# Methylenetetrahydrofolate reductase Gene Polymorphisms as a risk factor in Rheumatoid Arthritis patients from North Coastal Andhra Pradesh

Uma Bharathi Harikoti[1], Lakshmi Kalpana Veerathu[2], Ramakrishnam Naidu Adapa[3], Papa Kusuma Bunga[4]

[1]*Research scholars, department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India*

[2]*Associate professor, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India*

[3]*Head, Department of Rheumatology, King George Hospital Visakhapatnam, Andhra Pradesh India*

***Abstract*** **- Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory, autoimmune disorder of unknown epipathogenesis that can affect many tissues and organs but principally attacks synovial joints. RA affects approximately 0.5 to 1% of the population worldwide, with women 2 to 3 times as likely as men to develop the disease. The etiology of RA is not yet known and it is considered to be multifactorial. Genetic and environmental factors play an important role in development of RA. The methylenetetrahydrofolate reducatse (MTHFR) enzyme has several crucial cellular process and its deficiency can have many consequences of folate status. So the genetic defects in folate metabolizing MTHFR gene have potential to affect RA risk. The aim of the present study was to investigate the association of MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) gene polymorphisms in rheumatoid arthritis patients from North Coastal Andhra Pradesh. A total of 300 samples (150 RA patients and 150 controls) were included in the present study and genotyping was accomplished by using PCR - RFLP technique. The data was analyzed by SPSS 19 software. Chi square p values revels that MTHFR (C677T) and MTHFR (A1298C) polymorphisms does not shows association with RA. The odds ratio p values of the variants MTHFR (C677T) and MTHFR (A1298C) were statistically insignificant with RA. It was concluded from the present study that there was no significant association of MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) gene polymorphisms with RA.**

***Index Terms*** **- Rheumatoid Arthritis, Methylenetetrahydrofolate reducatse, Gene Polymorphism, Polymerase Chain Reaction, Restriction Fragment Length Polymorphism, Statistical Package for the Social Sciences**

## INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that primarily affects small joints, progressing to larger joints and gradually the skin, eyes, heart, kidneys and lungs. Frequently the bone and cartilage of joints are destroyed, tendons and ligaments weaken [1]. RA has a worldwide distribution and affects all ethnic groups. The disease can occur at any age but its prevalence increases with age, the peak incidence being between the fourth and sixth decades. RA affects approximately 0.5 to 1% of the population worldwide, with women 2 to 3 times as likely as men to develop the disease [2]. The etiology of RA is not yet known and it is considered to be multifactorial. Genetic and environmental factors play an important role in development of RA. The familial nature of rheumatoid arthritis suggests that genetic factors play role in susceptibility to rheumatoid arthritis [3]. Based on twin studies, the genetic contribution to rheumatoid arthritis susceptibility is estimated to be 60% [4]. Twin studies also show concordance rates of 15% to 30% between monozygotic twins and 5% among dizygotic twins, suggesting that 50% to 60% of RA cases are due to genetic factors [5,6].

The methylenetetrahydrofolate reducatse (MTHFR) enzyme is essential for intracellularfolate homeostasis and metabolism. It converts the 5 tetrahydrofolate to the 5,10-methyletetrahydrofolate (5,10-methyle-THF) which catalyzes the conversion of homocysteine to methionine, necessary to the methylation reactions of DNA, RNA and proteins [7,8]. The 5,10-methyle-THF is require for purine and pyrimidin synthesis. The MTHFR enzyme has several crucial cellular processes and its deficiency can have many consequences of folate status. So the genetic defects in folate metabolizing MTHFR gene have potential to affect RA risk [9, 10, 11]. Based on the above evidence, the present study was aimed to identify the association of methylenetetrahydrofolate reductase gene polymorphisms in rheumatoid arthritis patients from North Coastal Andhra Pradesh.

## MATERIALS AND METHODS

The present study was carried out with 150 rheumatoid arthritis patients (12 males and 138 females) from King George hospital, Visakhapatnam and 150 age and sex matched controls (10 males and 140 females) above 25 years from North Coastal Andhra Pradesh during the period 2017-2019.The sample size calculation was done by using sample size calculator.net. The study was approved by the institutional ethical committee for blood sample collection from the patients. The informed consent was obtained from each and every participant before collecting blood sample for the evaluation of MTHFR C677T and MTHFR A1298C polymorphisms. Ethical clearance number: ECR/197/Inst/KGH/2013/DCGI/ 20-04-2013.

## MOLECULAR ANALYSIS

DNA extraction: The DNA isolation was done by using salting out method. Genotyping was analysed by using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) techniques.

Genotyping of MTHFR C677T (rs1801133): One set of forward "5'- CATCCC TAT TGG CAG GTT AC-3" reverse "5'- GAC GGT GCG GTG AGA GTG-3" primers were used for PCR. The PCR profile was: initial denaturation at 94°C, 5 minutes, denaturation: 94°C, 1 minute, annealing: 58°C, 1 minute followed by 35 cycles each of 1 minute, and 72°C, then final extension at 72°C for 7 minutes. The amplification product was visualized in a 2% agarose gel under UV light. The PCR product amplification fragment of 198 base pair and then amplified fragments was digested with HinfI restriction enzyme.

The CC (homozygous type allele) genotype produces a single band of 198bp and CT (heterozygous type allele) genotype produces three bands of 198, 175 and 23bp. The TT (homozygous mutant type allele) genotype produces two bands of 175 and 23bp.

Genotyping of MTHFR A1298C (rs1801131): One set of forward "5'- CTT TGG GGA GCT GAA GGA CTA CTAC-3''and reverse "5'- CAC TTT GTG ACC ATT CCG GTT TG -3" primers were used for PCR. The PCR profile was: initial denaturation at 94°C, 5 minutes, denaturation: 94°C, 1 minute, annealing: 63°C, 1 minute followed by 35 cycles each of 1 minute and 72°C, then final extension at 72°C for 7 minutes. The amplification product was visualized in a 2% agarose gel under UV light. The amplification fragment of 265 base pairs and then amplified fragments was digested with MboII restriction enzyme.

The AA (homozygous type allele) genotype produces a single band of 265bp and AC (heterozygous type allele) genotype produces three bands of 265, 171 and 94bp. The CC (homozygous mutant type allele) genotype produces two bands of 171 and 94 bp.

Statistical Analysis: The data was analyzed by using Statistical Package of Social Sciences Software program (SPSS) 19 was used for calculating genotype and allele frequencies. Chi-square analysis was used to test for allele frequencies and odds ratio analysis was used to test for genotype frequencies in comparison of patients and healthy control groups. P value $\leq 0.05$ considered statistically significant.

## RESULTS

Tables

Table 1: Demographic variable of RA patients and controls

| Characteristics | RA patients (n=150) (%) | Controls (n=150) (%) |
|---|---|---|
| Male | 12 (8%) | 10(6.66%) |
| Female | 138 (92%) | 140 (93.3%) |
| Age years (M ±SD) | 45±16.61 | 51.3±6.2 |

Table 2: Genotype and allele frequencies of MTHFR CC677T (rs1801133) and A1298C (rs1801131) polymorphism in RA patients and controls

| Genes | Genotypes and alleles | RA patients N=150 (%) | Controls N=150 (%) | OR (95%CI) | P-value |
|---|---|---|---|---|---|
| MTHFR C677T | CC | 61 (40%) | 67 (44%) | 0.936 (0.579 – 1.513) | 0.789 NS |
| | CT | 69 (46%) | 71 (47%) | 0.583 (0.2650 –1.283) | 0.180 NS |
| | TT | 20 (13%) | 12 (8%) | 1.830 (0.8264 –4.055) | 0.136 NS |
| | C | 191 (63.66%) | 205 (68.33%) | 1.456 | 0.227 NS |
| | T | 109 (36.33%) | 95 (31.66%) | | |
| MTHFR A1298C | AA | 66 (44%) | 78 (52%) | 0.763 (0.4779–1.220) | 0.260 NS |
| | AC | 72 (48%) | 65 (43.33%) | 0.646 (0.239 – 1.740) | 0.387 NS |
| | CC | 12 (8%) | 7 (4.66%) | 2.026 (0.7542 -5.442) | 0.161 NS |
| | A | 204 (68%) | 221 (73.66) | 2.331` | 0.126 NS |
| | C | 96 (32%) | 79 (52.66%) | | |

**P≤0.01 – Highly Significant; *P≤0.05– Significant; NS – Not Significant

Table 3: MTHFR C677T (rs1801133) polymorphism and its interaction with MTHFR A1298C (rs1801131) polymorphism in RA patients and controls

| MTHFR C677T CC v/s (rs1801133) | RA patients N=150 | Controls N=150 | OR | 95% CI | P-value |
|---|---|---|---|---|---|
| MTHFR A1298C (rs1801131) | | | | | |
| AA | 32 | 34 | 7.843 | 2.156 - 28.527 | 0.001** |
| AC | 30 | 25 | 0.200 | 0.022 -1.773 | 0.148 NS |
| CC | 6 | 1 | 6.375 | 0.726 - 55.911 | 0.094 NS |
| MTHFR C677T CT+TT v/s (rs1801133) | | | | | |
| MTHFR A1298C (rs1801131) | | | | | |
| AA | 34 | 44 | 0.7752 | 0.416-1.443 | 0.422 NS |
| AC | 42 | 40 | 1.0750 | 0.320-3.607 | 0.906 NS |
| CC | 6 | 6 | 0.8333 | 0.246 – 2.815 | 0.769 NS |
| **P≤0.01 – Highly Significant; *P≤0.05– Significant; NS – Not Significant | | | | | |

Table 1 represents the Demographic variables of RA patients and controls. The frequencies of males were 12 (8%) females were 138 (92%) in RA patients and there was 10(6.66%) males and 140 (93.3%) females were in control group respectively. The mean age ±standard deviation (SD) was 45±16.61 in RA patients and 51.3±6.2 in control group.

Table 2 represents the genotype frequencies of MTHFR C677T (rs1801133) polymorphism in RA patients and controls. The genotype frequency of CC was higher in controls (44.6%) than RA patients (40.6%). The genotype frequencies of CT in RA patients and controls were (46%) and (47.3%) respectively. The genotype frequency of TT was higher in RA patients (13.3%) than controls (8%). The odds ratio of TT genotype was found to be showing small risk when compared to CC and CT genotypes.

The odds ratio p value of the genotypes CC, CT and TT were found to be statistically insignificant.

The frequency of C allele was 63.66% in RA patients and 68.33% in controls. The T allelic frequencies in RA patients and controls were 36.33% and 31.66% respectively. The chi-square p value reveals that MTHFR C677T (rs1801133) polymorphism does not shows association with RA.

The genotype frequencies of MTHFR A1298C (rs1801131) polymorphism in RA patients and controls were represented in table1. The genotype frequency of AA (52%) was higher in controls than RA patients (44%) whereas the frequency of genotype AC (48%) was higher in RA patients than controls (48%). The genotype frequency of CC (8%) was higher in RA patients than controls (4.66%). The odds ratio of CC genotype was found to be showing small risk when compared to AA and AC genotypes. The odds ratio p value of genotypes AA, AC and CC were found to be statistically insignificant.

The frequency of A allele was 68% in RA patients and 73.66% in controls. The C allelic frequencies in RA patients and controls were 32% and 52.66% respectively. The chi-square p value reveals that MTHFR A1298C (rs1801131) polymorphism does not shows association with RA.

Table 3 represents the MTHFR C677T (rs1801133) polymorphism and its interaction with MTHFR A1298C (rs1801131) polymorphism in RA patients and controls. From the above table it is evident, that the CC genotype of MTHFR C677T (rs1801133) polymorphism was statistically significant with AA genotype, whereas it is insignificant with AC and CC genotypes of MTHFR A1298C (rs1801131) polymorphism.

The CT and TT genotypes of MTHFR C677T (rs1801133) polymorphism were statistically insignificant with AA, AC and CC genotypes of MTHFR A1298C (rs1801131) polymorphism.

## DISCUSSION

During the past two decades many epidemiological studies have investigated the relationships of the MTHFR C677T and A1298C polymorphisms with various diseases including rheumatoid arthritis, birth defects, breast cancer, lymphoblastic leukemia, cardiovascular diseases, pregnancy complications and eventhough the results were still inconsistent [12,13,14,15,16,17,18,19,20]. Additionally, estimation of the genotype and haplotype distributions of the two polymorphisms in different populations has also been a focus of considerable interest from researchers worldwide.

Inanir et al., study suggested that there is no signifi¬cant association between genotypic frequencies of the MTHFR C677T polymorphism with risk of RA, but allele frequencies showed statistically significant association between patients and controls [21]. In addition, Brambila Tapia study demonstrated that the MTHFR C677T and A1298C polymorphisms in Mexican patients are significantly associated with RA risk [22].

Shaker et al., study shows the CT genotype and T allele of MTHFR C677 T are associated with RA [23]. On the other hand the different MTHFR A1298 C polymorphic forms were not associated with RA. The AC genotype of MTHFR A1298 C showed protective effect from the disease. This may be due to higher effect of MTHFR C677 T on MTHFR enzyme resulting in hyper homo cysteinaemia and subsequent cascade of cytokine activation. Results of previous studies were heterogeneous and some showed no association between risk of RA and different polymorphic forms of MTHFR C677 T and A1298 C [24, 25]. Others reported association of T allele but not any of the MTHFR C677 T polymorphic forms with RA [21]. Rubini study reported that the association of CC genotype of MTHFR A1298 C but not any of MTHFR C677 T polymorphic forms with susceptibility to RA in Italian population [26]. This heterogeneity in results may be attributed to racial variations in allele and genotype frequencies as reported by Hughes who found significant increase in T allele in MTHFR C677 T and C allele of MTHFRA1298 C (independent on disease status) in Caucasians when compared to African Americans [24].

## CONCLUSIONS

It was concluded from the present study absence of a confirmed direct functional effect of MTHFR C677T (rs1801133) and MTHFR A1298C (rs1801131) polymorphisms, the results indicate these polymorphisms have no role in RA susceptibility. Further studies with extended sample sizes (from the same population) are required to overcome the lack in power results and confirmed these results in the North

Coastal Andhra Pradesh population. In addition, further investigations of other polymorphisms and its association with RA susceptibility may be helpful to clarity the pathogenesis of the disease.

Acknowledgements: We would like to thank all the patients and individuals in this study for their participation and also thanks for the assistance of the clinicians, hospital staff and the department of Rheumatology in King George Hospital who contributed blood samples and data for this study. We are also very grateful for the Authors immense help received from the scholars whose articles are cited and included in references of this manuscript.

Conflict of Interest: No

Source of Funding: This work has been supported by University Grants Commission (UGC), New Delhi, India under Rajiv Gandhi National Fellowship to conduct the study.

REFERENCES

[1] Lee, J. E., Kim, I. J., Cho, M. S., & Lee, J. A case of rheumatoid vasculitis involving hepatic artery in early rheumatoid arthritis. J.of Korean Medical Science 2017; 32(7): 1207-1210.

[2] Tedeschi, S. K., Bermas, B., & Costenbader, K. H. Sexual disparities in the incidence and course of SLE and RA. Clinical Immunology 2013; 149(2): 211-218.

[3] Gabriel, S. E. The epidemiology of rheumatoid arthritis. Rheumatic Disease Clinics of North America 2001; 27(2):269-281.

[4] MacGregor, A. J., Snieder, H., Rigby, A. S., Koskenvuo, M., Kaprio, J., Aho, K., & Silman, A. J. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis & Rheumatism: Official J. of the American College of Rheumatology 2000; 43(1): 30-37.

[5] McInnes, I. B., & Schett, G. The pathogenesis of rheumatoid arthritis. New England J. of Medicine 2011; 365(23):2205-2219.

[6] Silman, A. J., & Pearson, J. E. Epidemiology and genetics of rheumatoid arthritis. Arthritis research & therapy2002; 4(S3): S265.

[7] Leclerc, D., & Rozen, R. Génétique moléculaire de MTHFR-Les polymorphismes ne sont pas tous bénins. médecine/sciences 2007; 23(3):297-302.

[8] Rozen, R. Annotation Molecular genetics of methylenetetrahydrofolate reductase deficiency. J. of inherited metabolic disease 1996; 19(5): 589-594.

[9] Song, G. G., Bae, S. C., & Lee, Y. H. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. Clinical rheumatology 2014; 31(12):1733-1739.

[10] Stamp, L., Roberts, R., Kennedy, M., Barclay, M., O'Donnell, J., & Chapman, P. The use of low dose methotrexate in rheumatoid arthritis—are we entering a new era of therapeutic drug monitoring and pharmacogenomics?. Biomedicine & pharmacotherapy 2006; 60 (10): 678-687.

[11] Manni, F. et al., Y-chromosome analysis in Egypt suggests a genetic regional continuity in Northeastern Africa. Human Biology 2002; 645-658.

[12] Bahari, G., Hashemi, M., Naderi, M., & Taheri, M. Association between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and susceptibility to childhood acute lymphoblastic leukemia in an Iranian population. Int. J. of hematology-oncology and stem cell research 2016; 10(3):130.

[13] Cen, H et al., Associations of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms with genetic susceptibility to rheumatoidarthritis: a meta-analysis. Clinical rheumatology 2017; 36(2): 287-297.

[14] Moll, S., & Varga, E. A. Homocysteine and MTHFR mutations. Circulation 2015; 132(1): e6-e9.

[15] Mollin, D. L., & Hoffbrand, A. V. The diagnosis of folate deficiency. Haematologica 1965; 3, 1.

[16] Mosaad, Y. M. et al., Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and susceptibility to acute lymphoblastic leukemia in a cohort of Egyptian children. Leukemia & lymphoma 2015; 56(9): 2699-2705.

[17] Ueland, P. M. MTHFR polymorphisms and disease. CRC Press 2005.

[18] Yang, B. 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).

[19] Zhong, S. et al,. A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in breast cancer. Molecular biology reports 2014; 41(9): 5775-5785.
[20] Kidd, K. K., et al., ALFRED: The Allele Frequency Database. 2012; URL: http://info. med. yale. edu/genetics/kkidd.
[21] Inanir, A., Yigit, S., Tekcan, A., Tural, S., & Kismali, G. IL-4 and MTHFR gene polymorphism in rheumatoid arthritis and their effects. Immunology letters 2013; 152(2): 104-108.
[22] Brambila-Tapia, A. J. L.,et al,. MTHFR C677T, MTHFR A1298C, and OPG A163G polymorphisms in Mexican patients with rheumatoid arthritis and osteoporosis. Disease markers 2012; 32(2):109-114.
[23] Shaker, O. G.,et al., Methylene tetrahydrofolate reductase, transforming growth factor-β1 and lymphotoxin-α genes polymorphisms and susceptibility to rheumatoid arthritis. Revista brasileira de reumatologia 2016; 56(5): 414-420.
[24] Hughes, L. B.,et al.,. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Annals of the rheumatic diseases 2006; 65(9):1213-1218.
[25] Palomino-Morales, R., et al.,. Research article A1298C polymorphism in the MTHFR gene predisposes to cardiovascular risk in rheumatoid arthritis. Heart failure 2010; 5: 2-3.
[26] Rubini, M., Padovan, M., Baricordi, O., Fotinidi, M., Govoni, M., & Trotta, F. The c. 1298A> C polymorphism in the methylenetetrahydrofolate reductase gene is associated with rheumatoid arthritis susceptibility in Italian patients. Endocrinology & Metabolism 2008; 8(6): 245-251.