GESA
GLOCAL ENVIRONMENT & SOCIAL ASSOCIATION

International Journal of Biological Innovations
http://ijbi.org.in | http://www.gesa.org.in/journals.php
https://doi.org/10.46505/IJBI.2023.5206
IJBI 5(2): 42-49 **(2023)**
E-ISSN: 2582-1032

MTHFR C677T POLYMORPHISM: A MATERNAL RISK FACTOR FOR HAVING A MENTALLY RETARDED CHILD IN INDIA

Vandana Rai*, Upendra Yadav and Pradeep Kumar

Human Molecular Genetics Laboratory, Department of Biotechnology
VBS Purvanchal University, Jaunpur (U.P.), India

**Corresponding author*: raivandana@rediffmail.com

Article Info:
Research Article
Received
20.08.2023
Reviewed
25.10.2023
Accepted
05.11.2023

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.

Cite this article as: Rai V., Yadav U. and Kumar P. (2023). MTHFR C677T polymorphism: A maternal risk factor for having a mentally retarded child in India. *International Journal of Biological Innovations*. 5(2): 42-49. https://doi.org/10.46505/ IJBI.2023.5206

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-methyltetrahydofolate, 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-

methylentetrahydrofolate) and DNA methylation (which involves 5,10-methyltetrahydrofolate).

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 frequncy varied in different population and is extensively studied (Rai *et al.,* 2010, 2012 a, 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 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 in mental retardation. In this work, authors investigated the effect of maternal C677T polymorphism of the MTHFR gene as risk factors for MR using cast 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

out in MJ thermal cycler (BioRad, USA). PCR amplification conditions included initial denaturation at 94^{0}C for 4 minutes followed by 30 cycles of denaturation at 94^{0}C for 1 minute, annealing at 65^{0}C for 1 minute and polymerization at 72^{0}C for 1 minute and a final polymerization at 72^{0}C for 10 minutes. The amplicons were digested with HinfI and resolved in a 3% agarose gel. The C→T substitution at nucleotide 677 creates a HinfI digestion site. The PCR product (198 bp) with T allele was digested in to 2 fragments (175 bp and 23 bp), whereas the PCR product with wild type C allele is not be cut by HinfI.

The Chi-square test was used to identify the departure from the Hardy-Weinberg equilibrium among the controls. To estimate the relative risk of MR in relation to SNP genotyped, Odds ratio (ORs) and 95% confidence intervals (CI) were calculated. The homozygote of the common allele (CC) in the subject was used as the reference and p value <0.05 was considered statistically significant. All statistical analyses were performed by online program available at ihg.gsf.de/cgi-bin/hw/hwa1.pl.

RESULT

The DNA from the controls and MR cases is PCR amplified and HinfI-digested product is resolved in 3% agarose gel. Figure 1 illustrates the three genotypes of MTHFR (CC, CT and TT) in MR case mothers. Authors performed three different types of statistical analyses: first, a comparison of allele frequencies between MR case and control individuals (C and T alleles); second, a comparison of the frequencies of the three genotypes (CC, CT and TT genotypes) among case and control individuals; and, third a comparison of the frequencies of 'susceptible' genotypes among case and control individuals (*i.e.* CT heterozygotes and TT homozygotes).

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.

Characters	CC		CT		TT		C		T	
	Number	%	No.	%	No	%	No.	%	No.	%
Case mothers	60	75%	17	21.25%	03	3.75%	137	85.62%	23	14.37%
Control mothers	72	90%	08	10%	00		152	95%	8	5%

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

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

p>0.05

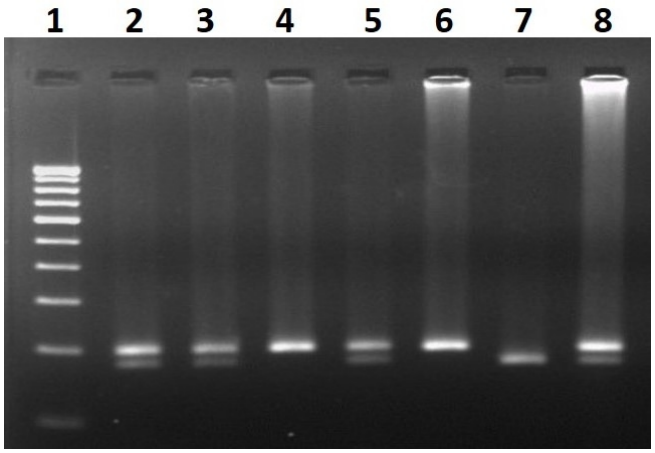

Figure 1: Hinf I digested amplicon, agarose gel picture showing different genotypes of MTHFR. Lane 1: 100bp marker; lane 2, 3, 4 and 8: heterozygous; lane 4 and 6 : homozygous wild; lane 7: homozygous mutant.

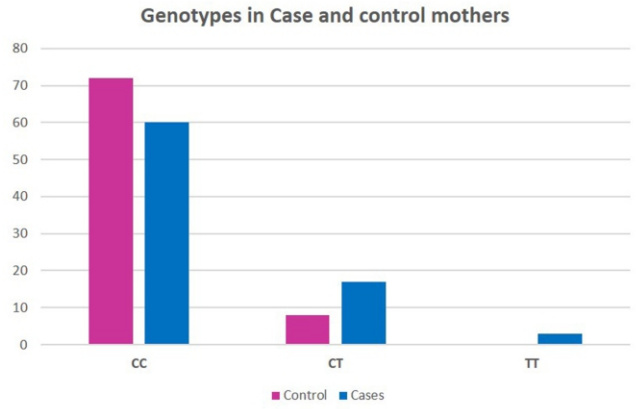

Figure 2: Bar diagrame showing CC, CT and TT genotypes distribution in cases and controls.

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 assocated 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 neuro-degenerative 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.

ACKNOWLEDGMENTS

The authors are grateful to all the patients, their families, and control volunteers for their participation in the study. The study was supported by the financial assistance from Department of Biotechnology, New Delhi (No BT/PR98887/SPD/11/1028/2007), and Department of Science and Technology, New Delhi (No. SR/SO/HS-61/2007) to Prof. Vandana Rai to conduct this exploration.

REFERENCES

1. **Bartlett J.M. and White A.** (2003). Extraction of DNA from Blood. In Methods in Molecular Biology, PCR Protocls, Bartlett, J.M.S. and D. Stirling (Eds.). 2nd Edn. Humana Press Inc., Totowa, NJ.
2. **Bottiglieri T., Parnetti L., Arning E., Ortiz T., Amici S., Lanari A. and Gallai V.** (2001). Plasma total homocysteine levels and the C677T mutation in the methyle netetrahydrofolate reductase (MTHFR) gene: a study in an Italian population with dementia. *Mechanism of Ageing and Development*. 122(16): 2013-2023. 10.1016/s0047-6374(01)00307-4.
3. **Frosst P., Blom H.J., Milos R., *et al.*** (1995). A candidate genetic risk factor for vascular disease: a common mutation in methyle netetrahydrofolate reductase. *Nature Genetics*. 10(1): 111-113. 10.1038/ng0595-111.
4. **Grandone E., Margaglione M., Colaizzo D., Marina d'Addedda, *et al.*** (1998). Methylene tetrahydrofolate reductase (MTHFR) 677T→C mutation and unexplained early pregnancy loss. *Thrombosis and Haemostasis.* 79(5): 1056-1057. 10.1055/s-0037-1615122
5. **Hobbs C.A., Cleves M.A., Lauer R.M., Burns T.L. and James S.J.** (2002). Preferential transmission of the MTHFR 677 T allele to infants with Down syndrome: implications for a survival advantage. *Am J Med Genet.* 113(1):9-14. 10.1002/ajmg.10704.
6. **Jacques P.F., Bostom A.G., Selhub J., *et al.*** (2003). Effects of polymorphisms of methionine synthase and methionine synthase reductase on total plasma homocysteine in the NHLBI Family Heart Study. *Atherosclerosis*. 166(1):49-55. 10.1016/s0021-9150(02)00204-6.
7. **Johnson M.H.** (2000). Functional brain development in infants: elements of an interactive specialization framework. *Child Dev.* 71(1):75-81. 10.1111/1467-8624.00120.
8. **Kluijtmans L.A., Young I.S., Boreham C.A., *et al.*** (2003). Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. *Blood*. 101(7): 2483-2488. 10.1182/blood.V101.7.2483.

9. **Kumar P. and Rai Vandana** (2018). Methylenetetrahydrofolate reductase C677T polymorphism and risk of esophageal cancer: An updated meta-analysis. *Egypt J Med Hum Genet*. 19(4): 273-284.

10. **Kumar P., Chaudhary A. and Rai V.** (2023). Evaluation of the Relationship between Dopamine Receptor D2 Gene TaqIA1 Polymorphism and Alcohol Dependence Risk. *Ind J Clin Biochem*. https://doi.org/10.1007/s12291-023-01122-7

11. **Mattson M.P. and Shea T.B.** (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. *Trends Neurosci*. 26(3):137-146. 10.1016/S0166-2236(03)00032-8.

12. **Molloy A.M., Mills J.L., McPartlin J., Kirke P.N., Scott J.M. and Daly S.** (2002). Maternal and fetal plasma homocysteine concentrations at birth: the influence of folate, vitamin B12, and the 5,10-methylenetetrahydrofolate reductase 677C→T variant. *Am J Obstet Gynecol*. 186(3):499-503. 10.1067/mob.2002.121105.

13. **Muntjewerff J.W., van der Put N., Eskes T., *et al.*** (2003). Homocysteine metabolism and B-vitamins in schizophrenic patients: low plasma folate as a possible independent risk factor for schizophrenia. *Psychiatry Res*. 121(1):1-9. 10.1016/s0165-1781(03)00200-2.

14. **Rai Vandana** (2011a). Evaluation of methylenetetrahydrofolate reductase gene variant (C677T) as risk factor for bipolar disorder. *Cell Mol Biol*. 57:1558-1566

15. **Rai Vandana** (2011b). Polymorphism in folate metabolic pathway gene as maternal risk factor for Down syndrome. *Int J Biol Med Res*. 2(4): 1055-1060.

16. **Rai V.** (2014a). Folate pathway gene MTHFR C677T polymorphism and risk of lung cancer in Asian populations. *Asian Pac J Cancer Prev*. 15: 9259-9264.

17. **Rai V.** (2014b). Genetic polymorphisms of methyle netetrahydrofolate reductase (MTHFR) gene and susceptibility to depression in Asian population: a systematic meta-analysis. *Cell Mol Biol*. 60 (3): 29-36.

18. **Rai Vandana** (2014c). Maternal methylene-tetrahydrofolate reductase (MTHFR) gene A1298C polymorphism and risk of nonsyndromic Cleft lip and/or Palate (NSCL/P) in offspring: A meta-analysis. *Asian J Medical Sciences*. 6 (1):16-21.

19. **Rai V.** (2014d). Methylenetetrahydrofolate reductase A1298C polymorphism and breast cancer risk: a meta-analysis of 33 studies. *Annals of Medical and Health Sciences Research*. 4 (6): 841-851.

20. **Rai Vandana** (2015). Evaluation of the MTHFR C677T polymorphism as a risk factor for colorectal cancer in Asian populations. *Asian Pac J Cancer Prev*. 16 (18): 8093-8100.

21. **Rai Vandana** (2016a). Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. *Metab Brain Disease*. 31: 727-735.

22. **Rai Vandana** (2016b). Folate pathway gene methylenetetrahydrofolate reductase C677T polymorphism and Alzheimer disease risk in Asian population. *Ind J Clin Biochem*. 31(3):245-52.

23. **Rai Vandana** (2016c). Methylene-tetrahydrofolate reductase C677T polymorphism and recurrent pregnancy loss risk in Asian population: a meta-analysis. *Ind J Clin Biochem*. 31:402-413.

24. **Rai Vandana** (2016d). Methylene-tetrahydrofolate reductase gene C677T polymorphism and its association with ovary cancer. *J Health Med Informat*. 7(3):226. 10.4172/2157-7420.1000226

25. **Rai Vandana** (2016e). The MTHFR C677T polymorphism and hyperuricemia Risk: a meta-analysis of 558 cases and 912 controls. *Metabolomics*. 6(1):166. 10.4172/2153-0769.1000166

26. **Rai Vandana** (2017a). Association of C677T polymorphism (rs1801133) in MTHFR gene with depression. *Cellular and Molecular Biology*. 63 (6): 60-67.

27. **Rai V.** (2017b). Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and

Alzheimer disease risk: a meta-analysis. *Mol Neurobio*. 54:1173-1186.

28. **Rai Vandana** (2018). Strong association of C677T polymorphism of methyle netetrahydrofolate reductase gene with nosyndromic cleft lip/palate (nsCL/P). *Ind J Clin Biochem*. 33(1):5-15.
29. **Rai Vandana and Kumar P.** (2017). Methylenetetrahydrofolate reductase C677T polymorphism and risk of male infertility in Asian population. *Ind J Clin Biochem.* 32(3): 253-260.
30. **Rai V. and Kumar P.** (2018a). Fetal MTHFR C677T polymorphism confers no susceptibility to Down syndrome: evidence from meta-analysis. *Egyptian J Med Hum Genet*. 19:53-58.
31. **Rai Vandana. and Kumar P.** (2018b). Methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to epilepsy. *Neurol Sci.* 39:2033-2041. 10.1007/s10072-018-3583-z.
32. **Rai Vandana and Kumar Pradeep** (2018c). Methylenetetrahydrofolate reductase A1298C polymorphism and autism susceptibility. *Austin Journal of Autism & Related Disabilities.* 4: 1048-1053.
33. **Rai Vandana and Kumar Pradeep** (2021). Methylenetetrahydrofolate reductase (MTHFR) gene C677T (rs1801133) polymorphism and risk of alcohol dependence: a meta-analysis. *AIMS Neurosci.* 8(2): 212-225.
34. **Rai V., Jamal F. and Kumar P.** (2022). Brain derived Neurotrophic Factor (BDNF) Val66Met Polymorphism is not risk factor for Bipolar Disorder. *International Journal of Biological Innovations*. 4(1): 16-28. https://doi.org/10.46505/IJBI.2022.4102.
35. **Rai V., Yadav U. and Kumar P.** (2012a). Prevalence of methylenetetrahydrofolate reductase C677T polymorphism in eastern Uttar Pradesh. *Indian J Hum Genet*. 18(1):43-46.
36. **Rai V., Yadav U. and Kumar P.** (2012b). Genotype prevalence and allele frequencies of 5,10-methylenetetrahydrofolate reductase (mthfr) C677T mutation in two caste groups of India. *Cell Mol Biol (Noisy-le-grand)*. 15 (58 Suppl): 1695-1701.
37. **Rai V., Yadav U. and Kumar P.** (2017a). Null association of maternal MTHFR A1298C polymorphism with Down syndrome pregnancy: An updated meta-analysis. *Egyptian J Med Hum Genet.* 18(1): 9-18. https://doi.org/10.1016/j.ejmhg.2016.04.003
38. **Rai V., Yadav U., Kumar P. and Yadav S.K.** (2010). Methylenetetrahydrofolate reductase polymorphism (C677T) in Muslim population of Eastern Uttar Pradesh, India. *Indian J Med Sci.* 64(5):219-223.
39. **Rai V., Yadav U., Kumar P., Yadav S.K. and Gupta S.** (2017b). Methylenetetrahydrofolate reductase A1298C genetic variant and risk of schizophrenia: an updated meta-analysis. *Indian J Med Res*. 145(4):437-447. 10.4103/ijmr.IJMR_745_14.
40. **Rizzi T.S., Beunders G., Rizzu P., *et al.*** (2012). Supporting the generalist genes hypothesis for intellectual ability/disability: the case of SNAP25. *Genes, Brain and Behavior*.11:767 - 771. https://doi.org/10.1111/j.1601-183X.2012.00819.x
41. **Ueland P.M., Refsum H., Beresford S.A. and Vollset S.E.** (2000). The controversy over homocysteine and cardiovascular risk. *Am. J. Clin. Nutr.* 72(2): 324-332. 10.1093/ajcn/72.2.324.
42. **Van der Put N.M., Eskes T.K. and Blom H.J.** (1997). Is the common 677C-->T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *Q J Med.* 90(2): 111-115. 10.1093/qjmed/90.2.111.
43. **Wouters M.G., Boers G.H., Blom H.J., Trijbels F.J., *et al.*** (1993). Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. *Fertility and Sterility*. 60(5): 820-825.
44. **Yadav U., Kumar P. and Rai V.** (2020). Maternal biomarkers for early prediction of the neural tube defects pregnancies. *Birth Defects Research*. 1-12. 10.1002/bdr2.1842.
45. **Yadav U., Kumar P. and Rai V.** (2011). Docking study of MTHFR with FAD by Hex.

J. Computational Intelligence in Bioinformatics. 4(1): 171-178.

46. **Yadav U., Kumar P. and Rai V.** (2016a). Role of MTHFR A1298C gene polymorphism in the etiology of prostate cancer: a systematic review and updated meta-analysis. *Egyp J Med Hum Genet.* 17(2): 141-148. https://doi.org/10.1016/j.ejmhg.2015.06.005

47. **Yadav U., Kumar P., Gupta S. and Rai V.** (2016b). Role of MTHFR C677T gene polymorphism in the susceptibility of schizophrenia: An updated meta-analysis. *Asian J Psychiatry.* 20: 41-51.

48. **Yadav U., Kumar P., Gupta S. and Rai V.** (2017). Distribution of MTHFR C677T gene polymorphism in healthy North Indian population and an updated meta-analysis. *Ind J Clinical Biochem.* 32(4):399-410. 10.1007/s12291-016-0619-0.