Genetic association between the cyclin-dependent kinase inhibitor gene \( p27/Kip1 \) polymorphism (rs34330) and cancer susceptibility: a meta-analysis

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The \( p27 \) rs34330 (-79C/T) polymorphism has been widely studied for human cancer susceptibility. The current findings, however, still remained controversial. Therefore, we performed the meta-analysis to provide a more accurate result. Eligible studies were identified from PubMed database up to June 2015. The association of \( p27 \) rs34330 polymorphism and cancer susceptibility was estimated with odds ratios and corresponding 95% confidence intervals. The meta-analysis was performed with Stata 12. A total of ten studies with 11,214 cases and more than 8,776 controls were included in the meta-analysis (including breast, lung, thyroid, endometrial, and hepatocellular cancer). In pooled analysis, \( p27 \) gene rs34330 polymorphism significantly increased the cancer susceptibility. Subgroup analysis indicated that the elevated risk was observed under all the genetic models for Asians and under three genetic models for Caucasians. Results of sensitivity analysis were similar to the overall results. The results suggested that the \( p27 \) rs34330 polymorphism increased the cancer susceptibility, especially in Asians. Further well-designed and large sample size studies are warranted to verify the conclusion.

Cancer is a leading cause of death and a major public health problem in both economically developed and developing countries. The occurrence of cancer is elevating because of the growth and aging of global population and environmental factors, especially in less developed countries, in which approximately 82% of the world’s population resides. Based on GLBOCAN estimates, about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide\(^1\). Many risk factors, such as lifestyle behaviors\(^2-^4\) and genetic factors\(^5-^9\), have been identified. However, cancer prevention is still a challenging project. Therefore, it is urgent to identify other risk factors for preventing cancers.

The \( p27/Kip1 \) (\( p27 \)) gene (also known as \( CDKN1B \)) is located on chromosome 12p13 and encodes cyclin-dependent kinase inhibitors (CDKIs) implicated in the negative regulation of the cell cycle\(^10,^11\). Cell cycle arrest allows cells to repair DNA damage and replication errors. Therefore, the loss of cell cycle control may contribute to the development of malignancies\(^12,^13\). Certain polymorphisms including rs2066827 (109T/G) and rs343330 (-79 C/T) of \( p27 \) gene have been identified as associated cancer susceptibility. In 2012, a meta-analysis had been performed to estimate the association between \( p27 \) gene rs2066827 polymorphism and cancer susceptibility\(^14\). The rs34330 polymorphism of \( p27 \) gene has also been widely studied for human cancer susceptibility. The existing evidence, however, still remains controversial and has not yet been investigated using meta-analytic methods. Therefore, we aimed in this study to investigate the association of \( p27 \) gene rs34330 polymorphism with cancer susceptibility.

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Results

Study characteristics. Figure 1 summarizes the detailed process of study selection. Based on the search strategy, 1,641 records were retrieved. In this meta-analysis, ten studies involving 11,214 cases and more than 8,776 controls were identified from the electronic databases according to the inclusion criteria. Characteristics of the identified studies are presented in Table 1. These case-controls studies were published between 2006 and 2014. Of them, three studies were conducted in China, two in the US, one in the UK, one in Australia, one in Turkey, one in Spain, and one in Brazil. Six types of malignant diseases were involved, shown as follows: breast cancer, lung cancer, bladder cancer, thyroid cancer, endometrial cancer, and hepatocellular cancer. The sample size in these studies ranged from 143 to 9,030 individuals. The genotype distributions of cases and controls were presented in seven studies. Other three studies only reported the ORs with 95% CIs in more than two of genetic models, such as homozygous model, heterozygous model, recessive model, or allele model; of these three studies, one study reported ORs and 95% CIs in two different populations, which was treated as two distinct reports in the combined analysis. All studies were consistent with HWE in controls except one study that did not provide the genotype distribution of controls or report any information for HWE.

Quantitative analysis. Table 2 shows the main results of summarized ORs and 95% CIs for all genetic models estimated in the present analysis of rs4330 polymorphism and cancer susceptibility. Overall, significantly
increased cancer susceptibility was observed in all the tested genetic models: homozygous model (TT vs. CC: OR = 1.30, 95% CI = 1.16–1.44, Fig. 2), heterogeneous model (CT vs. CC: OR = 1.13, 95% CI = 1.03–1.25, Fig. 3), dominant model (TT + CT vs. CC: OR = 1.21, 95% CI = 1.04–1.42, Fig. 4), recessive model (TT vs. CT + CC: OR = 1.18, 95% CI = 1.05–1.33, Fig. 5), allele model (T vs. C: OR = 1.10, 95% CI = 1.01–1.20, Fig. 6). Low to moderate between study heterogeneity was detected (I² = 15.8%, P = 0.293 for TT vs. CC; I² = 46.2%, P = 0.046 for CT vs. CC; I² = 58.3%, P = 0.025 for TT + CT vs. CC; I² = 7.7%, P = 0.369 for TT vs. CT + CC; I² = 47.0%, P = 0.057 for T vs. C).

Subgroup analysis showed increased cancer susceptibility under all tested genetic models (TT vs. CC: OR = 1.48, 95% CI = 1.24–1.78, Fig. 2; CT vs. CC: OR = 1.38, 95% CI = 1.17–1.61, Fig. 3; TT + CT vs. CC: OR = 1.35, 95% CI = 1.18–1.53, Fig. 4; TT vs. CT + CC: OR = 1.27, 95% CI = 1.10–1.46, Fig. 5; T vs. C: OR = 1.19, 95% CI = 1.01–1.40, Fig. 6). A fixed-effects model was assumed for all genetic models, except for the recessive model (TT vs. CT + CC), where a randomeffects model was used. The results of the meta-analysis are shown in Table 2.

Table 2. Meta-analysis of all studies and subgroups. aRemoving the study of Canbay 2009. HWE = Hardy-Weinberg equilibrium; OR = odds ratio; CI = confidence interval.
OR = 1.44, 95% CI = 1.17–1.78, Fig. 4; TT vs. CT + CC: OR = 1.21, 95% CI = 1.01–1.44, Fig. 5; T vs. C: OR = 1.22, 95% CI = 1.03–1.44, Fig. 6) for Asians and under three genetic models for Caucasians (TT vs. CC: OR = 1.21, 95% CI = 1.06–1.38, Fig. 2; TT + CT vs. CC: OR = 1.10, 95% CI = 1.02–1.19; T vs. C: OR = 1.08, 95% CI = 1.02–1.14) (Table 2). Pooled results of studies with controls in HWE were similar to the overall results. Sensitivity analysis was performed by removing the study of Canbay 2009 and the results were similar to the overall results under all the genetic models (Table 2).

Publication bias. According to funnel plots and Egger's test, no publication bias was observed in this meta-analysis (homozygous model: P = 0.339 for Egger's test, Fig. 7).

Discussion

As single studies may have inadequate statistical power to precisely estimate the association between p27 gene rs34330 polymorphism and cancer susceptibility, we performed this meta-analysis, which is a quantitative approach, to precisely estimate the true effects of gene polymorphism on cancer susceptibility. The present meta-analysis included ten case-control studies involving 11,214 cancers and more than 8,776 controls and suggested that p27 gene rs34330 polymorphism may increase the susceptibility to cancer, especially in Asian populations.

Cyclins and CDKs play crucial role in the cell cycle during cellular proliferation. These proteins regulate transitions between G1, S, G2 and M phases of the cell cycle, especially the transition from G1 to S. Results from experimental studies suggested that CDKIs could inhibit cellular proliferation through suppressing the kinase activity of the cyclin-CDK complexes and block the transition from G1 to S. Therefore, CDKIs have been identified as tumor suppressor proteins. The p27, which is a member of the CDKIs, has been postulated as a tumor suppressor gene. In 2012, a meta-analysis has been performed to investigate the association between rs2066827 polymorphism of p27 gene and cancer susceptibility, and they suggested that the p27 gene rs2066827 polymorphism did not associate with the overall cancer susceptibility in the general population. No meta-analysis was conducted investigating the association between other polymorphisms of p27 gene and cancer susceptibility.

It has been postulated that the rs34330 polymorphism, located in the 5'-untranslating region of p27 gene, might be correlated with a reduced production of p27 protein, and certain evidence indicated that rs34330 polymorphism could alter the transcription of p27. In recent decades, this mutation has been widely investigated concerning human cancer susceptibility and the current evidence is still inconclusive. Therefore, we performed the present meta-analysis, for the first time to the best of our knowledge, to summarize the true association between p27 gene rs34330 polymorphism and cancer susceptibility, and we found an elevated risk of cancer development associated with this polymorphism for overall populations. In this meta-analysis, certain evidence of low to moderate degree of between-study heterogeneity was detected in three genetic models (homozygous, dominant and allele model) and no evidence of heterogeneity was found in homozygous model and recessive model. The low to moderate between-study heterogeneity indicated acceptable credibility of the results of this meta-analysis. Moreover, the results of sensitivity analysis were robust when we excluded the studies with controls not in HWE. The results of subgroup analysis according to ethnicity showed that the elevated risk associated with
p27 gene rs34330 polymorphism were more predominant in Asians than Caucasians. The results indicated that ethnicity might play an important role in cancer susceptibility; however, the underlying mechanism is unclear.

Previously, two meta-analyses investigated the association of CDKN1B gene polymorphisms (including rs34330 and rs2066827) and CDKN1B rs2066827 polymorphism with susceptibility to breast cancer, respectively. The previous meta-analyses found that p27 gene rs2066827 polymorphism was not associated with breast cancer susceptibility. One meta-analysis comprehensively evaluated the association between polymorphisms of p27 gene and breast cancer susceptibility, and they found that p27 gene rs34330 polymorphism might be associated with breast cancer susceptibility. In this previous meta-analysis, the included studies with rs34330 polymorphism were four case-control studies, which were also included in our meta-analysis. Moreover, due to the quantity of included studies on different type of cancer, we did not perform subgroup analysis according to type of cancer.
In this study, certain limitations should be taken into consideration. First, confounding factors, such as selection bias and measurement bias, might distort the credibility of the result. Second, this meta-analysis only included Asian and Caucasian populations. Therefore, the external validity is relatively limited. Third, we could not eliminate the possibility of publication bias even though we detected no evidence of publication bias through Begg’s funnel plot and Egger’s linear regression method. In addition, the sample size and number of included studies is relatively small for gene-susceptibility investigation.

In conclusion, our meta-analysis suggests that the \( p27 \) gene rs34330 polymorphism might increase the cancer susceptibility, especially in Asians. Further well-designed and large sample size studies are warranted to verify the conclusion.
Methods

Literature search. A thorough literature search was performed using PubMed database up to June 2015. We used the following search terms to identify all potential relevant studies: (p27 OR p27Kip1 OR CDKN1B OR “cyclin-dependent kinase inhibitor 1B”) AND (cancer OR carcinoma OR adenocarcinoma OR neoplasm OR tumor) AND (gene OR allele OR polymorphism OR variation OR variant OR mutation). In addition, reference lists of retrieved articles were manually searched. No restriction was applied.

Inclusion criteria. All the studies included in the meta-analysis should meet the following inclusion criteria: (1) study design was case-control or cohort; (2) studies examined the association between p27 rs34330 polymorphism and cancer susceptibility; (3) studies presented detailed genotype counts or odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

Data extraction. Two reviewers independently extracted the following information from all identified studies according to a standardized data collection form: author name, publication year, ethnicity, location, number of cases and controls, type of cancer, source of control, Hardy-Weinberg equilibrium (HWE) in controls, genotyping method, genetic data, and ORs with corresponding 95% CIs. Any disagreements were resolved by discussion.

Statistical analysis. The OR and 95% CI were used to measure the association between p27 rs34330 polymorphism and cancer susceptibility. The significance of pooled ORs was determined by Z test at the P < 0.05 level of significance. We estimated the effect of p27 rs34300 polymorphism on cancer susceptibility using homozygous, heterogeneous, dominant, recessive, and allele models. Heterogeneity test was performed with the use of Q statistic at the P < 0.10 level of significance since the chi-square test has low power in the situation of a meta-analysis when numbers or sample sizes of included studies were small. We also calculated the I² metric, a quantitative measurement of heterogeneity among studies. The summary ORs were pooled using a fixed-effects model when the studies included were homogeneous and a random-effects model when statistical heterogeneity was detected (P < 0.1). Prespecified subgroup analyses according to HWE in controls, ethnicity, and source of control were performed to examine the impacts of these factors. Sensitivity analysis was performed by removing study which was an outlier. Potential publication bias was explored by Begg’s funnel plot and Egger linear regression test. All analyses were performed using Stata 12.0 (Stata, College Station, TX). The two-sided P values less than 0.05 were considered statistically significant, except where extra specified.

References
1. Torre, L. A. et al. Global cancer statistics, 2012. CA Cancer J Clin 65, 87–108 (2015).
2. Zeng, X. T. et al. Meta-analysis on the association between toothbrushing and head and neck cancer. Oral Oncol 51, 446–451 (2015).
3. Gawade, P. L. et al. Lifestyle, distress, and pregnancy outcomes in the Childhood Cancer Survivor Study cohort. Am J Obstet Gynecol 212, 47 e1–10 (2015).
4. Petersen, K. E. et al. The combined impact of adherence to five lifestyle factors on all-cause, cancer and cardiovascular mortality: a prospective cohort study among Danish men and women. Br J Nutr 113, 849–858 (2015).
5. Gao, X., Wang, J., Wang, W., Wang, M. & Zhang, J. eNOS Genetic Polymorphisms and Cancer Risk: A Meta-Analysis and a Case-Control Study of Breast Cancer. Medicine (Baltimore) 94, e972 (2015).
6. Geng, P. et al. Genetic Association Between NFKBIA -881A > G Polymorphism and Cancer Susceptibility. Medicine (Baltimore) 94, e1024 (2015).
7. Geng, P. et al. Genetic association between PER3 genetic polymorphisms and cancer susceptibility: a meta-analysis. Medicine (Baltimore) 94, e568 (2015).
8. Duan, Y. F. et al. Association between ABO gene polymorphism (rs505922) and cancer risk: a meta-analysis. Tumour Biol 36, 5081–5087 (2015).
9. Lu, L., Sun, Y., Li, Y. & Wan, P. The polymorphism MMP1 -1607 (1G > 2G) is associated with a significantly increased risk of cancers from a meta-analysis. Tumour Biol 36, 1685–1693 (2015).
10. Polyak, K. et al. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev 8, 9–22 (1994).
11. Polyak, K. et al. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. Cell 78, 59–66 (1994).
12. Sherr, C. J. Cancer cell cycles. Science 274, 1672–1677 (1996).
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Additional Information
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