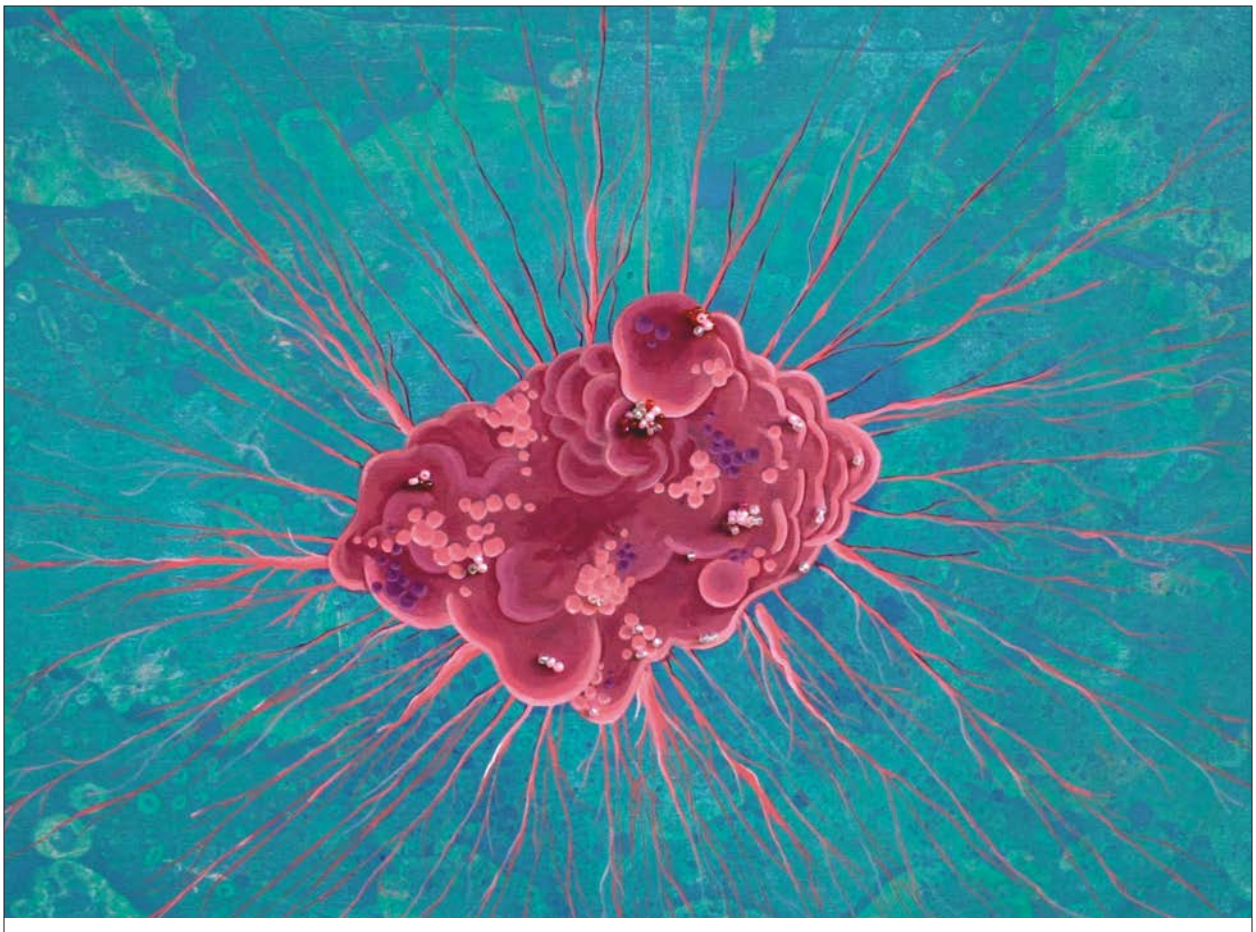

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Research Article
Single Nucleotide Polymorphism of *MSH3* Gene Alters Head and Neck Squamous-Cell Carcinoma Risk in North-India

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Abstract

Background and Objective: Squamous cell carcinoma of head and neck (SCCHN) is attributed by tobacco chewing and smoking which are known to cause DNA damage. Eventually such damage if get fixed as mutation can enhance the risk of SCCHN. These damaged are repaired by DNA repair protein such as MutS Homolog 3 (MSH3). Therefore, genetic polymorphisms in *MSH3* gene which affect its efficiency can modulate the risk of cancer including SCCHN. The present study examined the association of MSH3 rs26279 G>A polymorphism with the risk and clinical outcome of SCCHN. **Materials and Methods:** One Hundred and eighty six patients of SCCHN and 188 cancer free healthy control subjects were genotyped by PCR-RFLP method. Genotypes of SCCHN patients were further correlated with their disease status including stage, grade, tumour size, metastasis and lymph node involvement. **Results:** The risk of developing SCCHN was 2 times more with GG genotype compared to AA genotype for MSH3 G>A polymorphism (OR = 2.61, 95% CI 1.06-6.73, p-value = 0.05). The G allele itself imparted significantly higher risk for developing SCCHN compared to A allele (OR = 1.55, 95% CI 1.08-2.26, p-value = 0.01). Variant allele genotype (AG+GG) of MSH3 G>A polymorphism were associated with the development of high staged (III+IV) and large sized tumour (T3+T4). Compared to the common allele genotype (AA) the risk of developing high staged and large sized tumour were 2 times more with variant allele genotype (OR = 2.34, 95% CI = 1.19-4.58, p-value = 0.02 and OR = 2.14, 95% CI 1.32-4.40, p-value = 0.006, respectively). **Conclusion:** Result from the present study suggested that MSH3 rs26279 G>A polymorphism cannot only modify the risk for developing SCCHN but also the clinical status of tumour in SCCHN patients.

Key words: MSH3, polymorphisms, SNP, head and neck cancer, epidemiology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Squamous cell carcinoma of head and neck (SCCHN) develops from squamous cells present in mucous membranes and in the outer layer of skin. It can develop in mouth, naso-pharynx, throat, hypo-pharynx, larynx and trachea. It is the 7th most common cancer worldwide, with incidences of >30 per 100000 population in India. Risk of SCCHN increases with age as, individuals >50 years are comparatively more susceptible to develop SCCHN. Smoking tobacco, drinking alcohol and having a poor diet are important risk factors in the West and smoking bidis, chewing betel or areca nuts and taking snuff are important in the Indian subcontinent[1]. However, only a limited fraction of smokers and alcoholics develop SCCHN which is possibly due to a differential susceptibility to disease in overall population. Smoking induces DNA damage which if remains unrepaired due to faulty DNA repair system, can induce carcinogenesis. Polymorphisms in DNA repair gene which can alter the functions of the respective proteins can, therefore, alter the DNA repair efficiency as well as the susceptibility to cancer.

Mismatch repair (MMR) is a mechanism for identifying and repairing mis-incorporation of bases, insertion and deletion and thus plays crucial role in maintaining genomic stability[2]. Majority of mismatches are produced by replication errors. Mismatch repair consists of five major steps viz: Mismatch recognition, assembly of the repair complex, strand discrimination, degradation of the mismatch-containing strand and re-synthesis of the excised strand[3].

MSH3 is a protein coding gene located on chromosome number 5q14.1. MSH3 forms a heterodimer with MSH2 to form MutS beta which recognizes large insertion-deletion loops (up to 13 nucleotide long) and binds with mismatched DNA, thereby initiating DNA repair by forming ternary MutL alpha heterodimer complex. This MutL alpha is responsible for regulating mismatch repair (MMR) events like strand discrimination, excision and re-synthesis[4]. Any polymorphism in this gene may result the in the change of MSH3 protein formation which will eventually lead to the non-recognition of DNA mismatch, thereby not rectifying it and thus increasing the risk of cancer susceptibility. Several studies have shown association of MSH3 polymorphisms with several cancers including cancer of colon, breast and prostate[5,6]. In the present study, it was hypothesised that MSH3 rs26279 G>A polymorphisms may alter the risk and clinical outcome of SCCHN.

MATERIALS AND METHODS

Study subjects: The subjects in this study belonged to North Indian population. The cases (186) were registered in Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow (DRMLIMS) or King George's Medical University (KGMU), during 2013-2016, Lucknow for the treatment of SCCHN. The mean age of patients in this study was 52.13 (range 28-92 years). Cancer free healthy controls (188) were also selected from the same geographical area, ethnic background and approximately from similar age group as the cases. The studies have been approved by the appropriate institutional and/or national research ethics committees and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Sample collection: The blood samples of healthy controls and clinically and histologically confirmed SCCHN patients were collected after taking their consent. The venous blood was drawn in EDTA vials, thoroughly mixed and kept at -20°C.

Genomic DNA isolation and its quantification: Genomic DNA was isolated from collected blood samples using GenElute Blood Genomic DNA Miniprep kit (Sigma Aldrich, USA) as per manufacturer's protocol. The isolated DNA was quantified using QUBIT 2.0 Fluorometer (Invitrogen, USA). The quality of DNA was further visualized on 0.8% agarose gel having 0.5 μg mL^{-1} ethidium bromide.

Genotyping for MSH3: Genotyping for MSH3 polymorphism was performed by PCR-RFLP method. PCR was carried out in 10 μL volume reactions containing 20 ng DNA, 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.11 mM each DNTP, 0.3 μM each primer and 0.5 U *Taq* DNA polymerase (Sigma Aldrich, USA). Forward and reverse primer used for PCR were, F: 5'TCT AACAGG CAA GTA GGA AC 3' and R: 5' TAG CCA CAT TTA ATC CAT AAC 3'. PCR products were further digested with endonuclease Hha I (NEB, USA) for 1 h at 37°C. These digested products were further visualized on 2% agarose gel having 0.5 μg mL^{-1} ethidium bromide (Fig. 1).

Statistical analysis: The statistical significance for deviations from Hardy-Weinberg Equilibrium (HWE) was calculated using the Pearson Chi square-test. To determine the difference

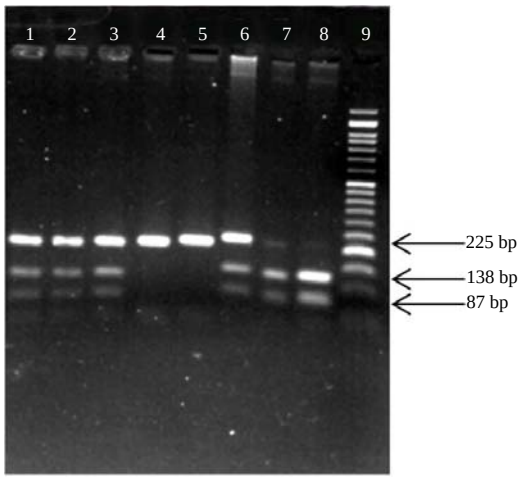

Fig. 1: Different genotypes of MSH3 polymorphism on 2% agarose gel
Lanes 4 and 5: AA genotype 225 bp, Lanes 1, 2, 3 and 6: AG genotype 225 bp, 138 bp, 87 bp, Lanes 7 and 8: GG genotype 138 bp, 87 bp, Lane 9: 50 base pair ladder

between the SCCHN cases and controls with respect to genotype distributions and allele frequencies, Chi square-test (Yates corrected) was used. It was also used to find out difference in distribution of genotype between different disease categories. Odds ratios (OR), 95% confidence interval (CI) and p-values for the assessment of associated risk due to genotypes and variant allele of studied polymorphisms were calculated by Epi-Info programme (http://wwwn.cdc.gov/ epiinfo/,center for disease control and prevention).

RESULTS AND DISCUSSION

Demographic detail of the study subjects including SCCHN cases and healthy control were listed in Table 1. All together 186 SCCHN cases and 188 healthy controls are included in the present study. Subjects of cases and control are matched for sex and ethnicity. The mean age for subjects for cases and control were 51 and 38 years, respectively. Smoking and tobacco consumption have been found to significantly increase the risk of SCCHN. Similar effect of smoking and tobacco chewing on SCCHN has been documented previously in oral and pharyngeal cancers in US[7]. The joint exposure of alcohol and tobacco has even higher risk[8]. The risk of cancer at larynx and hypo-pharynx was observed to be 10 times more in smokers and reduced after quitting[9]. Similarly more than joint, multiplicative risk of tobacco chewing and alcohol and consumption has also been

Table 1: Demographic characteristics of study subjects

Variable	Case	Control	p-value
Age			
Years	21-92	18-71	
Mean age	51.47	37.70	
Sex			
Female	37	29	Ref
Male	149	159	0.318
Smoking			
Non smoker	76	116	Ref
Smoker	110	71	<0.0001
Tobacco			
Non chewer	45	151	Ref
Chewer	141	36	<0.0001
Disease categories			
Stage			
High (III+IV)	126 (68%)		
Low(I+II)	60 (32%)		
Grade			
>1	100 (54%)		
1	86 (46%)		
T-status			
T3+T4	94 (51%)		
T1+T2	92 (49%)		
Lymph node			
N2+N3	53 (30%)		
N0+N1	123 (70%)		
Metastasis			
M1	30 (16%)		
M0	153 (84%)		
Site of cancer			
Alveolus	25 (16%)		
Tongue	33 (19%)		
Buccal mucosa	30 (17%)		
Supraglottic region	08 (5%)		
Submandibular lymph node	14 (8%)		
Pharynx	30 (17%)		
Larynx	33 (19%)		

reported for head and neck cancer[10]. Most of cancer patients had been presented with high stage (III+IV) and grade (>1) disease. However, proportion of patients without metastasis was lower than the patients with metastasis (Table 1).

Genotype distribution for MSH3 rs26279 G>A polymorphisms among the cases and control subjects were listed in Table 2. The frequency of GG genotype was significantly higher in cases than in control (p = 0.05) and the risk of developing SCCHN was 2 times more with GG genotype compared to AA genotype (OR = 2.67). In addition compared to A allele the risk of disease was significantly higher with G allele (Table 2). The GG genotype of MSH3 polymorphism has also been reported to increase the risk of oesophageal and colon cancer[11,12]. Similarly, the GG genotype of MSH3 polymorphism along with the GG genotype of MSH2 polymorphism have been previously reported to increase the

Table 2: Distribution of genotypes and allele from MSH3 A>G polymorphism among the SCCHN cases and control

Genotypes	Case (%)	Control (%)	p-value	OR	95% CI
AA	112 (60)	131 (70)	-	Ref	
AG	58 (32)	50 (26)	0.229	1.35	0.86-2.13
GG	16 (8)	7 (4)	0.052*	2.67*	1.06-6.73*
A allele	282 (76)	312 (83)			
G allele	90 (24)	64 (17)	0.019*	1.55*	1.08-2.26*

*Significant associations

Table 3: Distribution of genotypes for MSH3 A>G polymorphism among different disease categories

	Tumour stages				
Genotypes	High (III+IV) n (%)	Low (I+II) n (%)	p-value	OR	95%CI
AA	68 (54)	44 (73)		Ref	
AG+GG	58 (46)	16 (27)	0.02*	2.34*	1.19-4.58*
	Tumour grade				
	>1 n (%)	1 n (%)			
AA	62 (62)	50 (58)		Ref	
AG+GG	38 (38)	36 (42)	0.69	0.85	0.47-1.53
	Tumour T-status (size)				
	T3+T4 n (%)	T1+T2 n (%)			
AA	47 (50)	65 (71)		Ref	
AG+GG	47 (50)	27 (29)	0.006*	2.41*	1.32 - 4.40*
	Lymph node involvement				
	N(N1+N2+N3) n (%)	N0 n (%)			
AA	53(55)	49 (62)		Ref	
AG+GG	44 (45)	30 (38)	0.40	1.35	0.74-2.48
	Tumour metastasis				
	M n(%)	M0 n(%)			
AA	21 (70)	91 (59)		Ref	
AG+GG	9(30)	62 (41)	0.38	0.62	0.27-1.46

*Significant association

risk of laryngeal squamous cell carcinoma[13]. It is possible that G allele reduces the enzymatic activity of MSH3 and thus imparts risk for developing cancer. However, bioinformatics algorithm including SHIFT, PolyPhen and Allign-GVGD predicted neutral functional nature of amino acid change in MSH3 rs26279 G>A polymorphisms[12].

Distribution of genotypes for MSH3 rs26279 G>A polymorphisms among different disease categories were listed in Table 3. Since the frequency of GG genotype is very less among different disease categories, genotypes were dichotomised as common allele (AA) and variant allele (AG+GG) genotypes. Different genotypes of MSH3 polymorphism did not show any association with high or low grade diseases (Table 3). Similarly, none of the genotypes were associated with the risk of nodal involvement or tumour metastasis. However, risk for developing large sized tumour (T3+T4) was two times more with variant allele genotype (AG+GG) than the common allele genotype (AA) (Table 3). A recent report has also suggested development of lager size tumour in nasopharyngeal carcinoma with lack of function in MSH3[14]. Present study can possibly implicated to identify susceptible group in the population who are more prone to develop SCCHN. Not only that but information from present study can also be applied to predict clinical characteristics of tumour in patients once detected with SCCHN. However, before applying these results into clinical practices similar study with lager sample size is warned as small sample size is the main limitation of the present study.

CONCLUSION

This study suggested the alteration of risk for SCCHN with MSH3 rs26279 G>A polymorphisms. This polymorphism of MSH3 also found to influence the stage as well as size of

tumour in the present study. However, to establish our suggestions future study with larger sample size is warned.

SIGNIFICANCE STATEMENT

This study discovers the possible role of MSH3 rs26279 G>A polymorphism in the development of SCCHN in North Indian population. The study will help researchers to identify individual who are at higher risk to develop SCCHN and thus will help in the clinical management of this disease.

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