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ORIGINAL ARTICLE

Functional polymorphism of the MMP-1 promoter (-1607 1G/2G) in potentially malignant and malignant head and neck lesions in an Indian population

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Abstract

Matrix metalloproteinases (MMP) are a family of zinc-dependent proteases that degrade the entire component of the extracellular matrix. Our study explores the association of the *MMP1* gene promoter (-1607 1G/2G) polymorphisms in oral submucous fibrosis (OSMF) and head and neck squamous cell carcinoma (HNSCC) in an Indian population. The *MMP1*single-nucleotide polymorphism (SNP) was genotyped by polymerase chain reaction–restriction fragment length polymorphism analysis in 412 patients with OSMF, 422 with HNSCC and 426 controls. Our results showed that the frequency of 1G/2G or 2G/2G promoter genotypes having the 2G allele is associated with higher enzymatic activity and significantly increases in OSMF ($p<0.001$) and HNSCC cases ($p<0.00$). In this study, results concluded that SNPs in the *MMP1* promoter region may be associated with susceptibility to OSMF as well as HNSCC in an Indian population and addiction habits such as areca nut chewing and alcohol abuse may enhance the expression of the 2G allele of *MMP1* genes in OSMF and HNSCC cases.

Keywords: *Oral cancer; genetic polymorphisms; tobacco science*

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteases that are capable of degrading various components of the extracellular matrix (ECM). Degeneration of the matrix is a key event in the progression, invasion and metastasis of potentially malignant and malignant head and neck lesions and it might play an important role in polymorphic association. MMPs are counteracted by the tissue inhibitors of metalloproteinases (TIMPs), which inhibit the activity of MMPs and thereby restrict the degradation of ECM. The balance between the MMPs and TIMPs plays an important role in maintaining the integrity of healthy tissue. A disturbed balance of MMPs and TIMPs is found in various physiological and pathological conditions (Gomez et al. 1997, Lambert et al. 2004) and it affects the regulation of various cell behaviours such as cell proliferation, alteration of cell moiety, angiogenesis and apoptosis.

The *MMP1* geneis expressed in a wide variety of normal cells, such as stromal fibroblasts, macrophages, endothelial and epithelial cells and in various malignant cells (Brinkerhoff et al. 2000). Increased expression of *MMP1* has been associated with a poor prognosis in several malignancies such as oral carcinoma (Nishizawa et al. 2007, Vairaktaris et al. 2007, Cao et al. 2004, Lin et al. 2004), nasopharyngeal carcinoma (Nasr et al. 2007), head and neck carcinoma (Zinzindohoue et al. 2004, Hoshimoto et al. 2004, O-charoenrat et al. 2006) and tongue carcinoma (Shimizu et al. 2008). Author group recently reviewed the molecular functions and single-nucleotide polymorphic association of different MMPs

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such as MMP-1 (-1607 1G/2G), MMP-2 (-1306 C/T), MMP-3 (-1171 5A/6A), MMP-9 (-1562 C/T) and TIMP-2 (-418 G/C or C/C) and the possible therapeutic aspects of these proteases and concluded that MMPs may have a genotypic association with potentially malignant (oral submucous fibrosis, OSMF) and malignant head and neck lesions (head and neck squamous cell carcinoma, HNSCC) (Chaudhary et al. 2010).

In humans, 24 types of MMP genes are known and these are classified into five different groups based on their substrate specificity and domain structure. These include collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10 and MMP-11), matrilysins (MMP-7 and MMP-26) and membrane-type MMPs (MT-MMPs). The *MMP1* gene is located on chromosome 11q[22]. Among the MMPs, MMP-1 (interstitial collagenases) is a major proteinase of the MMP family that specifically degrades type I collagen, which is a major component of the ECM as well as other fibrillar collagens of type II, III, V, IV, IX and X (Ziober et al. 2000, Kerkela & Saarialho-Kere 2003).

The expression of MMP genes are transcriptionally induced by oncogenic transformation, cytokines, several growth factors including interleukins, interferons, epidermal growth factor (EGF), tumour necrosis factor (TNF)-α and transforming growth factor (TGF)-β (Westermarck & Kahari 1999). Many intracellular stimuli enhancethe expression of c-fos and c-jun proto-oncogene products and bind activator protein (AP)-1 at proximal promoter region of several MMPs such as MMP-1, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12 and MMP-13 types. Extracellular signals activate the dimeric AP-1 complex which is composed of jun and fos proteins. When these c-fos and c-jun proto-oncogenes are bound to the AP-1 element then the activity of the AP-1 element is mediated by mitogen-activated protein kinases (MAPKs), which are mitogen-activated intracellular signal-regulated kinase 1, 2 (ERK1, 2), stress activated jun N-terminal kinase and p38 MAPK (Overall & Lopez-Otin 2002). Therefore, MMPs are regulated at the transcriptional and post-transcriptional levels and their control at the protein level.

The level of expression of these genes can be influenced by single-nucleotide polymorphisms (SNPs) in the promoter region of their respective genes. Rutter et al. concluded that insertion of a G nucleotide at -1607 bp in the *MMP1* promoter region generates a new Ets binding site and increases the transcription of the *MMP1* gene (Rutter et al. 1998). The promoter region of *MMP1* contains a guanine insertion/deletion polymorphism (1G/2G polymorphism) at position -1607.Promoter assays have indicated that this is a functional polymorphism. Tower et al. reported that this 2G allele results in increased transcriptional activity because the guanine insertion creates a core-binding site (5'-GGA-3') for the Ets transcription factor family, leading to a higher expression of *MMP1* (Tower et al. 2002).

The presence of 2G alleles at the *MMP1* (-1607 1G/2G) promoter polymorphism are associated with the development and progression of OSCC as reported by Vairaktaris et al. in Greece and in cases of HNSCC reported by O-charoenrat et al. in Thailand (Vairaktaris et al.2007, O-charoenrat et al. 2006). Znzindohoue et al. in France and Hoshimoto et al. in Japan investigated the impact of functional polymorphism of *MMP1* (-1607 1G/2G) genotypes in HNSCC cases in a Caucasian population and in a Japanese population, respectively (Zinzindohoue et al. 2004, Hoshimoto et al. 2004). To the best of our knowledge, the relationship between the *MMP1* (-1607 1G/2G) SNP and the risk of development of lesions such as OSMF and HNSCC cases has not been investigated before in an Indian population. This study examine the association of *MMP1* gene promoter polymorphisms (-1607 1G/2G) in OSMF and HNSCC in an Indian population.

Materials and methods

Sample collection

A total of 412 OSMF and 422 HNSCC cases were recruited from the Department of Pathology and Otorhinolaryngology, MotiLal Nehru Medical College, Allahabad, India, after obtaining clearance from the institutional ethical committee. Detailed information of each patient was noted in a pretested proforma. Information regarding the patient's name, age, sex and addiction habits was gathered. Emphasis was given to addictions such as areca nut chewers (i.e. areca nut chewing with tobacco, Pan and slaked lime), never-areca nut chewers, smokers (i.e. cigarette and bidi smokers), never-smokers, alcohol drinker, never-alcohol drinker by the patients. Briefly, individuals who formerly or currently chewed four pouches of tobacco with areca nut and pan/day, smoked four cigarettes or bidi/day and consumed alcohol (~300 ml) three times/week for at least 2 years were defined as chewers, smokers and drinkers, respectively.

In addition, 426 healthy control subjects, with no history of any type of premalignancy and malignancy or genetic disorders, were recruited from individuals, who came for routine physical check-up in the same hospital and were matched for age, sex and addiction habit. This was a hospital-based case–control study and all samples were collected during May 2007 to April 2010 after the confirmation of potentially malignant and malignant head and neck diagnosis.

Detailed clinical examination of each OSMF patient was done to assess the site, size and type of lesion. Trismus was classified as stage I (>3cm), stage II (2–3cm) and stage III (<2cm). For confirmation of the clinical

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diagnosis, histopathological examination was carried out onbiopsy tissue and the grading for OSMF cases was done according to the classification given by Pindborg and Sirsat (1966). Likewise, for the HNSCC patients after the clinical diagnosis, a histopathological examination was carried out. The HNSCC cases were grouped on the basis of tumour site, lymph node position and metastasis (TNM) (Patel & Shah 2005).

All specimens were examined manually, independently by two different histopathologists in a double-blind fashion. If there were any discrepancies, a third opinion was obtained to reach a final diagnosis. As the histopathological slides were evaluated by histopathologists and the patients were examined by the otorhinolaryngology surgeons, there was no chance of bias. Then blood samples were taken from all the participants in this hospital-based case–control study after obtaining informed consent form. Blood (5 ml) was drawn from each subject into a vacutainer tube containing ethylenediaminetetra acetic acid (EDTA) and stored at 4°C until the samples were processed. None of the HNSCC patients gave a history of having OSMF prior to developing malignancy.

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Isolation of genomic DNA

Genomic DNA was extracted from the blood samples using the Qiagen QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA,USA). The extracted genomic DNA was quantified and checked for purity spectrophotometrically (Spectro UV-Vis Double Beam PC, UVD Model 2950; Labomed Inc., Culver City, CA, USA). Ethidium bromide (EtBr)-stained 0.8% agrose gel electrophoresis was used to confirm the presence of genomic DNA in patient and control samples.

Genotyping of the MMP1 promoter polymorphism

The *MMP1* genotype was determined by apolymerase chain reaction–restriction length fragment polymorphism (PCR-RFLP) assay. The PCR primers used for amplifying the *MMP1* polymorphism were: forward primer (FP) 5'- TGA CTT TTA AAA CAT AGT CTA TGT TCA-3' and reverse primer (RP) 5'-TCT TGG ATT GAT TTG AGA TAA GTC ATA GC- 3' (Zhu et al. 2001). PCR was performed in a 25 µl volume containing 50 ng of genomic DNA template, 2.5 µl of 10 × PCR buffer, 2.5 mmol of $MgCl_2$, 1 U of Taq DNA polymerase (Fermentas Inc., Glen Burnie, MD, USA), 200µmol of dNTPs and 200 nmol of forward and reverse primer. The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C and with a final step at 72°C for 15 min to allow for the complete extension of all PCR fragments. For a negative control, instead of the DNA sample, distilled water was used in a PCR reaction mixture.

Restriction enzyme digestion of MMP1 gene

A 10-ml aliquot of PCR product was digested at 37°C for overnight in a 15-ml reaction containing 5 U of *Alu*I and 1 × reaction buffer. After digestion, the products were separated on a 3.0% agarose gel stained with EtBr. After electrophoresis, homozygous 2G alleles were represented by a DNA band of 269 bp, homozygous 1G alleles were represented by DNA bands with sizes 241 and 28 bp, whereas heterozygotes displayed a combination of both alleles (269, 241 and 28 bp).

Statistical analysis

The distribution of the *MMP1* genotypes in healthy controls and patients did not significantly deviate from that expected for Hardy–Weinberg equilibrium. The χ^2 test was used to find out the difference in genotype distribution of *MMP1* promoters between the OSMF, HNSCC and control groups. Fisher's exact test was also applied in the statistical analysis. A p-value of <0.05 was considered to be statistically significant. For each parameter, the OSMF histopathological grades I, II, III and IV, and HNSCC cases risk were analysed by odds ratios (OR) and 95% confidence intervals (CI). The statistical analysis was performed using the SPSS 15.0 software package (SPSS Japan Inc., Tokyo, Japan).

Results

A total of 834 cases (average age 48.0 ± 17.6 years) were included in this study, of which 412 patients suffered from OSMF (252 males and 160 females) and 422 patients had HNSCC (292 males and 130 females). Four hundred and twenty-six (average age 47.01 ± 15.2 years, 297 male 129 female) were healthy controls. *MMP1* promoter polymorphism in patients with OSMF, HNSCC and healthy controls was analysed with respect to gender and age, and habits such as areca nut chewing, smoking and alcohol intake, etc. Histopathological grades of OSMF and TNM staging (tumour category, lymph node and metastasis of tumour) of HNSCC are shown in Table 1.

The association between *MMP1* promoter genotypes (1G/1G+1G/2G and 2G/2G) in the OSMF, HNSCC and control groups and various addiction habits are shown in Tables 2 and 3. In the OSMF group, the1G/2G genotype showed significantly fivefold (OR5.07, 95% CI2.827–9.115; p<0.00) risk in the case of areca nut chewers and nearly fourfold (OR3.67, 95%CI 1.923–7.007; p<0.001) risk in the case of smokers for developing OSMF,

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respectively. Similarly in the HNSCC group, the *MMP1* genotype showed significantly fivefold (OR 5.13, 95% CI 2.995–8.798; $p<0.00$) risk in the case of areca nut chewers and 3.5-fold (OR 3.53, 95% CI 1.892–6.547; $p<0.001$) risk in the case of smokers for developing HNSCC, respectively (Tables 2 and 3).

Table 1. Demographic distribution and clinical parameters among the oral submucous fibrosis (OSMF) and head and neck squamous cell carcinoma (HNSCC) cases.

Clinicopathological parameter	OSMF cases ($n=412$)	HNSCC cases ($n=422$)
Gender, *n* (%)		
Male	252(61.2)	292(69.2)
Female	160(38.8)	130(30.8)
Age (years), mean ± SD	42 ± 12.6	56 ± 15.6
Location, *n* (%)		
Lip (lower and upper)	77 (18.7)	52(12.3)
Tongue	108(26.3)	47(11.1)
Hard pallet	43 (10.4)	30(07.1)
Soft palate	82(19.9)	52(12.3)
Buccal mucosa	102 (24.7)	47(11.2)
Larynx	-	70(16.6)
Pharynx	-	54(12.8)
Hypopharynx	-	23(05.5)
Tonsil	-	47(11.1)
OSMF grade, *n* (%)		
I	126 (30.6)	-
II	117(28.4)	-
III	92(22.3)	-
IV	77(18.7)	-
T category, *n* (%)		
T1–2	-	273 (64.7)
T3-4	-	149 (35.3)
N category, *n* (%)		
N0	-	268 (63.5)
N1-3	-	154 (36.5)

The genotype distributions of promoter polymorphism in the *MMP1* gene in OSMF, HNSCC and controls were analysed with the Hardy–Weinberg equilibrium. The frequency of 1G/2G or 2G/2G promoter genotypes with the 2G allele that is associated with higher enzyme activity were significantly increased in OSMF cases (OR 1.18, 95% CI 0.96–4.45; $p<0.011$) and HNSCC cases when compared with controls (OR 1.51, 95% CI 1.23–1.85; $p<0.00$) (Tables 4 and 5). No significant association of MMP alleles was detected in relation to age and gender in either OSMF or HNSCC (Tables 6 and 7).

OSMF (n=412) and HNSCC (n=422) cases were stratified by clinicopathological parameters including location of the lesions and grading criteria. Their relationship with the *MMP1* polymorphism was evaluated by Fisher's exact test. As shown in Table 8, a significant association was found between the OSMF grading and distribution of 2G/2G genotype alone or in combination with 1G/2G genotype of *MMP1* ($p<0.00$). In cases of HNSCC, grading and distribution of *MMP1* genotypes were found significantly associated with tumour size ($p<0.0002$) as well as with advanced tumour stage ($p<0.004$). Therefore, we concluded that these results suggested that the *MMP1* (-1607 1G/2G) polymorphism may be associated with the development of OSMF as well as in HNSCC in higher grades (Tables 8 and 9).

Discussion

Carcinogenesis in the potentially malignant and malignant head and neck regions is a multistage

Table 2. Addiction habit distribution in relation to alleles in oral submucous fibrosis (OSMF) cases and healthy controls.

MMP1 genotype	OSMF cases (n=412) *n* (%)	Controls (n=426) *n* (%)	Trend test (p-value)	OR (95%CI)
Never areca nut chewer				
1G/1G,1G/2G	29(58.0)	20(54.1)	0.812	0.81 (0.332–1.197)
2G/2G	21(42.0)	17(45.9)		
Areca nut chewer				
1G/1G,1G/2G	72(73.5)	42(35.3)	<0.000	5.07 (2.827–9.115)
2G/2G	26(26.5)	77(64.7)		
Never smoker				
1G/1G,1G/2G	12(41.4)	18(46.2)	0.886	0.823 (0312–2.17)
2G/2G	17(58.6)	21(53.8)		
Smoker				
1G/1G,1G/2G	63(76.8)	47(47.5)	<0.001	3.668 (1.923–7.007)
2G/2G	19(23.2)	52(52.5)		
Never drinker				
1G/1G,1G/2G	19(32.2)	16(48.5)	0.188	0.504 (0.210–1.209)
2G/2G	40(67.8)	17(51.5)		
Alcohol drinker				
1G/1G,1G/2G	28(29.8)	79(79.8)	0.56	0.1074 (0.055–0.207)
2G/2G	66(70.2)	20(20.2)		

OR, odds ratio; CI, confidence interval.

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process which involves various genetic alterations, mutations in oncogenes, tumour suppressor genes and environmental factors such as areca nut chewing with tobacco, alcohol abuse and cigarette smoking (Hanahan & Weinberg 2000). The increased risk of HNSCC in relation to the 2G alleles of the *MMP1* promoter polymorphism includes genetic instability due to cell cycle activation and it increased the enzymatic activity of MMP-1 due to a disruption of the epidermal tissues. The relationship between *MMP1* (-1607 1G/2G) promoter polymorphism and the risk of HNSCC has been reported by Hashimoto et al. in a Japanese population (140 cases/223 controls) and by Zinzindohoue et al. in a Caucasian population (126 cases/249 controls) and they concluded that the *MMP1* promoter polymorphism may be associated with

Table 3. Addiction habits distribution in relation to alleles in head and neck squamous cell carcinoma (HNSCC) cases and healthy controls.

MMP1 genotype	HNSCC cases (n =422) n (%)	Controls (n = 426) n (%)	Trend test (p-value)	OR (95%CI)
Never areca nut chewer				
1G/1G,1G/2G	15(32.6)	20(54.1)	0.081	0.411 (0.168–1.004)
2G/2G	31(67.4)	17(45.9)		
Areca nut chewer				
1G/1G,1G/2G	98(73.7)	42(35.3)	<0.000	5.13 (2.995–8.798)
2G/2G	35(26.3)	77(64.7)		
Never smoker				
1G/1G,1G/2G	18(32.7)	18(46.2)	0.139	0.473 (0.198–1.128)
2G/2G	37(67.3)	21(53.8)		
Smoker				
1G/1G,1G/2G	70(76.1)	47(47.5)	<0.001	3.53 (1.892–6.547)
2G/2G	22(23.9)	52(52.5)		
Never alcohol drinker				
1G/1G,1G/2G	22(44.9)	16(48.5)	0.924	0.865(0.357–2.097)
2G/2G	27(55.1)	17(51.5)		
Alcohol drinker				
1G/1G,1G/2G	30(63.8)	79(79.8)	0.0646	0.446 (0.2066–0.9661)
2G/2G	17(36.2)	20(20.2)		

OR, odds ratio; CI, confidence interval.

Table 4. The genotype and allelotype frequency of *MMP1* in oral submucous fibrosis (OSMF) patients and healthy controls.

MMP1 genotype	Controls (n =426) n (%)	OSMF cases (n =412) n (%)	OR	95%CI	p-Value
2G/2G	204(47.8)	189(45.8)			
1G/2G	192(45.1)	172(41.8)	1.18	0.96-4.45	**<0.011**
1G/1G	30(07.1)	51(12.4)			

OR, odds ratio; CI, confidence interval.

Table 5. The genotype and allelotype frequency of *MMP1* in head and neck squamous cell carcinoma (HNSCC) patients and healthy controls.

MMP1 genotype	Controls (n =426) n (%)	HNSCC cases(n =422) n (%)	OR	95%CI	p-Value
2G/2G	204(47.8)	169(40.0)			
1G/2G	192(45.1)	178(42.2)	1.51	1.23-1.85	**<0.000**
1G/1G	30(07.1)	75(17.8)			

OR, odds ratio; CI, confidence interval.

Table 6. Allele distribution in relation to age and gender in oral submucous fibrosis (OSMF) cases and healthy controls.

	OSMF (n = 412)				Controls (n = 426)			
	2G/2G (n = 189)	1G/2G (n =172)	1G/1G (n =51)	p-Value	2G/2G (n =204)	1G/2G (n =192)	1G/1G (n =30)	p-Value
Age[a](years), mean ± SD	42.6 ± 12.6	46.5 ± 14.5	49.1 ± 11.2	0.248	51.8 ± 10.2	54.4 ± 12.5	46 ± 15.6	0.462
Gender[b], n (%)								
Males	112 (59.3)	103 (59.8)	37(72.5)		107 (52.5)	101 (52.6)	19 (63.3)	
Females	77(40.7)	69(40.1)	14(27.5)	0.202	97 (42.5)	91 (47.4)	11 (36.6)	0.519

[a]One-way ANOVA test; [b]χ^2test.

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HNSCC (Hashimoto et al. 2004, Zinzindohoue et al. 2004). In this case–control study, 412 patients with OSMF, 422 with HNSCC and 426 controls sample were included in order to obtain a more accurate result.

We examined the SNP association in the promoter region of the *MMP1* gene in the potentially malignant (OSMF) and malignant head and neck lesions (HNSCC) and found a significant association of 1G/1G, 1G/2G and 2G/2G alleles in relation to the addiction habits. In this study the frequency of 1G/2G or 2G/2G promoter genotypes with the 2G allele, was significantly increased in OSMF cases (p<0.001) and in HNSCC cases (p<0.00) compared with controls and we conclude that a guanine insertion/deletion polymorphism in the *MMP1* promoter region may be associated with the increased susceptibility for the development of cases of both OSMF and HNSCC. Similarly, Hashimoto et al. reported that the frequency of the *MMP1* 2G/2G genotype was significantly higher compared with the controls (p<0.042) whereas Zinzindohoue et al. reported that homozygous 2G/2G individuals

Table 7. Allele distribution in relation to age and gender in head and neck squamous cell carcinoma (HNSCC) cases and healthy controls.

	HNSCC (n = 422)				Controls (n = 426)			
	2G/2G (n = 169)	1G/2G (n = 178)	1G/1G (n = 75)	p-Value	2G/2G (n = 204)	1G/2G (n = 192)	1G/1G (n = 30)	p-Value
Age[a](years), mean ± SD	55.8 ± 14.8	56.5 ± 14.3	54.5 ± 11.2	0.248	51.8 ± 10.2	54.4 ± 12.5	46 ± 15.6	0.462
Gender[b], n (%)								
Males	115 (68.0)	124 (70.0)	53 (70.7)		107 (52.5)	101 (52.6)	19 (63.3)	
Females	54(32.0)	54(30.0)	22 (29.3)	0.905	97 (42.5)	91 (47.4)	11 (36.6)	0.519

[a]One-way ANOVA test; [b]χ^2test.

Table 8. Relationship of *MMP1* genotypes with clinicopathological parameters in oral submucous fibrosis (OSMF)cases.

Clinicopathological parameter	OSMF cases(n = 412), |n (%)	1G/1G,1G/2G, n (%)	2G/2G, n (%)	p-Value
Location				
Lip (lower and upper)	77(18.7)	35 (45.5)	42 (54.5)	
Tongue	108(26.3)	51 (47.2)	57 (52.7)	
Hard pallet	43(10.4)	23 (53.5)	20 (46.5)	0.05
Soft palate	82(19.9)	48 (58.5)	34 (41.5)	
Buccal mucosa	102(24.7)	66 (64.7)	36 (35.3)	
OSMF grade				
I	126(30.6)	44 (34.9)	82 (65.1)	
II	117(28.4)	66 (56.4)	51 (43.6)	
III	92(22.3)	66 (71.7)	26 (28.3)	<0.000
IV	77(18.7)	47 (61.0)	30 (39.0)	

Table 9. Relationship of *MMP1* genotypes with clinicopathological parameters in head and neck squamous cell carcinoma (HNSCC)cases.

Clinicopathological parameter	HNSCC cases (n = 422), n (%)	1G/1G,1G/2G, n (%)	2G/2G, n (%)	p-Value
Location				
Lip (lower and upper)	52(12.3)	27 (51.9)	25 (48.1)	
Tongue	47(11.1)	28 (59.6)	19 (40.4)	
Hard pallet	30(07.1)	21 (70.0)	09 (30.0)	
Soft palate	52(12.3)	27 (51.9)	25 (48.1)	
Buccal mucosa	47(11.2)	29 (61.7)	18 (38.3)	0.061
Larynx	70(16.6)	52 (74.3)	18 (25.7)	
Pharynx	54(12.8)	30 (55.5)	24 (45.5)	
Hypopharynx	23(05.5)	12 (52.2)	11 (47.8)	
Tonsil	47(11.1)	27 (57.4)	20 (42.5)	
T category				
T1–2	273 (64.7)	146 (53.5)	127 (46.5)	<0.0002
T3-4	149 (35.3)	107 (71.8)	42 (28.2)	
N category				
N0	268 (63.5)	147 (54.8)	121 (45.1)	<0.004
N1-3	154 (36.5)	106 (68.8)	48 (31.1)	

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were at lower risk of malignancy than the 1G/1G carriers alleles (p<0.003) and both suggested that the *MMP1* promoter polymorphism may be associated with HNSCC (Hashimoto et al. 2004, Zinzindohoue et al. 2004). In cases of OSCC, Nishizawa et al. and Rutter et al. reported that the frequency of the *MMP1* 2G allele was higher in oral malignancy (Nishizawa et al. 2007, Rutter et al. 1998).

Recently, Vairaktaris et al. examined possible interactions between nine MMPs such as MMP-1 (-1607 1G/2G), MMP-3 (-1171 5A/6A), MMP-9 (-1562C/T), TIMP-2 (-418C/G), VEGF (+936 C/T), GPI-alpha (+807 C/T), PAI-1 (4G/5G), ACE (interon 16 D/I) and TAFI (+325C/T) polymorphism in OSCC cases in early and advanced stages and reported that four (PAI-1, MMP-9, TIMP-2 and ACE) out of nine MMPs polymorphisms affected its expression (Vairaktaris et al 2009). The level of *MMP1* expression can be influenced by different SNPs in the promoter region. *In vitro*, the 2G allele of *MMP1* is associated with a higher promoter activity (Tower et al. 2002) and leads to an increase in the production of the MMP-1 proteins (Fujimoto et al. 2002), which is accompanied by genetic instability and activation of the cell cycle. An association between the *MMP1* 1G/2G polymorphism and OSCC has been investigated by many authors and suggests the significant involvement of the *MMP1* -1607 1G/2G polymorphism in an increasing risk for OSCC (Vairaktaris et al. 2007, Cao & Li 2006, Lin et al. 2004). In this study we concluded that these results suggest that the *MMP1* (-1607 1G/2G) polymorphism may be associated with development of OSMF as well as in HNSCC at higher grades. Similarly Nasr et al. suggested that the 2G (-1607) allele of the *MMP1* gene is highly associated with aggressive forms of nasopharyngeal carcinoma inpatients in Southwest of Tunisia (Nasr et al. 2007).

Shimizu et al. suggested that SNP in the promoter region of *MMP1* and interleukin-8 play an important role in tumour progression in tongue squamous cell carcinoma (p<0.003) (Shimizu et al. 2008). Przybylowska et al. suggested in breast carcinoma, that the 2G allele of the *MMP1* promoter polymorphism may be associated with the lymph node metastasis in patients with breast cancer and therefore it can be considered as a progression marker in this disease (Przybylowska et al. 2006). However, Lai et al. reported in cervical carcinoma that there was no significant correlation between human papilloma virus (HPV) status and MMP genotype in 135 high-grade squamous intraepithelial lesions (HSILs), but suggested that the heterozygous genotype of *MMP1* can be used as a prognostic marker in patients with invasive cervical carcinoma (Lai et al. 2005). Nishioka et al. also suggested that SNPs of the *MMP1* promoter influence invasion via transcriptional activity (Nishioka et al 1999). It has also been reported that ovarian tumours (Kanamori et al. 1999) and endometrial cancer tissues (Shan et al. 2005, Nishioka et al. 2000, Hettiaratchi et al. 2007, McColgan & Sharma 2009), idiopathic pulmonary fibrosis (Checa et al. 2008) and knee osteoarthritis (Barlas et al. 2009) samples from patients carrying the 2G allele contain higher levels of *MMP1* transcripts compared with those from patients not carrying this allele. In this case–control study, a significant association was found between the *MMP1* promoter 2G allele and an increased risk of these lesions, among 412 OSMF and 422 HNSCC cases in an Indian population.

OSMF and HNSCC may be induced by areca nut chewing and smoking, by producing disruption in the epithelial layer which can induce the expression of the MMP gene (Vairaktaris et al. 2007). In some cases, viral infections such as human papillomavirus (HPV) may cause potential malignancy (Llewellyn et al. 2004, Chaudhary et al. 2009). In addition, we observed a possible addictive interaction between this genetic polymorphism and areca nut chewing habits/smoking/alcohol use on the risk of head and neck lesions. A significant difference in *MMP1* genotypic polymorphism was found between controls and OSMF cases in areca nut chewers (OR5.07; p<0.00) and smokers (OR3.67; p<0.001). Similarly, in HNSCC cases, areca nut chewers and smokers had a fivefold increased risk (OR5.13; p<0.00) and threefold risk (OR3.53; p<0.0001), respectively. It is an attractive finding to conclude that areca nut chewers and smokers have an increased risk for the development of OSMF and HNSCC in an Indian population. Many reports have suggested similar findings, for example Vairaktaris et al. found that the *MMP1* 2G allele frequency was significantly increased in the OSCC group with tobacco abuse and without alcohol abuse (p<0.05) (Vairaktaris et al. 2007). Similarly O-charoenrat et al. reported that the 2G/2G frequency enhances HNSCC susceptibility especially in heavy smokers and drinkers (O-charoenrat et al. 2006) and also Cao et al. considered the relationship with smoking as a behavioural risk factor in OSCC (Cao et al. 2006). However, Hashimoto et al. reported that there was no significant association between the polymorphism and smokers using less that 40 packs/year or more than 40 packs/ year (Hashimoto et al 2004). Not only in *MMP1* gene expression but also in other MMPs, addiction plays an important role. Tu et al. reported that 5A alleles at -1171 in *MMP3* genes might have a greater than threefold risk in male areca nut chewers in relation to subjects having other genotypes (Tu et al. 2006). In another study, Tu et al. reported that functional *MMP9* -1562 C>T polymorphism is associated with OSCC risk only in younger areca chewers bearing the T allele (p<0.029) (Tu et al. 2007).

In conclusion, in this study, results show that SNPs in the *MMP1* promoter region may be associated with susceptibility to OSMF and HNSCC in an Indian population and that the addiction habits such as areca nut chewing and alcohol abuse may enhance the expression of the 2G

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allele of *MMP1*genes in OSMF and HNSCC cases. Studies with a larger number of subjects are needed to elucidate the polymorphic association of MMPs in these lesions in the Indian population.

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Declaration of interest

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