Impact of *PTEN* IVS4 Polymorphism (rs3830675) on Cancer Susceptibility: An Updated Meta-analysis

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Abstract. *Background/Aim: Phosphatase and tensin homolog (PTEN) acts as a tumor suppressor gene through the action of its phosphatase protein product. We performed a meta-analysis to evaluate their relationship. Materials and Methods: A comprehensive database search was performed. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the association between PTEN IVS4 polymorphism and cancer. Results: The meta-analysis indicated that PTEN IVS4 (–/–) genotype was significantly associated with the risk of cancer (OR=1.47, 95% CI=1.11-1.84), especially for digestive cancer (OR=1.67, 95% CI=1.28-2.18) compared to the (+/+) genotype. Moreover, the (–) allele of PTEN IVS4 polymorphism was also significantly associated with the risk of cancer (OR=1.27, 95% CI=1.14-1.41), especially for digestive cancer (OR=1.42, 95% CI=1.16-1.74) compared to the (+) allele. No significant association was observed between PTEN IVS4 (+/–) genotype and risk of cancer. Conclusion: PTEN IVS4 (–/–) genotype was significantly associated with increased risk of cancer especially for digestive tract cancer compared to the (+/+) genotype. A similar phenomenon was observed in the (–) allele of PTEN IVS4 polymorphism compared to the (+) allele and the recessive effect model.*

In 2012, about 14.1 million new cases of cancer occurred globally (1). This caused about 8.2 million deaths or 14.6% of all human deaths (2). With developing research, it is becoming clear that carcinogenesis is caused by mutation and epimutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death. Recently, it has become evident that genetic variation plays a significant role in the development and progression of cancer. More studies based on gene polymorphisms have proved that polymorphisms may contribute to cancer risk (3). Identification of the key gene polymorphisms that are associated with cancer risk is essential for predicting individuals at-risk.

Phosphatase and tensin homolog (PTEN) is a protein that, in humans, is encoded by the *PTEN* gene (4). Mutations of this gene are a crusial step in the development of many cancers. *PTEN* acts as a tumor suppressor gene through the action of its phosphatase protein product. This phosphatase is involved in the regulation of the cell cycle preventing cells from growing and dividing too rapidly (5). This gene was identified as a tumor suppressor that is mutated with high frequency in a large number of cancers. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase.

PTEN IVS4 polymorphism (rs3830675) with an ATCTT insertion at 109 bp downstream of exon 4 in intron 4 was one of the common *PTEN* polymorphisms. Recently, several studies continued to explore the association of *PTEN* IVS4 polymorphism with the risk of different cancers, but results were controversial. Aiming at elucidating the exact relation between *PTEN* IVS4 polymorphism with risk of cancer, we performed the current meta-analysis by collecting data from published case-control studies concerning the role of *PTEN* IVS4 polymorphism in carcinogenesis.

Materials and Methods

Literature search strategy. We performed a systematic review and meta-analysis on *PTEN* gene IVS4 polymorphism and cancer susceptibility in accordance with the PRISMA Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). We searched MEDLINE (PubMed), EMBASE, Web of Science and CNKI (China National Knowledge Infrastructure) without language restrictions to December, 2013, using the following search algorithms: ("PTEN" OR "phosphatase and tensin homolog" OR "polymorphism") AND ("polymorphism" OR "mutation") AND ("cancer neoplasms" OR "malignancy" OR "cancer carcinoma").

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In addition, the reference lists of retrieved articles were also checked to find additional relevant studies. No language restrictions were imposed.

Selection criteria. Study eligibility was determined independently by two reviewers (YW and JG). Disagreements were solved by consensus. Studies were considered for inclusion if they meet the following criteria: (i) studies evaluated *PTEN* gene IVS4 polymorphism (rs3830675) and cancer susceptibility, (ii) case–control studies and (iii) reported data necessary to calculate the odds ratio (OR) with corresponding 95% confidence interval (CI). If such data were unavailable, attempts were made to contact the first author and/or corresponding author *via* e-mail to provide the missing data before the study was excluded from the final analysis. When several reports were published on the same subject, only the most recent and informative one was included.

Data extraction. Two reviewers assessed articles for inclusion, extracted data and assessed quality independently. Any disagreement was presented to a third author and resolved by discussion among the investigators. The general information extracted included first author, publication year, ethnicity of the studied population, cancer type, numbers of each genotype in cases and controls, genotyping methods for *PTEN* gene IVS4 polymorphism and source of controls.

Statistical analysis. The statistical analysis was performed by the Stata software (Version 11.0; StataCorp, College Station, TX, USA). ORs and their 95% CI were used to assess the strength of association between *PTEN* gene polymorphisms and cancer risks. A *p*-value <0.05 was considered as statistically significant. Heterogeneity was measured by using Q statistic ($p<0.10$ indicates significant heterogeneity between studies) and I-squared (I^2) value (6)]. A fixed-effects model using the Mantel-Haenszel method (7)] was performed to calculate the pooled ORs when heterogeneity between studies was not significant. Otherwise, a random-effects model using the DerSimonian and Laird method (8)] was applied. Sensitivity analysis was performed to explore heterogeneity when significant heterogeneity was indicated. Subgroup analyses were performed to explore the effects of cancer type and source of controls. Additionally, publication bias was evaluated qualitatively by performing funnel plots and assessed quantitatively by the Begg's (9) and Egger's tests (10), respectively. A *p*-value<0.05 for Begg's and Egger's tests indicates significant publication bias.

Results

Study description. This meta-analysis was organized according to the PRISMA statement. Totally, 79 literature hits were indentified through electronic databases after duplicates removal. After reviewing the titles and abstracts of the potential available articles, 30 records were excluded mainly because of no relevance, *in vitro* or animal experiments, reviews or meta-analysis. The remaining 19 full-text articles were further assessed for eligibility. Finally, 6 full-text articles with eligibility were included in this meta-analysis (11-16) . The flow chart of article selection is presented in Figure 1. The main characteristics of the studies included in this meta-analysis are summarized in Table I.

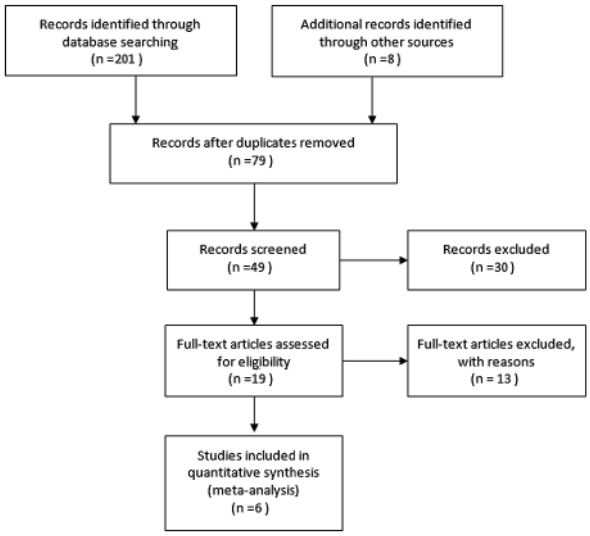

Figure 1. *The flow chart of literature selection.*

Associations of PTEN polymorphisms with cancer risks. Patients with *PTEN* IVS4 (–/–) genotype were significantly associated with increased risk of cancer (OR=1.47, 95% CI=1.11-.84, $p<0.01$, Figure 2) compared to the (+/+) genotype. Patients with *PTEN* IVS4 (+/–) genotype were not significantly associated with risk of cancer (OR=0.92, 95% CI=0.70-1.13, $p<0.01$, Figure 3) compared to the (+/+) genotype. The recessive effect model (–/–) *vs*. (–/+ and +/+) indicated a statistically significant association between *PTEN* IVS4 polymorphism and increased cancer risk with an OR=1.45 (95% CI=1.21-1.70, $p<0.01$, Figure 5). The dominant effect model (–/+ and –/–) *vs*. (+/+) showed no statistically significant association between *PTEN* IVS4 polymorphism and increased cancer risk with an OR=1.13 (95% CI=0.90-1.37, $p<0.01$, Figure 6). *PTEN* IVS4 (rs3830675) polymorphism with (–) allele was significantly associated with the risk of cancer (OR=1.27, 95% CI=1.14-1.41, $p<0.01$, Figure 4), compared to the (+) allele.

Sub-group analysis. As for cancer type, *PTEN* IVS4 (–/–) genotype was observed to be associated with increased risk of digestive tract cancer (OR=1.67, 95% CI=1.28–2.18, $p<0.01$) but no significant association was found with breast cancer (OR=1.27, 95% CI=0.79–2.04, $p=0.32$) or prostate cancer (OR=1.20, 95% CI=0.83–1.73, $p=0.34$). The same relationship was observed in *PTEN* IVS4 (+/–) genotype. The recessive effect model and dominant effect model also demonstrated a significant association between *PTEN* IVS4 polymorphism and risk of digestive breast cancer, prostate cancer, especially for digestive tract cancer. Compared to the (+) allele, allele analysis revealed that the (–) allele of *PTEN* IVS4 polymorphism was only significantly associated with

Table I. *Characteristics of studies*.

First author	Year	Ethnicity	Cancer	Source control	Case				Control				Genotyping method
					n	(+/+)	(+/–)	(–/–)	n	(+/+)	(+/–)	(–/–)	
Ozturk O. (11)	2013	Turkish	Breast cancer	PB	118	6	55	57	128	8	79	41	PCR-RFLP
Canbay E. (12)	2013	Turkish	Colorectal cancer	PB	203	7	81	115	245	15	144	86	PCR-RFLP
Canbay E. (13)	2013	Turkish	Gastric cancer	HB	93	7	29	57	113	10	60	43	PCR-RFLP
Zhao Z.H. (14)	2012	Chinese	Breast cancer	HB	210	63	80	67	210	53	109	48	PCR-RFLP
Ge H. (15)	2008	Chinese	Gastric cancer	PB	257	48	146	63	634	167	329	138	PCR-RFLP
Ge H. (16)	2007	Chinese	Esophageal cancer	PB	350	73	183	94	634	167	329	138	PCR-RFLP

PCR-RFLP, Polymerase chain reaction- restriction fragment length polymorphism; PB, population based; HB, hospital based.

digestive tract cancer (OR=1.42, 95% CI=1.16–1.74, $p<0.01$); no relationship was found in the sub-groups of breast or prostate cancer (Table II).

As for ethnicity type, patients of *PTEN* IVS4 (–/–) genotype were significantly associated with increased risk of cancer in the Turkish and Chinese but not in the American ethnic group. Individuals with *PTEN* IVS4 (+/–) genotype, showed no significant association in the different sub-groups based on ethnicities. In stratified analysis of different ethnicities, consistently increased risk of cancer for *PTEN* IVS4 (–) allele was observed in the Turkish and Chinese populations but no significant relation was found in the American group, compared to the (+) allele (Table II).

As for source control, individuals with *PTEN* IVS4 (–/–) genotype exhibited an increased cancer risk in the population-based (PB) sub-group rather than the hospital-based (HB) sub-group. It is worth noting that sub-group analysis of PB and HB individuals with *PTEN* IVS4 (+/–) genotype demonstrated controversial outcomes (PB: OR=1.31, 95% CI=1.07-1.59, $p<0.01$; HB: OR=0.63, 95% CI=0.41–0.96, $p=0.03$), which suggested that the selection of the controls might influence the result of the relation between *PTEN* IVS4 (+/–) genotype and cancer risk (Table II).

Publication bias. Among techniques to minimize the effects of publication bias, we performed a thorough search for unpublished studies, and used analytical tools, such as a funnel plot, to quantify the potential presence of publication bias. The Begg's test was carried-out to access the publication bias in our studies. In our study, slight publication bias was observed in the recessive effect model (Egger's test $p=0.04$; Begg's test $p=0.02$) and allele analysis of *PTEN* gene IVS4 polymorphism (Egger's test $p=0.04$; Begg's test $p=0.02$).

Sensitivity analyses. To explore potential source of heterogeneity across studies, we carried-out several sensitivity analyses. When one study was removed, the rest were analyzed sequentially by meta-analysis. Any study in overweight or obesity group was omitted and the pooled ORs were not materially altered with the overall pooled OR, suggesting that the overall results were not sensitive to any single research (data not shown).

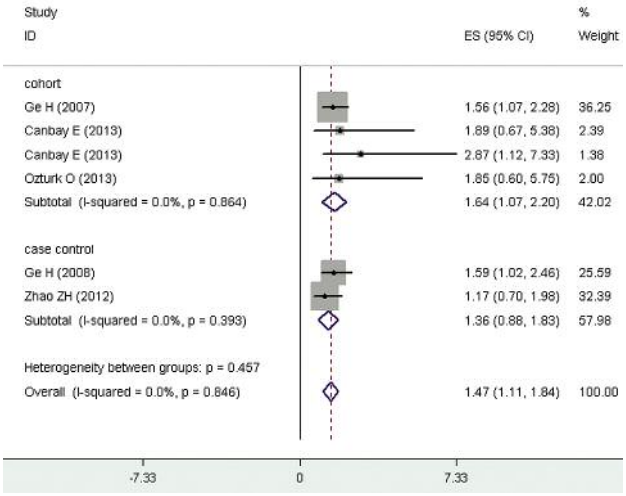

Figure 2. *Forest plot between PTEN IVS4 polymorphism and cancer risk (–/– vs. +/+).*

Discussion

The identification of genetic variants capable of modulating cancer development could be helpful for the early detection and design of targeted-treatment and prevention strategies. With high interest in gene susceptibility to carcinogenesis, increasing efforts have been devoted to the study of genetic variants and cancer risk. Most recently, increasing number of studies investigated the association between *PTEN* IVS4 polymorphism (rs3830675) and risk of various types of cancer. For instance, in a study of Ozturk *et al*. (11), the presence of ATCTT insertion (+/+) genotype at downstream of exon 4 in intron 4 of *PTEN* IVS4 gene was also associated with 1.83-fold decreased risk of breast cancer development ($p<0.033$; OR=1.83, 95 % CI=1.11-3.03).

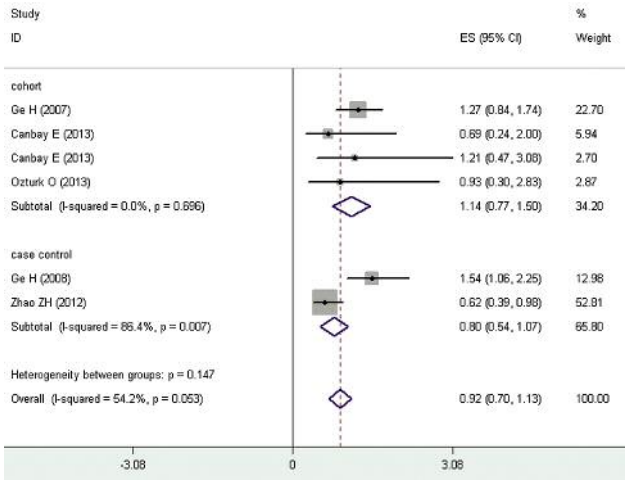

Figure 3. *Forest plot between PTEN IVS4 polymorphism and cancer risk (+/– vs. +/+).*

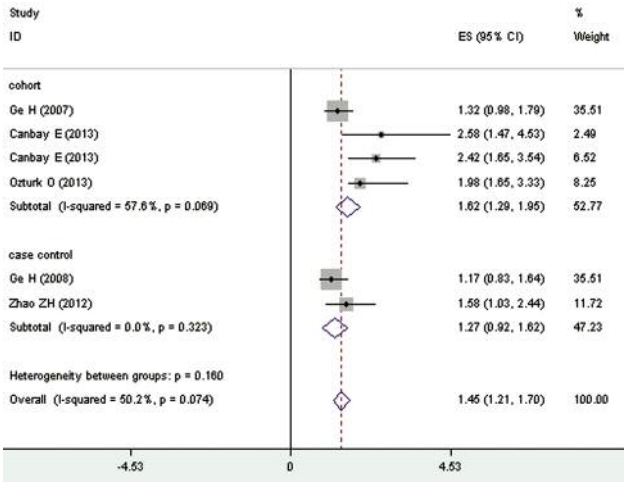

Figure 5. *Funnel plot between PTEN IVS4 polymorphism and cancer risk (–/– vs. [–/+ and +/+]).*

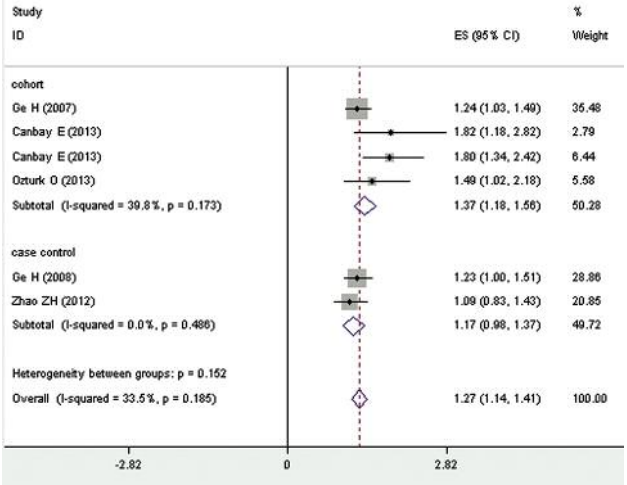

Figure 4. *Funnel plot between PTEN IVS4 polymorphism and cancer risk (– allele vs. + allele).*

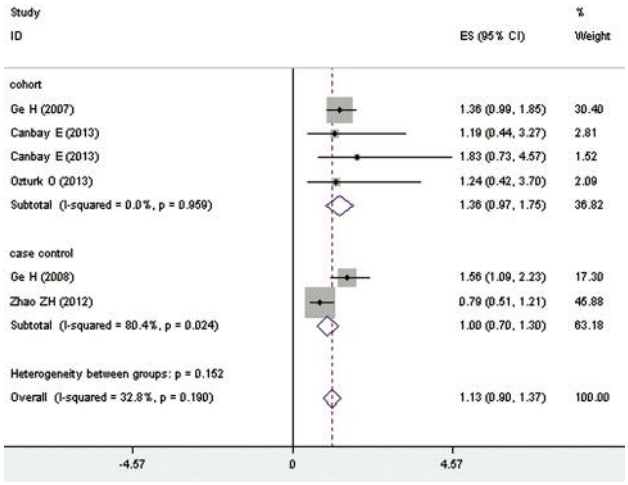

Figure 6. *Funnel plot between PTEN IVS4 polymorphism and cancer risk ([–/– and –/+] vs. +/+).*

Subsequently, a research by Canbay *et al*. (12) proposed that the *PTEN* IVS4 (–/–) genotype exhibited a significantly elevated risk for GC compared to controls ($p<0.005$; OR=1.6, 95% CI=1.19-2.14), which was consistent with the suggestion of Zhuang *et al*. (14) who revealed the (–/–) genotype of *PTEN* IVS4 that appeared to be absence of ATCTT insertion at downstream of exon 4 in intron 4 of the *PTEN* gene was found to be associated with 1.55-fold increased risk of colon cancer ($p<0.005$; OR=1.55, 95% CI=1.24-1.94) and 1.4-fold increased risk of rectum cancer ($p<0.005$; OR=1.4, 95% CI=1.08-1.82). Later, the results of the study by Ge *et al*. (15) confirmed that, compared to the *PTEN* IVS4–/– genotype, the IVS4+/+ genotype significantly decreased the risk of esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma development; the adjusted OR was 0.64 (95% CI=0.44-0.94) and 0.63 (95% CI=0.41-0.98), respectively.

In this comprehensive meta-analysis for the association between *PTEN* IVS4 polymorphism and cancer risk, a pooled analysis suggested a major role of the polymorphism in shaping over cancer risk. Specifically, the *PTEN* IVS4 polymorphism (–/–) genotype was shown to confer 47% and

Table II. *Association between PTEN IVS4 polymorphism and cancer risk.*

Genetic model	Subgroup	N	Overall effect	
			OR (95%CI)	*p*-Value
(–/–) *vs*. (+/+)	Overall	6	1.47 (1.11-1.84)	<0.01
	Digestive tract cancer	4	1.67 (1.28-2.18)	<0.01
	Breast cancer	2	1.27 (0.79-2.04)	0.32
	Turkish	3	2.23 (1.24-4.03)	<0.01
	Chinese	3	1.47 (1.14-1.89)	<0.01
	Population based	5	1.49 (1.20-1.85)	<0.01
	Hospital based	2	1.29 (0.82-2.06)	0.27
(+/–) *vs*. (+/+)	Overall	6	0.92 (0.70-1.13)	0.46
	Digestive tract cancer	4	1.33 (1.05-1.68)	0.01
	Breast cancer	2	0.66 (0.43-1.01)	0.06
	Turkish	3	0.95 (0.52-1.71)	0.76
	Chinese	3	1.09 (0.67-1.78)	0.74
	Population based	5	1.31 (1.07-1.59)	<0.01
	Hospital based	2	0.63 (0.41-0.96)	<0.01
Recessive effect model	Overall	6	1.46 (1.21-1.69)	<0.01
	Digestive tract cancer	4	1.70 (1.16-2.49)	<0.01
	Breast cancer	2	1.74 (1.24-2.42)	<0.01
	Turkish	3	2.32 (1.77-3.04)	<0.01
	Chinese	3	1.32 (1.08-1.61)	<0.01
	Population based	5	1.45 (1.06-1.98)	0.03
	Hospital based	2	1.90 (1.35-2.67)	<0.01
Dominant effect model	Overall	6	1.13 (0.89-1.37)	<0.01
	Digestive tract cancer	4	1.45 (1.16-1.81)	<0.01
	Breast cancer	2	0.84 (0.56-1.25)	0.45
	Turkish	3	1.44 (0.81-2.54)	0.32
	Chinese	3	1.21 (0.84-1.75)	0.41
	Population based	5	1.38 (1.14-1.66)	<0.01
	Hospital based	2	0.84 (0.57-1.25)	0.24
(–) allele *vs*. (+) allele	Overall	6	1.27 (1.14-1.41)	<0.01
	Digestive tract cancer	4	1.42 (1.16-1.74)	<0.01
	Breast cancer	2	1.21 (0.97-1.51)	0.13
	Turkish	3	1.71 (1.39-2.10)	<0.01
	Chinese	3	1.20 (1.06-1.36)	<0.01
	Population based	5	1.29 (1.09-1.53)	<0.01
	Hospital based	2	1.37 (0.83-2.26)	0.24

OR, Odds ratio; CI, confidence interval.

67% increases in whole cancer risk and digest cancer compared with the (+/+) genotype. Moreover, the recessive effect model (–/–) *vs*. (–/+ and +/+) also demonstrated significant association between *PTEN* IVS4 polymorphism and cancer risk (OR=1.46) and digestive tract cancer (OR=1.70). Similarly, the (–) allele of *PTEN* IVS4 polymorphism was significantly associated with 1.27-fold increase of cancer development, especially for digestive tract cancer with 1.42-fold compared to the (+) allele.

Heterogeneity analysis. We conducted a meta-regression analysis to investigate the impact of heterogeneous factors on the OR estimates. The cancer type, ethnicity type and source of control were chosen as potential heterogeneous factors (Table II).

In multivariate meta-regression analysis of *PTEN* IVS4 (–/–) genotype with (+/+) genotype, none of these factors were significant in the heterogeneous estimate (p=0.85 for cancer type; p=0.73 for ethnicity type; p= 0.62 for source of control).

In the comparison of *PTEN* IVS4 (+/–) genotype with (+/+) genotype, cancer type and source of control did not show any heterogeneity. However, we observed heterogeneity in the Chinese ethnicity (p<0.01). The meta-regression analysis indicated that Chinese ethnicity might not be a major source contributing to the heterogeneity presented in the overall analyses (I^2=54.2%, p=0.05).

As for the comparison of recessive effect model (–/–) *vs*. (–/+ and +/+), cancer type and source of control showed

heterogeneity, which could explain the overall heterogeneity ($p<0.01$ for digestive cancer; $p<0.01$ for HB), whereas ethnicity type did not indicate any heterogeneity ($p=0.77$ for Turkish; $p=0.56$ for Chinese).

As for the comparison of dominant effect model (–/+ and –/–) *vs.* (+/+), although we observed heterogeneity in the Chinese ethnicity analysis (I2=67.5%, $p=0.05$), no heterogeneity was found in the overall analyses. Maybe the weight of Chinese ethnicity is so small to affect the robustness of the results.

As for the comparison of allele analysis, the overall heterogeneity could be explained by source of control ($p=0.03$ for PB; $p=0.05$ for HB).

Mechanism. *PTEN* is one of the most commonly lost tumor suppressors in human cancer. In fact, up to 70% of men with prostate cancer are estimated to have lost a copy of the *PTEN* gene at the time of diagnosis (17). During tumor development, mutations and deletions of *PTEN* occur that inactivate its enzymatic activity leading to increased cell proliferation and reduced cell death. Frequent genetic inactivation of *PTEN* occurs in glioblastoma, endometrial cancer and prostate cancer, while reduced expression is found in many other tumor types, such as lung and breast cancer. Furthermore, *PTEN* mutation also causes a variety of inherited predispositions to cancer.

The structure of PTEN reveals that it consists of a phosphatase domain and a C2 domain: the phosphatase domain contains the active site, which carries-out the enzymatic function of the protein, while the C2 domain binds the phospholipid membrane. Thus, PTEN binds the membrane through its C2 domain, bringing the active site to the membrane-bound PIP3 to de-phosphorylate it. When the PTEN enzyme is functioning properly, it acts as part of a chemical pathway that signals cells to stop dividing and can cause cells to undergo programmed cell apoptosis when necessary. These functions prevent uncontrolled cell growth that can lead to formation of tumors. There is also evidence that the protein made by the *PTEN* gene may play a role in cell movement (migration) and adhesion of cells to surrounding tissues. *PTEN* orthologs have been identified in most mammals for which complete genome data are available.

In recent years, the intron polymorphism of *PTEN* drew considerable attention.

A research from the University of Toledo (18) showed that some intron sequences possess important functions, although introns were originally believed to be non-functional because they do not code for proteins. Furthermore, researches from the University of California (19) and the University of Southern California (20) proposed that some introns could regulate expression of genes, while others could be further processed after splicing to generate non-coding RNA molecules. Important intron sites with polymorphisms could disrupt the splicing process during transcription. The *PTEN* IVS4 (rs3830675) polymorphism may lead to a splicing error or may be by linkage disequilibrium with another locus affecting the expression and function of the PTEN. The alternation of *PTEN* expression would inevitably change the role of *PTEN* in maintaining genome stability, whereas loss of function of this tumor suppressor might, therefore, lead to carcinogenesis. Although the above-mentioned possible mechanism might partially explain the observed association between *PTEN* IVS4 (rs3830675) polymorphism and cancer susceptibility, a rare functional study has been carried out but the exact mechanism remains largely elusive (21). Future functional studies are required to illuminate the detailed mechanism regarding the role of PTEN IVS4 (rs3830675) polymorphism in carcinogenesis.

Limitations. There were, however, several limitations of the present meta-analysis. First, some residual confounding is inevitable. For instance, we were unable to investigate the underlying effect of the covariates in the original studies, such as living environment, educational background, family history, age and sex, which may influence the results. Second, publication bias is always an important issue in the meta-analyses. Publication bias is a problem when interpreting our results. Negative studies are less likely to be published in indexed journals, leading to potential publication bias. We saw no evidence of such publication bias in the Egger's linear regression test but the funnel plot seemed asymmetrical. However, according to the Cochrane Handbook for Systematic Reviews of Interventions, Egger's test typically has low power. Finally, although we made our best to track and acquire unpublished work and grey literature, especially university theses or conference proceedings, there were inevitability some investigations left out. As a result, publication bias may have influenced the results. Of note, only English literature studies were included in the present study implying the possibility that our findings were biased for many non-English literature articles.

Conclusion

In conclusion, this meta-analysis indicated that the *PTEN* IVS4 (–/–) genotype was significantly associated with increased risk of cancer, especially for digestive tract cancer compared to the (+/+) genotype. A similar phenomenon was observed in the (–) allele of *PTEN* IVS4 (rs3830675) polymorphism compared to the (+) allele and the recessive effect model (–/–) *vs.* (–/+ and +/+). Future large-scale studies performed in multiple populations are warranted to confirm our results.

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