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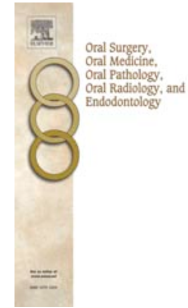

MutSα and MutLα immunoexpression analysis in diagnostic grading of oral epithelial dysplasia and squamous cell carcinoma

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Abstract:

Objectives: This study explored the expression of DNA mismatch repair (MMR) proteins in a range of oral biopsies. We furthermore evaluated the significance of MMR protein expression combined with basic demographic data in differentiating grades of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC).

Study Design: Immunohistochemical expression of MutSα (hMLH1 and hPMS2) and MutLα (hMSH2 and hMSH6) was compared in 98 formalin fixed paraffin embedded oral biopsies: 21 normal, 24 mild-dysplasia (MD), 8 moderate/severe-dysplasia (SD) and 45 OSCC.

Results: Expression of hMLH1, hPMS2 and hMSH2 was reduced in MD, SD and OSCC compared to normal. Reduced hMSH2 immunoreactivity discriminated poorly-differentiated OSCC from well-differentiated OSCC. The diagnostic model correctly classified 71.4% of cases and revealed that hPMS2 negative biopsies were more likely to be cancerous (OR[95% CI] 0.11[0.000-0.813] P=0.040).

Conclusion: The results suggest a diagnostic role for MMR proteins in OED and OSCC.

Keywords: Mismatch Repair, Oral Epithelial Dysplasia, Oral Squamous Cell Carcinoma, Immunohistochemsitry, Diagnostic Model.

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Introduction:

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of oral cavity and oropharynx malignancies.[1] Oral malignant transformation is a multi-step process[2] that can initially manifest as oral potentially malignant disorders (OPMD).[3] The most important prognostic factor for malignant transformation is the degree of epithelial dysplasia,[4] with lesion site, patient age, gender and alcohol / smoking habits also considered. The concept of field cancerization was first discussed in 1953 and is likely to have a molecular foundation in DNA repair mechanisms which are susceptible to both genetic and epigenetic compromise, since surveillance of DNA integrity is a most fundamental defence against localised mutation, clonal selection and malignancy.

The human DNA mismatch repair (MMR) genes are pivotal in preserving genomic integrity by repairing base substitution mismatches and insertion/deletion mismatches that evade the "proof-reading" functionality of DNA polymerases. Defective MMR mechanisms generate novel genomic microsatellites, otherwise known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs), which can be passed clonally throughout cellular progeny. Cumulative acquisition of DNA microsatellites (termed microsatellite instability) is the hallmark of defective MMR pathways and has been associated with numerous malignancies.[7-9]

Nomenclature and structure of two MMR proteins (MutS and MutL) are conserved from prokaryotes to eukaryotes, but in contrast to bacterial Mut proteins, eukaryotic Mut proteins are heterodimers of two related but distinct proteins.[10] The current study focused on one form of both the major Mut proteins: MutSα and MutLα, in each case using antibodies against both of their constituent proteins. MutSα proteins are a heterodimer of hMSH2 and hMSH6 and discriminate between mismatched and perfectly paired DNA. Similarly, MutLα proteins are a heterodimer of hMLH1 and hPMS2, and are believed to repair post-replication errors.[11]

Hereditary non-polyposis colorectal cancer (HNPCC), otherwise known as Lynch syndrome, is an autosomal dominant syndrome caused by mutations in MutSα and MutLα genes.[12] A diagnosis of Lynch Syndrome is reached through a combination of clinical and tumour biomarker assessments. Semiquantitative assessment of immunohistochemical staining for MutSα and MutLα genes has been shown to be a useful asset in screening for carriers of these gene mutations in Lynch syndrome patients.[13] Similarly, expression of hMLH1 and hMSH2

is either lost or significantly reduced in head and neck squamous cell carcinoma and lesions with a high degree of dysplasia.[14-20] Allelic imbalance in hMLH1 has been suggested to be an aetiological factor in head and neck carcinogenesis [20] and promoter methylation of this gene has been shown to be an early event in oral carcinogenesis.[14] Previous studies in this area have been limited to expression of hMLH1 and hMSH2 in either OPMD or squamous cell carcinoma.

In the current study, we hypothesised decreased MMR protein expression to be associated with oral epithelial dysplasia (OED) and OSCC. We explored MMR protein expression based on immunohistochemistry (IHC) staining of hMLH1, hPMS2, hMSH2, and hMSH6 across a range of normal, dysplastic and cancerous oral biopsies. Furthermore we combined MutSα and MutLα expression as a panel with minimal patient demographic co-factors (age and sex) in a multinomial logistic regression model to evaluate their statistical significance in differentiating grades of OED and OSCC.

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Materials and Methods:

Ethical review: This study was approved by the Ethics Committees of the University of Queensland (2007001478) and the Royal Brisbane Hospital (HREC/10/QRBW366).

Patient Samples: A total of 98 formalin-fixed paraffin-embedded (FFPE) specimens were chosen randomly from tissue archives of the Oral Oncology Research Group, the University of Queensland. Samples were collected between 1947 and 2012 and classified histologically as 21 negative for dysplastic changes (referred to herein as normal), 24 mild dysplasia (MD), 8 moderate to severe dysplasia (SD), and 45 oral squamous cell carcinoma (OSCC) to form a cohort of 98 patients with a male to female ratio of 1.27 (Table 1). A Haematoxylin and Eosin (H&E) stained section from the samples was assessed by an oral pathologist (CSF) according to the World Health Organization (WHO) classification system for oral epithelial dysplasia.[21] OSCC cases were further graded into well and moderately/poorly differentiated groups according to WHO criteria.[21]

Immunohistochemical staining: Immunohistochemical staining was performed as described by us previously.[22] Briefly, 5-µm sections were dewaxed and dehydrated in xylene and graded ethanol. Heat-induced epitope retrieval (HIER) utilised Diva decloaker solution (DV2004MX Biocare Medical, Concord CA, USA) in a Decloaking Chamber™ (Model DC2002 Biocare Medical, Concord CA, USA) at 125ºC for 30 seconds then returned to 90°C, followed by a 5-minute wash in distilled water. Sections were incubated in endogenous peroxidase blocker (Peroxidazed1 PX968M Biocare Medical, Concord CA, USA), then non-specific background stain blocking reagent (Background Snipper, BS966G, Biocare Medical, Concord CA, USA). Slides were incubated for 15 minutes with primary monoclonal anti-human antibodies (Biocare Medical Inc, Concord, CA, USA) as follows: hMLH1 (G168-15), hPMS2 (A 16-4), hMSH2 (FE11) and hMSH6 (BC/44). Secondary antibody and revealing agent was obtained from Biocare Medical (MACH1 Universal HRP polymer kit, M1U539L10, Biocare Medical, Concord, CA, USA).Sections were revealed using Biocare Betazoid DAB (BDB900G, Biocare Medical, Concord CA, USA). Sections were counterstained with CAT haematoxylin (CATH-M Biocare Medical, Concord CA, USA) and mounted with Leica CV mount (Leica Microsystems, Wetzlar, Germany). Positive (colon) and negative staining controls were conducted.

H-score of positive immunoreactivity in stained sections: Immunoreactivity of hMLH1, hPMS2, hMSH2 and hMSH6 was analysed semi-quantitatively. Epithelial cells with brown

nuclear staining, regardless of intensity, were considered positive. Both negative and positive cells were counted in 16 randomized high power (x400) microscopic fields [23] using light microscopy and an eyepiece grid (Standard 20, Olympus, Japan). Epithelial cells from the invasive front of OSCC cases were included in the pool of all epithelial cells and also recorded separately for further analysis. In small samples where there were less than sixteen fields per slide, all fields were evaluated. The percentage of positively stained cells was scored as described by Muller et al.[24] A score of 0 indicated no cell immunopositivity, score 1 represented 1-10% positive cells, 2; 11-50% positive cells, 3; 51-80% positive cells and 4; more than 80% positive cells.[24]

The immunoreactivity intensity was scored on a 0-3 scale based on comparison of epithelial cell staining intensity with positive control cells (Figure 1). A score of 3 indicated immunoreactivity equivalent to that of positive control and a score of 0 denoted no immunoreactivity. The H-score, which ranged from 0 to 12, was the product of categorised percentage positive score and the scaled stain intensity score.[13]

In order to perform multinomial logistic regression, patients were further categorized into two groups based on immunoexpression thresholds as per Barrow et al. where the genetic alterations in each of the MMR genes was associated with their immunohistochemical expression.[13] Although immunoexpression of hMSH6 did not meet the minimum requirements for carrier mutation detection in Barrow's study, an H-score of 5 or less was considered a carrier for hMSH6 mutations (sensitivity: 81.3%, specificity: 80.85). [13]

Statistical Analysis:

Non-parametric analysis was performed using IBM SPSS Statistics V.20 software (IBM Corporation, Armonk, NY, USA). H-score data normality was established using the Kolmogorov-Smirnov test. Immunolocalisation H-score data for disease groups were compared to normal using two-tailed Mann-Whitney U tests with Bonferroni correction for n=6 comparisons (*$p<0.008$) unless stated otherwise. The relationship between expression of MMR protein expression (H-score) and lesion severity was established by Spearman's product-moment correlation coefficient after ordinal grading of lesions: normal, 0; mildly dysplastic, 1; severely dysplastic, 2; OSCC, 3. Multinomial logistic regression was used to identify predictors of dysplasia/neoplasia in comparison to normal tissue, with H-score

thresholds for the mismatch repair panel,[13] and sex of the participants as independent variables while adjusting for age.

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Results:

MMR protein intra-cellular distribution.

Figure 2 shows typical immunostaining of 4 mismatch repair genes (hMLH1, hPMS2, hMSH2 and hMSH6) in normal oral tissue, mild dysplasia, moderate-severe dysplasia, and oral squamous cell carcinoma (OSCC). Immunoreactivity for these proteins was localised predominantly in cell nuclei, with a small number of samples exhibiting cytoplasmic expression of MMR proteins (hMLH1 in 2 OSCC and 1 moderate-severe dysplasia; hPMS2 in 2 OSCC, 3 mild dysplasia, 1 normal; hMSH2 in 3 OSCC; hMSH6 in 12 mild dysplasia and 10 normal; all 4 proteins in 3 OSCC and 1 moderate-severe dysplasia).

MMR protein association with oral disease severity.

Figure 3 provides a non-parametric representation of H-score distribution grouped by lesion classification. Relative to normal tissue, the overall trend was for all lesion groups to exhibit reduced expression of hMLH1, hPMS2 and hMSH2 which was supported in most cases (6/9) by Mann-Whitney U tests, although large inter-quartile ranges of H-score impaired attainment of statistical significance for severe dysplasia lesions stained for hMLH1 and hPMS2 and for OSCC lesions stained for hMSH2. Interestingly, the H-scores that describe expression of hMSH6 showed equivalent median values and very high inter-quartile ranges for all lesion groups. OSCC cases were graded into well and moderately/poorly differentiated groups according to WHO criteria.[21] Median H-scores for hMSH2 expression were higher in well differentiated (median score = 7; IQR = 3.7 to 9; n = 26) compared to poorly differentiated cases (median score = 4; IQR = 4 to 6; n = 19), ($U = 159.500$, $z = -2.051$, $p = 0.040$, Mann-Whitney U test without Bonferroni correction (*$p<0.05$)).

Correlation of MMR protein expression with oral disease severity.

The relationship between MMR protein expression and disease severity was investigated by Spearman's product-moment correlation coefficient of H-score data versus an ordinal grading of lesion severity. Relatively weak negative correlations with graded lesion severity were observed for hPMS2, hMSH2 and hMLH1, but not hMSH6 (Table 2). The strongest relationship between MMR protein expression and disease severity was observed for hPMS2 ($\rho = -0.514$, $n = 98$, $p<0.0001$), the only other statistically significant correlation being for hMLH1 ($\rho = -0.311$, $n = 98$, $p<0.002$).

Co-expression correlations for MMR protein expression.

Based on what is known about the four MMR proteins under investigation, one might expect co-expression of hMSH2 with hMSH6 (to comprise the MutSα heterodimer) and co-expression of hMLH1 with hPMS2 (to comprise the MutLα heterodimer). Co-expression correlation coefficients for each antigen are also presented in Table 2. All MMR proteins exhibited weak but statistically significant positive co-expression correlations. As anticipated, the strongest co-expression correlations were between hMSH2 and hMSH6 ($\rho = 0.457$, $n = 98$, $p<0.0001$) and between hMLH1 with hPMS2 ($\rho = 0.403$, $n = 98$, $p<0.0001$).

Construction of a diagnostic model of oral carcinogenesis based on DNA mismatch repair protein immunoexpression.

Multinomial logistic regression was performed to assess the impact of independent variables (sex, mismatch repair pathway protein H-scores) on the likelihood and degree of dysplasia/neoplasia in participants, adjusted for age. Due to the small number of samples, site of the lesion and history of smoking were not included in this model. The model was able to differentiate between the different degrees of dysplasia/neoplasia χ^2 (18, n = 98) = 114.409, $p<0.0001$. The model classified 71.4% of cases correctly (Table 3) and explained between 68.9% (Cox and Snell R square) and 75.2% (Nagelkerke R square) of variance in diagnosis, with the best prediction power for normal (95.2%) and the weakest prediction power for severe dysplasia at 37.5%. The strongest association for MMR proteins was between hPMS2 and ability of the model to differentiate between normal and OSCC (Table 4). Here, the model identified cases with loss of hPMS2 as being more likely to have OSCC (OR[95% CI] 0.011[0.000-0.813] P=0.040) compared to normal. The general formula by which the model was designed is as follows:

$$Ln\left(\frac{p(Dx)}{p(Normal)}\right) = \mathrm{b} + \sum(\beta_{xi} \times xi)$$

in which *p*= probability, *Dx*= Diagnosis of sample (mild dysplasia, moderate/severe dysplasia and OSCC), b= interface, β= Coefficient for each independent variable, *x*= independent variable and *i*= score for which model is calculated.

Below are the three equations used for data shown in Table 4.

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$$Ln\left(\frac{\text{probability(mild dysplasia)}}{\text{probability(normal)}}\right)$$

$$= -0.255 + 0.154(\text{age}) - 3.215(\text{sex} = 1.0) - 0.1566(\text{hMLH1}_{\text{Score}} = 1.00) - 0.4641(\text{hMSH2}_{\text{Score}} = 1.00) - 1.220(\text{hMSH6}_{\text{Score}} = 1.00) - 0.2.915(\text{hPMS2}_{\text{Score}} = 1.00)$$

$$Ln\left(\frac{\text{probability(moderate to severe dysplasia)}}{\text{probability(normal)}}\right)$$

$$= -2.673 + .172(\text{age}) + (-5.144)(\text{sex} = 1.0) + (1.278)(\text{hMLH1}_{\text{Score}} = 1.00) + (-2.054)(\text{hMSH2}_{\text{Score}} = 1.00) + (-1.484)(\text{hMSH6}_{\text{Score}} = 1.00) + (-2.502)(\text{hPMS2}_{\text{Score}} = 1.00)$$

$$Ln\left(\frac{\text{probability(OSCC)}}{\text{probability(normal)}}\right)$$

$$= -2.104 + 0.202(\text{age}) - 4.377\ (\text{sex} = 1.0) - 1.746\ (\text{hMLH1}_{\text{Score}} = 1.00) + 1.058\ (\text{hMSH2}_{\text{score}} = 1.00) - 1.413\ (\text{hMSH6}_{\text{Score}} = 1.00) - 4.554\ (\text{hPMS2}_{\text{Score}} = 1.00)$$

Discussion:

This study analysed immunoreactive protein expression of the 4 sub-units that comprise the 2 major MMR proteins, MutSα and MutLα, in an archive of 98 FFPE oral tissue biopsies bearing a spectrum of disease ranging from normal through mild and moderate/severe dysplasia to oral squamous cell carcinoma (OSCC). Our principal aim was to demonstrate alterations in expression of MMR proteins in oral dysplasia and neoplasia, as previously reported for other cancer types. This was achieved by construction of a predictive model of OSCC that was based on IHC staining of hMLH1, hPMS2, hMSH2, and hMSH6 combined with minimal patient demographic co-factors (age and sex). A trend between oral disease severity and reduced expression of hMLH1, hPMS2 and hMSH2 (but not hMSH6) was evident, and interestingly, reduced hMSH2 immunoreactivity discriminated poorly differentiated OSCC from well differentiated OSCC. In addition, this study investigated co-expression correlations for the 4 MMR associated proteins and documented sporadic anomalies in their intracellular distribution. The results affirm the role of genetic instability in oral carcinogenesis by demonstrating a negative trend between MutSα and MutLα immunoscore data and diagnostic grading of dysplasia and OSCC severity. These findings suggest potential application of MutSα and MutLα expression analysis to diagnostic grading of dysplasia and OSCC, and support the hypothesis that defective MMR pathways play a functional role in oral cancer. At this stage, however, there is no suggestion that MutSα and MutLα are potential therapeutic targets for OSCC.

Under normal conditions, expression of MMR proteins has been associated with a proliferative epithelial compartment (transient amplifying cells adjacent to basement membrane with an active replication process).[25] The proliferative region within epithelial dysplasia or neoplasia however is more difficult to define. Therefore the scoring of these proteins as a comparative evaluation of DNA repair in epithelial samples must encompass the entire epithelium. The design, implementation and interpretation of our semiquantitative assessment of MutSα and MutLα drew upon the seminal work of Barrow et al. which found immunohistochemical staining for MutSα and MutLα to be an asset for identification of carriers of mutated MMR genes in patients with Lynch syndrome.[13] Since its discovery a century ago, Lynch syndrome has been associated with inherited genetic mutations in MMR proteins and a higher risk for acquiring several forms of malignancy including colorectal tumours, breast cancer,[26] lung cancer, adrenal cortical neoplasm and pancreatic acinar cell carcinoma.[27] Only a limited number of studies have focused on association and pathologic

features of MMR related tumours in the upper gastrointestinal tract and specifically oral squamous cell carcinoma.

The work of Barrow et al. is significant as it used receiver operating characteristic (ROC) curves to link MMR gene mutations to their histochemical immunoreactivity in Lynch syndrome. The current study utilised the immunoexpression thresholds that identify MMR gene mutations in Lynch syndrome to categorise oral biopsies into two independent groups that became the nominal dependent variables in multinomial logistic regression analysis of immunoreactivity H-scores, age and gender.

The diagnostic model of oral carcinogenesis presented here was effective in distinguishing oral lesion disease severity ($\chi2$ (18, n=98) = 88.543, $p<0.0001$), correctly identifying 71.4% of cases correctly and explaining 68.9% to 75.2% of diagnostic variance. Subsequent work is intended to validate this model in a larger independent cohort of dysplastic and OSCC patient biopsies.

Defective MMR pathways are associated with several pathological conditions.[7-9] Phenotypic consequences of microsatellite accumulation in coding and non-coding regions of DNA may account for the observation that genomic instability is an early event in carcinogenesis.[6] The role of MMR proteins in oral carcinogenesis has not yet been studied extensively,[18, 28] tending to be limited to the presence or absence of hMLH1 and hMSH2 genes in dysplastic or neoplastic oral lesions. The current study is more inclusive, encompassing loss, reduction and over expression of hMLH1, hMSH2, hMSH6, hPMS2 in a range of normal, dysplastic and neoplastic oral samples.

We provide co-expression correlations for the four MMR proteins under investigation. These data support known heterodynamic associations between hMSH2 and hMSH6 within the MutSα complex, and between hMLH1 and hPMS2 within the MutLα complex. Weaker correlations that cross MutSα and MutLα homologs are also of interest since they suggest that MutSα and MutLα expression levels become compromised in oral lesions simultaneously.

Both MutLα subunits, hMLH1 and hPMS2, exhibited reduced expression with increased disease severity. This is the first study of hPMS2 expression in a range of oral tissues, whereas expression of hMLH1 in oral lesions has been the subject of prior publications. Reduced immunoreactivity of hMLH1 in dysplastic lesions and OSCC complements previous

reports of negative correlations between hMLH1 expression and disease severity for OPMD and OSCC.[16-18] Promoter methylation and subsequent loss of hMLH1 expression has previously been associated with oral carcinogenesis and tumour progression.[14, 29] Positive immunoreactivity for hMLH1 has great specificity and sensitivity for microsatellite instability detection,[30] and it is possible that previously reported intra-tumour heterogeneity in microsatellite instability for OSCC[31] may correlate with similar hMLH1 heterogeneity. However, our study did not demonstrate a significant difference between hMLH1 expression at the invasive tumour front compared to central/superficial areas, and only 4 OSCC cases showed intra-tumour heterogeneity in hMLH1 expression.

For the MutSα subunits, hMSH2 and hMSH6, reduced expression with increased disease severity was only observed for hMSH2. There were no noteworthy trends between hMSH6 immunoexpression and lesion grading. Favourable oral disease stratification was achieved with hMSH2 which distinguished mild and moderate-severe dysplasia from normal tissue and distinguished well differentiated from poorly differentiated OSCC. Similar studies in colon tissues have shown hMSH2 to be a good marker of MMR mutation carriers.[24, 32] Although hMSH2 and hMSH6 contribute to the MutSα complex, a previous study in head and neck cancer indicated a low level (12%) of allelic imbalance at hMSH2/hMSH6 loci. While expression of hMSH6 did not correlate with disease severity, inclusion of this protein in an associate covariate model of MMR proteins improved the ability of the model in classifying moderate-severe dysplasia samples by 13%. Hence we believe that the decision to exclude hMSH6 from this panel should be postponed until replication of our findings is undertaken.

The present results affirm the role of genetic instability in oral carcinogenesis by demonstrating a negative trend between MutSα and MutLα immunoscore data and diagnostic grading of oral epithelial dysplasia and OSCC severity. These findings are concluded from a small sample size with limited number of dysplastic cases, particularly moderate and severe dysplasia. Further validation in an independent cohort of samples and patients would be recommended.

This is a retrospective study performed on randomly selected representative oral biopsies collected over an extended period of time and inevitably moderate/severely dysplastic cases are under-represented. Through a mathematical model we proposed a potential diagnostic

role for MMR proteins as an immunohistochemical panel; however this model is only an adjunct to the diagnosis made by a trained pathologist and should be considered within its innate limitations. In conclusion, our data support the hypothesis that defective MMR pathways play a potentially functional role in oral cancer. Although there is no suggestion that MutSα and MutLα are potential therapeutic targets for OSCC, application of MutSα and MutLα expression analysis to diagnostic grading of oral epithelial dysplasia and OSCC has potential to promote early detection and improved management of OPMD and oral cancer.

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Figure legends

Figure 1

Representative immunostaining of the positive control for hMLH1 (i), hPMS2 (ii), hMSH2 (iii) and hMSH6 (iv).

Figure 2

Representative photomicrographs showing A) Normal samples for ii) hMLH1, iii) hPMS2 , iv) hMSH2 and v) hMSH6 (H-score=12 across the board, B) mild dysplasia for ii) hMLH1 (H-score=8), hPMS2 (H-score=9), hMSH2 (H-score=8) and hMSH6 (H-score=9), C) moderate dysplasia for ii) hMLH1 (H-score=2), hPMS2 (H-score=4), hMSH2 (H-score=6) and hMSH6 (H-score=9), D) epithelium from well differentiated OSCC samples for hMLH1 (H-score=6), hPMS2 (H-score=9), hMSH2 (H-score=4) and hMSH6 (H-score=6), E) well differentiated OSCC cell nests and the underlying connective tissue for hMLH1 (H-score=6), hPMS2 (H-score=4), hMSH2 (H-score=6) and hMSH6 (H-score=9) and F) poorly differentiated OSCC for hMLH1 (H-score=0), hPMS2 (H-score=1), hMSH2 (H-score=0) and hMSH6 (H-score=2). Relevant haematoxylin and eosin photomicrographs for each sample are shown in column i (Ai to Fi).

Figure 3

Distribution of immunohistochemical expression scores for mismatch repair proteins in normal, mild dysplasia (MD), moderate-severe dysplasia (SD) and OSCC samples. (* P <0.0125)

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Table1: Characteristics of patient samples (n=98).

	Normal mucosa	Dysplasia		OSCC	
		Mild	Moderate/ severe	Well differentiated	Moderately/ poorly differentiated
Gender					
Male	8	13	6	19	9
Female	13	11	2	7	10
Age					
< 45 (range 18-45)	20	4	2	3	1
≥45 (range 45-73)	1	20	6	23	18
Biopsy site					
Tongue	4	8	1	3	1
Palate	2	1	1	11	2
Gingiva	9	5	2	1	4
Labial mucosa	0	6	1	7	4
Buccal mucosa	6	4	3	4	8
Smoking history					
Positive	1	15	3	15	12
Negative	20	1	0	3	2
Unavailable	0	8	5	8	5

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Table 2: Correlation of MMR gene expression with each other and patient's graded diagnosis.

	MutLα		MutSα		Diagnosis
	hMLH1	hPMS2	hMSH2	hMSH6	
MutLα					
hMLH1	-	0.403**	0.396**	0.131	-0.311**
hPMS2		-	0.214*	0.191	-0.514**
MutSα					
hMSH2			-	0.457**	-0.47
hMSH6				-	0.34

** Correlation is significant at $P < 0.01$ (2 tailed)

* Correlation is significant at $p < 0.05$ (2 tailed)

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Table 3. Predicted vs observed diagnosis according to multinomial logistic regression adjusted for age of the participants.

	Predicted				
Observed	Normal	Mild dysplasia	Mod/Severe dysplasia	OSCC	% Correct
Normal	20	0	0	1	95.2%
Mild dysplasia	0	9	1	14	37.5%
Mod./Severe dysplasia	1	1	4	2	50.0%
OSCC	1	6	1	37	82.2%
Overall Percentage	22.4%	16.3%	6.1%	55.1%	71.4%

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Table 4: Environmental and genetic predictors of diagnosis according to multinomial logistic regression model.

	Lesions compared to normal cohort OR (CI 95%)		
	Mild dysplasia	Severe dysplasia	OSCC
Demographic			
Age	1.167 (1.045-1.303)*	1.188 (1.052-1.340)*	1.223 (1.088-1.375)*
Sex	0.040 (0.000- 4.773)	0.006 (0.000-1.147)**	0.013 (0.000-1.697)
Expression level			
hMLH1	0.209 (0.007-6.531)	3.589 (0.078-164.260)	0.175 (0.005-6.162)
hMSH2	0.629 (0.015-26.617)	0.128 (0.002-8.054)	2.879 (0.061-136.511)
hMSH6	0.295 (0.010-9.125)	0.227 (0.006-8.930)	0.243 (0.007-8.052)
hPMS2	0.054 (0.001-3.859)	0.082 (0.001-7.840)	**0.011 (0.000-0.813)***

* $p < 0.05$
** p= 0.056

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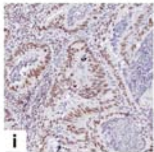
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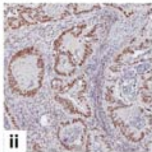
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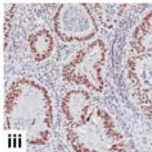
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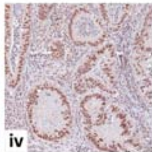
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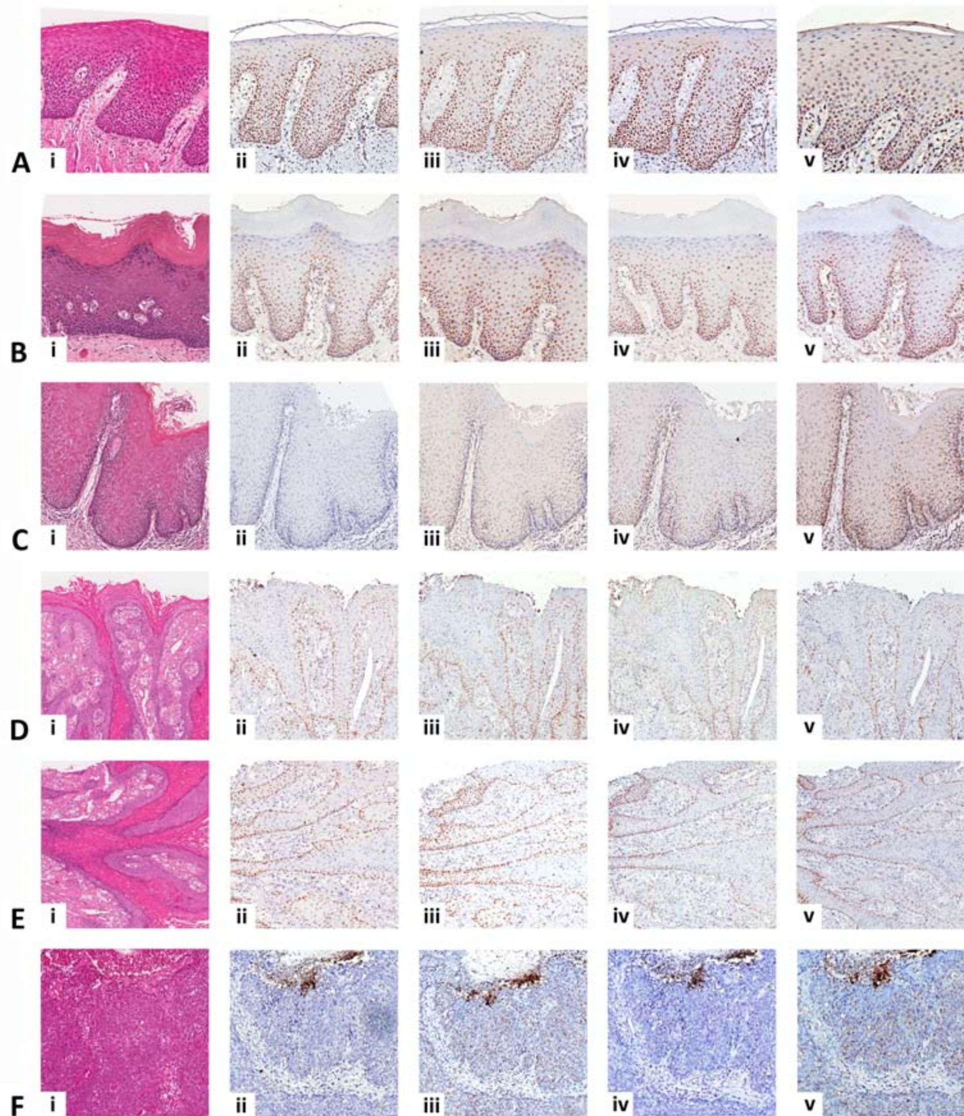
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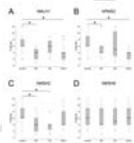
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