Original Article

Polymorphic variants of mismatch repair gene human MutS homologue-2 influence oral squamous cell carcinoma in a South Indian population

Kanaram Choudhary, Sanish Sathyan[1], V. T. Beena[2], Swagatika Panda[3], R. Sivakumar[2], Anil Ahuja[4], Moinak Banerjee[1]

Dental Officer, [1]Department of Human Molecular Genetics, Sanish Sathyan, Rajiv Gandhi Centre for Biotechnology, [2]Department of Oral and Maxillofacial Pathology, Government Dental College, Trivandrum, Kerala, [3]Department of Oral and Maxillofacial Pathology, Institute of Dental Sciences, SOA University, Bhubaneswar, Odisha, [4]Command Military Dental Centre (CC), Lucknow, India

ABSTRACT

Background: Oral cancer is the third most common cancer in India. It is a multifactorial disease. Cells with defective mismatch repair (MMR) mechanisms cannot correct genetic errors that occur during cellular replication, resulting in a reduction in the fidelity of DNA repair. The objective of this study is to investigate the role of polymorphic variants in MMR gene human MutS homolog-2 (*hMSH2)* in oral squamous cell carcinoma (OSCC) and associated clinicopathological features in Malayalam speaking population from Kerala. **Materials and Methods:** Patients diagnosed with OSCC, without superimposed premalignant and other malignant conditions were selected for polymorphism screening of *hMSH2* gene. Two single nucleotide polymorphisms (SNPs), rs2303428 (*hMSH2*-6C/T) located at −6 of the 3' splice acceptor site of exon 13 and rs61756463 (*hMSH2* 471C/A) located in exon 3 of *hMSH2* were selected based on their functional relevance. **Results:** Polymorphism screening of *hMSH2* gene suggests that rs2303428 was associated with both allelic and genotypic combinations with OSCC. In allelic level, the T allele was associated ($P = 0.009$) with OR of 2.86, whereas in genotypic level the TT genotype was found to be significantly associated ($P = 0.012$). *In silico* prediction of functional implication of this SNP indicated altered transcriptional regulation with functional significance score of 0.176. Although assessing the intergroup comparison within OSCC patients for age (≤50 and >50), gender and histo-differentiation grading, we could not find any association with any of these variables. **Conclusion:** Certain polymorphic variants in the MMR gene *hMSH2* can result in OSCC in Malayalam speaking south Indian population and could indicate defective repair mechanism or Microsatellite instability. Distribution of rs2303428 allele had clear ethnic distribution across world population.

Key words: Mismatch repair gene, oral squamous cell carcinoma, polymorphism

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a multifactorial disease, and it considered as major public health burden. All cancers are polygenic in nature where tumor progression carries a seemingly endless combination of genetic and epigenetic alteration. In men, oral cancers are considered the leading fatal cancers in both rural and urban areas in India. The number of oral cancers was more than twice the number of lung cancers in individuals aged 30-69 years, indicating that the range of fatal cancers caused by tobacco in India.[1] Oral cancer is often diagnosed only after it has advanced to an untreatable stage where the cancer cells have become aggressive and immune to therapeutic drugs. More than half of oral cancer cases diagnosed annually die during the same year owing to the difficulties in detecting the disease at an early treatable stage. In parts of India, oral cancer represents more than 50% of all cancers.[2] Carcinogenesis is characterized by an accumulation of mutations in DNA that causes cellular dysfunction leading to uncontrolled growth. The etiology of oral cancer is multifactorial. There

Access this article online	
Quick Response Code:	Website: www.ccij-online.org
	DOI: 10.4103/2278-0513.150614

Address for correspondence: Dr. Kanaram Choudhary, B-8, 201, Sidhivinayaka, Shantividyanagri Hatkesh, Mira Road East, Maharastra - 401 107, India. E-mail: drk84choyal@gmail.com

are four major classes of genes involved in the initiation, promotion and progression of cancer; these are oncogenes, tumor suppressor genes, apoptosis regulating genes and DNA repair genes. Recent studies have focused on the impact of defective mismatch repair gene (MMR) genes in the pathogenesis of malignancy, particularly in hereditary nonpolyposis colorectal cancer (HNPCC).[3-5] The theory suggests that cells with defective MMR mechanisms cannot correct genetic errors that occur during cellular replication, e.g. point mutations, deletions, insertions and strand slippage.[5] Thus, there is a reduction in the fidelity of DNA repair. When errors occur in proto-oncogenes and anti-oncogenes, loss of control over cell growth and proliferation may happen. Thus, MMR defects have been discussed recently as a mechanism of carcinogenesis equal in importance to primary mutation of proto-oncogenes and anti-oncogenes.[3,5]

The human homolog of the bacterial *MutS* gene, human MutS homologue-2 (*hMSH2)* was the first MMR gene to be mapped and isolated on chromosome 2p16.[6] Until date, very few studies have investigated whether polymorphism in MMR gene might causes microsatellite instability. Previous oral cancer allelotype studies have analyzed only a small number of microsatellite markers on each chromosome, but studies regarding the polymorphism in MMR genes in oral cancer are lacking. Hence, the study was undertaken to evaluate whether OSSC can be caused by polymorphism in MMR gene *hMSH2*.

MATERIALS AND METHODS

A prospective case–control study based design was conducted for 1 year starting from December 2010 to January 2012. Based on strict selection criteria, 49 subjects and 49 ethnically matched Malayalam speaking controls from the state of Kerala, India, were selected for the study. Patients diagnosed with OSCC, without superimposed premalignant and other malignant conditions and undergoing chemotherapy as well radiotherapy were excluded from study, were recruited. A proforma was designed to record patient's socio-demographic details, such as: Name, age, gender, habits, site, and histopathological grading was collected. The study was approved by the institutional ethical committee of Govt. Dental College, Trivandrum. Peripheral blood sample (3 ml) was collected and stored at −70°C till DNA isolation. DNA was extracted using conventional phenol-chloroform method. The concentration of the genomic DNA isolated from each blood sample was checked by measuring its absorbance at 260 nm using a spectrophotometer (BioSpec-1601, Shimadzu). The ratio of absorbance at 260 nm and 280 nm (A260/A280) was used to estimate the purity of the DNA. A260/A280 between 1.7 and 1.8 indicated good quality DNA, relatively free from protein contamination.

Polymorphism analysis of cases and control groups

For polymorphism analysis we have selected two single nucleotide polymorphism (SNP) rs2303428 (*hMSH2*-6C/T) located at −6 of the 3′ splice acceptor site of exon 13 and rs61756463 (*hMSH2* 471C/A) located in exon 3 of *hMSH2*. rs2303428 was amplified using Forward (5′-TGTGGGCAGGCTGTGGTTC-3′) and reverse (5′-CTCCCATATTGGGGCCT GCA-3′) primers flanking the exon 13 while rs61756463 was amplified using a forward (5-TTAGGCTTCTCCTGGCAATC-3′) and reverse (5′-CCTTTCCTAGGCCTGGAA TC-3′) primers flanking the exon 3. Polymerase chain reaction (PCR) conditions were standardized with 95°C for initial denaturation of 5 min, followed by cycle denaturation at 94°C for 30 s annealing for 30 s and extension at 72°C for 30 s. Annealing temperature for rs2303428 and rs61756463 was standardized at 60.5°C and 64.6°C respectively. Both the SNPs were detected using PCR restriction fragment length polymorphism, while rs2303428 was determined using PstI restriction enzyme with fragment sizes of mutant allele 130 + 20 and wild-type allele 150 bp. rs61756463 was determined using HaeIII restriction site with wild-type C allele 213 + 108 and mutant A allele 321bp.

Case-control genetic comparisons were performed using the Chi-square test and allelic and model based odds ratios (OR), and 95% confidence intervals (CI) were calculated by Fisher's exact test (two-tailed). Further stratification of the patients was done to understand the role of *hMSH2* variants with the gender, age and histopathological gradients. All statistical analyses were performed using the Graph Pad Prism 5.01, (GraphPad software Inc. San Diego, CA, USA). We considered $P < 0.05$ as significant. Functional prediction of the deleterious effect if any, of the associated SNP with respect to the functional categories such as protein coding, splicing regulation, transcriptional regulation, and posttranslation was assessed *in silico* using F-SNP program (http://www.compbio.cs.queensu.ca/F-SNP/). F-SNP extracts information from a large number of resources such as PolyPhen, SIFT, SNPeffect, SNPs3D, LS-SNP, Ensembl, ESEfinder, RescueESE, ESRSearch, PESX, TFSearch, Consite, GoldenPath, KinasePhos, OGPET, Sulfinator to generate a functional significance (FS) score.

RESULTS

Demographic variables of the study are presented in Table 1. The OSCC patient population comprised of 61% males and 39% females, who fell in two age groups, of which 82% subjects were above 50, and 18% were below 50 age group. Data on habits shows 74% of OSCC patients with tobacco chewing habit, 18% patients with tobacco smoking habit and 8% patients were without tobacco habit. rs61756463 (*hMSH2* 471C/A) was observed to be monomorphic in cases and controls

while rs2303428 (*hMSH2*-6C/T) was observed to be polymorphic [Figure 1]. Allele and genotype comparison of cases and control indicated that rs2303428 was associated with both allelic and genotypic combinations with OSCC. In allelic level, the T allele was associated with *P* -0.0099 with an OR of 2.86 (CI: 1.321 to 6.176) while in genotypic level the TT genotype was found to be significantly associated with *P* - 0.012 [Table 2]. The T and C allele frequency in OSCC patients was 87 (88.77%) and 11 (11.22%), while in controls it was 72 (73.46%) and 26 (26.53%) respectively. At genomic level, TT genotype was found to be 79.59% in OSCC patients, whereas in controls it was 51.02%. *In silico* prediction of functional implication of this SNP indicated altered transcriptional regulation with FS score of 0.176. We subsequently assessed the role of *hMSH2* variants with various demographic and pathological variables such as age, gender, tobacco habits and histo-differentiation of the disease. While assessing the intergroup comparison within OSCC patients for age, gender and histo-differentiation grading, we could not find any association with any of these variables. While assessing the allelic variants extracted from 1000 genome projects, we observe that C allele is the minor allele across ethnicity, but tends to vary across ethnicities with Asians representing more or less similar frequencies, that are in sharp contrast to African and Europeans population frequencies [Figure 2].

Table 1: Demographic details of the study group

Variables	Sample No.	%
Sample size		
OSCC patients	49	
Controls	49	
Gender %		
Male	30	61
Female	19	39
Age %		
≤50	9	18
>50	40	82
Tobacco usage %		
Smoking	9	18
Chewing	36	74
No habit	4	8
Alcohol consumption %		
Yes	12	24
No	37	76
Histo-differentiation %		
Poor	13	28
Moderate	21	43
Well differentiated	15	28

OSCC: Oral squamous cell carcinoma

DISCUSSION

Genomic instability is a broad name, which includes chromosomal instability and microsatellite alterations.

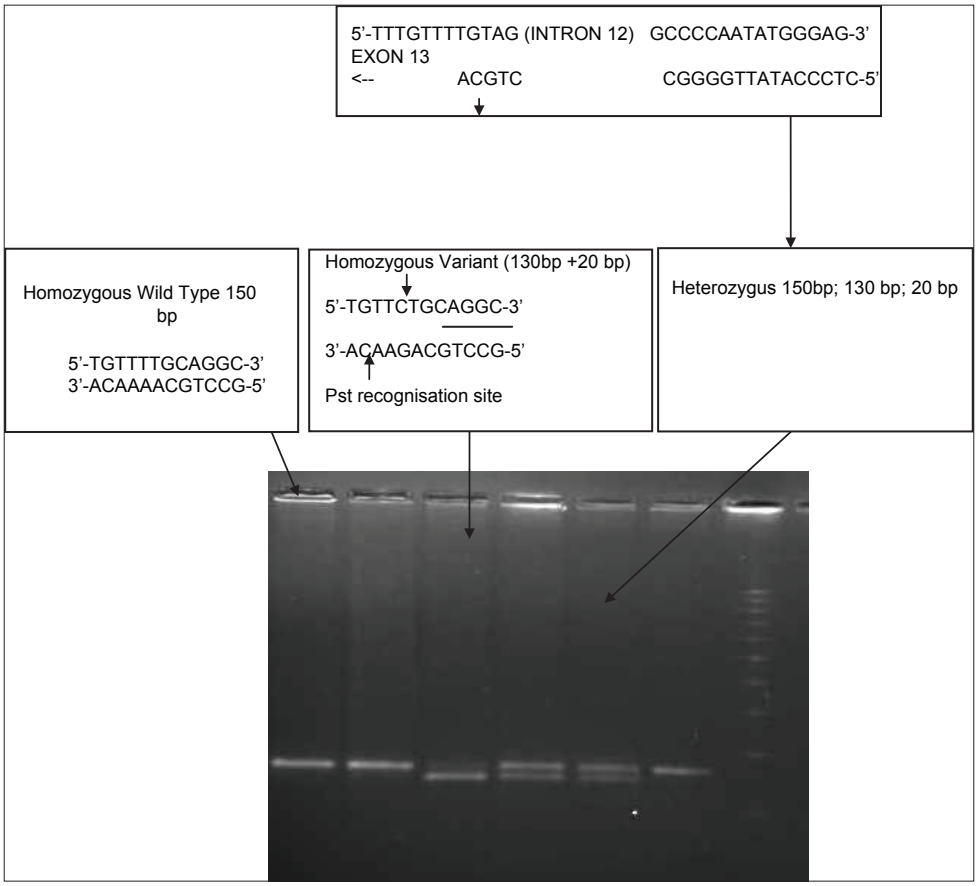

Figure 1: Polymerase chain reaction restriction fragment length polymorphism pattern of rs2303428

Table 2: Frequency distribution of rs2303428 level in OSCC cases and control group

rs2303428	TT	CT	CC	*P* value	T	C	OR (95% CI)	*P*
OSCC cases	39	9	1		87	11	2.856 (1.321-6.176)	0.0099
	0.796	0.184	0.020	0.012	0.888	0.112		
Controls	25	22	2		72	26		
	0.510	0.449	0.041		0.735	0.265		

OR: Odds ratio, CI: Confidence interval, OSCC: Oral squamous cell carcinoma

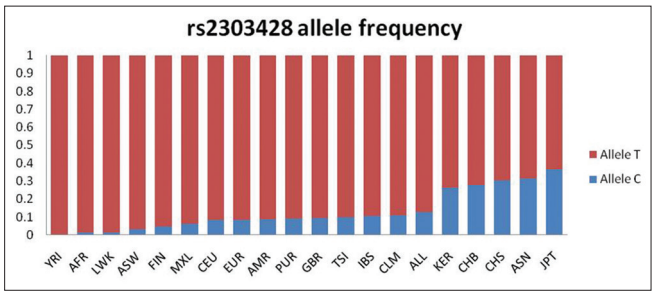

Figure 2: Distribution of rs2303428 across ethnicities extracted from 1000 genome project including Kerala population

Microsatellite alterations could be explained on the basis of defective MMR process. In the present study, we analyzed the polymorphism status of *hMSH2* gene and relationship with clinicopathological data in Kerala population. We report a strong allelic and genotypic association with rs2303428 in OSCC. Previous studies have identified a T > C transition in rs2303428 polymorphism at position −6 of the 3′ splice acceptor site of exon 13.[6,7] Polymorphisms within the 3′ splice junction consensus sequence between +1 and −16 may alter the efficiency of RNA splicing.[7] The −6 exon 13 polymorphism lies within a short poly (T) tract forming an atypical 3′ splice acceptor sequence, which may weaken splice site recognition. The location of the −6 exon 13 T > C polymorphism has led to suggestions that the variant *hMSH2* sequence may result in alternatively spliced mRNA.

Data on the mismatch gene alterations in head and neck cancer is rare. There are not many studies regarding microsatellites in the flanking region of *hMSH2* gene or about the role of polymorphism in *hMSH2*. *hMSH2* gene present in chromosome 2p is responsible for DNA MMR and inactivation of this gene is associated with increased instability in simple repeats in genomic DNA and microsatellite instability.[7] Microsatellite instabilities have been reported in many cancers, but with inconsistent observation. Boland *et al*. demonstrated that HNPCC and sporadic gastric and endometrial tumors, which show instability mostly at the smaller (mono- and dinucleotide) repeats was in sharp contrast to elevated levels of instability in tri- and tetra-nucleotide repeats in other sporadic tumors.[8] While examining the frequency of MSI in HNSCC, no instability was observed using the 2 mononucleotide markers BAT25 and BAT26 in 56 patients of head and neck SCC.[9] In sharp contrast in another study on colorectal cancer patients, MSI was reported in BAT 26 in all tumor tissue.[10] Retention of heterozygous pattern in D2S123, along with no MSI in BAT 26 were observed in HNSCC patients of Thailand population. These MSI correlated with mutational status of *hMSH2*.[11] A striking contrast observation was reported in the Indian population which reported no noticeable instability at mononucleotide markers in HNSCC tumors.[12] In the same Indian population, an hypermethylated state was observed in *hMSH2* and *hMLH1* gene which correlated with increased MSI.[13] However, mutational status of *hMSH2* in these Indian patients was not assessed. It has been suggested that the profile of target gene mutations in MSI-H tumors is tissue-specific, with both qualitative and quantitative differences between gastrointestinal and endometrial MSI-H cancers.[14] The report on MSI status of BAT 26 contradicted by many earlier studies clearly indicates that routine evaluation of both normal, as well as tumor specimens on each patient, have to be done to avoid mistakes in classifying an allelic variation as MSI in both tumorigenic and nontumorigenic samples of each individual.[15] It often becomes difficult to have both tumorigenic and nontumorigenic samples from the same patient, therefore, if a clear role of MMR gene in MIN is established then possibly any sample of the patient can be utilized for diagnostic and prognostic purposes.

Some *in vitro* and animal work on different tumor types indeed suggested that MMR-deficient cells have a poorer response to cisplatin, carboplatin and methylating agent.[16,17] Therefore knowledge on the status of polymorphism in MMR gene in oral cancer could have a therapeutic implication. The present study does highlight a significant role of *hMSH2* mutation in oral cancers that is germline in nature. This could indicate a higher incidence of MSI in OSCC, that can further be aggravated with other influencing factors such as tobacco or smoking. Influence of this polymorphism in MSI needs to be studied further. However, the role of this polymorphism is not associated with the prognosis of the disease. A larger sample size with more precise clinical data can provide greater insight into the pathogenicity and prognosis of the disease.

CONCLUSION

Oral cancer is the third most common cancer in India. It is a multifactorial disease, and tobacco, alcohol consumption are predisposing factors. The present study indicates higher practice of tobacco chewing in OSCC patients, but

this did not associate with *hMSH2* polymorphism profile. The polymorphisms in the MMR gene *hMSH2* can result in OSCC in Malayalam speaking south Indian population and could indicate defective repair mechanism or MSI. The study could implicate in diagnostic or therapeutic capability. However, this needs to be validated further in increased sample size and across different ethnic population.

ACKNOWLEDGMENTS

We thank HMG lab of Rajiv Gandhi center for Biotechnology, Trivandrum in assisting and helping in the entire process of our work.

REFERENCES

1. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, *et al*. Cancer mortality in India: A nationally representative survey. Lancet 2012;379:1807-16.
2. Khandekar SP, Bagdey PS, Tiwari RR. Oral cancer and some epidemiological factors: A hospital based study. Indian J Comm Med 2006;31:1-4.
3. Arzimanoglou II, Gilbert F, Barber HR. Microsatellite instability in human solid tumors. Cancer 1998;82:1808-20.
4. Loeb LA. Microsatellite instability: Marker of a mutator phenotype in cancer. Cancer Res 1994;54:5059-63.
5. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, *et al.* Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993;75:1215-25.
6. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, *et al*. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75:1027-38.
7. Brentnall TA, Rubin CE, Crispin DA, Stevens A, Batchelor RH, Haggitt RC, *et al*. A germline substitution in the human MSH2 gene is associated with high-grade dysplasia and cancer in ulcerative colitis. Gastroenterology 1995;109:151-5.
8. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, *et al.* A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248-57.
9. Blons H, Cabelguenne A, Carnot F, Laccourreye O, de Waziers I, Hamelin R, *et al.* Microsatellite analysis and response to chemotherapy in head-and-neck squamous-cell carcinoma. Int J Cancer 1999;84:410-5.
10. Ki-Hyuk S, Joo-Ho S, Jung-Hwa K, Jae-Gahb P. Mutational analysis of promoters of mismatch repair genes *hMSH2* and *hMLH1* in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: Identification of three novel germ-line mutations in promoter of the *hMSH2* gene1. Cancer Res 2002;62:38-42.
11. Sanguansin S, Petmitr S, Punyarit P, Vorasubin V, Weerapradist W, Surarit R. HMSH2 gene alterations associated with recurrence of oral squamous cell carcinoma. J Exp Clin Cancer Res 2006;25:251-7.
12. Chakrabarti S, Dasgupta S, Roy S, Bhar A, Sengupta A, Roy A, *et al.* Microsatellite instability in squamous cell carcinoma of head and neck from the Indian patient population. Int J Cancer 2001;92:555-61.
13. Sengupta S, Chakrabarti S, Roy A, Panda CK, Roychoudhury S. Inactivation of human mutL homolog 1 and mutS homolog 2 genes in head and neck squamous cell carcinoma tumors and leukoplakia samples by promoter hypermethylation and its relation with microsatellite instability phenotype. Cancer 2007;109:703-12.
14. Duval A, Reperant M, Compoint A, Seruca R, Ranzani GN, Iacopetta B, *et al*. Target gene mutation profile differs between gastrointestinal and endometrial tumors with mismatch repair deficiency. Cancer Res 2002;62:1609-12.
15. Salahshor S, Kressner U, Fischer H, Lindmark G, Glimelius B, Påhlman L, *et al*. Microsatellite instability in sporadic colorectal cancer is not an independent prognostic factor. Br J Cancer 1999;81:190-3.
16. Papouli E, Cejka P, Jiricny J. Dependence of the cytotoxicity of DNA-damaging agents on the mismatch repair status of human cells. Cancer Res 2004;64:3391-4.
17. Berry SE, Davis TW, Schupp JE, Hwang HS, de Wind N, Kinsella TJ. Selective radiosensitization of drug-resistant MutS homologue-2 (MSH2) mismatch repair-deficient cells by halogenated thymidine (dThd) analogues: Msh2 mediates dThd analogue DNA levels and the differential cytotoxicity and cell cycle effects of the dThd analogues and 6-thioguanine. Cancer Res 2000;60:5773-80.

Cite this article as: Choudhary K, Sathyan S, Beena VT, Panda S, Sivakumar R, Ahuja A, *et al.* Polymorphic variants of mismatch repair gene human MutS homologue-2 influence oral squamous cell carcinoma in a South Indian population. Clin Cancer Investig J 2015;4:170-4.

Source of Support: Nil, **Conflict of Interest:** None declared.