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Genetic Association Of Aurora Kinase Gene (Aukra T>A) Polymorphism With Oscc

Ashwin Krishna B[1], Karthikeyan Murthykumar[2]

[1]Undergraduate, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,

Email id - 152001067.sdc@saveetha.com

[2]Senior lecturer, Department of Pedodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai-600077,

Email - karthikeyanmurthykumar@gmail.com

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ABSTRACT

Background: Aurora kinase A (AURKA) is a serine/threonine kinase that plays a pivotal role in mitotic spindle assembly, centrosome maturation, and chromosomal segregation. Disruption or upregulation of AURKA checkpoints can induce genetic instability and aneuploidy, serving as a critical driver in tumorigenesis. This study aimed to investigate the genetic association between the AURKA gene (T>A) polymorphism (rs2273535) and Oral Squamous Cell Carcinoma (OSCC) in a South Indian population.

Materials and Methods: A cross-sectional study was conducted utilizing venous blood samples from 100 participants partitioned into clinical groups, including a group of cases and periodontally healthy controls (n=25 per evaluated sub-cohort). Genomic DNA was isolated using a modified Miller's protocol. Polymerase Chain Reaction (PCR) amplification was executed using custom primers spanning the polymorphic site, yielding a 165 bp amplicon, followed by restriction fragment length polymorphism (RFLP) digestion utilizing the ApoI endonuclease. Genotype frequencies, allele distributions, and Hardy-Weinberg Equilibrium (HWE) compliance were statistically analyzed using Chi-Square tests and Odds Ratios (OR) via SPSS software. Results: The distribution of the AURKA rs2273535 polymorphism conformed to HWE in both cohorts (P > 0.05). Genotype frequencies for the case group were 0\% for AA, 52\% (n=13) for AT, and 48\% (n=12) for TT. For the control group, frequencies were 0\% for AA, 60\% (n=15) for AT, and 40\% (n=10) for TT. Statistical evaluation revealed no significant variation in overall genotype distribution between cases and controls (\chi^2, P = 0.5688). Furthermore, allele frequency shifts (T vs. A) showed no statistically significant correlation with elevated risk profile under dominant (P = 1.0000), recessive (P = 0.5693), or individual allelic (P = 0.6562) models. Conclusion: The findings demonstrate that the AURKA (rs2273535) gene polymorphism is not significantly associated with a susceptibility to oral lesions within the evaluated sample size. While AURKA aberrations remain a critical hallmark in broad human malignancies, larger multi-ethnic studies with expanded cohorts are required to fully elucidate its explicit synergistic impact on OSCC risk progression...

Keywords: Aurora Kinase A (AURKA), Gene Polymorphism, Single Nucleotide Polymorphism (SNP), Oral Squamous Cell Carcinoma (OSCC), rs2273535, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) , Genetic Susceptibility

INTRODUCTION

Aurora kinases are serine/threonine kinases that are essential for cell proliferation. They are phosphotransferase enzymes that help the dividing cell dispense its genetic materials to its daughter cells(1). More specifically, Aurora kinases play a crucial role in cellular division by controlling chromatid segregation. Aurora A and Aurora B kinases play important roles in mitosis. The Aurora A kinase is associated with centrosome maturation and separation and thereby regulates spindle assembly and stability(2). The Aurora B kinase is a chromosome passenger protein and regulates chromosome segregation and cytokinesis.Abnormal expressions of aurora kinase may disturb checkpoints function in mitosis(3). This can cause genetic disability and also triggers development of cancer. Aurora kinase A (AURKA) is present at 20q13 chromosome is also present along with Aurora kinase B which is AURKB and C which is AURKC. It acts as the checkpoint in the kinase cell cycle and also influences the transformation of phases from G2 in which preparation for division takes place to M phase where mitosis takes place in the cell cycle(4) . Self renewal and new programming of stem cells also have the functions shown by these genes. Increased expression of Aurora kinases leads to disturbance in the checkpoints in the mitosis which could be a cause of the OSCC. To our knowledge no investigation has been conducted on genetic association of aurora kinase polymorphism with chronic periodontitis(5).

Oral squamous cell carcinoma is a malignant tumour that may occur anywhere within the oral cavity. It is the most common oral cancer, representing about 80%–90% of all malignant neoplasms of the mouth(6). Its prevalence varies in different parts of the world; Among all countries, the Asian continent has the highest incidence and mortality rates for oral cavity and oropharynx cancers. It occurs in the oral cavity and oropharynx and can be caused by a variety of etiological factors, but smoking and alcohol are considered to be the most common risk factors, particularly in the Western world(7)(8–12).The main etiological variables linked to OSCC in South Asian nations are the use of smokeless tobacco and areca nut products. It is locally invasive, infrequently metastasises to ipsilateral regional lymph nodes, and rarely spreads to distant sites. It is the world's sixth leading cause of cancer death with a mortality rate of greater than 50%. These types of cancers have high rates of systemic dissemination(13)..

Aurora Kinase A (AURKA) is a low-penetrance tumour susceptibility gene that plays a role in centrosome maturation, centrosome separation, which encodes a centrosome related serine/threonine kinase, is amplified and highly expressed in several human malignancies, including advanced OSCC(14). Other factors that have been linked include human papillomavirus (HPV) and Candida infections, dietary inadequacies, and genetic predisposition. The majority of cases of oral and oropharynx OSCC occur in elderly male patients, with tongue and tonsils being the most commonly affected sites. The presence of advanced disease is one of the most important prognostic variables in OSCC patients. As a result, early detection of oral cancer is a critical strategy for lowering morbidity and mortality. Surgical resection with or without adjuvant therapy is the standard of care for OSCC(15). Patients with oral squamous cell carcinoma (OSCC) account for more than 90% of all malignant tumours in the head and neck. Apart from cell division, stem cell self-renewal and reprogramming have also been emphasised. Upregulation of AURKA leads to an increase in centrosome numbers and an increase in aneuploidy, which is a pretty typical occurrence in head and neck squamous cell carcinoma, with up to 90% of all tumours showing this(16).

Taken together, our findings strongly show that AURKA plays a key role in tumorigenesis, indicating that AURKA is a promising target for cancer treatment. Some researchers demonstrated that the Caucasians harbouring AURKA rs1047972 T>C was associated with an increased risk of breast cancer which shows that there are links between AURKA genes with major cancers but studies have proven, to have this link of AURKA with OSCC(17). So the primary goal of this study is to see if there is a link between all types of Aurora Kinase gene polymorphism and oral squamous cell cancer with consideration that there are no studies investigating the influence of the AURORA kinase polymorphism on expression of OSCC. The present study was carried out with the aim of getting the results to express the genetical association of AURORA kinase polymorphism with OSCC.

MATERIALS AND METHODS:

This study employed a cross-sectional design involving individuals from the South Indian population. A total of 100 individuals who reported to the Department of Periodontics, Tamil Nadu, were included in this study. The participants were divided into a control (Group A, n=25) and CP (Group B, n=25) based on the clinical examination of probing pocket depth, clinical attachment loss, and bleeding on probing. The CP group contained 25 patients (male – 17 and female – 8) with the mean age of 39.02±8.22. The CP patients were recruited based on the criteria of American Academy of Periodontology-2008. The control group contained 25 periodontally healthy participants (male – 17 and female – 8) with mean age of 41.34±7.49. A detailed history of dental treatment, family history of periodontal diseases, smoking habits, as well as general health concerns were obtained from the participants. Except for the presence of periodontitis, the patients included in this study were systemically healthy. Smokers, pregnant or lactating mothers, immunocompromised individuals, and participants who underwent periodontal therapy within the past 6 months were excluded from this study. The study was approved by the Institutional Ethical Committee (SRB/MDS/PERIO/18-19/0004).

Sample Collection and Deoxyribonucleic Acid Extraction

A volume of 2 ml of venous blood was collected from antecubital fossa and dispersed into a sterile tube containing a pinch of ethylene diamine tetraacetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller et al. 1998 protocol.

Polymerase Chain Reaction and Restriction Endonuclease Digestion

MMP8 (-799C/T) gene (SfcI) polymorphisms were assessed by PCR amplification and restriction digestion. The following primers, forward primer: 5'-CTGTTGAAGGCCTAGAGCTGCTGCTCC-3' and reverse primer: 5'-GATCTTCTCTTCAAACTCT ACCC-3', were used for amplification of DNA spanning the SfcI polymorphic site, of the MMP8 gene. The amplification of DNA was performed in 20µl volumes using 10ng of genomic DNA and 5 pmol/µl each of forward and reverse primers along with PCR master mix (Takara, Japan). The cycling conditions were as follows: Initial denaturation at 94ºC for 5 min, denaturation at 94ºC for 35 s, annealing at 60ºC for 35 s, extension at 72ºC for 35 s, and a final extension at 72ºC for 5 min. 5 µl of PCR product was checked on a 1% agarose gel. 15 µl of PCR product was digested using SfcI restriction enzyme procured from New England Biolabs, England. Digestion was carried out at 37ºC for 2 h. The digested product was visualized on a 2% agarose gel, and the results were documented.

Statistical Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, IL). The distribution of genotypes and allele frequencies in the CP and control groups was compared using the Chi Square test. The risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence intervals. Statistical significance in all tests was determined at $P < 0.05$.

RESULTS:

Table 1: Genotype frequencies of AURKA gene polymorphism (rs2273535) among the cases and controls

Groups	AA	AT	TT	A	T	HWE (p value)*
Case (N=25)	0	13	12	0.26	0.74	0.07
Control (N=25)	0	15	10	0.30	0.70	0.211

*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 $\chi 2df$ ($P = 0.5688$).

Table 2: Overall genotype distribution of the AURKA gene polymorphism (rs2273535) in cases and controls

Genotypes	Case	Control	Unadjusted OR [95% CI]	P value
Dominant				
AA	0	0	1.0000 [0.0191 - 52.365]	1.0000
AT + TT	25	25		
Recessive				
AT + AA	13	15	0.7222 [0.2355 - 2.215]	0.5693
TT	12	10		
Allele				
A	13	15	0.8198 [0.3418 - 1.9662]	0.6562
T	37	35		

Figure 1: Agarose gel electrophoretogram showing partial amplification of site spanning AURKA gene polymorphism (rs2273535) run along with standard DNA ladder [Lane M = 100 bp DNA marker]

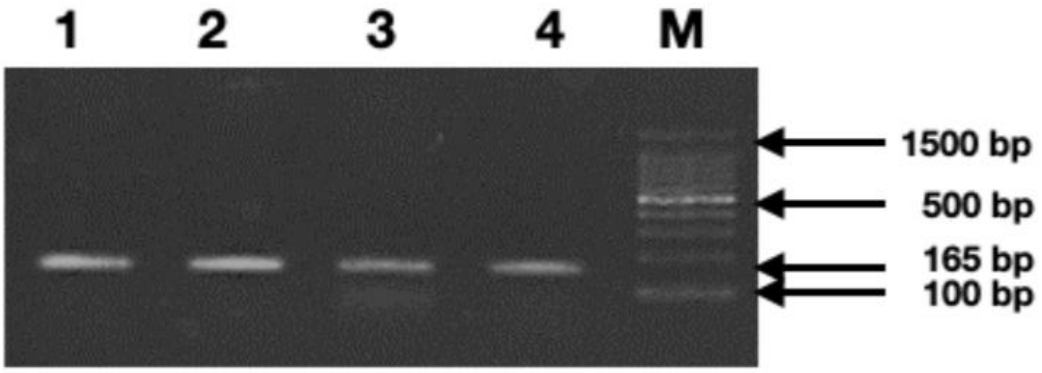
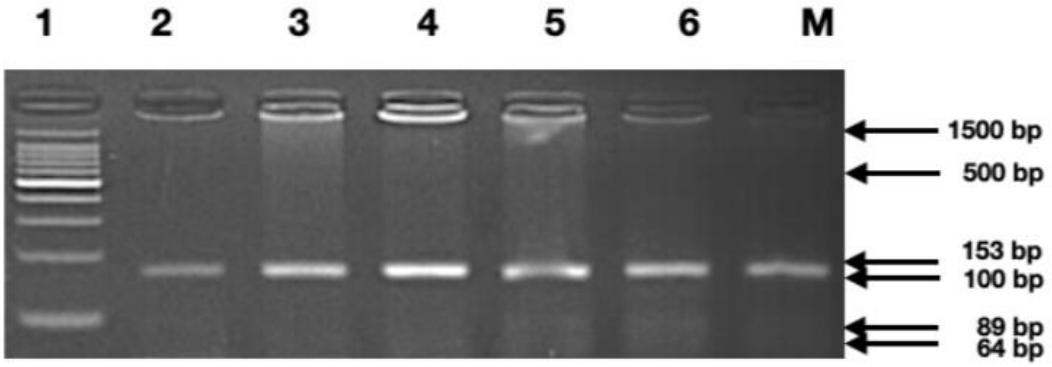
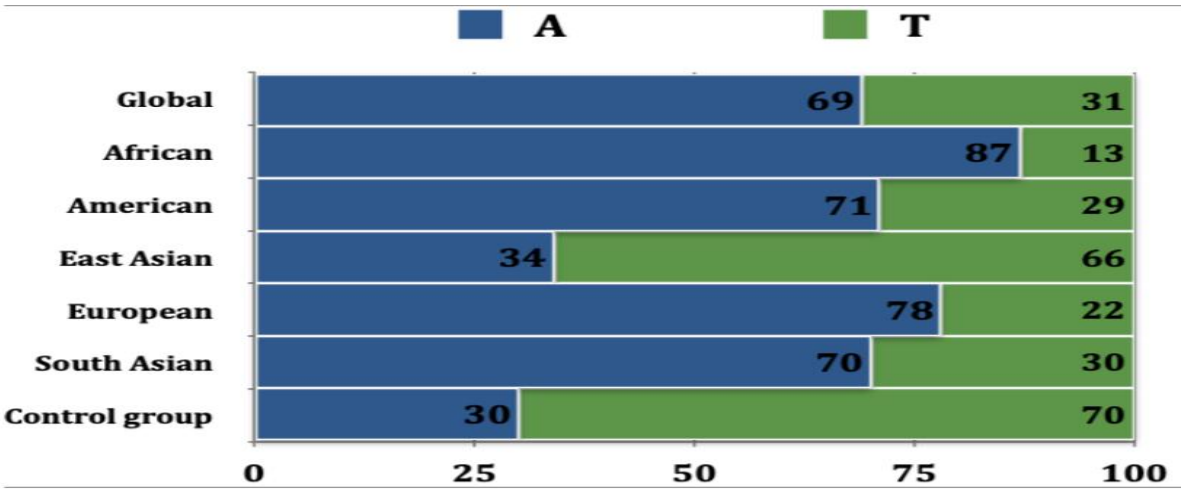


Figure 2: Agarose gel electrophoretogram showing ApoI digested amplicon of spanning site of AURKA gene polymorphism (rsrs2273535) (Homozygous AA - 89+64+12 bp; Heterozygous TA - 153 + 89 + 64 + 12 bp; Homozygous TT - 153+12 bp) [Lane M = 100 bp DNA marker] Only heterozygous genotype was observed. NC - Negative control (Non-template control)



Graph 1: The graph depicts the allele frequency of AURKA gene polymorphism (rs2273535) in different population [Data acquired from Ensembl database]



PCR information:

Primer sequence:

AURKA TA-F: 5'-CTTTCATGAATGCCAGAAAGTT -3'

AURKA TA-R: 5'-CTGGGAAGAATTTGAAGGACA-3'

Amplicon size: 165 bp,

Annealing temperature: 54 degree C for 20 seconds.

DISCUSSION:

AURKA is a serine-threonine kinase that seems to be necessary for the maturation of centromeres and plays a role in cytokinesis and mitosis that follow. AURKA inhibited the transcription factor N-MYC that promotes degradation in a prior study, accelerating the G1-S transition(18). However, AURKA is a crucial component of T cell activation, which mostly happens via the Lck signal and includes T cells in the immune response to cancer. AURKA's activity may be influenced by its genetic polymorphism; for example, a reduced kinase activity of AURKA brought on by a different SNP may result in genomic instability and tumour. Genetic vulnerability is important in different cancer types, and mitosis-regulating AURKA overexpression is highly connected with cancer progression(19). It may be possible to manage the risk of developing cancer by identifying the specific AURKA gene involved in cancer susceptibility. AURKA is regulated by genotypic polymorphisms in mitosis, which have been previously reported. While Caucasian carriers are not at an increased risk of breast cancer, Asian carriers of AURKA rs2273535 are. AURKA rs2273535 might make people more vulnerable to UTUC(20). Additionally, patients who received alisertib for metastatic urothelial cancer and who have the AA genotype of

rs1047972 have longer progression-free survival than patients who have the wild-type TT genotype. Oral cancer risk is elevated by the AURKA rs2273535 (T91A) polymorphism(21).

Since the beginning of the twentieth century, there has been extensive interest in the relationship between centrosome abnormality and malignant tumours. Chromosomes must be separated properly, which is dependent on the centrosome's role in guaranteeing symmetry and dual polarity during cell division. The aberrant chromosomal separation in malignant tumours can be traced back to centrosome dysfunction, it has been demonstrated. There have been claims linking AURKA to a number of cancers, including pancreatic and colorectal cancer(22). Promoter regions, exons, introns, and 3'-untranslated regions may all be affected by genetic variations, which may then impact how a gene is expressed. In mammalian cells, increased centrosome amplification and chromosomal instability may result from increased AURKA expression. No studies have looked into the connections between AURKA SNPs and the emergence of oral cancer as of yet. In order to reveal the role of AURKA rs2273535 in cancer risk, Wang et al. performed a systematic review using meta-analysis. They found that the allele shift from T to A in rs2273535 was associated with an overall higher risk of tumours, specifically breast cancer(23).

An earlier investigation established the role of the LIN28B-RAN-AURKA axis in the development of neuroblastoma. Aurora Neuroblastoma A overexpression is linked to advanced clinical stages, MYCN amplification, disease progression, and recurrence. The heterozygous AurkA type (Phe/Ile) was more strongly expressed in tumour samples than in the control cohort, according to PCR examination of HNSCC and benign tissues(24). Concurrently, a higher incidence of the heterozygous AurkA genotype in esophageal carcinomas has been discovered, which has been linked to accelerated tumour development(25). Additionally, the AurkA polymorphism has been linked to various cancers, such as breast cancer and hepatocellular carcinomas. Patients who have UCC and elevated AURKA mRNA expression in tumour tissues not only have significant rates of cancer metastasis and high-grade malignancies, but also have comparatively low overall and UCC-specific survival rates(21). Overexpression of AURKA promotes cancer spread, increases medication resistance, and is linked to a poor prognosis.

CONCLUSION:

The susceptibility to and clinicopathologic status of OSCC are related with a number of SNP variations of AURKA. The results suggest that the clinical course and progression of OSCC can be affected by the association between the EGFR genotype and the AURKA SNP. Future research with bigger sample numbers and diverse ethnic groups is necessary to fully understand whether AURKA SNPs affect OSCC risk. The outcome of the treatment could be improved by carefully defining patient classification..

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