ORIGINAL ARTICLE

Study of Association of Methylenetetrahydrofolate Reductase C677T Polymorphism with Essential Hypertension in Eastern India

Rituparna Maji[1], Kazi Ashique Firdoush[2], Anindya Dasgupta[3]

Authors' affiliations: [1]Assistant Professor, Dept. of Biochemistry, Calcutta National Medical College, Kolkata; [2]Assistant Professor, Dept. of Biochemistry, Coochbehar Government Medical College, Coochbehar; [3]Professor, Dept. of Biochemistry, Calcutta National Medical College, Kolkata

Correspondence: Kazi Ashique Firdoush, Email: ashiquefirdoush@gmail.com, Mobile No.: +91 9830513460

ABSTRACT

Introduction: Essential Hypertension (EH) is a complex disease, resulting from the interaction of multiple genetic & environmental factors. Mutation of Cytosine to Thymine at position 677 of methylenetetrahydrofolate reductase (MTHFR) gene causes decreased activity of the enzyme and it has been attributed to essential hypertension in many studies. There is limited data from Indian population on this topic.Hence a case-control study was designed to assess the association MTHFR C677T polymorphism with EH in the eastern Indian subpopulation.

Methodology: Polymerase chain reaction using suitable primer, followed by restriction fragment length polymorphism analysis using *Hinf 1* enzyme was used to identify MTHFR C677T genotypes in 207 diagnosed hypertensive patients and 210 matched controls.

Results: Not a single mutant TT genotype was found in either case or control group in our study population. Frequency of heterozygote CT was higher in case group (23.1%) than control (18.2%) but the difference was statistically non-significant (OR: 0.741, 95% CI: 0.430-1.275). Also, no significant difference of allele frequency between the two groups was observed for the polymorphisms studied (OR: 0.767, 95% CI: 0.460-1.278).

Conclusion: Our data shows that MTHFR C677T polymorphism is not associated with the risk of EH in this population.

Key words: Single nucleotide polymorphism, Methylenetetrahydrofolate reductase, Restriction fragment length polymorphism, Essential hypertension, rs1801133, C677T

INTRODUCTION

Hypertension is a major contributor to the global burden of diseases and death, causing almost 13-15% of total deaths worldwide.[1] The majority (80-90%) of hypertension is due to unknown etiology and is known as Essential Hypertension (EH). EH is a complex disease resulting from the interaction of multiple genetic & environmental factors. Polymorphism of several genes like CYP17A1, CYP1A2, FGF5, SH2B3 etc. has been associated with EH in several studies.[2]

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in folate metabolism and in remethylation of homocysteine into methionine. Two common single nucleotide polymorphisms, C677T and A1298C are known to affect the enzyme function. For the MTHFR C677T polymorphism, a C to T transition at nucleotide position 677 in exon 4 generates an alanine to valine change at amino acid 222. As a result, the homozygous MTHFR 677TT genotype possesses a thermolabile enzyme with reduced activity, which results in enhanced plasma homocysteine concentration.[3]. Elevated plasma Homocysteine has been found in hypertensive patients and has shown a positive association with blood pressure.[4,5] Thus we hypothesised that there is a role of MTHFR polymorphism in the development of essential hypertension.

Over the last few years, a number of studies examined the association between MTHFR C677T polymorphisms and EH risk among different populations.[6-9] However, these results were controversial. Even studies on Indian population, though few in number, have failed to establish the relation conclusively.[10]

With this background, we proposed to study the association MTHFR C677T polymorphism with Essential Hypertension in eastern Indian subpopulation.

MATERIALS AND METHODS

An observational, case-control hospital based study was conducted at the Department of Biochemistry, Calcutta National Medical College commencing from December 2016 to June 2018. 207 female patients in the age group of 25 to 55 years with clinically diagnosed hypertension or on anti-hypertensive medications for at least one month were included in the study. Patients with secondary hypertension like pregnancy induced hypertension, renal diseases, endocrine diseases like primary hyper-aldosteronism, hypothyroidism, Cushing's syndrome etc. were excluded from the study. Subjects with history of stroke, coronary artery disease, peripheral vascular disease, regular consumers of oral contraceptive pills or multi-vitamin were also excluded. For comparison, 210 age and sex matched healthy females with no history of hypertension and abiding by the same exclusion criteria were selected as controls. For ethnicity purpose, only subjects residing in this region for at least two generations were considered eligible for the study. Informed consents were obtained from all participants. The study followed all guidelines of the Helsinki declaration, 1975, revised in 2000 and was initiated only after obtaining the approval of institutional ethical committee.

Study parameters: 7 ml. of venous blood sample was collected from each subject. Fasting plasma glucose (FPG), serum urea, serum creatinine, serum cholesterol, serum triglyceride (TG), and serum High Density Lipoprotein (HDL) were quantitatively measured as a part of the routine investigations for the hypertensive patients and also the non-hypertensive controls. Anthropometric measurements (Height, Weight, Waist circumference, Hip circumference) were done and Body Mass Index (BMI) and Waist Hip Ratio (WHR) were calculated.

Identification of MTHFR C677T polymorphism

DNA was isolated from 3 ml of whole blood by phenol-chloroform extraction method. After assessment of quality and quantity of isolated DNA, amplification of exon 4 of MTHFR gene was done using forward primer 5'-TTT GAG GCT GAC CTG AAG CAC TTG AAG GAG-3' and reverse primer 5'-GAG TGG TAG CCC TGG ATG GGA AAG ATC CCG-3'.[11].Following reaction conditions were used: primary denaturation at 94ºC for 7 minutes, followed by 35 cycles of denaturation at 94° C, annealing at 61.5° C and extension at 72° C for 30 seconds each; final extension was carried out at 72° C for 7 minutes. The 173bp long PCR product thus obtained was digested with *Hinf I* restriction enzyme according to the protocol supplied by the manufacturer. C677T polymorphism creates a restriction site for *Hinf I* causing cleavage of the 173 bp product into 125 bp and 48 bp fragments. The digestion products were electrophoretically run in 3% agarose gel and visualized with ethidium bromide for identification of the polymorphism. However, the visualization of 48 bp was not consistent and we relied on following protocol for genotypic identification: single band of 173bp as CC, two bands of 173bp & 125bp as CT and single band of 125 bp as TT (Fig. 1).

Analysis: Data obtained was summarized and analyzed using IBM SPSS Statistics (version 20.0). Test of significance was done by Student's t-test and Odds Ratios were calculated by Chi square test. P-value <0.05 was taken as significant. Genotypes were checked for the conformance of Hardy Weinberg Equilibrium.

RESULTS

The present case–control study assessed the distribution of the C677T polymorphism of MTHFR in 207 patients with hypertension and 210 controls without any history of hypertension. Table 1 shows the baseline characteristics of the subjects. When hypertensive (HT) and non-HT groups were compared based on clinical parameters, there were significant difference in WHR and BMI. When comparing biochemical parameters, FPG and serum TG were significantly higher but serum Cholesterol and HDL was significantly lower among hypertensive than non-hypertensive.

Complete genetic work up was possible in only 156 samples out of 207 hypertensive cases and 165samples out of 210 controls. Table 2 shows the comparison of C677T genotype and allele in the HT and non-HT group. No homozygote TT genotype was found in either group. 81.8% of non-hypertensive had homozygote CC genotype compared to 76.9% among hypertensive. Frequency of heterozygote CT was higher in HT group (23.1%) than non-HT group (18.2%) but the difference was statistically non-significant (OR: 0.741, 95% CI: 0.430 to 1.275). In the non-hypertensive group, 90.91% had C allele and just 9.09% T allele, while among hypertensive 88.46% had C allele compared to 11.54% who had T allele. No significant difference of allele frequency between the two groups was observed for the polymorphisms studied (OR: 0.767, 95% CI: 0.460 to 1.278). Both case & control groups are maintaining Hardy-Weinberg equilibrium.

Even after excluding diabetic patients, the difference of both genotype frequency (OR: 0.733, 95% CI: 0.415 to 1.295) and allelic frequency (OR: 0.972, 95% CI: 0.919 to 1.028) between the two groups were not statistically significant.

Table 1: Comparison of baseline characteristics between case and control groups

Parameters	HTN (n=207) Mean ± SD	Non-HTN (n=210) Mean ± SD	P value
BMI	27.39±5.43	25.26±3.90	0.000$
WHR	1.00±0.07	0.95±0.07	0.000$
SBP	137.70±15.97	114.14±16.22	0.000$
DBP	89.37±9.93	79.00±8.60	0.000$
FPG	109.34±37.53	94.64±16.59	0.001$
Ur	26.65±7.65	25.43±9.35	0.156#
Cr	1.08±0.30	1.07±0.21	0.880#
UA	5.27±1.48	5.18±0.84	0.430#
BIT	0.77±0.38	0.73±0.47	0.424#
Chol	192.62±50.74	205.21±42.44	0.006$
TG	157.65±70.96	143.71±65.37	0.038$
HDL	50.78±13.00	58.79±7.13	0.000$

Table Legends: HTN: Hypertensive; BMI: Body Mass Index; WHR: Waist Hip Ratio; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; Ur: Serum Urea; Cr: Serum Creatinine; UA: Serum Uric Acid; BIT: Serum Total Bilirubin; Chol: Serum Total Cholesterol; TG: Serum Triacylglycerol; HDL: Serum High Density Lipoprotein ($Significant; #Not Significant)

Table 2: Distribution of C677T genotype and allele among case and control group

Frequency		HTN(n=156)	Non HTN(n=165)	P value	Odds Ratio	95% Confidence Interval	
						Lower	Upper
Genotype*	CC	120 (76.9%)	135 (81.8%)	0.278#	0.741	0.430	1.275
	CT	36 (23.1%)	30 (18.2%)				
Allelic	C	276 (88.46%)	300 (90.91%)	0.307#	0.767	0.460	1.278
	T	36 (11.54%)	30 (9.09%)				

(*Numbers of TT genotype were zero in both groups; #Not Significant)

DISCUSSION

Human MTHFR is located on the short arm of chromosome1 (position 36.3). Eleven exons join to form a mature mRNA that encodes the 77 kDa MTHFR protein. This protein, active only in dimer form, catalyses the conversion of 5, 10-methylenetetrahydrofolate to 5-methyl tetrahydrofolate and thus lead to the remethylation of Homocysteine to Methionine. Any polymorphism affecting MTHFR protein function may lead to hyperhomocysteinemia, especially in suboptimal folate status. Likewise, in many studies homocysteine has been found to have a positive association with elevated BP [12]. The possible pathogenesis may be that plasma Homocysteine induces arteriolar constriction, increases Na+ reabsorption and also causes arterial stiffness.[13]

18 types of mutation of MTHFR gene have been identified in cases of homocysteinuria but the most commonly studied is C677T and A1298C [11]. In 677T polymorphism (rs1801133), there is Ala to Val substitution at codon 222 on exon 4 which encodes a thermolabile enzyme with dissociation of dimer and loss of FAD binding capacity.[14] This leads to about 30% reduction in MTHFR enzyme activity in heterozygotes (CT) and 60% in homozygotes (TT).[15] In contrast, A1298C polymorphism (Ala to Glu at codon 429) shows just 10% and 35-45% reduction in enzyme activity among heterozygotes and homozygotes respectively.[14]. Although both these polymorphisms may elevate plasma homocysteine levels and cause EH, C677T lies in the catalytic domain (whereas A1298C which lies in the C-terminal end of the regulatory domain) and its effect on enzyme function is expected to be much more dramatic. In fact, few studies also support the fact that MTHFR C677T polymorphism plays a role in developing EH but MTHFR A1298C polymorphism may not be associated with an increased risk of EH.[16] So in our study we preferred C677T as a potential genetic candidate of EH over A1298C.

As the decrease in MTHFR 677T enzyme activity has been recognised to be due to the loss of FAD, some studies have shown that Riboflavin supplementation contributes to the reduction of hyperhomocysteinemia in hypertensive patients with the variant of MTHFR.[17] A recent study has further shown that supplementation with Riboflavin in hypertensive patients carrying the homozygous genotype mutant 677TT of MTHFR, without other cardiovascular diseases, has considerably improved the value of systolic blood pressure.[18]. These observations have paved the way for personalized medicine and underline the importance of MTHFR polymorphism determination in our population.

We chose our study and control population carefully and tried to include a homogenous population as much as possible. Out of the limited data available on the prevalence of MTHFR polymorphism in our country, sex bias of the polymorphism was found,

females had a much significantly higher T allele frequency than males (Odds Ratio: 2.67, p >0.05).[11] That is why we chose only females for our study. Also, stressing on the importance of ethnicity in polymorphism studies, only subjects who had two generations living in the same area were included. None of our subjects had consanguineous marriage. Subjects were included irrespective of their diabetic history. A study from China found no association between the genotype at rs1801133 polymorphism of the MTHFR gene and the risk of Type 2 DM [19]. Though the FPG in the non-HT group was significantly lower than the HT group, the mean FPG values in both the groups were within normal range. Even after excluding diabetic patients (FPG 126 or more was taken as diagnostic cut-off values), the difference of both genotype frequency (OR: 0.471, 95% CI: 0.146 to 1.525) and allelic frequency (OR: 0.506, 95% CI: 0.165 to 1.553) between the two groups were statistically non-significant.

In our study, SBP, DBP, WHR and BMI were significantly higher in the hypertensive group than non-hypertensive as per expectation. Serum cholesterol was a bit lower in HT group, though non-significantly (Table No 1). This might be due to the fact that 40% of our HT group were on hypolipidaemic drugs compared to our control group where there were none.

Homozygous T genotype was found in neither the HT group nor the non-HT group. This was in coherence with the very low prevalence finding of TT genotype in some studies in the Indian subcontinent.[11, 20, 21] T allele frequency in both case group (11.54%) and healthy controls (9.09%) were much less than in UK (18.6%) and USA (32.2%) but was similar to that found in a prevalence study in South India (10.12%).[11]

Both allelic and genotype frequencies of variant were higher in hypertensive than non-hypertensive but the difference was non-significant (Table no. 2). Various articles reported similar findings worldwide.[6, 9, 22]. However, our results were in contrast to the observations of a meta-analytical study by Boyi Yang et al, who showed that overall, the C677T polymorphism was significantly associated with EH (OR = 1.36, 95% CI = 1.20–1.53). However, stratified analysis by ethnicity within the same meta-analysis revealed a non-significant association among Indians, Sri Lankans, Latinos and Black Africans.[23] Another article from North India also reported significantly higher genotype frequency of 677CT in EH compared to controls but found no association between plasma homocysteine and serum folate with 677CT genotype.[24] Though limited data was available on MTHFR polymorphism in Eastern India, none of them is related to the association of the polymorphism with EH.[25, 26]. A study on MTHFR polymorphism among CVD patients in native inhabitants of Kolkata, West Bengal, India also showed no significant difference between genotype and allelic frequencies from the control group.[26] This discrepancy of data among the Northern and eastern region of our country might be due to the fact that though the MAF (minor allele frequency) of C677T was less than 0.1 in most of the populations; region-wise, the highest MAF was observed in the North Indian population, where of the four populations studied three had a MAF of more than 0.1.[27] In sync with the above finding, the allelic frequency of 677T was 0.909 in our control group and was 0.115 among hypertensive.

Our study failed to prove an association between MTHFR C677T and development of EH. This implies that the effect of polymorphism at rs1801133 might not be a major regulatory factor for maintaining normal blood pressure in our population. This calls for a large population based study of MTHFR C677T polymorphism and a thorough search of other variants of MTHFR like A1298C, G1793A, T1317C[24] and other candidate genes related to EH in the East Indian region. Our results must be interpreted with caution keeping in mind the limitations of our study: low sample size, inability to measure plasma homocysteine and folate levels and confounding effect of other polymorphisms or candidate genes of EH.

REFERENCES

1. Longo DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, et al. editors. Harrison's Principles of Internal Medicine. 18th ed. New York, NY, USA: McGraw-Hill Professional; 2011.
2. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature Genetics 2009; 41 (6): 666–676. doi: 10.1038/ng.361
3. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genetics 1995; 10 (1): 111–113. doi:10.1038/ng0595-111
4. Lim U, Cassano PA. Homocysteine and blood pressure in the Third National Health and Nutrition Examination Survey, 1988-1994. American Journal of Epidemiology 2002; 156 (12): 1105–1113
5. Sundström J, Sullivan L, D'Agostino RB, Jacques PF, Selhub J, et al. Plasma Homocysteine, Hypertension Incidence, and Blood Pressure Tracking. Hypertension 2003; 42 (6): 1100–1105. doi: 10.1161/01.HYP.0000101690.58391.13
6. Ilhan N, Kucuksu M, Kaman D, Ilhan N, Ozbay Y. The 677 C/T MTHFR Polymorphism is Associated with Essential Hypertension, Coronary Artery Disease, and Higher Homocysteine Levels. Archives of Medical Research 2008; 39 (1): 125–130. doi: 10.1016/j.arcmed.2007.07.009
7. Heux S, Morin F, Lea RA, Ovcaric M, Tajouri L, Griffiths LR. The Methylenetetrahydrofolate Reductase Gene Variant (C677T) as a Risk Factor for Essential Hypertension in Cau-

casians. Hypertension Research 2004; 27 (9): 663–667. doi:10.1291/hypres.27.663

8. Nishio H, Lee MJ, Fujil M, Kario K, Kayaba K, et al. A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population. Japanese Journal of Human Genetics 1996; 41 (2): 247–251. doi:10.1007/BF01875985

9. Fridman O, Porcile R, Morales AV, Gariglio LO, Potenzoni MA, et al. Association of methylenetetrahydrofolate reductase gene 677C>T polymorphism with hypertension in older women in a population of Buenos Aires City. Clinical and Experimental Hypertension 2013; 35 (3): 159–166. doi:10.3109/10641963.2012.690471

10. Markan S, Sachdeva M, Sehrawat BS, Kumari S, Jain S, et al. MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians. Molecular and Cellular Biochemistry 2007; 302 (1–2): 125–131. doi:10.1007/s11010-007-9434-5

11. Radha Rama Devi A, Govindaiah V, Ramakrishna G, Naushad SM. Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population. Current Science 2004; 86(3): 440–443. doi:10.4103/0971-6866.96645

12. Stehouwer CDA, Guldener CV. Does Homocysteine Cause Hypertension? Clinical Chemistry and Laboratory Medicine 2003;41 (11): 1408–1411. doi:10.1515/cclm.2003.216

13. Guldener CV, Nanayakkara PWB, Stehouwer CDA. Homocysteine and Blood Pressure. Current Hypertension Reports 2003; 5: 26–31. doi:10.1007/s11906-003-0007-z

14. Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proceedings of the National Academy of Sciences of the United States of America 2001; 98 (26):14853–14858. doi:10.1073/pnas.261469998

15. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A Second Genetic Polymorphism in Methylenetetrahydrofolate Reductase (MTHFR) Associated with Decreased Enzyme Activity. Molecular Genetics and Metabolism 1998; 64 (3): 169–172. doi:10.1006/mgme.1998.2714

16. Wu YL, Hu CY, Lu SS, Gong FF, Feng F, et al. Association between methylenetetrahydrofolate reductase (MTHFR) C677T/A1298C polymorphisms and essential hypertension: A systematic review and meta-analysis. Metabolism: Clinical and Experimental 2014; 63 (12): 1503–1511. doi: 10.1016/j.metabol.2014.10.001

17. McNulty H, Dowey LRC, Strain JJ, Dunne A, Ward M, et al. Riboflavin Lowers Homocysteine in Individuals Homozygous for the MTHFR 677C→T Polymorphism. Circulation 2006; 113 (1): 74–80. doi:10.1161/CIRCULATIONAHA.105.580332

18. Wilson CP, McNulty H, Ward M, Strain JJ, Trouton TG, et al. Blood Pressure in Treated Hypertensive Individuals with the MTHFR 677TT Genotype Is Responsive to Intervention with Riboflavin. Hypertension 2013; 61 (6): 1302–1308. doi: 10.1161/HYPERTENSIONAHA.111.01047

19. Zhong J-H, Rodríguez AC, Yang N-N, Li L-Q. Methylenetetrahydrofolate Reductase Gene Polymorphism and Risk of Type 2 Diabetes Mellitus. PLoS One. 2013; 8 (9): e74521. doi: 10.1371/journal.pone.0074521

20. Gupta SK, Kotwal J, Kotwal A, Dhall A, Garg S. Role of homocysteine & MTHFR C677T gene polymorphism as risk factors for coronary artery disease in young Indians. Indian Journal of Medical Research 2012; 135 (4): 506–512.

21. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, et al. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. Journal of Human Genetics 2005; 50(12): 655–663. doi:10.1007/s10038-005-0313-1

22. Deshmukh A, Rodrigue KM, Kennedy KM, Land S, Jacobs BS, et al. Synergistic effects of the MTHFR C677T polymorphism and hypertension on spatial navigation. Biological psychology 2009;80 (2): 240–245. doi: 10.1016/j.biopsycho.2008.10.003

23. Yang B, Fan S, Zhi X, Li Y, Liu Y, et al. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: A meta-analysis from 114 studies with 15411 cases and 21970 controls. PLoS One 2014; 9 (2): e87497. doi: 10.1371/journal.pone.0087497

24. Poduri A, Kumari S, Jain S, Khullar M. A case-control study of the association between the MTHFR gene and essential hypertension in Asian Indians. Journal of Human Hypertension 2009; 23 (2): 140–142. doi:10.1038/jhh.2008.107

25. Dhar S, Chatterjee S, Ray S, Dutta A, Sengupta B, et al. Polymorphisms of methylenetetrahydrofolate reductase gene as the genetic predispositions of coronary artery diseases in eastern India. Journal of Cardiovascular Disease Research 2010; 1 (3): 152–157. doi:10.4103/0975-3583.70922

26. Mukhopadhyay K, Dutta S, Das Bhomik A. MTHFR gene polymorphisms analyzed in population from Kolkata, West Bengal. Indian Journal of Human Genetics 2007;13 (1): 38. doi:10.4103/0971-6866.32035

27. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, et al. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. Journal of Human Genetics 2005; 50 (12): 655–663.