RESEARCH PAPER

Genetic Association Of Aurka Gene Polymorphism (Rs6024836) With Susceptibility To Oral Squamous Cell Carcinoma– A Case Control Study

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ABSTRACT:

INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is the world's sixth leading cause of cancer death with a mortality rate of more than 50%.Many human malignancies, particularly advanced OSCC, have AURKA, which encodes a centrosome-related serine/threonine kinase, amplified and overexpressed gene .Aurora Kinase A, also known as AURKA has been identified as a definite low-penetrance tumor susceptibility gene is involved in several mitotic events including centrosome maturation.

AIM: The aim of the study is to evaluate the genetic association of AUKRA gene polymorphism with susceptibility to oral squamous cell carcinoma- a case control study in south indian population.

MATERIALS AND METHODS:

A total of 50 individuals who reported to the Department of oral surgery, Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N = 25) and CP group B (N =25) based on the clinical examination .

RESULTS:

The results of the study reveal that the AURKA gene is associated with susceptibility and clinicopathologic status of oral squamous cell carcinoma.

CONCLUSION:

From the study it has been concluded that there is a significant association of AURKA gene polymorphism with susceptibility to oral squamous cell carcinoma.

KEYWORDS: Aurora kinase, cancer,genetic association,malignancy

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INTRODUCTION

The Aurora kinases are importantly involved in cell cycle and they exhibit most of their known functions in mitosis. They are involved in some checkpoint regulation pathways including spindle assembly checkpoint, alignment of metaphase chromosomes and chromosomal bi- orientation(1). Aberrant expression of Aurora kinases may disturb checkpoint functions particularly in mitosis and this may lead to genetic instability and trigger the development of tumors(1,2). Aurora kinases have gained much attention since they were identified as bona fide oncogenes and play important functions during mitosis, so their aberrant expression can lead to changes in the cells underlying the cancer.(3)

Oral squamous cell carcinoma (OSCC) is the world's sixth leading cause of cancer death with a mortality rate of more than 50% respectively.Despite recent breakthroughs in surgery and adjuvant treatment choices, total cure is obtained in only around half of the patients(4).Unlike many other cancers, HNSCC rarely has distant metastases at the time of diagnosis, although it does have a higher rate of systemic dissemination(2). Many human malignancies, particularly advanced OSCC, have AURKA, which encodes a centrosome-related serine/threonine kinase, amplified and overexpressed.Patients with oral squamous cell carcinoma (OSCC) account for more than 90% of all malignant tumors in the head and neck(5).Aurora Kinase A, also known as AURKA has been identified as a definite low-penetrance tumor susceptibility gene is involved in several mitotic events including centrosome maturation, centrosome separation, and mitotic entrance during the G2 to M phase.Members of Aurora kinase family have been found over- expressed in various types of commonly occurring epithelial carcinomas.Indeed, both Aur-A and -B have been found to be overexpressed in oral cancers..The activity of aurora A is dependent on p53, as p53 can inhibit the function of aurora by directly binding to its catalytic domain.

In addition to specializing in cell division,it's been pronounced to additionally modify the self-renewal and reprogramming of stem cells. Upregulation of AURKA causes an increase in centrosome numbers and an induction in aneuploidy and is a fairly common occurrence in head and neck squamous cell carcinoma and can be observed in up to 90% of all tumors. Moreover overexpression of Aur-A promotes Ras-induced oncogenic transformation. Taken together these results strongly suggest the important role of AURKA in tumorigenesis.Thus the prime objective of the present study is to evaluate the association of aurora kinase gene polymorphism with oral squamous cell carcinoma.

MATERIALS AND METHODS:

This study employed a cross-sectional design involving individuals from Chennai, Tamil Nadu, India. A total of 50 individuals who reported to Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N = 25) and OSCC group B (N = 25) based on clinical examination. The OSCC group contained 26 patients (17 male, 8 female) between 40 to 60 years. The control group contained 25 healthy subjects (17 male, 8 female) between 40 to 60 years.

A detailed history of dental treatment, family history of OSCC, smoking habits as well as general health concerns were obtained from the subjects. Except for the presence of OSCC, the patients included in this study were systemically healthy. Pregnant or lactating mothers, immunocompromised individuals and subjects who had undergone surgery within the past 6 months were excluded from this study. The study was approved by the institutional ethics committee.

Sample collection and DNA extraction

A volume of 5 mL of venous blood was collected from the antecubital fossa and dispersed in a sterile tube containing a pinch of ethylenediaminetetraacetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller et al 1998 protocol (22) Polymerase chain reaction and restriction endonuclease digestion Estrogen receptor gene (ESRI) polymorphisms were assessed by polymerase chain reaction(PCR) amplification and restriction digestion.

The primers forward 5'-TTCTAGGCTACAGCTCCAGTT -3' and reverse 5'-GTTTACCAGGTGCCGATG -3'were used for amplification of DNA spanning the ESRI polymorphic site of the ESR gene. The amplification of DNA was performed in 20-L volumes using 10 ng of genomic DNA, 5 pmol/L each of the forward and reverse primers along with PCR Master Mix (Takara, Shiga, Japan). The cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 35 seconds, annealing at 60°C for 35 seconds, extension at

72°C for 35 seconds and a final extension at 72°C for 5 minutes. A 5-uL volume of PCR product was checked on a 1% agarose gel, and 15 L of PCR product was digested using a ESRI restriction enzyme (New England Biolabs, Hitchin, UK). Digestion was carried out at 37°C for 2 hours.The digested product was visualized on 2% agarose gel and the results were documented.

Statistical analysis:

All statistical analyses were performed using SPSS version 23.0 for Windows (SPSS, Chicago, IL, USA). The distribution of genotypes and allele frequencies in the OSCC and control groups were compared using the - 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 set at $P < .05$.

Demographic details of OSCC patients and normal healthy subjects

Details	OSCC Cases (N=25)	Normal healthy subjects (N=25)
Gender ratio (Male: Female)	2: 1 Male: 17 Female: 8	2: 1 Male: 17 Female: 8
Age Range	40 - 60 years	40 - 60 years
Smoking habits	N = 17	N = 5
Alcoholic	N = 6	N = 0
Tumor grade	Well differentiated = 8 Moderately differentiated = 9	NA

RESULT:

Table 1: Genotype frequencies of AURKA gene polymorphism (rs6024836) among the cases and controls

Groups	GG	AG
Case (N=25)	0	25
Control (N=25)	0	25

For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freed

Figure 1: Agarose gel electrophoretogram showing partial amplification of site spanning AURKA gene polymorphism (rs6024836) run along with standard DNA ladder [Lane M = 100 bp DNA marker

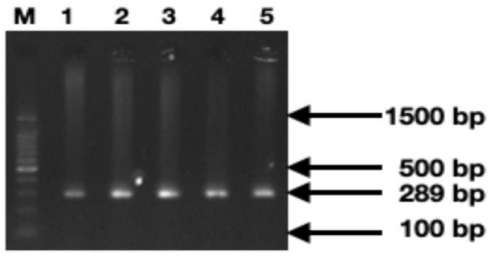
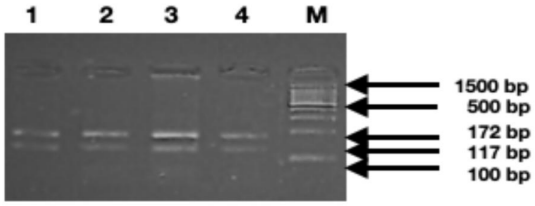
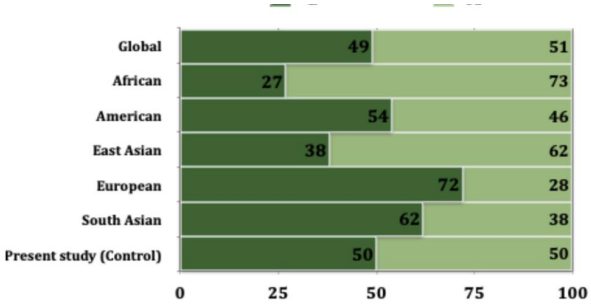


Figure 2: Agarose gel electrophoretogram showing ApoI digested amplicon of spanning site of AURKA gene polymorphism (rs6024836) (Homozygous GG - 289 bp; Heterozygous AG - 289 + 172+ 117 bp; Homozygous AA - 172 + 117 bp) [Lane M = 100 bp DNA marker] Only heterozygous genotype was observed.



Graph 1: The graph depicts the allele frequency of AURKA gene polymorphism (rs6024836) in different population [Data acquired from Ensembl database]



PCR information:

Primer sequence:

AURKA GA-F: 5'- TTCTAGGCTACAGCTCCAGTT -3' AURKA GA-R: 5'- GTTTACCAGGTGCCGATG -3'

Amplicon size: 289 bp, Annealing temperature: 58 degree C for 30 seconds.

DISCUSSION:

In this study we observed the association of aurora kinase gene polymorphism (G>A) with oral squamous cell carcinoma.Mitosis-regulating AURKA overexpression is strongly associated with cancer

progression ,and genetic susceptibility is critical in various cancer types. Identifying the specific AURKA gene involved in susceptibility to cancer may aid the management of cancer risk.Overexpression of AURKA has been demonstrated in a variety of human cancers,including breast cancer,head and neck squamous cell carcinoma, colorectal cancer,ovarian cancer, and advanced OSCC.(6)Aberrant expression of AURKA may lead to a high degree of chromosomal instability in tumors, further susceptibility to malignant transformation.(7)AURKA has been shown to be associated with progression, survival, histologic differentiation, and metastasis of a variety of tumors. In head and neck cancers, AURKA overexpression is significantly associated with progression or survival.(8) In addition,previous studies reported that HNSCC cells and tissues overexpressed AURKA, and knockdown of AURKA by siRNA alone or in combination with paclitaxel significantly increased HNSCC cell proliferation in vitro.A previous study showed that the allelic variant AURKA 91A (rs2273535) was associated with a higher risk of oral cancer and also show that the AURKArs2273535 polymorphism is associated with an increased risk of breast cancer, especially in Asians.

Despite advances in treatment over the past two decades, OSCC remains one of the tumors with a poor prognosis. Considering the amplification/overexpression of Aurora Kinases in many cancers, many small molecule inhibitors of Aurora kinases have recently been developed, such as ZM447439, 41 Hesperidin, 42 VX-680 (MK-0457), and AZD1152.A study found that the *AURKA* rs2273535 polymorphism increases the risk of breast cancer, and that the rs1047972 polymorphism is a protective factor for breast cancer .A study conducted in Korea revealed that the *AURKA* rs2273535 polymorphism was in strong LD with the rs1047972 genotype, and that patients with the *AURKA* haplotype variants had high kinase activity and a high risk of progression to advanced stage gastric cancer . These results indicate that the *AURKA* gene variants might have different functional roles in different cancers.

Similarly *AURKA* overexpression also promotes cancer metastasis, increases drug resistance, and is associated with poor prognosis. In a study on 786 men with oral cancer, patients with the GG genotype and G allele of *AURKA* rs2064863 were at a 1.365-fold higher risk of stage III or IV oral cancer than were those with the wild-type AA genotype. Other reports on hepatocellular carcinoma have indicated that patients with at least one A allele (A/T or A/A genotype) in *AURKA* rs2273535 were less likely progress to stage III or IV (0.593-fold) and develop large tumors (0.591-fold).

CONCLUSION:

From the study it has been concluded that there is a significant association of AURKA gene polymorphism with susceptibility to oral squamous cell carcinoma.In conclusion, several variants of AURKA are associated with susceptibility to and clinicopathologic status of OSCC. Our data provided evidence that carriers of the G>A allele of *AURKA* rs6024836 were at a higher risk of OSCC. Thus, the AURKA SNPs may be significant predictors of Oral squamous cell carcinoma occurrence and reliable biomarkers of disease progression and metastasis in patients with OSCC.

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CONFLICTS OF INTEREST:

The authors declare that there are no conflicts of interest in the present study

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