RESEARCH NOTE

Methylenetetrahydrofolate reductase (*MTHFR*) *C677T* gene polymorphism and alcohol consumption in hyperhomocysteinaemia: a population-based study from northeast India

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Introduction

Chronic alcohol consumption is often found to be associated with hyperhomocysteinaemia through inhibiting methionine synthase. Moreover, single nucleotide change in *MTHFR C677T* gene also leads to hyperhomocysteinaemia. A total of 598 unrelated individuals (case = 451; control = 147) aged 25–70 years were screened for *MTHFR C677T* polymorphism by polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion. Plasma homocysteine (Hcy) levels were measured using chemiluminescence technique to understand the role of both alcohol consumption and *677T* allele independently and in combination in the causation of hyperhomocysteinaemia. Results indicate a relatively higher frequency of *677T* allele among cases. Both cases and controls show higher mean plasma Hcy levels (>25 μmol/L), however, difference was not statistically significant. Individuals carrying mutant *677T* allele did not show significant difference for Hcy level in both the groups. However, cases with mutant *TT* genotype presented significant increase in Hcy level. Neither 677*T* allele, nor alcohol consumption is found to predispose individuals to higher homocysteine levels. However, *TT* genotype coupled with alcohol consumption leads to significant increase risk of Hcy levels.

Chronic alcohol consumption is one of the leading health risks and according to WHO (2011) it is world's third largest risk factor for disease and disability. More than 60 disease conditions have been reported to be associated with alcohol consumption (Gmel and Rehm 2003). Alcohol consumption is often found to be associated with hyperhomocysteinaemia, i.e., elevation of plasma Hcy levels through inhibiting methionine synthase (Bleich *et al.* 2005). Several studies investigating the relationship of alcohol and Hcy levels have shown that alcohol consumption, particularly in actively drinking alcoholics, is positively associated with elevated plasma Hcy levels (Bleich *et al.* 2000a, b; Nienaber Rousseau *et al.* 2013). It could be most likely a result of either direct effect (Barak *et al.* 1993) or through its metabolite acetaldehyde (Hidiroglou *et al.* 1994). Elevation of plasma Hcy levels may also be impeded by polymorphisms in some of the genes involved in 1-carbon metabolism, which help in catabolising Hcy or deficiencies in the micro-nutrients like folate, vitamin B12 and riboflavin that are required in Hcy metabolic pathway. Gene that encodes the methylenetetrahydrofolate reductase (*MTHFR*) enzyme that irreversibly converts 5, 10-methylenetetrahydrofolate (5, 10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), is reported to be an important genetic determinant of plasma Hcy level. Most of the available literature has reported that a nonsynonymous single-nucleotide polymorphism (SNP) C to T transition at 677 bp of *MTHFR* gene is associated with hyperhomocysteinaemia (Frosst *et al.* 1995). Individuals carrying *TT* genotype have significantly higher Hcy than those with the *CC* genotype (Husemoen *et al.* 2003). Moreover, some studies also reported that the *TT* genotype was found to be more prevalent among the alcohol dependent (AD) cases suggesting positive association of mutant *677T* allele with alcoholic patients (Benyamina *et al.* 2009).

Pathophysiologically it is well known that hyperhomocysteinaemia is associated with several complex diseases, including cardiovascular diseases, pregnancy complications, neural tube defects, Alzheimer disease, schizophrenia, cancer, etc. Available literatures elucidated that both gene and

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environment play a crucial role in elevation of plasma Hcy levels causing several complications. However, inconsistent results of the combined effect of both have been reported and association between alcohol intake and Hcy is unclear (Ganji and Kafai 2003). We therefore believe that interaction of gene and environment might have synergistic effects. Thus, in the present study an attempt was made to understand the combined effect of *MTHFR C677T* gene polymorphism and alcohol consumption in causation of hyperhomocysteinaemia among Meiteis of Manipur, geographically and linguistically a well-defined population. Meitei population belonged to a Mendelian population having common ethnic background (Saraswathy *et al.* 2009a, b; Singh *et al*. 2013). They marry among the same community with clan exogamy and hence preserved common gene pool. Moreover, occurrence of alcohol consumption is very high in Manipur.

Material and methods

Subjects

The present study is conducted among Meitei community (a Mendelian population) from Manipur, a northeastern state of India (Singh *et al*. 2013). All the subjects were recruited randomly from four districts of Manipur (Imphal East, Imphal West, Bishnupur and Thoubal), where Meiteis are predominantly found. A total of 598 male individuals (case = 451 and control = 147) aged 25–70 years unrelated up to 1st cousin were included in the present study. Cases include individuals consuming alcohol occasionally and moderately, whereas controls are absolute nonalcohols. None of the cases were alcohol dependent according to DSM-IV criteria (American Psychiatric Association 1994). Prior written consent was obtained from all the subjects and ethical clearance was also obtained from the Department of Ethical Committee, Department of Anthropology, University of Delhi.

Biochemical and genetic analyses

Intravenous blood samples (5 mL) were collected in EDTA coated vacutainers from all the individuals using disposable single use syringes. DNA was isolated from the lymphocytes using the conventional salting-out method (Miller *et al.* 1988). *MTHFR C677T* gene polymorphism was screened among all the individuals using standard protocol (Frosst *et al.* 1995). Briefly, the presence of mutation was determined by PCR amplification of genomic DNA using oligonucleotides F: 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and R: 5′-AGGACGGTGCGGGAGAGTG-3′ which yielded a fragment of 198 bp. It was followed by digestion with *Hin*fI restriction enzyme which digests the 198 bp fragment into 175 and 23 bp fragments. The digested DNA fragments were observed on 3% agarose gel electrophoresis stained with ethidium bromide. Plasma Hcy levels were measured only in a subset of samples using standard Chemiluminescence technique among cases ($N = 69$) and controls ($N = 29$). Individuals with plasma Hcy level >15 μmol/L were identified as having hyperhomocysteinaemia.

Statistical analysis

Allele frequencies were calculated by gene counting method and Hardy–Weinberg equilibrium (HWE) was determined using the χ^2 goodness-of-fit test using PopGene 1.31 (Yeh and Yang 1999). Differences in means of Hcy levels between alcohol consumers and alcohol nonconsumers groups were compared using Student's *t*-test and χ^2-test. Association of drinking status as well as *MTHFR C677T* allelic and genotypic counts with hyperhomocysteinaemia were evaluated by Pearson's χ^2-test followed by odds ratio (OR) at 95% confidence interval (CI), using the freely available 2 × 2 contingency table (http://vassarstats.net/odds2x2.html).

Results and discussion

MTHFR (*C677T*) gene is found to be polymorphic in both cases and controls. *CT* genotype and *677T* allele are more frequent among cases as compared to controls, however the observed difference is not found to be statistically significant. Hcy level is exceeding the normal range in both cases and controls, reaching as high as 25.75 μM/mL. But the frequency of hyperhomocysteinaemic individuals is relatively higher among the cases as compared to controls. Relative risk analysis also reveals that individuals who consume alcohol show more than one fold increased risk for hyperhomocysteine (OR = 1.203, CI = 0.48–3.02, $P = 0.698$). However the risk was not found to be statistically significant (table 1).

In both cases and controls, the mean values of Hcy level did not differ significantly between individuals carrying *CC* and *CT* genotypes ($P>0.05$). However, individuals carrying *TT* genotype evidenced significant increase of Hcy level only among cases and not among the controls ($P = 0.0017$) (figure 1). It agrees with previous studies that support the risk-conferring nature of mutant *677T* allele for elevation of plasma Hcy levels (Kumar *et al.* 2009).

Thus, the results of present study suggest that individuals with *TT* genotype when consume alcohol are more likely to be hyperhomocysteinaemic. Findings of the present study are also supported by other gene and environmental interaction study which show combined effects of alcohol intake and low *MTHFR* activity associating with adverse effects on Hcy (Chiuve *et al.* 2005; Yang *et al.* 2005; Nienaber-Rousseau *et al.* 2013). The present study throws light on the role of alcohol consumption in enhancing the risk for hyperhomocysteinaemia specifically with respect to *TT* genotype of *MTHFR C677T* polymorphism. Interestingly, individuals with normal Hcy levels are found to have complete absence of mutant homozygous *TT* genotype in both the studied groups. However, *TT* genotype is observed among hyperhomocysteinemic individuals in both cases and controls.

Table 1. Individual characteristics and distribution of genotypic and allelic frequencies of *MTHFR C677T* gene polymorphism and homocysteine level among cases and controls.

Characteristics		Case ($n = 451$**)	Control ($n = 147$**)	*P* value
Age (year), mean±SD*		52.21±10.04 (33–73)	51.56±10.04 (32–75)	0.49[a]
MTHFR C677T, *n* (%)	*CC*	312 (69.18)	107 (72.79)	0.65[b]
	CT	125 (27.72)	35 (23.81)	
	TT	14 (3.10)	5 (3.40)	
	C allele	0.83	0.85	0.51[b]
	T allele	0.17	0.15	
Homocysteine, μ mol/L*		2575 (2.88–50)	2575 (2.8–50)	1.00[a]
	Normal, *n* (%)	21 (30.43)	10 (34.48)	0.69[b]
	Hyperhomocysteinaemia, *n* (%)	48 (69.56)	19 (65.52)	

*Values shown are median/mean (range); **sample size for Hcy level (control = 29; case = 69).
[a]Unpaired *t*-test.
[b]Likelihood ratio χ^2 test.

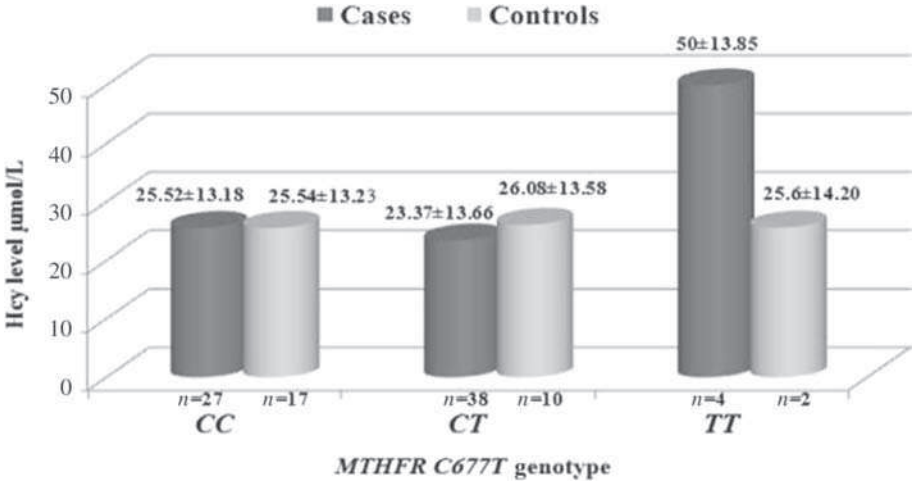

Figure 1. Distribution of mean values of plasma homocysteine level among cases and controls with respect to *MTHFR C677T* polymorphism.

Higher Hcy levels among cases could be attributed to folate deficiency induced by ethanol, as alcohol consumption is reported to have folate deficiency due to various factors such as intestinal malabsorption, altered hepatobiliary metabolism and increased renal excretion (Hamid *et al.* 2009). Further studies are needed to explain higher mean Hcy levels among the control groups. On the other hand, elevation in the plasma Hcy levels could also be due to other environmental variables like deficiencies in the micro-nutrients like vitamin B_{12} as evidenced from several studies (Mann *et al.* 1999). Though, it could be ruled out as majority of the Manipuri population adhered to nonvegetarian diet which contains high vitamin B_{12}. However, smaller sample size and lack of data on related biochemical variables like folate and vitamin B_{12} could be major limitations of the present study.

In conclusion, neither 677*T* allele nor alcohol consumption leads to hyperhomocysteinaemia in the present study. However, *TT* genotype in combination with alcohol consumption is causing an abrupt increase in Hcy level which could be a public health concern. The present study is first of its kind where both cases and controls are selected from a single Mendelian population of specific geographical region where in most factors like climate, food habits, lifestyle and genetic makeup are common. Validation of the results of the present study in different ethnic groups with larger sample sizes may be more relevant for management of the problem of alcoholism.

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References

American Psychiatric Association 1994 *Diagnostic and statistical manual of mental disorders,* 4th edn. American Psychiatric Press, Washington, DC, USA.

Barak A. J., Beckenhauer H. C., Hidiroglou N., Camilo M. E., Selhub J. and Tuma D. J. 1993 The relationship of ethanol feeding to the methyl folate trap. *Alcohol* **10**, 495–497.

Benyamina A., Saffroy R., Blecha L., Pham P., Karila L., Debuire B. *et al.* 2009 Association between *MTHFR* 677C-T polymorphism and alcohol dependence according to Lesch and Babor typology. *Addict. Biol.* **14**, 503–505.

Bleich S., Carl M., Bayerlein K., Reulbach U., Biermann T., Hillemacher T. *et al.* 2005 Evidence of increased homocysteine levels in alcoholism: the Franconian alcoholism research studies (FARS). *Alcohol. Clin. Exp. Res.* **29**, 334–336.

Bleich S., Degner D., Javaheripour K., Kurth C. and Kornhuber J. 2000a Homocysteine and alcoholism. *J. Neural. Transm. Suppl.* **60**, 187–196.

Bleich S., Degner D., Wiltfang J., Maler J. M., Niedmann P., Cohrs S. *et al.* 2000b Elevated homocysteine levels in alcohol withdrawal. *Alcohol Alcohol.* **35**, 351–354.

Chiuve S. E., Giovannucci E. L., Hankinson S. E., Hunter D. J., Stampfer M. J., Willett W. C. *et al.* 2005 Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. *Am. J. Clin. Nutr.* **82**, 155–162.

Frosst P., Blom H. J., Milos R., Goyette P., Sheppard C. A., Matthews R. G. *et al.* 1995 A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* **10**, 111–113.

Ganji V. and Kafai M. R. 2003 Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988–1994. *Am. J. Clin. Nutr.* **77**, 826–833.

Gmel G. and Rehm J. 2003 Harmful alcohol use. *Alcohol Res. Health* **27**, 52–62.

Hamid A., Wani N. A. and Kaur J. 2009 New perspectives on folate transport in relation to alcoholism-induced folate malabsorption –association with epigenome stability and cancer development. *FEBS J.* **276**, 2175–2191.

Hidiroglou N., Camilo M. E., Beckenhauer H. C., Tuma D. J., Barak A. J., Nixon P. F. *et al.* 1994 Effect of chronic alcohol ingestion on hepatic folate distribution in the rat. *Biochem. Pharmacol.* **47**, 1561–1566.

Husemoen L. L. N., Thomsen T. F., Fenger M., Jørgensen H. L. and Jørgensen T. 2003 Contribution of the thermolabile methylenetetrahydrofolate reductase variant to total plasma homocysteine levels in healthymen and women. Inter99 (2). *Genet. Epidemiol.* **24**, 322–330.

Kumar J., Garg G., Sundaramoorthy E., Prasad P. V., Karthikeyan G., Ramakrishnan L. *et al.* 2009 Vitamin B12 deficiency is associated with coronary artery disease in an Indian population. *Clin. Chem. Lab. Med.* **47**, 334–338.

Mann N. J., Li D., Sinclair A. J., Dudman N. P., Guo X. W., Elsworth G. R. *et al.* 1999 The effect of diet on plasma homocysteine concentrations in healthy male subjects. *Eur. J. Clin. Nutr.* **53**, 895–899.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 215.

Nienaber-Rousseau C., Pisa P. T., Venter C. S., Ellis S. M., Kruger A., Moss S. J. *et al.* 2013 Nutritional Genetics: The case of alcohol and the MTHFR C677T polymorphism in relation to homocysteine in a black South African population. *J. Nutrigenet. Nutrigenomics* **6**, 61–72.

Saraswathy K. N., Kiranmala N., Murry B., Sinha E., Saksena D., Kaur H. *et al.* 2009a A genomic insight into the diversity among tribal and non-tribal population groups of Manipur. *Biochem. Genet*. **47**, 694–706.

Saraswathy K. N., Sanjenbam Y. M., Murry B., Devi K. N., Sinha E., Gupta V. *et al.* 2009b Population severance in manipur at DRD2 locus. *Genet. Test. Mol. Biomarkers* **13**, 831–839.

Singh H. S., Ghosh P. K. and Saraswathy K. N. 2013 DRD2 and ANKK1 gene polymorphisms and alcohol dependence: a case-control study among a mendelian population of east Asian ancestry. *Alcohol Alcohol*. **48**, 409–414.

World Health Organization. 2011 Global status report on alcohol and health. WHO Geneva (http://www.who.int/substance_abuse/publications/global_alcohol_report/msbgsruprofiles.pdf).

Yang C.-X., Matsuo K., Ito H., Shinoda M., Hatooka S., Hirose K. *et al.* 2005 Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. *Carcinogenesis* **26**, 1285–1290.

Yeh F. C. and Yang R. 1999 Popgene version 1.31. Microsoft window based freeware for population genetic analysis. University of Alberta and Tim Boyle, Centre for International Forestry Research, Canada.

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