CANCER GENOMICS & PROTEOMICS *19*: 456-463 (2022)
doi:10.21873/cgp.20332

The Contribution of PDCD6 Polymorphisms to Oral Cancer Risk

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Abstract. *Background/Aim: Programmed cell death 6 (PDCD6) is up-regulated and highly expressed in early apoptotic cells. In several types of cancer, such as cervical, breast and lung cancers, the association of PDCD6 genotypes have been investigated. However, the contribution of PDCD6 variant genotypes to oral cancer has never been examined. The current study aimed to evaluate the contribution of the PDCD6 rs4957014 and rs3756712 genotypes to the risk of oral cancer in Taiwan. Patients and Methods: The contribution of PDCD6 genotypes to oral cancer risk was examined among 958 patients with lung cancer and 958 age- and sex-matched healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: The data showed that the hetero-variant GT and homo-variant GG genotypes of PDCD6 rs4957014 were associated with a decreased risk of oral cancer [odds ratio (OR)=0.81 and 0.39, 95% confidence interval (CI)=0.67-0.97 and 0.27-0.56, respectively]. The recessive and dominant models also showed that G carriers have protective effects (OR=0.43 and 0.72, 95% CI=0.30-0.61 and 0.61-0.87, respectively). The analysis of allelic frequency distributions showed that the G allele of PDCD6 rs4957014 was associated with reduced oral cancer risk (OR=0.71, 95% CI=0.62-0.82). There was no significant association between any PDCD6 rs3756712 genotype and oral cancer risk. In addition, the GG genotype at PDCD6 rs4957014 significantly decreased the risk of oral cancer among both males (adjusted OR=0.31, 95%CI=0.24-0.56) and females (adjusted OR=0.44, 95% CI=0.22-0.91). Furthermore, the GG genotype at PDCD6 rs4957014 significantly decreased the risk of oral cancer among smokers (adjusted OR=0.35, 95% CI=0.22-0.58), alcohol drinkers (adjusted OR=0.33, 95% CI=0.18-0.49), non-betel quid chewers (adjusted OR=0.33, 95% CI=0.17-0.81), betel quid chewers (adjusted OR=0.34, 95% CI=0.21-0.59), but not among never-smokers and non-alcohol drinkers. Conclusion: The G allele carriers of PDCD6 rs4957014 may have protective effects on oral cancer risk and serve as a practical marker for early detection of oral cancer in Taiwan.*

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Key Words: Alcohol drinking, betel quid, genotype, oral cancer, PDCD6, polymorphism, smoking.

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Oral cancer is the fourth most common male cancer and the fourth leading cause of male cancer death in Taiwan, where the density of oral cancer is the top of the world (1-3). There are already several environmental factors known to contribute to the development of oral cancer, including

alcohol, tobacco and betel quid consumption, bad brushing, dental implanting habits, and viral infectious status (4, 5). From the molecular epidemiological viewpoint, several studies have provided evidence that specific genotypes may also contribute to oral cancer risk (6-11). A combined analysis of genomic, environmental, and personal behavioral aspects to oral cancer development may greatly help in precise and personalized therapy for oral cancer patients.

PDCD6 (also known as apoptosis-linked gene 2, ALG-2), is located on the human chromosome 5p15.33 encoding for a 22-kD calcium-binding protein (12, 13). PDCD6 is a multiple binding protein that is capable of interacting with Alix/AIP1 (14), ASK1 (15), and Sec31A (16), participating in the T cell receptor-, Fas-, glucocorticoid- and endoplasmic reticulum stress-induced programmed cell death (12, 17). PDCD6 does not seem to be essential for T-cell maturation, since *PDCD6* knockout mice exhibit normal T-cell development and function (18). On the contrary, *PDCD6* deficiency cells were defective in blocking the apoptosis signals induced by TCR, FAS and dexamethasone (18). In the literature, PDCD6 expression levels and *PDCD6* genotypes are reported to be associated with cancer risk (19-21). As early as 2003, it was found that PDCD6 was expressed significantly higher in the tumor sites compared to the non-tumor sites among patients of hepatoma and lung cancer (20). In 2012, the G allele at *PDCD6* rs4957014 was reported to be associated with decreased lung cancer risk (21). In 2013, the GG genotype at *PDCD6* rs4957014 together with the GT and GG genotypes at *PDCD6* rs3756712 were associated with decreased leiomyoma risk (19). In 2014, GT genotype at *PDCD6* rs4957014 was reported that it may contribute to decreased bladder cancer risk (22). One year later, the GG genotype at *PDCD6* rs4957014 was found to be associated with increased cervical cancer risk (23). In 2017, the GT genotype at *PDCD6* rs4957014 was found to decrease endometrial cancer risk (23) while in 2019, Shen and colleagues found that the GG genotype at *PDCD6* rs4957014 contributed to lower risk of lung cancer (24). So far, there hasn't been any report examining the contribution of *PDCD6* genotypes to oral cancer susceptibility up. Thus, in the current study, we aimed at investigating the contribution of *PDCD6* rs3756712 and rs4957014 single-nucleotide polymorphic (SNP) genotypes to the risk of oral cancer and examining the joint effect of environmental factors and *PDCD6* genotypes on oral cancer risk in Taiwan.

Patients and Methods

Population collecting methodology. Originally, cases diagnosed with oral cancer were recruited at the Outpatient Clinics of General Surgery in China Medical University Hospital in Taichung, Taiwan. Characteristics of the cases, including histological details, were all graded and defined by experienced clinical-pathological experts. Patients with history of other cancers were excluded, and a final of 958 cases were recruited. All participants were Taiwanese, providing 3-5 ml of blood sample for further genotyping studies. The same amounts of healthy volunteers as controls were selected by matching for age, sex, and behavioral factors such as alcohol, tobacco and areca-nut consumption, after initial random sampling from the Health Examination Cohort of China Medical University Hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin, any genetic or familial diseases, and other nationalities. The study was approved by the Institutional Review Board of the China Medical University Hospital (DMR101-IRB1-306). Selected characteristics of all participants are summarized in Table I.

Table I. *Characteristics of the investigated 958 oral cancer patients and 958 non-cancer healthy subjects*.

Characteristic	Controls (n=958)	Cases (n=958)	*p*-Value
Age (years)	56.8±8.7	56.4±7.5	0.3755[a]
Sex, n (%)			1.0000[b]
Male	728 (76.0%)	728 (76.0%)	
Female	230 (24.0%)	230 (24.0%)	
Personal habit, n (%)			
Cigarette smoking	668 (69.7%)	718 (74.9%)	**0.0107**[b]
Alcohol drinking	642 (67.0%)	684 (71.4%)	**0.0377**[b]
Betel quid chewing	508 (53.0%)	773 (80.7%)	**<0.0001**[b]
Primary tumor site, n (%)			
Tongue		397 (41.4%)	
Buccal mucosa		356 (37.2%)	
Mouth floor		39 (4.1%)	
Retromolar trigone		33 (3.4%)	
Alveolar ridge		29 (3.0%)	
Palate		27 (2.8%)	
Lip		39 (4.1%)	
Other		38 (4.0%)	

SD: Standard deviation; [a]Based on Student's *t*-test; [b]Based on chi-square test. Significant *p*-values ($p<0.05$) are shown in bold.

PDCD6 genotyping. Total DNA from the blood of each participant was extracted using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) and further processed as previously published (25-27). Briefly, the polymerase chain reaction (PCR) programs were set as: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 30 s; and a final elongation at 72˚C for 10 min. The sequences of paired primers for *PDCD6* rs4957014 were 5'-TGGTGTTTCATACCATTGACACTTGC-3' and 5'-CTCAGAA CCAAGCAGGTTCCTTCA-3', respectively. For *PDCD6* rs3756712, the sequences of paired primers were 5'- TACAGTGG CAAAGGACCACA-3' and 5'-CACATTCCAGCACTCACCAC-3', respectively. Following the PCR reaction, the products for *PDCD6* rs4957014 and rs3756712 were subject to *Hph* I and *Rsa* I (New England BioLabs, Ipswich, MA, USA) digestion, respectively. As for *PDCD6* rs4957014, the digestable G allele was cut into 113 and 13 base pair products, while the indigestable T allele was 126 base pairs long. As for *PDCD6* rs3756712, the digestable G allele was cut into 113 and 13 base pair products, while the indigestable T allele was 126 base pairs long. Overall, the genotypic process was conducted by two researchers independently. Three percent of the PCR products

Table II. *Distribution of PDCD6 rs4957014 genotypes among the oral cancer patients and non-cancer healthy subjects.*

Genetic model	Genotype	Controls		Cases		OR (95% CI)	*p*-Value[a]
		n	%	n	%		
rs4957014							
Codominant	TT	436	45.5%	513	53.6%	1.00 (reference)	
	GT	417	43.5%	397	41.4%	**0.81 (0.67-0.97)**	**0.0268**
	GG	105	11.0%	48	5.0%	**0.39 (0.27-0.56)**	**0.0001**
	P_{trend}						**0.0001**
	P_{HWE}						0.7237
Recessive	TT+GT	853	89.0%	910	95.0%	1.00 (reference)	
	GG	105	11.0%	48	5.0%	**0.43 (0.30-0.61)**	**0.0001**
Dominant	TT	436	45.5%	513	53.5%	1.00 (reference)	
	GT+GG	522	54.5%	445	46.5%	**0.72 (0.61-0.87)**	**0.0004**

[a]Based on chi-square test without Yates' correction; bold values indicate statistical significance, $p<0.05$. P_{trend}, *p*-Value for trend; P_{HWE}, *p*-Value for Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.

were selected for direct sequencing and the results from PCR-RFLP and sequencing were 100% concordant with each other.

Statistical analysis. The sample sizes of the case and control groups were both 958. To examine the controls for being representative of the general population, the distribution of *PDCD6* genotype frequencies among the controls under the Hardy-Weinberg equilibrium was checked by applying the goodness-of-fit test. At the same time, the Student's *t*-test was applied to compare the distribution of age between case and control groups. In addition, Pearson's Chi-square test was applied to compare the distributions of the *PDCD6* genotypes between the case and control groups in Table II and Table III, or between other counterpart subgroups in Table IV, Table V, Table VI, and Table VII. Further, the associations between the *PDCD6* genotypes and oral cancer risk are estimated by odds ratios (ORs) and corresponding 95% confidence intervals (CIs) before and after adjusting for confounding factors, such as age, sex, smoking, alcohol drinking and betel quid chewing behaviors, as indicated. A test for trend was based on chi-square test without Yates' correction checking the relationships of adding a variant genotype on the oral cancer risk. Results with *p*-Value less than 0.05 were considered statistically significant.

Results

The characteristics such as age, sex, smoking, alcohol drinking and betel quid chewing habits of the 958 oral cancer patients and the 958 controls are listed and compared in Table I. Additionally, the primary tumor sites of the oral cancer patients were summarized in the bottom of Table I. Firstly, no difference was found concerning age and sex between the case and control groups (p=0.3755 and 1.0000, respectively) (Table I, top part). Secondly, there are more smokers, alcohol drinkers and betel quid chewers in the oral cancer than the control group (p=0.0107, 0.0377 and 0.0001, respectively), indicating that these behaviors are oral cancer risk factors for Taiwanese (Table I, middle part). Lastly, tongue (41.4%) and buccal mucosa (37.2%) are the most prevalent primary tumor sites among the Taiwan oral cancer population (Table I, bottom part).

The distribution of genotypic frequencies of *PDCD6* rs4957014 and rs3756712 are compared in codominant, recessive, and dominant models in Table II and Table III, respectively. Firstly, the genotypic frequencies of the *PDCD6* rs4957014 and rs3756712 both fit well with the Hardy–Weinberg equilibrium in the control group (p=0.7237 and 0.1181, respectively). Secondly, there is a significant difference in the distribution of *PDCD6* rs4957014 genotypes between the oral cancer and control groups (p for trend=0.0001). In detail, the frequencies of the heterozygous variant GT and homozygous variant GG of *PDCD6* rs4957014 are 41.4 and 5.0% in the oral cancer group, significantly lower than those (43.5 and 11.0%, respectively) in the control group (p= 0.0268 and 0.0001, respectively). These results indicate that GT (OR=0.81, 95% CI=0.67-0.97) and GG (OR=0.39, 95% CI=0.27-0.56) genotypes at *PDCD6* rs4957014 can serve as a protective predictor of oral cancer (Table II, top panel). In the recessive model, GG genotype at *PDCD6* rs4957014 is also associated with a decreased oral cancer risk, compared to the TT+GT genotypes (OR=0.43, 95% CI=0.30-0.61, p=0.0001) (Table II, middle panel). In the dominant model, GT+GG genotypes at *PDCD6* rs4957014 are also associated with a decreased oral cancer risk, compared with the wild-type TT genotype (OR=0.72, 95% CI=0.61-0.87, p=0.0004) (Table II, bottom panel). Overall, *PDCD6* rs4957014 genotypes can serve as a novel biomarker for oral cancer in the Taiwanese population.

Similarly, the *PDCD6* rs3756712 genotypic frequency was examined about its contribution to oral cancer in each model. However, no significant differences were found in codominant, recessive, or dominant models (Table III).

Table III. *Distribution of PDCD6 rs3756712 genotypes among the oral cancer patients and non-cancer healthy subjects.*

Genetic model	Genotype	Controls		Cases		OR (95% CI)	*p*-Value[a]
		n	%	n	%		
rs3756712							
Codominant	TT	545	56.9%	521	54.4%	1.00 (reference)	
	GT	343	35.8%	348	36.3%	1.06 (0.88-1.29)	0.5424
	GG	70	7.3%	89	9.3%	1.33 (0.95-1.86)	0.0948
	P_{trend}						0.2409
	P_{HWE}						0.1181
Recessive	TT+GT	888	92.7%	869	90.7%	1.00 (reference)	
	GG	70	7.3%	89	9.3%	1.30 (0.94-1.80)	0.1156
Dominant	TT	545	56.9%	521	54.4%	1.00 (reference)	
	GT+GG	413	43.1%	437	45.6%	1.11 (0.92-1.33)	0.2698

[a]Based on chi-square test without Yates' correction. P_{trend}, *p*-Value for trend; P_{HWE}, *p*-Value for Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.

Table IV. *Distribution of PDCD6 rs4957014 and rs3756712 allelic frequencies among the oral cancer patients and non-cancer healthy subjects.*

	Controls	%	Cases	%	OR (95% CI)	*p*-Value[a]
rs4957014						
Allele T	1289	67.3%	1423	74.3%	1.00 (reference)	
Allele G	627	32.7%	493	25.7%	**0.71 (0.62-0.82)**	**0.0001**
rs3756712						
Allele T	1433	74.8%	1390	72.5%	1.00 (reference)	
Allele G	483	25.2%	526	27.5%	1.12 (0.97-1.30)	0.1148

[a]Based on chi-square test; bold values indicate statistical significance, $p<0.05$. OR, Odds ratio; CI, confidence interval.

To validate our findings in *PDCD6* rs4957014 and rs3756712 genotypes, we also conducted allelic frequency analysis. Consistent with the findings in Table II, the G allele of *PDCD6* rs4957014 is associated with a decreased risk of oral cancer, compared with the wild-type T allele (OR=0.71, 95% CI=0.62-0.82, p=0.0001) (Table IV, top panel). On the contrary, the variant G allele of *PDCD6* rs3756712 is not associated with any significantly altered risk for oral cancer (Table IV, bottom panel).

The oral cancer prevalence among Taiwanese is extremely high and there is a sex difference, the male *versus* female is about 9 to 1. In Table V, we have stratified the distribution of *PDCD6* rs4957014 among oral cancer patients and controls according to sex. The *PDCD6* rs4957014 genotype is differentially distributed among oral cancer patients and controls both in males (adjusted OR=0.31, 95% CI=0.24-0.56) and females (adjusted OR=0.44, 95% CI=0.22-0.91) (Table V).

Since betel quid chewing behavior together with smoking and alcohol drinking behaviors are well accepted as contributors to oral cancer risk, we are also interested in accessing the combinative effects of the *PDCD6* genotype and these personal behaviors on oral cancer susceptibility. The joint effects of *PDCD6* rs4957014 with smoking status are presented in Table VI. For never-smokers, the genotypes of *PDCD6* rs4957014 are not associated with oral cancer. On the contrary, the GG genotype of *PDCD6* rs4957014 is associated with decreased oral cancer risk (adjusted OR=0.35, 95% CI=0.22-0.58) (Table VI). The joint effects of *PDCD6* rs4957014 with alcohol drinking status are presented in Table VII. For the non-alcohol drinkers, the genotypes of *PDCD6* rs4957014 are not associated with oral cancer. On the contrary, the GG genotype of *PDCD6* rs4957014 is associated with decreased oral cancer risk (adjusted OR=0.33, 95% CI=0.18-0.49) (Table VII). The joint effects of *PDCD6* rs4957014 with betel quid status are presented in Table VIII. For the non-betel quid chewers, the GG genotype of *PDCD6* rs4957014 is associated with decreased oral cancer risk (adjusted OR=0.33, 95% CI=0.17-0.81). Similarly, the GG genotype of *PDCD6* rs4957014 is also associated with decreased oral cancer risk (adjusted OR=0.34, 95% CI=0.21-0.59) (Table VIII).

Table V. *Odds ratios for PDCD6 rs4957014 genotype and oral cancer after stratified by gender.*

Genotypes	Males		aOR (95% CI)[a]	Females		aOR (95% CI)[a]
	Controls	Cases		Controls	Cases	
TT	335 (46.0%)	392 (53.8%)	1.00 (Reference)	101 (43.9%)	121 (52.6%)	1.00 (Reference)
GT	313 (43.0%)	302 (41.5%)	0.86 (0.69-1.08)	104 (45.2%)	95 (41.3%)	0.74 (0.48-1.13)
GG	80 (11.0%)	34 (4.7%)	**0.31 (0.24-0.56)**	25 (10.9%)	14 (6.1%)	**0.44 (0.22-0.91)**
Total	728 (100%)	728 (100%)		230 (100%)	230 (100%)	

aOR, Adjusted odds ratio; CI, confidence interval. [a]The aORs were estimated with multivariate logistic regression analysis after adjustment for age, smoking, alcohol drinking and areca chewing habits. Bold values indicate statistical significance, $p<0.05$.

Table VI. *Odds ratios for PDCD6 rs4957014 genotype and oral cancer after stratified by smoking status.*

Genotypes	Never-smokers		aOR (95% CI)[a]	Smokers		aOR (95% CI)[a]
	Controls	Cases		Controls	Cases	
TT	129 (44.5%)	125 (52.1%)	1.00 (Reference)	307 (46.0%)	388 (54.0%)	1.00 (Reference)
GT	130 (44.8%)	98 (40.8%)	0.79 (0.46-1.08)	287 (43.0%)	299 (41.7%)	0.84 (0.65-1.04)
GG	31 (10.7%)	17 (7.1%)	0.55 (0.27-1.06)	74 (11.0%)	31 (4.3%)	**0.35 (0.22-0.58)**
Total	290 (100%)	240 (100%)		668 (100%)	718 (100%)	

aOR, Adjusted odds ratio; CI, confidence interval. [a]The aORs were estimated with multivariate logistic regression analysis after adjustment for age, gender, alcohol drinking and betel quid chewing habits. Bold values indicate statistical significance, $p<0.05$.

Discussion

PDCD6 is an apoptosis-linked gene (12) and its encoded protein plays a role in T cell receptor-induced programmed cell death in a Ca^{2+}-dependent manner as a pro-apoptotic factor (28). On the contrary, PDCD6 also has an anti-apoptotic capacity via promoting HeLa cell passing through G2/M checkpoints, resulting in enhanced proliferation (29). This contrasting character of PDCD6 may be explained by its multiple binding capacity with various Pro-rich proteins, such as Alix (ALG-2-interacting protein X) (30), annexins VII/XI (31), Sec31A (SEC31 homolog A) (16), and TSG101 (tumor susceptibility gene 101) (30). In mice, knockout of PDCD6 did not hinder apoptotic processes (18). In the literature, there have only been a few studies examining the association of *PDCD6* genotypes with several types of cancer, including lung (21, 24), ovarian (32), endometrial (33), and cervical cancer (23). We have summarized all of the studies reporting *PDCD6* rs4957014 genotypes with cancer types, for the overview of its association with several types of cancer (Table IX). Up to now, there is neither investigation of *PDCD6* genotype contribution to oral cancer determination, nor the conclusive role of *PDCD6* genotypes in carcinogenesis.

In the current study, the contribution of *PDCD6* genotypes, in addition to smoking, alcohol drinking, and betel quid chewing status to oral cancer risk are firstly examined. The highlights of the study include that the heterozygous GT and homozygous GG genotypes at *PDCD6* rs4957014 are significantly associated with a decreased risk of oral cancer in codominant, recessive, and dominant models (Table II). In addition, the variant G allele of *PDCD6* rs4957014 was associated with a decreased oral cancer risk (Table IV). Further, the protective effects of *PDCD6* rs4957014 GG genotype on oral cancer risk is obvious among both males and females (Table V), smokers (Table VI), alcohol drinkers, and non-betel quid chewers and betel quid chewers (Table VII). The protective effect of rs4957014 GG genotype on oral cancer risk is also in line with He's and Zhou's findings in lung and cervical cancer, respectively (21, 23). On the other hand, it has been reported that *PDCD6* rs3756712 and rs4957014 polymorphisms are associated with increased endometrial cancer risk (33). Further studies in various and larger populations are needed for understanding the role of *PDCD6* in oral carcinogenesis.

Sex preference is a potential risk factor for oral cancer. The ratio of male to female is about 9 to 1 in Taiwan. Although the possible mechanisms are still largely unknown, it is thought that endocrine-related factors play a critical part in the sex difference for oral cancer risk. While our oral cancer patients were stratified by sex, *PDCD6* genotypes significantly associate with oral cancer risk among both men and women, thus no sex difference was found (Table V).

Table VII. *Odds ratios for PDCD6 rs4957014 genotype and oral cancer after stratification by alcohol drinking status.*

Genotypes	Non-alcohol drinkers		aOR (95% CI)[a]	Alcohol drinkers		aOR (95% CI)[a]
	Controls	Cases		Controls	Cases	
TT	146 (46.2%)	144 (52.6%)	1.00 (Reference)	290 (45.2%)	369 (45.2%)	1.00 (Reference)
GT	136 (43.0%)	110 (40.1%)	0.84 (0.60-1.21)	281 (43.7%)	287 (43.8%)	0.77 (0.56-1.09)
GG	34 (10.8%)	20 (7.3%)	0.55 (0.29-1.13)	71 (11.1%)	28 (11.0%)	**0.33 (0.18-0.49)**
Total	316 (100%)	274 (100%)		642 (100%)	684 (100%)	

aOR, Adjusted odds ratio; CI, confidence interval. [a]The aORs were estimated with multivariate logistic regression analysis after adjustment for age, gender, smoking, and betel quid chewing habits. Bold values indicate statistical significance, $p<0.05$.

Table VIII. *Odds ratios for PDCD6 rs4957014 genotype and oral cancer after stratified by betel quid chewing status.*

Genotypes	Non-betel quid chewers		aOR (95% CI)[a]	Betel quid chewers		aOR (95% CI)[a]
	Controls	Cases		Controls	Cases	
TT	207 (46.0%)	98 (53.0%)	1.00 (Reference)	229 (45.1%)	415 (53.7%)	1.00 (Reference)
GT	198 (44.0%)	80 (43.2%)	0.88 (0.61-1.33)	219 (43.1%)	317 (41.0%)	0.78 (0.61-1.02)
GG	45 (10.0%)	7 (3.8%)	**0.33 (0.17-0.81)**	60 (11.8%)	41 (5.3%)	**0.34 (0.21-0.59)**
Total	450 (100%)	185 (100%)		508 (100%)	773 (100%)	

aOR, Adjusted odds ratio; CI, confidence interval. [a]The aORs were estimated with multivariate logistic regression analysis after adjustment for age, gender, smoking and alcohol drinking habits. Bold values indicate statistical significance, $p<0.05$.

Table IX. *Summary of findings about PDCD6 rs4957014 genotype with cancer risk.*

Author (Ref)	Year	Population	Cancer type	Controls	Cases	Highlights
Shih *et al*. (present study)	Current	Taiwanese	Oral	958	958	GT and GG genotype may decrease oral cancer risk
Shen *et al*. (24)	2019	Taiwanese	Lung	716	358	GG genotype may decrease lung cancer risk
Yuan *et al*. (33)	2017	Chinese	Endometrial	518	238	GT genotype may decrease endometrial cancer risk
Zhou *et al*. (23)	2015	Chinese	Cervical	541	328	GG genotype may increase cervical cancer risk
Zhou *et al*. (22)	2014	Chinese	Bladder	509	332	GT genotype may decrease bladder cancer risk
Zhang *et al*. (19)	2013	Chinese	Leiomyoma	436	295	GG genotype may decrease leiomyoma risk
He *et al*. (21)	2012	Chinese	Lung	306	302	G allele may decrease lung cancer risk[a]

[a]No specific genotype was associated with cancer risk.

As mentioned above, cigarette smoking, alcohol drinking and betel quid chewing are well-known behavioral risk factors for oral cancer. For instance, tobacco carcinogen-induced DNA adducts are reported to greatly enhance the probability of cancer (34). In this regard, the interaction of the genotype of *PDCD6* with cigarette smoking, alcohol drinking, and betel quid chewing status were concisely discussed. Firstly, concerning smoking, the results showed that the genotypic distribution of the variant genotypes of *PDCD6* rs4957014 were significant among those who were ever smokers, but not among never-smokers (Table VI). There was no literature reporting that *PDCD6* rs4957014 T allele attenuated cells' DNA repair capacity with too many DNA adducts left unrepaired as those cells with G allele. Secondly, concerning alcohol drinking, the results showed that the genotypic distribution of the variant genotypes of *PDCD6* rs4957014 were significantly different among those who were ever alcohol drinkers, but not that for never drinkers (non-alcohol drinkers) (Table VII). Lastly, concerning betel quid chewing, the results showed that the genotypic distribution of the variant genotypes of *PDCD6* rs4957014 was significantly different among both ever

chewers and non-chewer subgroups (Table VIII). Further investigations using cells from patients with different genotypes of *PDCD6* rs4957014 should be conducted to explore the joint effects of tobacco smoking, alcohol drinking, and betel quid chewing with *PDCD6* rs4957014 genotypes on cancer risk.

There has been no phenotypic evidence up to now showing that the *PDCD6* rs4957014 T allele attenuates the capacity to promote those cells with high DNA damage or leave too many DNA adducts unrepaired as those cells with G allele. Further investigations using cells from patients with different genotypes of *PDCD6* rs4957014 should be conducted to explore the joint effects of gender, personal smoking, drinking, betel quid chewing status, and other oral cancer risk factors. From the molecular viewpoint, the methylation status (35) and cooperation with C-Raf (36) may alter some oncogenic signaling and promote carcinogenesis. More recently, a novel Circ-Calm4/miR-124-3p/PDCD6 axis has been reported to regulate pyroptosis, another type of cell death, in hypoxia-Induced pulmonary arterial smooth muscle cells (37).

In conclusion, the current study provides solid evidence that the G allele of *PDCD6* rs4957014 can serve as a protective biomarker, specifically interacting with smoking, alcohol drinking and betel quid chewing behaviors for oral cancer risk among Taiwanese. These novel findings should be validated in larger and different populations to understand the role of PDCD6 in carcinogenesis.

Conflicts of Interest

The Authors declare no conflicts of interest with any company or person.

Authors' Contributions

Research design was done by LCS, CWT and JLH. Patient and questionnaire collections were conducted by LCS and CLH. Experimental work was done by WSC, JSY, CLH, and YCW. Statistical analysis was done by TCH, WSC and JLH, while DTB and CWT wrote the manuscript, whereas DTB, CWS, and WSC reviewed it and are responsible for the revision.

Acknowledgements

We are grateful to Tissue-bank of China Medical University Hospital and doctors/nurses for their excellent sample collection and technical assistance. The technical assistance from Yu-Chen Hsiau, Yi-Ru Huang, Yu-Hsin Lin were very critical in manuscript preparation. This study was supported mainly by China Medical University and Asia University (CMU110-ASIA-01).

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Received March 7, 2022
Revised April 20, 2022
Accepted April 26, 2022