Methylenetetrahydrofolate Reductase Gene (MTHFR_677C→T) Associated with the development of depression

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Abstract— Globally, Depression is widespread neuropsychiatric disorders affecting around 5% of the population and has been described as millennia linked with neurobiology showing association with direct neurochemicals and biochemical incredible factors, interact with “gene-environment” as long as a scaffold potential for better exploration. The aetiology of depression is still unknown but believes to be the interaction between gene and environment including some of the other factors responsible for development of depression. The PCR-RFLP analysis of MTHFR (C677T) gene showed 0.45% in CT (heterozygous) genotype in patients of depression in comparison to controls (0.15%), suggesting increased risk of depression in those individuals. However, the odd ratio was also calculated at 95% confidence interval for MTHFR C677T gene which revealed non-significant difference between cases and control, may be because of small sample size.

Keywords: Depression, Genetics, MTHFR gene, folate, Polymorphism

I. INTRODUCTION
Depression is a heterogeneous disorder that affects mood, physical health and behaviour. One of the most common outcomes of depression is suicidal propensity, being responsible for 60% of the death. Epidemiological study suggests that frequency of depression is 5.8% of men and 9.5% of women in any given year and this prevalence varies in relation to different population. Recently, more than 300 million people suffer from depression in India. It is estimated that depression will become the second leading cause of premature disability or death worldwide by the year 2020 (1,2). Several clinical manifestations of depression like alternation in cognitive, psychomotor, biological, motivational, behavioural and emotional process (3,4) are reported in depressed condition. No single factor is responsible for causing depression. Several psychosocial factors like adverse living conditions, genetic and biological factors also play a part in the etiopathogenesis of depression. The genetic susceptibility and interaction between “gene-gene” & “gene-environment” are influenced by the severity of depression and commonly combined with other psychiatric disorders contributing in development of many other neurodegenerative disorders (5-8).

Folic acid is an crucial component of DNA synthesis, cell replication, DNA excision, repair and DNA methylation (9) whereas folate deficiency can contribute to depressed mood, increases the risk of many other disorders through impaired DNA repair and synthesis, disruption of DNA methylation and chromosome breaks (10-11). The 5,10- methylenetetrahydrofolate reductase (MTHFR) regulate folic acid metabolism through conversion of 5, 10- methylenetetrahydrofolate to 5-methyltetrahydrofolate and its polymorphism has been reported to be associated with depression and other psychiatric disorders. This gene is located on chromosome 1p36.3, with two common single nucleotide polymorphism (SNPs) or alleles i.e. C677T and A1298C to facilitate the enzymatic actions and involved in the metabolism of folate (12-16). However, apart from its biological implications and neuropsychiatric disorder development, it is associated with gene mutation resulting in polymorphic variation of alleles that arises the possibility of enhancement of depression. Thus MTHFR could be used as non-invasive tool or genetic marker for diagnosis of depression. The present study dealt with the evaluation of the possible gene-environment interaction including MTHFR C677T gene mutation in the patients of depression to explore the aetiology of affective disorder.

II. MATERIALS AND METHODS
A. Collection of blood sample
Blood was collected from clinically diagnosed cases of depression (n = 15) on the basis of the inclusion and exclusion criteria described by DSM IV, age 25-60 of both gender (male n=6 & female n=9) with their respective controls (8 male & 7 females) attending O.P.D of S.S. Hospital I.M.S, B.H.U and SRM Hospital, Chennai. Blood samples were utilized for genetic studies of depressed patients. The subjects were recruited considering ethical guidelines. The study was approved by ethical committee of both the organizations (registration. no. dean/2009-10/1412 at 11.2.2010).

B. Isolation of Genomic DNA and PCR based RFLP analysis
Genomic DNA was isolated from peripheral blood, as described previously by Miller, et al. (17) and samples were kept at -20°C till further analysis. In the present study depressed patients were selected characterizing the folate metabolism markers namely MTHFR C677T. MTHFR Primers C677T (F-5'TGAAGGAGAAGGTTGTCCTGCGGGA3') & (R-5'AGGACGGTGCGGTTAGATG3').Restriction fragment length polymorphism (RFLP) analysis was carried out to determined missence mutation in the presence of Hinfl (15). PCR product (6μl), digested at 37°C for 3hr. in reaction volume of 25 μl containing 1U of Hinfl-I restriction enzyme (New England, Biolabs) and NEB buffer (2.5 μl). Digested product of RFLP was separated on 3% agarose gel stained with EtBr and visualized on Gel Doc system.

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C. Statistical analysis
Genotype and allele frequencies in study cases and controls were determined by Hardy - Weinberg equilibrium equation. Statistical analysis was carried out using X2 - test comparing with controls and depressive group of patients to determine the level of significance. The odd ratio at 95% confidence interval was calculated to determine the genetic risk makers between depressive patients and their respective control study subjects.

III. RESULT
The statistical analysis showed no significant difference with respect to control subjects evaluated on chi square test. Table- 1 and fig.1 depicted the detail findings of RFLP analysis of MTHFRC677T gene polymorphism in depression cases as well as control group. Frequency of genotypic variants i.e. CC homozygous genotype (1.65%) wild type and CT genotype (0.45%) heterozygote condition in depressive patients, whereas in controls the genotype frequency varies between 1.90% (CC) to 0.15% (CT). The homozygous mutant TT (0.15%) genotype was higher in depressed patients as compared with normal subjects.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% frequency (n=15)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>11(1.65)</td>
<td>0.4(0.04-3.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>CT</td>
<td>3(0.45)</td>
<td>3.5(0.2-100.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>TT</td>
<td>1(0.15)</td>
<td>Inf (0.05,-inf)</td>
<td>3.2</td>
</tr>
<tr>
<td>C</td>
<td>0.8</td>
<td>0.4(0.04-4.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>T</td>
<td>0.1</td>
<td>Inf (0.03,-inf)</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table 1: MTHFR C677T showing genotype variation and calculated odd ratio at 95 % C.I. between study cases and controls

IV. DISCUSSION
The present study shows an association between low folate intake and an increased risk for the development of depressive mood disorder. In this study, we have undertaken a case-control study to investigate the role of MTHFR (C677T) gene polymorphisms and their susceptibility for development of depression. MTHFR, enzymes play a crucial role in the folate metabolic pathway due to involvement in both DNA synthesis and DNA methylation. In one of the studies conducted by us, observed the genetic susceptibility in various types of psychiatric disorders leading to a growing attention to the MTHFR C677T gene polymorphisms which has been proven as a major risk factor for neuropsychiatric diseases including mental disability (16).

Although enzyme involved in the folate dependant homocysteine pathway are strongly involved with depression, due to wide ethnic variation & population diversity which have shown difference in allele frequency of their polymorphic variation. Therefore, apart from genetic factor, environmental factors are also responsible for depression (4-8). The present study showed that the 5’-10 MTHFR is strongly associated with increased risk of depression. Deficiency of folate and cobalamin also can lead to depression because its deficiency impairs the activity of enzyme involved in the folate metabolism and simultaneously, increases the level of homocysteine and decreases the vitamin B12 as compared to normal control. Thus, elevated homocysteine is independently associated with depression. This condition is able to cause mutation in the MTHFR C677T gene. However, MTHFR gene polymorphism is also affected by level of RBC, folate and plasma homocysteine. Therefore, depression have multifactor origin of CNS disorders with common variant in more than one gene involved in folate and homocysteine metabolism could interact to increase depression level as well as development of depressive disorders.

Our study showed high percentage frequency (1.90%) of homozagyosity of CC genotype (wild type) in comparison to control i.e. 1.65%. We also observed TT genotype (rare type) in depressive patients. The CT genotype (0.45%) in heterozygous condition was quite high when compared with control group suggesting increased risk of development of depression. A common C to T transition at nucleotide 677 (C→T) associated as a risk for various clinical lesions including neuropsychiatric disorders has also been reported (11-18). Although, both depression cases and controls belonged to the same ethnic background and shared a common geographic origin of various parts of India. However, in the present study, there was no statistically significant association of the genotypes studied. In the multivariate analysis additionally adjusted for smaller sample size, patient’s age and gender, the MTHFR 677T allele was associated with reduced frequency of enzymatic activity. Further, based on present finding it is speculated that CC genotype is more associated with mild depression, CT genetic variant with moderate degree of depression and...
TT variant has more association with patients suffering for severe depression.

V. CONCLUSION

It is concluded that present case controlled study has significant association with low folate intake as well as impaired folate metabolism that play major role in the aetiology of depression. The study confirms role of folic intake and “gene-gene” interaction acting as risk factor in the development of depression. Therefore, folate supplement has a potential effect on prevention and management of depression that reduces the risk of the MTHFR variant. Thus, the present study has important implications in the assessment of potential “risk factor” for development of depression either due to folate deficient diet (nutritional factor) or unknown environmental factors responsible for depression.

REFERENCES