RESEARCH PAPER

GENETIC ASSOCIATION OF RFC1 (rs1051266) POLYMORPHISM WITH SUSCEPTIBILITY TO ORAL CANCER

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ABSTRACT

Background

Oral cancer is a major public health concern with a multifactorial etiology involving both environmental and genetic factors. The Reduced Folate Carrier 1 (RFC1) gene plays a crucial role in folate transport and metabolism, which are essential for DNA synthesis, repair, and methylation. Genetic variations in RFC1 may influence individual susceptibility to oral cancer.

Aim

To evaluate the association between the RFC1 gene polymorphism (rs1051266) and susceptibility to oral cancer in a South Indian population.

Materials and Methods

A hospital-based case–control study was conducted among 50 participants, including 25 histopathologically confirmed oral cancer patients and 25 healthy controls. Genomic DNA was isolated from peripheral blood samples. Genotyping of the RFC1 rs1051266 polymorphism was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Genotype and allele frequencies were compared between cases and controls using the Chi-square test. Hardy–Weinberg equilibrium (HWE) was assessed for both groups. Statistical analysis was carried out using SPSS software, and a p-value <0.05 was considered statistically significant.

Results

The genotype frequencies among cases were TT (36%), TC (16%), and CC (48%), while among controls they were TT (40%), TC (12%), and CC (48%). The T allele frequency was 44% in cases and 46% in controls, whereas the C allele frequency was 56% and 54%, respectively. No statistically significant difference was observed in genotype distribution between oral cancer patients and healthy controls ($\chi^2 = 0.071$, $p = 0.7893$). Similarly, allele frequencies did not differ significantly between the two groups.

Conclusion

The findings of the present study indicate that the RFC1 rs1051266 polymorphism is not significantly associated with oral cancer susceptibility in the studied South Indian population. Further studies with larger sample sizes and diverse populations are warranted to clarify the role of RFC1 genetic variants in oral carcinogenesis.

KEYWORDS: Genes, RFC1, polymorphism, oral cancer, human, health, disease.

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.INTRODUCTION:

Oral cancer poses a considerable global health challenge, imposing a substantial burden on individuals and healthcare systems worldwide. The intricate origin of oral cancer involves a dynamic interplay between genetic predisposition and environmental factors like tobacco use and alcohol consumption. Emerging evidence suggests that genetic variations play a significant role in an individual's susceptibility to oral cancer.

A genetic element currently under scrutiny is the rs1051266 polymorphism within the Reduced Folate Carrier 1 (RFC1) gene. RFC1 plays a critical role in folate metabolism, a pathway integral to DNA synthesis, repair, and methylation. Considering the fundamental role of folate in maintaining genomic stability, genetic variations affecting RFC1 function may potentially impact an individual's vulnerability to cancer, including oral cancer(2).

Previous research has linked RFC1 polymorphisms to various cancer types, emphasising the need to investigate their association with oral cancer susceptibility. The rs1051266 polymorphism, situated within the RFC1 gene, has demonstrated particular relevance to cancer risk across diverse populations(3). Understanding the genetic underpinnings of oral cancer is crucial not only for unravelling the complexities of its development but also for devising targeted prevention and intervention strategies(4)

This study endeavours to explore the genetic association between the RFC1 rs1051266 polymorphism and susceptibility to oral cancer. Employing a comprehensive case-control analysis, we aim to clarify whether specific alleles or genotypes of this polymorphism confer an elevated risk of developing oral cancer(5)(6). Additionally, we will examine the potential impact of this genetic variant on clinicopathological features, providing insights into its relevance to disease progression.

The identification of genetic markers linked to oral cancer susceptibility holds significant implications for personalised medicine and public health. The insights gleaned from this study may contribute to the development of innovative approaches for risk assessment, early detection, and tailored prevention strategies. By advancing our comprehension of the genetic factors influencing oral cancer, we aspire to refine clinical management precision and pave the way for more effective and targeted interventions against this formidable disease.

1. MATERIALS AND METHODS:

1.1 Study Design

The present study was conducted at Saveetha Dental College and Hospitals, Chennai, India. A case–control study was conducted to investigate the association between the RFC1 gene polymorphism (rs1051266) and susceptibility to oral cancer in a South Indian population.

1.2 Study Population

The study included a total of 50 individuals, comprising 25 histopathologically confirmed oral cancer patients (cases) and 25 healthy controls. Patients diagnosed with oral cancer were recruited from the outpatient and inpatient departments. Healthy individuals without a history of oral cancer or other malignancies were included as controls.

1.3 Sample Collection and DNA Extraction

Approximately 2–3 mL of peripheral venous blood was collected from each participant in EDTA-containing tubes after obtaining informed consent. Genomic DNA was extracted from peripheral blood leukocytes using standard protocols and stored until further analysis.

1.4 Genotyping of RFC1 Gene Polymorphism (rs1051266)

Genotyping of the RFC1 rs1051266 polymorphism was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method.

1.5 PCR Amplification

The target region encompassing the polymorphic site was amplified using the following primers:

Forward Primer: 5′-AGGCTGGGCTCAAACTACAG-3′ Reverse Primer: 5′-TCCTTGGCAGATTCCATAGC-3′

PCR amplification was performed under standard reaction conditions with an annealing temperature of 56°C for 1 minute. The amplified PCR product yielded a fragment of 759 bp.

1.6 Restriction Enzyme Digestion

The PCR products were digested with HhaI restriction enzyme according to the manufacturer's instructions. The digested products were separated by agarose gel electrophoresis and visualized under ultraviolet transillumination.

Genotypes were determined based on the observed restriction patterns. Representative PCR and RFLP products are shown in Figures 1 and 2.

1.7 Statistical Analysis

Genotype and allele frequencies were calculated by direct counting. Differences in genotype

distribution between cases and controls were analyzed using the Chi-square test. Hardy–Weinberg equilibrium (HWE) was assessed separately in cases and controls. Statistical analysis was performed using SPSS software. A p-value less than 0.05 was considered statistically significant.

2. Results:

2.1 Demographic Characteristics

A total of 50 subjects were included in the study, comprising 25 oral cancer cases and 25 healthy controls. Among the cases, 21 (84%) were males and 4 (16%) were females, whereas the control group consisted of 16 (64%) males and 9 (36%) females. Tobacco-related habits, including smoking and pan chewing, were more prevalent among oral cancer patients than controls. Alcohol consumption was reported by 7 patients (28%) in the case group, while none of the control participants reported alcohol consumption. Histopathological examination revealed that 10 cases (40%) were moderately differentiated squamous cell carcinoma (MDSCC), 8 cases (32%) were well-differentiated squamous cell carcinoma (WDSCC), and 7 cases (28%) belonged to other histopathological variants.

2.2 CR Amplification of RFC1 Gene

PCR amplification of the RFC1 gene successfully generated a 759 bp amplicon in all samples analyzed. Representative amplification products are shown in Figure 1.

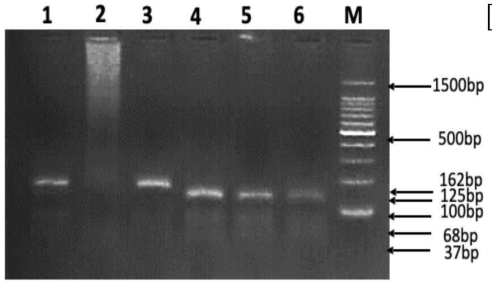


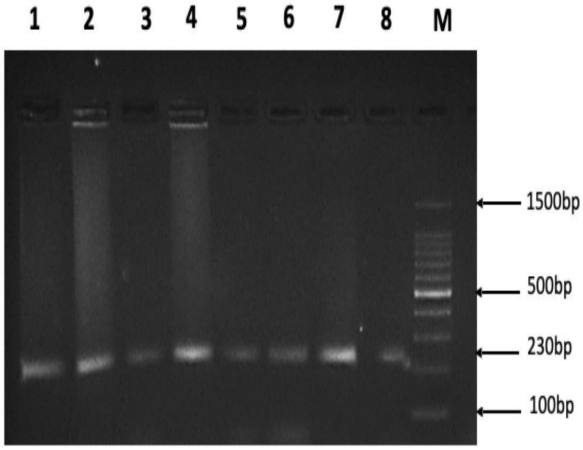
Figure 1: Agarose gel electrophoretogram showing partial amplification of *RFC1* gene polymorphism *(rs1051266)* spanning polymorphic run along with standard DNA ladder [Lane M = 100 bp DNA marker]

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly χ2df (P =0.7893).

3.2 PCR-RFLP Analysis of RFC1 Gene Polymorphism

Restriction digestion of the amplified PCR products using HhaI enzyme generated distinct banding patterns corresponding to different genotypes. The representative digestion patterns are shown in Figure 2.

Figure 2: Agarose gel electrophoretogram



showing *HhaI* digested amplicon of *RFC1* gene (Homozygous AA- 162+68bp; Heterozygous TC = 271+488+759 bp; Homozygous CC - 759 bp) [Lane M = 100 bp DNA marker]

3.3 Genotype and Allele Frequency Distribution

The genotype and allele frequencies of the RFC1 rs1051266 polymorphism among oral cancer cases and controls are presented in Table 1.

Table 1: Genotype frequencies of *RFC1* gene polymorphism *(rs1051266)* among the cases and controls

Groups	TT	TC	CC	T	C	HWE (p value)*
Case (N=25)	9	4	12	0.44	0.56	<0.0001
Control (N=25)	10	3	12	0.46	0.54	<0.0001

The TT genotype was observed in 36% of cases and 40% of controls. The TC genotype was observed in 16% of cases and 12% of controls, while the CC genotype was present in 48% of both groups. The T allele frequency was 44% among cases and 46% among controls, whereas the C allele frequency was 56% and 54%, respectively.

No statistically significant difference was observed in genotype distribution between oral cancer patients and controls (χ^2 = 0.071, p = 0.7893), indicating that the RFC1 rs1051266 polymorphism was not significantly associated with oral cancer

susceptibility in the studied South Indian population.

3.4 Population-Based Allele Frequency Distribution

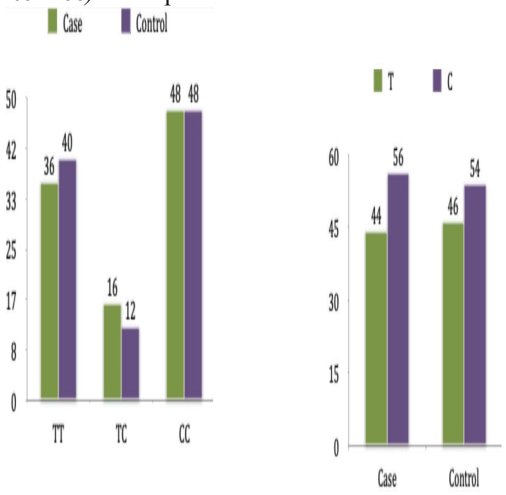
The distribution of T and C alleles of the RFC1 rs1051266 polymorphism across different global populations obtained from the Ensembl database is illustrated in Graph 1. The frequency of the T allele was highest in the African population (67%) and lowest in the South Asian population (41%), while the C allele showed the opposite trend.

Graph 1: The graph depicts the allele frequency of *RFC1* gene polymorphism *(rs1051266)* in different populations obtained from the Ensembl database.

3.5 Comparison of Genotype and Allele Frequencies Between Cases and Controls

The genotype and allele frequencies of the RFC1 rs1051266 polymorphism among oral cancer cases and healthy controls is represented in Graph 2. Similar genotype and allele distributions were observed in both groups, further supporting the absence of a significant association between the RFC1 polymorphism and oral cancer risk.

Graph 2: The graph demonstrates the genotype and allele frequency of *RFC1* gene polymorphism *(rs1051266)* in the present dataset



PCR information:

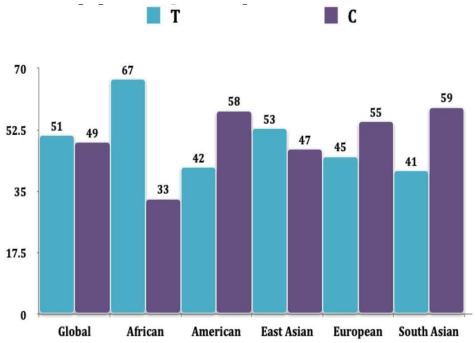
Primer sequence:
Forward: 5'- AGGCTGGGCTCAAACTACAG - 3'
Reverse: 5'- TCCTTGGCAGATTCCATAGC- 3'

Amplicon size: 759 bp, **Annealing temperature:** 56 degree C for 1 minute

3. DISCUSSION:

RFC1, a crucial enzyme in folate metabolism, may not play a prominent role in colorectal cancer (CRC) development through its polymorphisms. While various studies have explored the association between the RFC rs1051266 polymorphism and cancer, no significant link with CRC risk has been established. Similarly, the RFC1 80G>A polymorphism and the RFC1 −43T>C and 696C>T polymorphisms do not appear to show a significant association with CRC risk(7).

Despite the impact of RFC1 polymorphisms on plasma folate levels, it is argued that they may not exert a dominant influence on folate metabolism in CRC cases. Previous research suggests that RFC1 primarily facilitates the transport of naturally-occurring folate rather than folic acid, the synthetic form commonly found in supplements.



Since folic acid is more efficiently absorbed by the body and constitutes a major portion of total folate intake, the potential effect of genetic variants of RFC1 polymorphisms on CRC risk may be limited(8,9). Furthermore, the interaction between RFC1 polymorphisms and plasma folate levels did not demonstrate a significant influence on CRC risk in the present study.

The primary obstacle lies in translating observations derived from laboratory research into practical applications within a clinical setting. This translation is essential for utilizing the information either as a basis for predictive testing or for individuals to make informed decisions about lifestyle changes or medical interventions aimed at reducing disease risk(10,11). While individual odds ratios are valuable for assessing relative risk, focusing solely on single genetic loci fails to effectively distinguish between those who will develop
the disease and those who won't, especially given that these polymorphisms are prevalent in healthy individuals at a frequency exceeding 1%(12,13).

The advantage of multi-locus investigations becomes apparent when compared to
single-locus inquiries. In multi-locus studies, the genetic risk factor profile can be employed to derive sensitivity versus 1-specificity graphs for various combinations of alleles(14,15). This approach allows for the creation of receiver operating characteristic (ROC) curves, and the area under these curves serves as a metric to validate the discriminatory ability of a continuous

biomarker (16,17,18). In summary, multi-locus investigations offer a more comprehensive understanding of genetic contributions to disease risk and enhance the ability to assess and validate the predictive potential of genetic markers in a clinical context (19).The significance of the present study underscores the necessity to communicate disease risk based on genetic profiles beyond mere odds ratios. This emphasis arises from prior research that, despite revealing a robust association with a disease, failed to provide indicative predictive value.

4. CONCLUSION:

In current clinical practice, only a limited number of specific pharmacogenetic tests are employed to facilitate dose adjustments. Typically, these tests rely on identified genetic variants that influence the pharmacokinetics of drugs, resulting in changes in the plasma levels of active drugs and/or metabolites. Nevertheless, pharmacogenetic testing is commonly conducted in response to medication-related issues experienced by patients. As a result, some experts in this field advocate for a proactive approach, asserting that "pre-emptive testing is the preferred strategy."

5. ACKNOWLEDGMENT:

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6. CONFLICT OF INTEREST:

The authors declared that there was no conflict of interest in the present study

7. REFERENCES:

1. Means RJ Jr, Rodgers G, Glader B, Arber DA, Appelbaum FR, Dispenzieri A, et al. Wintrobe's Clinical Hematology. Lippincott Williams & Wilkins; 2023. 6360 p.
2. Galbiatti ALS, Ruiz MT, Rezende Pinto D, Raposo LS, Maníglia JV, Pavarino-Bertelli EC, et al. A80G polymorphism of reduced folate carrier 1 (RFC1) gene and head and neck squamous cell carcinoma etiology in Brazilian population. Mol Biol Rep. 2010 Jul 27;38(2):1071–8.
3. Association between 11 genetic polymorphisms in folate-metabolising genes and head and neck cancer risk. Eur J Cancer. 2012 Jul 1;48(10):1525–31.
4. Soghani B, Ebadifar A, Khorshid HRK, Kamali K, Hamedi R, Moghadam FA. The study of association between reduced folate carrier 1 (RFC1) polymorphism and non-syndromic cleft lip/palate in the Iranian population. BioImpacts : BI. 2017;7(4):263.
5. Sage Journals [Internet]. [cited 2023 Nov 25]. Sage Journals: Your gateway to world-class journal research. Available from: https://journals.sagepub.com/action/cookieAbsent
6. Kim JW, Jeon YJ, Jang MJ, Kim JO, Chong SY, Ko KH, et al. Association between folate metabolism-related polymorphisms and colorectal cancer risk. Molecular and Clinical Oncology. 2015 May 1;3(3):639–48.
7. Naushad SM, Alrokayan SA, Almajhdi FN, Hussain T. Influence of RFC1 c.80A>G Polymorphism on Methotrexate-Mediated Toxicity and Therapeutic Efficacy in Rheumatoid Arthritis: A Meta-analysis. Ann Pharmacother [Internet]. 2021 Mar 22 [cited 2023 Nov 25]; Available from: https://journals.sagepub.com/doi/abs/10.1177/10600280211002053
8. Mansoori N, Tripathi M, Alam R, Luthra K, Sharma S, Lakshmy R, et al. Serum Folic Acid and RFC A80G Polymorphism in Alzheimer's Disease and Vascular Dementia. Am J Alzheimers Dis Other Demen [Internet]. 2014 Feb 19 [cited 2023 Nov 25]; Available from: https://journals.sagepub.com/doi/full/10.1177/1533317513505131
9. Website [Internet]. Available from: https://academic.oup.com/jn/article-abstract/143/12/2007/4614932
10. Hu YH, Zhou L, Wang SS, Jing X, Guo HL, Sun F, et al. Methotrexate Disposition in Pediatric Patients with Acute Lymphoblastic Leukemia: What Have We Learnt From the Genetic Variants of Drug Transporters. 2019 [cited 2023 Nov 25]; Available from: http://dx.doi.org/10.2174/1381612825666190329141003
11. Muralidharan N, Mariaselvam CM, Cb M, Negi VS. Reduced folate carrier-1 80G > A gene polymorphism is not associated with methotrexate treatment response in South Indian Tamils with rheumatoid arthritis. Clin Rheumatol. 2015 Mar 15;35(4):879–85.
12. Future Medicine [Internet]. [cited 2023 Nov 25]. [No title]. Available from: https://www.futuremedicine.com/doi/abs/10.2217/pgs.15.1

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13. den Hoed MAH, Lopez-Lopez E, te Winkel ML, Tissing W, de Rooij JDE, Gutierrez-Camino A, et al. Genetic and metabolic determinants of methotrexate-induced mucositis in pediatric acute lymphoblastic leukemia. Pharmacogenomics J. 2014 Nov 4;15(3):248–54.
14. Kodidela S, Dorababu P, Thakkar DN, Dubashi B, Sundaram R, Muralidharan N, et al. Association of NUDT15 c.415C>T and FPGS 2572C>T Variants with the Risk of Early Hematologic Toxicity During 6-MP and Low-Dose Methotrexate-Based Maintenance Therapy in Indian Patients with Acute Lymphoblastic Leukemia. Genes . 2020 May 28;11(6):594.
15. Farimani RM, Azimi-Nezhad M, KhorramKhorshid HR, Ebadifar A, Tohidkhah S, Jafarian Z, et al. Association between PTCH1 and RAD54B Single-Nucleotide Polymorphisms and Non-syndromic Orofacial Clefts in the Northeast Population of Iran. Avicenna J Med Biotechnol. 2022;14(4):310.
16. Natarajan SR et al. Identification of FOXM1 as a novel protein biomarker and therapeutic target for colorectal cancer progression: evidence from immune infiltration and bioinformatic analyses. International Journal of Biological Macromolecules. 2024;282(Pt 4):137201.doi: 10.1016/j.ijbiomac.2024.137201.
17. Yadalam PK et al. Prediction of interactome hub genes in oral cancer and chronic inflammatory periodontitis. Dental and Medical Problems. 2025;62(6):1099-1108.doi: 10.17219/dmp/18594.
18. Selvam SP, Pandiar D.Role of *Candida* in the Pathogenesis of Oral Squamous Cell Carcinoma: A Systematic Review. *J Clin of Diagn Res*.2025; 19(12):ZC05-ZC11. https://www.doi.org/10.7860/JCDR/2025/76916/22071
19. Balabadra KM, Panneer Selvam S, Ramadoss R, Sundar S. Hydroxyapatite synthesis and characterization from marine sources: A comparative study. *J Oral Biol Craniofac Res.* 2024;14(6):706-711. doi:10.1016/j.jobcr.2024.09.009.