中文題目：使用四種上遺傳性指標基因架構上消化呼吸道癌症之風險預測模型與模型之驗證

英文題目：Deviation and validation of a risk assessment model for the upper aerodigestive tract cancer using a four-gene panel of epigenetic markers

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Background: Squamous cell carcinoma (SCC) is the major phenotype of esophageal cancer in Taiwan. Survival of this cancer is decreased because of field cancerization, in which synchronous and metachronous cancers occur in the upper aerodigestive tract (UADT). Screening and surveillance measures currently aid physicians in starting early intervention. However, the populations of patients exposed to well-established risk factors may outnumber the capacity of endoscopists to screen them. Following a negative endoscopy, an efficient risk-assessment method is needed to design surveillance programs while past efforts have not produced effective models. Aberrant DNA methylation in histologically normal mucosae has attracted attention as an indicator of past exposure to carcinogens and as a marker for future risk prediction. We quantified field cancerization of SCC in the UADT with epigenetic markers and evaluated their performance for risk assessment.

Materials and Methods: Methylation levels were analyzed by quantitative methylation-specific polymerase-chain-reaction (qMSP) analysis of biopsied specimens from a deviation set of 255 patients and a validation set of 224 patients with or without cancers during a three-year period. We analyzed four epigenetic markers: homeobox A9 (*HOXA9*), neurofilament heavy polypeptide (*NEFH*, 200 kDa), ubiquitin carboxyl-terminal esterase L1 (*UCHL1*), and metallothionein 1M (*MT1M*). These four markers, whose promoter CpG islands were methylated in esophageal SCCs, were isolated by a genome-wide screening of genes re-expressed after esophageal SCC cell lines were treated with the demethylating agent 5-aza-2'-deoxycytidine. They were also methylated at low levels in adjacent esophageal mucosae. We also measured traditional risk factors, including demographic characteristics of age, sex, and body mass index; lifestyle risk factors of alcohol drinking, betel quid chewing, and cigarette smoking (briefly as "ABC"); polymorphisms in genes encoding enzymes involved in the metabolism of alcohol, including aldehyde dehydrogenase (*ALDH*) *2*, alcohol dehydrogenase (*ADH*) *1B*, and *ADH 1C*; polymorphisms in genes encoding enzymes involved in the metabolism of xenobiotics, including glutathione S transferase (*GST*) *P1*, *GST M1*, and *GST T1*; serological markers, including increased mean corpuscular volume (MCV), *Helicobacter pylori* infection, and human papillomavirus infection; and endoscopic findings of numerous irregular-shaped multiform unstained areas over the background esophageal mucosae. We used the measurement of DNA methylation levels in normal-appearing esophageal mucosae as a starting point for the following analyses: (1) comparison of the methylation levels according to different stages of carcinogenic sequence and different levels of carcinogen exposure; (2) building logistic-regression models to determine the likelihood of UADT cancers based on epigenetic markers, based on traditional risk factors, and based on both together; and (3) comparison of the predictive values of epigenetic markers on a validation set of patients not used in the initial model building phase.

Results: Methylation levels of four markers increased stepwise, with the lowest levels in normal esophageal mucosae from healthy subjects without carcinogen exposure, then normal mucosae from healthy subjects with carcinogen exposure, then normal mucosae from cancer patients, and the highest levels were in cancerous mucosae ($P<0.05$). Cumulative exposure to alcohol increased methylation of *HOXA9* in normal esophageal mucosae ($P<0.01$). Drinkers had higher methylation levels of *UCHL1* and *MT1M* ($P<0.05$), and users of betel quid had higher methylation levels of

HOXA9 (*P*=0.01). Smokers had increased methylation levels of all four markers (*P*<0.05). Methylation levels in the normal-appearing esophageal mucosae were significantly associated with risk of SCC for all the four epigenetic markers. To predict head-and-neck cancer, AUCs for each of the epigenetic markers were 83% for *HOXA9* (bootstrap 95% CI: 77–88%), 69% for *NEFH* (60–74%), 67% for *UCHL1* (59–74%), and 74% for *MT1M* (67–81%). To predict esophageal cancer, similarly, the AUCs were 78% for *HOXA9* (73–83%), 71% for *NEFH* (65–77%), 73% for *UCHL1* (67–78%), and 71% for *MT1M* (65–77%). Overall, the areas under the curve (AUCs) for each of the markers were 80% for *HOXA9* (75–84%), 69% for *NEFH* (64–75%), 71% for *UCHL1* (66–76%), and 72% for *MT1M* (66–77%) in predicting UADT cancer. Regarding traditional risk factors, patients with SCC tended to be male, lower in BMI, and more likely to have the ABC habits. Serologically, their MCVs were higher, but their rate of *H. pylori* and human papillomavirus infections was similar to that of the controls. Endoscopy found that the cancer patients were more likely to have numerous LVLs in the background mucosae. A significant interaction was found between carriers of the genetic polymorphisms of an inactive *ALDH2*2* allele and levels of alcohol consumption, indicating that this genotype modified alcohol-related cancer risk. The performance of risk-assessment models was compared using ROC curve analyses. Overall exposure to ABC had a higher sensitivity (93%, 95% CI: 90–96%) but a lower specificity (45%, 95% CI: 35–54%), while the presence of endoscopic LVLs had a lower sensitivity (50%, 95% CI: 44–56%) but a higher specificity (92%, 95% CI: 88–98%). The AUCs increased as the models were based on MCV; then the overall exposure to ABC, endoscopic LVLs, and MCV (the traditional model); then the methylation levels of *HOXA9* and *NEFH* (the epigenetic model); and then the overall exposure to ABC, endoscopic LVLs, and methylation levels of *HOXA9* and *NEFH* (the combined model), which was the most accurate. The sensitivity/specificity pairs of the optimal cutpoints were 56% (95% CI: 48–64%) / 57% (48–66%), 74% (67–81%) / 74% (66–82%), 74% (69–79%) / 75% (67–83%), and 82% (76–88%) / 81% (74–88%), respectively. The performance of epigenetic model (bootstrap AUC: 83%, 95% CI: 79–87%) was similar to that (AUC: 80%, 95% CI: 67–91%) of traditional model (*P*=0.51). After adding epigenetic markers, the combined model (AUC: 91%, 95% CI: 88–94%) were more accurate than the traditional model (*P*<0.001). Epigenetic markers performed well in the validation set with 80% (73–85%) AUC.

Conclusions: We successfully quantified the field for cancerization using a four-gene panel of epigenetic markers in the small biopsied samples. Our work may improve the risk assessment and may potentially be generalized across high-risk regions to allocate limited endoscopic resources for the surveillance and early detection of UADT cancers.