J. Biol. Sci., 16(16): 788795.

Soriente, L., A. coppola, P. Madonna, A.M. cerbone, G. Di Minno, G. Orefice and A. D'Angelo, 1998. Homozygous C677T mutation of the 5, 10 methylenetetrahydofolate reductase gene and hyperhomocysteinemia in Italian patients with a history of early- onset ischemic stroke. *Stroke*, 29: 869-87.

Szczeklik A, Sanak M, Jankowski M, *et al*. 2001. Mutation A1298C of methylenetetrahydrofolate reductase: risk for early coronary disease not associated with hyperchomocysteinemia. *Am J Med Genet*; 101:36-9.

Tripathi,R., Tewari,S., Singh,P.K., and Agarwa,S. 2010. Association of homocysteine and methylene tetrahydrofolate reductase (MTHFR C677T) gene polymorphism with coronary artery disease (CAD) in the population of North India. *Genetics and MolecularBiology,* 33, 2, 224-228.

Van Bockxmeer FM, Mamotte CDS, Vasikaran SD and Taylor RR., 1997. Methylenetetrahydrofolate reductase gene and coronary artery disease. *Circulation* 95:21-23

Vander Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, *et al*.1998. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neuraltube defects? *Am J Hum Genet*; 62:1044–51.

Vasisht S., Gulati R., Narang R., Srivastava N., Srivastava L.M., Manchanda S.C. and Agarwal D.P. 2002. Polymorphism (C677T) in the 5, 10-Methylenetetrah ydrofolate reductase (MTHFR) gene: a preliminary study on north Indian men. Indian Journal of clinical biochemistry, 17(1) 99-107.

Verhoef P, Kok FJ, Kluijtmans LA, Blom HJ, Refsum H, Ueland PM, Kruyssen DA. 1997. The 677C3T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. *Atherosclerosis*.;132: 105–113.

Vijaya Lakshmi, S.V., Naushad, S.M., Rupasree, Y., *et al*, 2011. Interactions of 50-UTR thymidylate synthase polymorphism with 677CfiT methylene tetrahydrofolate reductase and 66AfiG methyltetrahydrofolate homocysteine methyl-transferase reductase polymorphisms determine susceptibility to coronary artery disease. *J. Atheroscler. Thromb*. 18, 56–64.

W. 1999. 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;* 20:584-592.

Wald, D.s., J.P. Bestwick and N.J. Wald, 2012. Homocysteine as a cause of ischemic heart disease: The door remains open. *Clin. Chem*.., 58: 1488-1490.

Wald, D.S., M. Law and J.K. Morris, 2002. Homocysteine and cardiovascular disease: Evidence on causality from a meta-analysis. *BMJ, Vol.* 325.

Wang, W., Wang, Y., Gong, F., *et al*, 2013. MTHFR C677T polymorphism and risk of congenital heart defects: evidence from 29 case-control and TDT studies. PLoS One 8, e58041

Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. 1998. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab; 64:169–72.

Yamanda, K. *et al.,* 2001. Effects of common polymorphisms on the properties of recombinant human methylenetet rahydrofolate reductase. *Proc. Natl. Acad. Sci. USA*, 98, 14853–14858.

Zee RYL, Mora S, Cheng S *et al*. 2007. Homocysteine, 5,10-methylenetetrahydrofolate reductase 677CT polymorphism, nutrient intake, and incident cardiovascular disease in 24,968 initially healthy women. Clinical Chemistry; 53:5845–5851.

Zheng YZ, Tong J, Do X.P,*et al*. 2000. Prevalence of methylenetetrahydrofolate reductase C677T and its association with arterial and venous thrombosis in the Chinese population. *Br J Haematol*; 109:870-4.

****