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Research Article

See Perspectives on p. 1073 and p. 1075

Loss of Heterozygosity (LOH) Profiles—Validated Risk Predictors for Progression to Oral Cancer

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Abstract

A major barrier to oral cancer prevention has been the lack of validated risk predictors for oral premalignant lesions (OPL). In 2000, we proposed a loss of heterozygosity (LOH) risk model in a retrospective study. This paper validated the previously reported LOH profiles as risk predictors and developed refined models via the largest longitudinal study to date of low-grade OPLs from a population-based patient group. Analysis involved a prospective cohort of 296 patients with primary mild/moderate oral dysplasia enrolled in the Oral Cancer Prediction Longitudinal Study. LOH status was determined in these OPLs. Patients were classified into high-risk or low-risk profiles to validate the 2000 model. Risk models were refined using recursive partitioning and Cox regression analyses. The prospective cohort validated that the high-risk lesions (3p and/or 9p LOH) had a 22.6-fold increase in risk ($P = 0.002$) compared with low-risk lesions (3p and 9p retention). Addition of another 2 markers (loci on 4q/17p) further improved the risk prediction, with five-year progression rates of 3.1%, 16.3%, and 63.1% for the low-, intermediate-, and high-risk lesions, respectively. Compared with the low-risk group, intermediate- and high-risk groups had 11.6-fold and 52.1-fold increase in risk ($P < 0.001$). LOH profiles as risk predictors in the refined model were validated in the retrospective cohort. Multicovariate analysis with clinical features showed LOH models to be the most significant predictors of progression. LOH profiles can reliably differentiate progression risk for OPLs. Potential uses include increasing surveillance for patients with elevated risk, improving target intervention for high-risk patients while sparing a large number of low-risk patients from needless screening and treatment. *Cancer Prev Res; 5(9); 1–9. ©2012 AACR.*

Introduction

Oral squamous cell carcinoma (SCC) has high global public health impact, incurring an estimated 263,900 new cases and 128,000 deaths in 2008 (1). The ability to detect the disease in the premalignant stage could have a significant impact on outcome. The challenge has been to differentiate premalignant lesions at high-risk from those at low-risk of undergoing progression to better target interventions that improve patient well-being as well as cost and health resource efficiency. The presence of dysplastic areas provides an indication of risk, especially for higher grades of dysplasia and severe dysplasia/carcinoma *in situ* (2). However, histology is a relatively poor predictor for lesions with lower grade dysplasia (mild or moderate), which represents the majority of oral dysplasia.

The search for additional markers for malignant progression has spanned decades; however, there are no validated markers to date. Loss of heterozygosity (LOH) in key chromosomal loci represents one of the more promising markers in the literature consistently identified as a potentially independent risk predictor, supported by data from several laboratories, including hallmark studies by Sidransky, Califano, Mao, Hong, Lippman, and Lee (3–6). In 2000 (7), we used a retrospective analysis of oral premalignant lesions (OPL) with known outcome to develop a model for oral cancer progression. That model showed a greater than 20-fold increase in progression risk for lesions with 3p and/or 9p LOH compared with lesions with retention of these 2 regions. In this paper, we report the validation of the LOH profiles as risk predictors in the previous model using a new prospective cohort obtained from the Oral Cancer Prediction Longitudinal (OCPL) Study. The OCPL is the largest longitudinal study attempted to date, following patients with primary mild or moderate oral dysplasia and is unique in that it draws from a community-based rather than a high-risk population. We also report

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on the use of these new samples to further refine our LOH model. Subsequently, the new model was "reverse" validated with samples used in our original retrospective study. We show that both previous and current LOH models are strong predictors of progression for low-grade dysplasia in multicovariate analyses with clinical and molecular features. This validated molecular model holds great promise for improving the clinical management of oral precancers.

Materials and Methods

Patient population

This population-based study involved patients who prospectively enrolled in the ongoing OCPL Study in Vancouver (British Columbia, Canada) between January 1, 1997 and December 31, 2007. Accrual to this cohort was from community practices across British Columbia (population, 4.1 million in 2011). Patients were identified primarily through a centralised pathology service, the BC Oral Biopsy Service, which receives biopsies from dentists and ENT surgeons across the province. This population-based biopsy service receives 250 to 300 dysplasia cases annually. Patients with dysplastic lesions were referred to 5 Oral Dysplasia Clinics in Greater Vancouver where they were accrued to the study using written informed consent and a study protocol approved by the Institutional Research Board. Study eligibility required a histologic diagnosis of mild or moderate dysplasia in the oral cavity with no prior history of oral cancer. A total of 296 patients met these study criteria with a median follow-up time of 44·6 months (25th and 75th percentiles, 29.3 and 63.9). During the study period, 41 (13.9%) of these cases progressed, 17 to severe dysplasia (8), 2 to carcinoma *in situ* (CIS; ref. 2), and 22 to SCC (9).

Also included in the study analysis were data from the retrospective study reported in 2000 (7) that gave rise to the previous LOH model. That study included 116 individuals with OPLs (39 hyperplasia and 77 mild/moderate dysplasia) with no prior history of oral cancer identified between 1971 and 1997. The median follow-up time was 43.5 months (25th and 75th percentiles, 36.0 and 103.3). Twenty-nine of these patients (25.0%) underwent malignant progression, 5 to CIS, and 24 to SCC. In both the retrospective and prospective studies, primary OPLs were followed without any definitive treatment. The retrospective and the current prospective cohorts did not overlap.

No statistically significant differences were observed between the 2 cohorts in sex and tobacco exposure. The retrospective cohort was younger (median age = 48.8) with less patients in the ventral tongue/floor of mouth site (41.4%), and more hyperplasia but less mild and moderate dysplasia (33.6%, 34.5%, and 31.9%, respectively). To address the potential confounding of the difference in the patient characteristics and the risk assessment, we added the covariates which were significantly different between the 2 cohorts in the multicovariate Cox model analysis along with other significant risk predictors such as the LOH patterns. Only significant covariates were retained in the final model after adjusted with other covariates.

Clinical pathologic data and follow-up

The OCPL study collects demographic data, clinical information, as well as tobacco and alcohol habits at study entry. Patients were followed at 6 month intervals. At each follow up, oral lesions were examined and any worrisome changes were rebiopsied; repeat biopsies of the index site were scheduled for 2-year intervals if no biopsy was taken for that site in the intervening interval. The primary endpoint of this study was time from index biopsy to histologically confirmed progression to severe dysplasia or higher, occurring at the same anatomical site as the index biopsy. Inclusion of severe dysplasia as the progression endpoint was based on our findings that without treatment, progression occurred in 46% of patients in 3 years; 54% in 5 years (unpublished data).

Eighty-two of the 296 (27.7%) patients in the prospective cohort had multiple lesions. These were defined by biopsy to be true leukoplakia (i.e., histologically confirmed as hyperplasia, mild or moderate dysplasia with exclusion of confounding lesions such as reactive hyperplasia/trauma, candidiasis, lichen planus) and with at least 3 cm of clinically normal mucosa separating them from other lesions. Of cases with multiple lesions, 55 (18.5%) had 2 or more OPLs at study entry and 33 (11.1%) developed additional OPLs during follow-up. Six of the 33 cases had already had multiple OPLs at entry.

In multiple lesion cases, the choice of OPL for inclusion in this analysis was the one with poorest outcome, (i.e., those that progressed). In cases without progression, the OPL present at entry was used if only a single lesion was present at that time. If multiple lesions were present at entry, the one with the highest histologic grade was chosen for analysis. Histologic diagnoses were reviewed by at least 2 of the study pathologists (L. Zhang, C.F. Poh, and K. Berean) and a consensus diagnosis used in data analysis.

Assessment of molecular risk pattern

Areas of dysplasia were microdissected for microsatellite analysis. The same protocol for analyzing the LOH markers in chromosome regions 3p14.2; 4q26, 4q31.1; 8p21.3, 8p23.3; 9p21; 11q13.3, 11q22.3; 13q12.3-13, 13q14.3; 17p11.2, and 17p13.1 was applied in this prospective study as was described in the aforementioned retrospective study (7). LOH analysis was done as a blind analysis on coded samples.

Statistical analysis

For both the retrospective cohort and the prospective cohort, the main analyses were based on the time-to-event outcome because every patient had a different length of follow-up. Time to endpoint was calculated from date of the index biopsy to endpoint date or to last follow-up date before May 31, 2010 if no progression occurred. Time-to-progression curves were estimated using Kaplan–Meier analysis. HRs and the corresponding 95% confidence intervals (95% CI) were determined using Cox regression analysis with the Wald test. Associations between patient prognostic factors and outcome were tested using the

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univariate Cox model. The proportional hazard assumption was tested by the cox.zph function, which tests for zero slope in the regression line in the plot of Schoenfeld residual versus log (time). Recursive partitioning using RPART with exponential scaling for survival data was also used as a method for classifying patients according to progression risk. In addition, the 5-year progression rate and its corresponding 95% CI were calculated for each risk group in the prospective cohort. Note that the retrospective cohort was based on a case-control design. Hence, the estimated progression rate from the survival analysis might not reflect the population progression rate. However, assuming that the risk predictors (e.g., LOH profiles, high-risk site, and smoking status) and the follow-up time are independent, HRs can still provide useful estimates for the progression risk for the risk predictors. *C*-index and its 95% CI were calculated to measure the prediction accuracy for time-to-event data. It was defined as the proportion of patient pairs in which the predictions and outcomes are concordant (10). *C*-index is equivalent to the area under the curve of a time-dependent ROC analysis with a value of 0.5 corresponding to a prediction by chance alone and 1.0 corresponding to perfect prediction. The validation of the risk models was based on confirming that the LOH profiles derived from one cohort were associated with progression risk in a different cohort. The validation was not based on the individual risk model, which accounts for the baseline risk as well as the absolute magnitude of the risk predictions. All tests were 2-sided with $P \leq 0{\cdot}05$ considered to be statistically significant. Statistical analyses were carried out using SPSS and the R language/package (11).

Results

Characteristics of patients in the current OCPL cohort

Table 1 shows demographics, lifestyle habits, and histology for all patients in the current study by outcome. Progression was associated with smoking status and site of the lesions but not with gender, age, race, and histologic diagnosis at entry. A univariate Cox analysis showed that lesions from never-smokers had a 2.1-fold increase in risk compared with ever (former/current)-smokers (95% CI, 1.1–3.9; $P = 0.02$) and that lesions from high-risk site (tongue and floor of mouth; ref. 12) had a 3.2-fold increase in risk compared with other oral sites (95% CI, 1. 5–6.7; $P = 0.002$). Hence, smoking status and high-risk site were used for further risk modeling.

Validation of the previous model

As done in the retrospective study, the initial characterisation of the lesions in the current study involved assessment of LOH on 7 chromosome arms. All progressing lesions showed LOH on least one of the arms. (Table 2)

We then validated the previous model of 3p and 9p retention as low-risk of progression and 3p and/or 9p LOH as high-risk using the current data (Table 2, shaded rows). Only one out of every 100 lesions with the low-risk pattern progressed. The high-risk pattern was present in virtually all progressing cases (39/40, 97.5%) and was associated with a 22.6-fold increase in progression risk (95% CI, 3.1–164.5; $P = 0.002$; Fig. 1A). These data were very similar to that observed in the previous retrospective study.

Of interest, when we examined the relative contribution of 3p and 9p to this progression model, some differences were observed between prospective and retrospective cohorts. In the prospective cohort, LOH at 3p was not a significant predictor for progression by itself (HR = 1.3, $P = 0.48$, Table 2). However, 9p LOH showed strong associations with progression in both cohorts (HR for the prospective cohort: 17.0; 95% CI, 4.1–70.8; $P < 0.001$; for retrospective cohort, 3.8; 95% CI, 1.6–8.9; $P = 0.002$, Table 2).

Development of the current model

To further refine the LOH model, we used recursive partitioning analysis to construct a new classification model that used the current data from regions on all 7 arms. The model chose 3 arms as covariates: 9p, 17p, and 4q (Fig. 2A). LOH status of 9p was the first most significant split. For cases showing 9pLOH, a second split involved 17p status, whereas among cases with 17p LOH, a third split involved 4q status (Fig. 2A). On the basis of this analysis, patients were placed into 3 categories with respect to the risk of progression: low-risk lesions (9pR, 46.8% of informative cases); intermediate-risk lesions (9pLOH only or 9pLOH with either 17pLOH or 4qLOH but not both; 43.2% of informative cases); and high-risk lesions (LOH on all 3 arms, 10.1% of informative cases). The 5-year progression rates for the low-, intermediate-, and high-risk groups along with their 95% CI shown in the parentheses were 3.1% (0%, 7.6%), 16.3% (8.2%, 23.4%), and 63.1% (29.5%, 80.7%), respectively. Compared with low-risk lesions, the HR for intermediate-risk lesions was 11.6 (95% CI, 2.7–49.9; $P = 0.001$) and for high-risk 52.1 (95% CI, 11. 8–230.6; $P < 0.001$; Fig. 2B). Time to progression was significantly shortened for the high-risk category compared with intermediate- and low-risk groups (Fig. 2B).

To validate the LOH profiles as the risk predictor in the new model, we classified cases from the previous retrospective cohort into the same 3 groupings and conducted a further Kaplan–Meier analysis and a Cox model analysis with the Wald test (Fig. 2C). A similar trend was observed with HRs for intermediate- and high-risk categories of 3.4 (95% CI, 1.4–8.2; $P = 0.006$) and 11.2 (95% CI, 3.3–38.6; $P<0.001$) over the low-risk lesions, respectively.

Multicovariate analysis including clinical variables

Multicovariate analysis was conducted on both previous and current LOH risk models, incorporating statistically significant clinical features associated with progression in univariate analysis: the location of lesion at high-risk site (yes or no) and smoking status (non-smoker, yes or no; Table 3). Histology was initially included but later removed due to nonsignificant results in both univariate and multivariate analyses. The analysis used the retrospective and prospective datasets, as described above. For each data set, we fitted 4 different Cox proportional hazards

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Table 1. Patient characteristics in the prospective cohort for all patients, nonprogressing patients, and progressing patients

Characteristic	All $N = 296$	Nonprogressing ($n = 255$)	Progressing ($n = 41$)	HR (95% CI)	P[a]
Age[b]					
Young	149	129 (86.6)[c]	20 (13.4)	1	0.76
Old	147	126 (85.7)	21 (14.3)	1.1 (0.59–2.0)	
Sex, *n* (%)					
Female	142	121 (85.2)	21 (14.8)	1	0.95
Male	154	134 (87.0)	20 (13.0)	1.0 (0.5–1.8)	
Race, *n* (%)[d]					
White	247	216 (87.4)	31 (12.6)	1	0.10
Non-white	49	39 (79.6)	10 (20.4)	1.8 (0.90–3.76)	
Tobacco exposure, *n* (%)[e]					
Never smoked	91	71 (78.0)	20 (22.0)	1	
Former smoker	125	107 (85.6)	18 (14.4)	0.6 (0.33–1.18)	0.15
Current smoker	80	77 (96.3)	3 (3.8)	0.2 (0.06–0.67)	0.009
Tobacco exposure, *n* (%)[e]					
Ever smoked	205	184 (89.8)	21 (10.2)	1	
Never smoked	91	71 (78.0)	20 (22.0)	2.1 (1.1–3.9)	0.02
Tobacco-smoking history, *n* (%)[f]					
Light smoker	111	98 (88.3)	13 (11.7)	1	0.15
Heavy smoker	88	81 (92.0)	7 (8.0)	0.5 (0.20–1.3)	
Alcohol consumption[g]					
Never or light drinker	229	199 (86.9)	30 (13.1)	1	0.58
Heavy drinker	53	45 (84.9)	8 (15.1)	1.2 (0.57–2.7)	
Site, *n* (%)					
Remaining sites	130	121 (93.1)	9 (6.9)	1	0.002
Ventrolateral tongue/floor of mouth	166	134 (80.7)	32 (19.3)	3.2 (1.53–6.74)	
Histology of index biopsy, *n*(%)					
Hyperplasia[h]	34	28 (82.4)	6 (17.6)	1	
Mild dysplasia	127	111 (87.4)	16 (12.6)	0.9 (0.33–2.22)	0.76
Moderate dysplasia	135	116 (85.9)	19 (14.1)	1.0 (0.39–2.46)	0.96
Duration of follow-up, mo					
Median	44.6	46.5	32.0		

[a]*P* values were calculated with the use of the univariate Cox model.
[b]Old was defined as age above median (57.6 years).
[c]Row percentage is reported.
[d]Race was self-reported.
[e]Smoker was defined as consumption of more than 100 cigarettes in life time
[f]Heavy smoker was defined as a pack-year above the median (22.2). A pack-year is defined as the equivalent of smoking one pack of cigarettes per day for 1 year.
[g]Alcohol information is only available for 282 patients. Heavy drinker is defined as consumption of more than 14 drinks per week for women and 21 drinks per week for men. 1 drink = 8oz beer = 4oz wine = 1oz spirits.
[h]Hyperplasia were from site of previous dysplasia.

regression models with follow-up time as the response variable and outcome as the censoring indicator. The 4 models used the following sets of covariates, respectively: model 1—previous LOH risk pattern; model 2—previous LOH risk pattern, high-risk site, and smoking status; model 3—current LOH risk pattern; and model 4—current LOH risk pattern, high-risk site, and smoking status. We then measured and compared the prediction accuracy of the 4 models based on the *C*-index. The analysis shows that the LOH patterns (previous or current) are the most significant covariates ($P < 0.05$ in all models). For the prospective cohort, high-risk site is significant in model 2. Nonsmoking status is significant in model 4 and is approaching significance at the 5% level in model 2. Neither was significant for the retrospective dataset.

C-index for model 4 with the current LOH model, high-risk site, and smoking status as covariates was 0.81 for the prospective cohort and was the highest in all models. For the

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Table 2. LOH patterns in prospective cohort and retrospective cohort

Study	LOH patterns[a]	All patients	Nonprogressing cases	Progressing cases	HR (95% CI)	*P*
Prospective cohort		296	255 (86.1)[b]	41 (13.9)		
	Presence of LOH[c]					
	No LOH	35	35 (100.0)	0 (0.0)	1	
	Any LOH	252	211 (83.7)	41 (16.3)	NA	
	3p and 9p					
	3p and 9p R	100	99 (99.0)	1 (1.0)	1	0.002
	3p and/or 9p LOH	191	152 (79.6)	39 (20.4)	22.6 (3.1—164. 5)	
	9p					
	9p R	130	128 (98.5)	2 (1.5)	1	<0.001
	9p LOH	161	124 (77.0)	37 (23.0)	17.0 (4.1–70.8)	
	3p					
	3p R	185	163 (88.1)	22 (11.0)	1	0.48
	3p LOH	106	90 (84.9)	16 (15.1)	1.3 (0.7–2. 4)	
Retrospective cohort		116	87 (75·.0)	29 (25.0)		
	Presence of LOH[c]					
	No LOH	36	36 (100.0)	0 (0.0)	1	
	Any LOH	68	39 (57.4)	29 (42.6)	NA	
	3p and 9p[d]					
	3p and 9p R	49	48 (98.0)	1 (2.0)	1	0.003
	3p and/or 9p LOH	60	32 (53.3)	28 (46.7)	21.1 (2.9—155.8)	
	9p					
	9p R	66	59 (89.4)	7 (10.6)	1	0.002
	9p LOH	47	25 (53.2)	22 (46.8)	3.8 (1.6–8.9)	
	3p					
	3p R	74	64 (86.5)	10 (13.5)	1	<0.001
	3p LOH	36	18 (50.0)	18 (50.0)	3.9 (1.8–8.4)	

[a]LOH, loss of heterozygosity; R, retention (no LOH); NI, non-informative. Due to NI, total numbers of cases for each reported LOH pattern may not add up to the total number of subjects.

[b]Row percentage is reported.

[c]For the "No LOH" category, subjects need to have retention in all chromosome arms tested. For the "Any LOH" category, subjects can have some chromosome arms to be non-informative as long as at least one chromosome arm was LOH.

[d]Ref. 7. Values in the Table differ slightly from original publication due to additional analyses of samples since publication of study. The previous publication included 7 cases that we categorised as low-or high-risk with NI on either 3p or 9p.

retrospective cohort, the *C*-index for model 2 is slightly better than that in model 4. Using model 4 on the prospective cohort showed a 9.7-fold increase for patients with the intermediate risk-pattern and a 41.7-fold increase in risk for patients with the high-risk pattern (i.e., 9pL/4qL/17pL) compared with patients with the low-risk pattern (9pR). The corresponding fold increases were 3.2 and 7.6 in the retrospective cohort, respectively.

To further examine the robustness of the risk models, we also examine the effect of LOH and other potential risk factors using only invasive cancer as the event outcome. On the basis of the LOH profiles, the LOH low-risk group defined in models 1 or 2 had no cancer developed for both retrospective and prospective cohorts. In addition, the LOH low-risk group defined in models 3 or 4 also had no cancer developed for the prospective cohort. In both cohorts and for all models, the oral cancer percentages were consistently higher in groups with higher risks. Note that the designation of "no cancer" or "cancer" is subject to the length of follow-up for each patient. When using only cancer as the event endpoint, we also analyzed the retrospective cohort using the covariates given in models 3 and 4. The results were consistent with what were reported in Table 3 (data not shown). We also tested the proportional hazards assumption in all the Cox model analyses. No apparent violations of the model assumption were noted.

Discussion

The oral cancer disease burden presents both a challenge and an opportunity to the clinical and scientific communities: adequate prevention and proper disease management remains difficult, but improvement is crucial. The oral cavity is a site that is readily amenable to clinical examination, with knowledge of both premalignant lesions and risk factors, for which histologic progression is well-defined. Indeed, some of the earliest studies on chemoprevention

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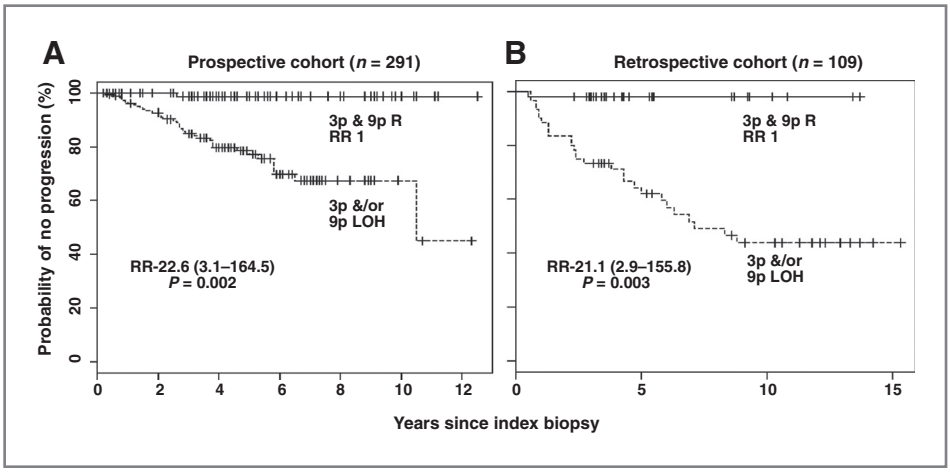

Figure 1. Kaplan–Meier plot of time to progression for the prospective cohort and the retrospective cohort with risk stratification by the previously reported model (7). A, Kaplan–Meier estimates plot in the prospective cohort using the previous model derived in the retrospective study of 2000, i.e., with a high-risk pattern of 3p and/or 9p LOH and a low-risk pattern of 3p and 9p retention (R): N = 191 for high-risk pattern (of whom 39 progressed); N = 100 for low-risk pattern (of whom 1 progressed). As a comparison, (B) shows Kaplan–Meier estimates in the earlier retrospective cohort (high-risk pattern, N = 60, of whom 28 progressed; low-risk pattern, N = 49, of whom 1 progressed). For all panels, total numbers are adjusted to reflect informative cases.

were done at this site (13–16). However, a main barrier continues to be the lack of validated markers that can stratify premalignant lesions into those at low- and high-risk for progression. It has been very difficult to study low-grade OPLs, as patients with OPLs are typically seen in community dentists' offices instead of research hospitals, making potential study participants difficult to identify and recruit. In addition, the retention of patients for longitudinal study can be quite challenging. The data presented in this paper provide the first independent validation for a group of LOH markers that predict progression for such lesions, confirming the importance and independency of these markers in

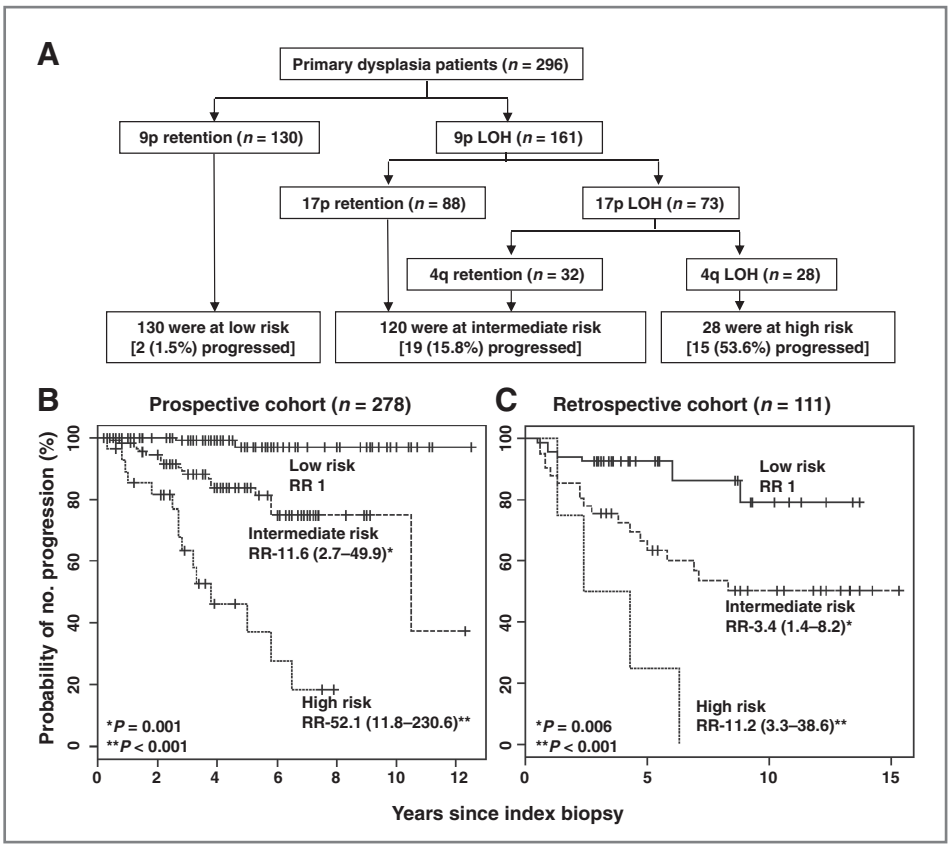

Figure 2. Classification of study patients into risk of progression categories and time to progression curves according to such categories. Recursive partitioning analysis was used to identify progressing factors with the most influential predictive significance in a proportional-hazards model for time to progression and to classify patients into categories of low, intermediate, or high risk of progression. Covariates in the analysis were 3p, 4q, 8p, 9p, 11q, 13q, and 17p. A, resulting classifications. Note that 5 patients were noninformative for 9p and an additional 13 were noninformative for 4q. (B) and (C) show data for the Kaplan–Meier plots of time to progression in the classified patients in prospective cohort and retrospective cohort, respectively. For all panels, total numbers are adjusted to reflect informative cases.

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Table 3. Cox proportional hazards models on time to progression

		Prospective cohort ($N = 296$)			Retrospective cohort ($N = 116$)		
Analysis	**Variable**	**HR (95% CI)**	***P***	***C*-index (95% CI)**	**HR (95% CI)**	***P***	***C*-index (95% CI)**
Previous models							
Model 1	3p and/or 9p LOH[a]	22.6 (3.1–164.5)	0.002	0.66 (0.47–0.81)	21.1 (2·9–155·8)	0.003	0.70 (0.48–0.86)
Model 2	3p and/or 9p LOH[a]	18.5 (2.5–135.3)	0. 004	0.75 (0.56–0.87)	14.4 (1.9–111.7)	0.01	0.77 (0.53–0.91)
	High-risk site	2.5 (1.2–5.6)	0.02		1.9 (0.8—4.7)	0.16	
	Non-smoker	1.8 (1.0—3.4)	*0.06*		1.8 (0.8–4.2)	0.17	
Current models							
Model 3	Intm-risk LOH[b]	11.6 (2.7–49.9)	0.001	0.79 (0.59–0.91)	3.4 (1.4–8.2)	0.006	0·68 (0·46–0·84)
	High-risk LOH[c]	52.1 (11.8–230.6)	<0.001		11.2 (3.3–38.6)	<0.001	
Model 4	Intm-risk LOH[b]	9.7 (2.2–42.0)	0.003	0.81 (0. 61–0.92)	3.2 (1.1–9.4)	0.04	
	High-risk LOH[c]	41.7 (9.3–187.6)	<0.001		7.6 (1.8–32.6)	0.007	0.75 (0·51–0.90)
	High-risk site	1.8 (0.8–4.0)	0.16		2.2 (0.8–5.7)	0.12	
	Nonsmoker	2.0 (1.0–3.8)	0.05		1.7 (0.7–4.1)	0.21	

[a]High-risk LOH: (3p &/or 9p LOH) compared with low-risk (3p and 9p R).
[b]Intm-R (Intermediate-risk LOH): (9pLOH only or with either 17pLOH or 4qLOH but not both) compared with low-risk (9pR).
[c]High-risk LOH: (9pL and 4qL and 17pL).

comparison to the clinical and pathologic features using both univariate and multicovariate analysis.

We present 2 LOH models in this study, each of which have potential clinical use. In the current study, retention of 3p14 and 9p21 loci was validated as a low-risk profile with only 1% of cases with this pattern showing progression. Because at least a third of cases in both prospective and retrospective cohorts had this pattern (34.4% and 45.0%, respectively), a significant proportion of individuals could be spared morbidity from more aggressive interventions using this indicator, enabling a targeted allocation of resources that will improve health system and cost efficiency. In contrast, LOH on 3p and/or 9p (the high-risk profile of the previous study) was present in virtually all progressing cases (39/40, 97.5%) with a significant elevation in HR, supporting the profile as a reliable high-risk indicator. However, as only 20% of cases with LOH on 3p and/or 9p underwent progression, there is a need for the development of more sophisticated markers to examine this subgroup of individuals to further stratify the risk (and to increase the specificity). Indeed, the strength of the refined model is its ability to better identify individuals with an increased likelihood of progression to cancer and represents a first step in this direction. When we input data from regions on 7 arms into a recursive-partitioning analysis of data from the prospective cohort, we identified 4q and 17p as containing important predictors of progression when used in conjunction with 9p. We confirmed the predictive value of the LOH profiles of this new model using data from the retrospective study. This analysis identified LOH on 3 arms (9p, 4q, and 17p) as having a 52.1-fold and 11.2-fold increase in progression risk compared with 9p retention in the prospective and retrospective cohort, respectively. More than 60% of such lesions showing progression within 5 years compared with approximately 30% for LOH on 3p and/or 9p, representing a significant improvement in risk prediction of high-risk lesions. This new model could in turn facilitate the identification of patients requiring aggressive monitoring or for accrual to a chemoprevention strategy. The higher risk of these patients points to the need for development of strategies that would improve outcome.

Alteration of the 9p21 locus, which contains $p16^{INK4a}$ and $p14^{ARF}$, has been receiving growing attention in recent years. Not only do these genes play an important role in halting cell-cycle progression through inhibition of phosphorylation of retinoblastoma protein and in causing cell-cycle arrest and apoptosis through stabilising of *p53* (8, 17, 18), but recent findings suggest a potential role for this locus in cellular senescence and tissue stem cell behavior (9, 19–23). The validation of the importance of 9p21 in both of our previous and current studies lends support for this locus as a driving force in progression of OPLs. In the current study, 3p14 had relatively little impact on progression risk by itself. This was unexpected given the aforementioned associations of this locus with outcome and suggests the need to revisit other markers on 3p to identify further candidates associating with risk. However, there is not yet a clear confirmed tumor suppressor gene target in the region. Thus, LOH on 3p may represent a passenger alteration rather than a driving force for progression, especially as this region has a well-known fragile site within it. The importance of genes *p53* and *CHRNB1* in the 17p region has been shown by numerous studies. The 4q region is fairly wide and requires fine mapping to better localize genes of interest and to reduce noninformativity.

Oral cancer and leukoplakia occur mostly in smokers; however, when leukoplakia does occur in nonsmokers, some studies showed that they are at higher risk for cancer progression (24–27). Our findings support these studies with significantly elevated risk for progression of lesions in

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nonsmokers compared with smokers with nearly half of the progressing lesions (48.8%) occurred in nonsmokers. One could postulate that these lesions have genetic underpinning. Furthermore, although smokers with leukoplakia could stop smoking to reduce the cancer progression risk, this is not possible for the nonsmokers. This fact highlights the need for clinicians to carefully assess the molecular profiles of such lesions with increased surveillance and appropriate management in accordance with their risk for progression to cancer.

In summary, this study provides the first validated molecular models for use in differentiating low-grade oral dysplasia at low-risk for progression from those with greater risk, via the largest longitudinal study of low-grade OPLs from a population-based patient group. Currently, 2 chemoprevention studies are using LOH markers to stratify patients at risk for multi-institutional trials in the United States—the phase III Erlotinib Prevention of Oral Cancer study (28) and the Phase II Cetuximab for Treatment of High-Risk Pre- Malignant Upper Aerodigestive Lesions trial (29). Results from these studies should add to our understanding of the use of these markers. The validation of the 2 risk models presented in this paper represents a significant first step in the evolution of a systematic decision-making process for this very heterogeneous group of lesions and an important move towards clinical application of these markers in a way that minimizes patient morbidity while maximising health system and cost efficiency.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Zhang, C.F. Poh, J.J. Lee, M.P. Rosin
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Zhang, C.F. Poh, M. Williams, D. Laronde, K.W. Berean, P. Gardner
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Zhang, C.F. Poh, D. Laronde, K.W. Berean, H. Jiang, L. Wu, J.J. Lee
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Zhang, H. Jiang, M.P. Rosin
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