Abstract: The present case-control study examined the association of genetic polymorphism with mental retardation. One common polymorphism (SNPs), C677T in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene involved in folate metabolism, is known to lower the activity of this enzyme. One hundred sixty mothers (with MR and normal children), from the Eastern Uttar Pradesh, India, were genotyped for MTHFR C677T SNP. Significant association with this SNP was detected, more specifically, with T allele in the mothers of MR children. The relative risk of T (C677T) in mothers for MR-affected pregnancy was 3 (OR 3.179, 95% CI 1.40-7.78). The results indicated statistically significant associations between maternal MTHFR C677T polymorphism and congenital heart defects in foetus under different genetic models.

Keywords: Homocysteine, Mental Retardation, Methylenetetrahydrofolate reductase, MTHFR.

INTRODUCTION
Mental retardation is defined as a significant impairment of cognitive and adaptive functions with onset before age 18 years. Depending on the intelligence quotient (IQ), which is measured by standardized age-dependent tests, several degrees of severity are distinguished, with mild (IQ 50–70) and moderate to severe (IQ lower than 50) being used most commonly. The prevalence of mental retardation in developed countries is generally estimated to about 2-3% but varies in different studies from 1 to 10% (Rizzi et al., 2012).

The methylenetetrahydrofolate reductase gene (MTHFR) encodes an enzyme that produces 5-methyltetrahydrofolate, which is the methyl donor to homocysteine for synthethizing methionine. MTHFR, a key regulatory enzyme in one-carbon metabolic cycles, is required for the synthesis of 5-methyltetrahydrofolate, the primary circulatory form of folate and the carbon donor for homocysteine remethylation to methionine. It in general plays a central role in balancing DNA synthesis (which involves 5,10-
folate in midtrimester, could be teratogenic both through hyperhomocysteinemia and also through folate deficiency, causing the developmental brain damage (Johnsson, 2000).

Abnormal folate metabolism and C677T polymorphism has been reported as a risk factor for several diseases and/or clinical conditions including neural tube defects (Yadav et al., 2016a), Down syndrome (Hobbs et al., 2000), orofacial clefts (Rai, 2017a), psychiatric disorders and cancer (Rai, 2014a) but not a single study is carried out to evaluate MTHFR C677T polymorphism as maternal risk factor for mental retardation. In this work, authors investigated the effect of maternal C677T polymorphism of the MTHFR gene as risk factors for MR using case control approach.

MATERIALS AND METHODS
Total 80 case mothers with MR children and 80 control mothers with normal children that have no reported abnormalities were recruited for the study. The study was carried out between January 2010 and October 2011 and was approved by the Institutional Ethics Committee (IEC) of VBS Purvanchal University, Jaunpur (U.P.). Informed written consent was taken from each subject after fully verbal explanation of the nature of the study.

To be assured that the control samples were appropriate for this study, authors compared the case group with control group with respect to the three major factors that could theoretically influence the analysis: maternal, caste and socioeconomic background. After obtaining informed consent, 3 ml peripheral blood was collected in EDTA tubes from all subjects.

DNA was extracted according to the method of Bartlett and White (2003). Genotyping for the MTHFR point mutation C677T was carried out by PCR-RFLP method of Frosst et al. (1995). PCR was carried out in a total reaction volume of 15 l consisting of 1.5 l of PCR buffer (10X), 1.5 l of dNTPs mix (2.5mM), 4pM of each of the forward (5’TGAAGGAGAAGGTGTCTGCGGGA-3’) and reverse (5’AGGACGGTGCGGTGAGAGTG-3’) primers, 1.5 units of taq DNA polymerase enzyme (Genei) and 200ng genomic DNA. PCR was carried

Frosst et al. (1995) reported a common and clinically important substitution (C→T) 667th position in this gene. The C677T (677C→T; Ala→Val) polymorphism occurs in exon 4 and results in an alanine-to-valine substitution at codon 222. Individuals with the MTHFR C677T TT genotype have been shown to have 30 percent in vitro MTHFR enzyme activity compared with the wild type, whereas those with the heterozygous (CT) genotype have been found to have 70 percent wild-type MTHFR enzyme activity (Frosst et al., 1995). The homozygous state (677TT) is associated with hyperhomocysteinemia and decreased levels of S-adenosylmethinine (SAM). The SAM is the main methyl group donor for all the cellular methylation processes. The C677T mutation changes the secondary structure of the peptide and interactions between monomers. The variant protein loses its cofactor FAD more quickly and has lower stability (Yadav et al., 2011). The mutation effect can be suppressed by addition of folate, which causes a higher FAD affinity and an increase in MTHFR stability. The C677T polymorphism frequency varied in different population and is extensively studied (Rai et al., 2010, 2012a, 2012b; Yadav et al., 2017).

Individuals with the MTHFR C677T genotype are considered to have increased dietary requirements because they have lower red cell folate levels compared with those without this variant (Molloy et al., 1997). Homozygosity for this variant of MTHFR is associated with mild hyperhomocysteinemia, particularly in individuals with low folate intake (Frosst et al., 1995; vander Put et al., 1995; Jacques et al., 2003; Kluijtmans et al., 2003). Maternal impairments in folate metabolism and elevated homocysteinemia are known risk factors for having a child with Down syndrome (DS) and NTD. Moderate hyperhomocysteinemia is toxic to adults and toxic to the foetus in early gestation (Wouters et al., 1993), and possibly teratogenic in the first trimester, causing neural tube defects (van der Put et al., 1995). Thus, the MTHFR heat-labile mutation, in the presence of decreased dietary methylentetrahydrofolate) and DNA methylation (which involves 5,10-methyltetrahydrofolate).
The allele frequencies of MTHFR in case (women with MR child) and control mother populations are listed in Table 1. MTHFR 677T allele frequency was 14.37% (23/160 allele) in case mothers and 5% in control mothers (8/160). The odds ratio for T allele in cases against controls has been calculated (OR= 3.19; 95% CI: 1.38-7.36; p= 0.004) and showed significant association between T allele and MR (p>0.05) (Table 2).

The frequencies of MTHFR CC, CT and TT genotypes (CC, CT, and TT) among case mothers were 0.75, 0.212, and 0.037, respectively. The corresponding frequencies among control mothers were 0.90 and 0.08 respectively (Table 1). TT genotype was not found in control samples. There were significant differences in genotype frequencies between the two groups. Authors tested also other three models viz-dominant (TT + CT vs CC), codominant (CT vs CC) and recessive (TT vs CC or CT) models (Table 2). The homozygous mutant (TT) genotype difference was observed in MR cases and controls, therefore odd ratio has been calculated for TT versus CC genotypes between cases and controls (OR= 8.38; 95% CI: 0.42- 165; p= 0.61). Highest difference was found in percentage of heterozygous i.e. in case mothers CT percentage was 21.2% whereas in control mothers CT percentage is only 10%. Odds ratio for CT versus CC genotype was 2.55 (95%CI: 1.02-6.31;p= 0.038). Authors also tested dominant model and combined odds ratio of CC versus CT and TT (combined mutant) against the control has been also calculated (OR= 3.0; 95% CI: 1.23-7.29; p= 0.012). All these statistical analyses demonstrated that T allele might be a risk factor for being DS individuals.

Table 1: Distribution of MTHFR genotypes in MR case and control mothers.

<table>
<thead>
<tr>
<th>Characters</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Case mothers</td>
<td>60</td>
<td>75%</td>
<td>17</td>
<td>21.25%</td>
<td>03</td>
</tr>
<tr>
<td>Control mothers</td>
<td>72</td>
<td>90%</td>
<td>08</td>
<td>10%</td>
<td>00</td>
</tr>
</tbody>
</table>
involved in the pathogenesis of mental retardation. The MTHFR gene has a critical role in (i) determining folate and homocysteine levels, and (ii) regulation of the availability of methyl groups, which are required for epigenetic control of gene expression. It is also worth to be mentioned that MTHFR gene has a significant role in early ontogeny and variant MTHFR (C677T) gene affects normal fetal development either by inadequate methylation process or by toxic effect of homocysteine on developing embryo.

However, a number of factors have been shown to influence plasma homocysteine levels and the most important genetic factor is a polymorphism in the MTHFR gene, an essential enzyme in homocysteine metabolism. Several reports

Table 2: Odd Ratios with confidence interval in different genotypes between case and control mothers.

<table>
<thead>
<tr>
<th>Model</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive (TT vs. CC or CT)</td>
<td>8.388</td>
<td>0.42-165.60</td>
<td>p=0.061</td>
</tr>
<tr>
<td>Co-dominant (CT vs. CC)</td>
<td>2.550</td>
<td>1.02-6.31</td>
<td>p=0.038</td>
</tr>
<tr>
<td>Dominant (TT + CT vs. CC)</td>
<td>3.000</td>
<td>(1.23-7.29)</td>
<td>p=0.012</td>
</tr>
<tr>
<td>Additive (T vs. C)</td>
<td>3.190</td>
<td>(1.38-7.36)</td>
<td>p=0.004</td>
</tr>
</tbody>
</table>

p>0.05

Figure 1: Hinf I digested amplicon, agarose gel picture showing different genotypes of MTHFR. Lane 1: 100bp marker; lane 2, 3, 4 and 8: heterozygous; lane 4 and 6: homozygous wild; lane 7: homozygous mutant.

Figure 2: Bar diagram showing CC, CT and TT genotypes distribution in cases and controls.

DISCUSSION

In comparison with controls, the T allele is overexpressed in MR mothers. MTHFR 677T allele frequencies, particularly the TT genotype frequency, were higher in the case mothers than in the control mothers. There were significant differences in the frequency distributions of the T allele and the CT and TT genotypes between the patient and control mothers. In comparison with the CC genotype, the OR for the 677TT genotype was 8.388 and that for the 677CT genotype was 2.55. This indicated that the risk of the disease in subjects with the TT and CT genotypes was 8.388 and 2.55 times higher than in those with the CC genotype. This indicated that the MTHFR 677C→T gene variation is associated with increased risk of mental retardation. These results strongly suggest that the MTHFR gene is involved in the pathogenesis of mental retardation.

The MTHFR gene has a critical role in (i) determining folate and homocysteine levels, and (ii) regulation of the availability of methyl groups, which are required for epigenetic control of gene expression. It is also worth to be mentioned that MTHFR gene has a significant role in early ontogeny and variant MTHFR (C677T) gene affects normal fetal development either by inadequate methylation process or by toxic effect of homocysteine on developing embryo. However, a number of factors have been shown to influence plasma homocysteine levels and the most important genetic factor is a polymorphism in the MTHFR gene, an essential enzyme in homocysteine metabolism. Several reports
indicate that a high plasma level of homocysteine may be a risk factor for various pathological conditions, such as pregnancies complicated by neural tube defects (Yadav et al., 2020), pregnancy complications (Grandone et al., 1998; Ueland et al., 2001), depression and dementia (Bottiglieri et al., 2001), schizophrenia, autism etc. (Muntjewerff et al., 2003) and several neurodegenerative disorders (Mattson and Shea, 2003).

MTHFR gene C677T polymorphism is reported as risk factor for several diseases like schizophrenia (Yadav et al., 2016b; Rai et al., 2017b), bipolar disorder (Rai, 2011a and 2011b), depression (Rai, 2014b, 2014c and 2017b), autism (Rai, 2016a, 2016b; Rai and Kumar, 2018a), alcoholism (Rai and Kumar, 2021), migraine (Rai et al., 2022), epilepsy (Rai and Kumar, 2018b), Alzheimer’s disease (Rai, 2016c), Down syndrome (Rai and Kumar, 2018c), cleft lip and palate (Rai, 2015, 2018), male infertility (Rai and Kumar, 2017), recurrent pregnancy loss (Rai, 2016d), breast cancer (Kumar et al., 2023), esophageal cancer (Kumar and Rai, 2018), lung cancer (Rai, 2014d), colorectal cancer, ovary cancer (Rai, 2016e), prostate cancer (Yadav et al., 2016a) etc.

CONCLUSION
In conclusion, authors confirm a possible association between genetic polymorphisms in the folate/homocysteine pathway and MR. Present study suggests that deficient MTHFR enzyme activity in pregnant women, related to the C677T variant, is associated with a higher risk of having offspring affected with mental retardation. Given the low sample size in this study, the present results seem tentative and need further studies to replicate.

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