Risk classification has a performance comparable to biomarkers

A second analysis was made categorizing lesions as either benign/mild grade dysplasia, or moderate or severe grade dysplasia/cancer. To analyze the predictive power of expert risk classification, the 161 samples were analyzed according to histopathological diagnosis (benign/mild grade dysplasia n= 140 vs. moderate or severe dysplasia/cancer n= 21) having excluded patients that had known cancer at first presentation (n=30). None of the predictors (e.g. age, sex, tobacco, and alcohol consumption) reached statistical significance on univariate analysis. Risk classification, described as low risk or high risk, was associated with histopathological diagnosis, but with a high confidence interval (OR=5.9, 95%CI= 2.1-17.2; p=0.001). *DCC* and *HOXA9*, as single biomarkers, were associated with histopathological diagnosis (OR= 3.9, 95%CI= 1.4-10.7; p= 0.008; OR=3.3, 95%CI=1.3-8.4; p= 0.015; respectively). In this analysis, *EDNRB* was not associate with histopathological diagnosis.

A multivariate model analyzed risk classification and *EDNRB, HOXA9* and *DCC* methylation status. Risk classification was again independently associated with histopathological diagnosis in 3 genes analysis after adjusting either of them (OR=6.7, 95%CI=2.2-20.1;p=0.0007, OR=7.1, 95%CI=2.3-22.0;p=0.0006, and OR=6.6, 95%CI=2.2-20.2; p=0.0009, respectively), but again the CI was high for all of them. *DCC* and *HOXA9* methylation status had again a significant association with moderate or severe dysplasia/invasive cancer diagnosis (OR=4.1, 95%CI=1.3-12.5; p=0.014 and OR=3.6, 95%CI=1.3-10.1; p=0.017, respectively) after adjusting for risk classification. Once more, for this analysis, EDNRB did not reached statistical significance (OR=2.6, 95%CI=0.9-7.5; p=0.068).

To predict the accuracy of risk classification and biomarkers, sensitivity and specificity were calculated using ROC analysis (Table S1). The AUC was also calculated with a 95% CI. *DCC* as a sole biomarker, had 38% (95% CI= 18-62) sensitivity and 86% (95% CI= 80-92) specificity, with AUC of 0.62 (95% CI= 0.51-

0.73), *EDNRB* had 43% (95% CI=22-66) sensitivity and 76% (95% CI=68-83) specificity, with AUC of 0.59 (95% CI=0.48-0.71) and *HOXA9* had 62% (95% CI=38-82) sensitivity and 67% (95% CI=58-74) specificity, with AUC of 0.64 (95% CI=0.53-0.76) when treated as a binary variable (methylation versus no methylation). The combination of the three genes, *EDNRB, HOXA9* and *DCC,* improved performance somewhat (sensitivity 76%; 95% CI= 53-92 and specificity 65%; 95% CI= 56-73), with AUC of 0.76 (95% CI= 0.66-0.86). Risk classification, when analyzed as a single predictor for histopathologic diagnosis, had 76% (95% CI= 53-92) sensitivity and 65% (95% CI= 56-73) specificity, with AUC of 0.71 (95% CI= 0.60-0.81). Using logistic regression analysis, we combined risk classification and each gene methylation status. *DCC* and *EDNRB,* individually associated with risk classification, presented the same sensitivity and specificity (76%, 95% CI= 53-92; 65%, 95% CI= 56-73) but different AUC (0.76, 95%CI= 0.66-0.86; 0.75, 95%CI= 0.65-0.85, respectively) and *HOXA9* presented same sensitivity, and a discrete difference in specificity (64%, 95%CI=56-72), with a better AUC (0.77, 95%CI= 0.68-0.87).

Finally the combination of risk classification, *DCC, HOXA9* and *EDNRB* showed same sensitivity and specificity as risk classification alone, with AUC of 0.76 (95%CI= 0.66- 0.86).

Figure S1 – ROC curves corresponding to the use of gene signatures, clinical exam and a combination of these. A: Risk Classification; B: EDNRB; C: DCC; D: EDNRB and DCC; E: EDNRB and Risk Classification; F: DCC and Risk Classification; G: EDNRB, DCC and Risk Classification