RESEARCH COMMUNICATION

Effects of the Cyclin D1 Polymorphism on Lung Cancer Risk - a Meta-analysis

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Abstract

<u>Background</u>: Cyclin D1 (CCND1) is critical in the transition of the cell cycle from G1 to S phases and unbalanced cell cycle regulation is a hallmark of carcinogenesis. A number of studies conducted to assess the association between CCND1 G870A polymorphism and susceptibility to lung cancer have yielded inconsistent and inconclusive results. In the present study, the possible association above was assessed by a meta-analysis. <u>Methods</u>: Eligible articles were identified for the period up to November 2011. Pooled odds ratios (OR) with 95% confidence intervals (95%CI) were appropriately derived from fixed effects or random-effects models. Sensitivity analysis excluding studies whose genotype frequencies in controls significantly deviated from the Hardy-Weinberg equilibrium (HWE) was performed. <u>Results</u>: Ten case-control studies with a total of 10,548 subjects were eligible. At the overall analysis the CCND1 870A allele appeared to be associated with elevated lung cancer risk (for allele model, pooled OR = 1.24, 95% CI: 1.08-1.44, P = 0.004; for homozygous model, pooled OR = 1.45, 95% CI: 1.14-1.84, P = 0.003; for recessive model, pooled OR = 1.29, 95% CI: 1.06-1.58, P = 0.013; for dominant model, pooled OR = 1.33, 95% CI: 1.08-1.65, P = 0.009). Subgroup analyses by ethnicity and sensitivity analysis further pointed to associations, particularly in Asians. <u>Conclusion</u>: This meta-analysis suggests that the A allele of CCND1 G870A polymorphism confers additional lung cancer risk.

Keywords: Lung cancer - CCND1 - meta-analysis - polymorphism

Introduction

Lung cancer is one of the leading causes of cancer deaths in the world (Herbst et al., 2008). Epidemiological evidence has suggested that genes controlling the metabolism of carcinogens are associated with the risk of lung cancer, possibly through their ability to modulate DNA damage by carcinogens (Herbst et al., 2008; Salim et al., 2011). Recent molecular biological studies have shown that lung cancer may be caused by the accumulation of multiple genetic defects including these of tumor suppressor genes, oncogenes, and DNA repair genes (Brennan et al., 2011; Xiao et al., 2011). A frequent target in lung cancer is the deregulation of G1-S phase progression in the cell cycle, where the transition through G1 to S phase is regulated by cyclins, cyclin-dependent kinases, and their inhibitors (Knudsen et al., 2006, Brennan et al., 2011). The G1/Scheckpoint arrests the cell cycle to prevent replication of damaged DNA and allow DNA damage to be repaired (Knudsen et al., 2006). Considering the important role in the transition from G1 to S phase o the cell cycle, cyclin D1 (CCND1) is considered as an essential regulator for this process, whose deregulation has been implicated in pathogenesis of several types of cancers, including lung cancer (Knudsen et al., 2006;

Musgrove et al., 2011). The CCND1 gene has a G to A polymorphism (G870A) at codon 242 in exon 4, which increases the frequency of alternative splicing, leading to an altered protein that does not contain the sequence involved in protein turnover and then has a longer half-life (Knudsen et al., 2006). To date, many epidemiological studies have been conducted to explore the relationship between the CCND1 G870A polymorphism and lung cancer risk (Qiuling et al., 2003; Gautschi et al., 2006; Hung et al., 2006; Sobti et al., 2006; Hsia et al., 2011). However, the findings were inclusive, partially due to the possible small effect of the polymorphism on lung cancer risk and the relatively small sample size in each of published studies. In order to reduce the probability of false-negative results and derive a more precise estimation of the association of CCND1 G870A polymorphism with lung cancer risk, we therefore performed a meta-analysis based on the data from all related published literature.

Materials and Methods

Search strategy

In our meta-analysis, we searched the articles using the search terms "CCND1," "cyclin D1," "lung cancer" and "polymorphism" in the PubMed, Embase and CNKI

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Table 1. Summary ORs and 95%CIs of CCND1 Polymorphism and Lung Cancer Risk

Comparisons	N	Allele model (A versus G)			Homozygous model (AA versus GG)			Recessive model (AA versus AG/GG)			Dominant model (AA/AG versus GG)		
		OR(95%CI)	P	I^2	OR(95%CI)	P	I^2	OR(95%CI)	P	I^2	OR(95%CI)	P	I^2
Total studies	10	1.24 (1.08, 1.44)	0.003	78%	1.45 (1.14, 1.84)	0.003	66%	1.29 (1.06, 1.58)	0.013	68%	1.33 (1.08, 1.65)	0.009	74%
Caucasians	4	1.23 (0.97, 1.57)	0.088	91%	1.37 (0.94, 1.99)	0.097	83%	1.21 (0.94, 1.56)	0.145	72%	1.33 (0.93, 1.92)	0.118	90%
Asians	5	1.29 (1.14, 1.46)	<0.001	16%	1.65 (1.27, 2.14)	<0.001	7%	1.56 (1.28, 1.90)	<0.001	44%	1.29 (1.04, 1.61)	0.022	0%
Studies with HWE	8	1.08 (1.01, 1.14)	0.014	15%	1.49 (1.06, 2.09)	0.012	6%	1.15 (0.95, 1.39)	0.23	51%	1.07 (0.97, 1.17)	0.154	0%
Caucasians with HWE	3	1.06 (0.99, 1.13)	0.094	0%	1.13 (0.99, 1.28)	0.073	0%	1.13 (1.01, 1.26)	0.034	46%	1.04 (0.94, 1.15)	0.5	0%
Asians with HWE	4	1.23 (1.05, 1.46)	0.013	26%	1.49 (1.06, 2.09)	0.021	13%	1.47 (0.97, 2.24)	0.072	50%	1.26 (0.96, 1.65)	0.099	0%

N, number of relevant studies; I^2, I^2 value for heterogeneity

databases, and the last search updated on November 2011. Additional studies were identified by a hand search of references of original studies or review articles on the association between CCND1 G870A polymorphism and lung cancer. The following inclusion criteria were used for the literature selection for our meta-analysis: (a) the diagnosis of lung cancer patients was confirmed histopathologically; (b) a case–control study of the CCND1 G870A polymorphism and lung cancer; (c) sufficient available data for estimating an odds ratio (OR) with 95% confidence interval (CI). For republished studies, the largest one was selected.

Data extraction

Two investigators independently extracted data and reached consensus on the following characteristics of the selected studies: the first author's name, year of publication, country, sources of controls, ethnicity (categorized as Asian, European, and mixed populations), genotyping method, numbers of cases and controls with various genotypes, Hardy-Weinberg equilibrium (HWE) and minor allele frequency (MAF) in controls.

Statistical analysis

The strength of association between the CCND1 G870A polymorphism and lung cancer risk was accessed by calculating crude odds ratios (ORs) with 95% confidence intervals (CIs). We evaluated the allele model (A versus G), homozygous model (AA versus GG), the dominant model (GA/AA versus GG) and the recessive model (AA versus GG/GA), respectively. Heterogeneity assumption was checked by the I2 statistic to quantify the proportion of the total variation due to heterogeneity, and a I^2 value less than 50% indicates a lack of heterogeneity among studies (Higgins and Thompson, 2002; Higgins et al., 2003). Then, the fixedeffects model (the Mantel–Haenszel method) was used to pool the data (Mantel and Haenszel, 1959). If $I^2 > 50\%$, the random-effects model (the DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). To validate the credibility of outcomes in this meta-analysis, sensitivity analysis was performed by sequential omission of individual studies or by omitting studies without HWE. The potential

publication bias was estimated by visual inspection of the funnel plot (Egger et al., 1997). All statistical tests were performed with Review Manage (v.4.2; Oxford, England), All P values were two-sided, and a P value < 0.05 was considered statistically significant.

Results

Eligible studies

A total of 10 case-control studies with a total of 10, 548 subjects on the association between CCND1 G870A polymorphism and lung cancer risk were included for this meta-analysis (Qiuling et al., 2003; Wang et al., 2003; Buch et al., 2005; Gautschi et al., 2006; Hung et al., 2006; Sobti et al., 2006; Wang et al., 2007; Wang et al., 2008; Li et al., 2009; Hsia et al., 2011). There were four studies of European population (Buch et al., 2005; Gautschi et al., 2006; Hung et al., 2006; Wang et al., 2007), five studies of Asians (Qiuling et al., 2003; Wang et al., 2003; Wang et al., 2008; Li et al., 2009; Hsia et al., 2011) and one study of mixed population (Sobti et al., 2006). There were about 80.0% studies using the classic PCR-RFLP assay to genotype the CCND1 G870A polymorphism. All studies indicated that the distribution of genotypes in the controls was consistent with HWE except two (Buch et al., 2005; Hsia et al., 2011).

Meta-analysis

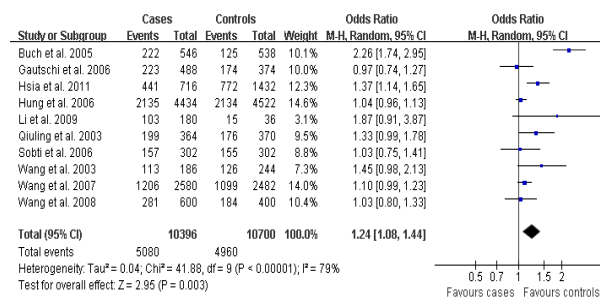Table 1 listed the results of the association between the CCND1 G870A polymorphism and lung cancer, and

Figure 1. OR of Lung Cancer Associated with CCND1 G870A Polymorphism for A Allele Compared with G Allele (Allele Contrast Model)
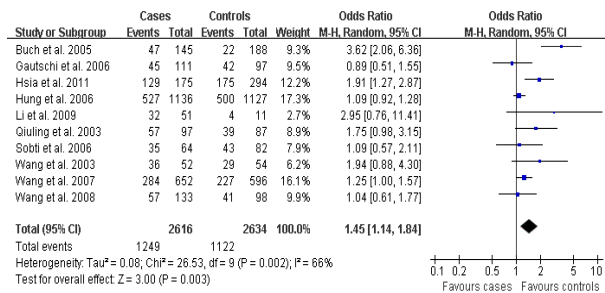
Figure 2. OR of Lung Cancer Associated with CCND1 G870A Polymorphism for AA Genotype Compared with GG Genotype (Homozygote Model)
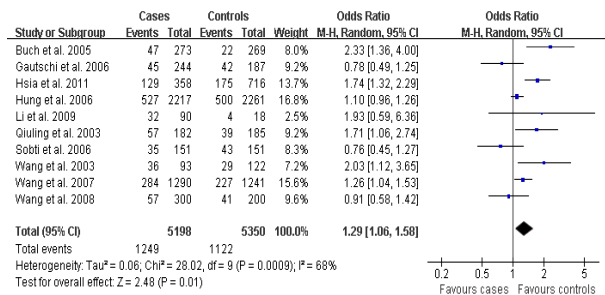

Figure 3. OR of Lung Cancer Associated with CCND1 G870A Polymorphism for AA Genotype Compared with AG/GG Genotype (Recessive Model)

the heterogeneity test. Overall, At the overall analysis the CCND1 870A allele seemed to be associated with elevated lung cancer risk (for Allele model, pooled OR = 1.24, 95% CI: 1.08-1.44, P = 0.004; for Homozygous model, pooled OR = 1.45, 95% CI: 1.14-1.84, P = 0.003; for Recessive model, pooled OR = 1.29, 95% CI: 1.06-1.58, P = 0.013; for Dominant model, pooled OR = 1.33, 95% CI: 1.08-1.65, P = 0.009) (Figure 1, Figure 2, Figure 3). Sensitivity analysis by sequential omission of individual studies or by omitting studies without HWE didn't alter the significance of ORs above and validated the credibility of ORs (Data not shown). Besides, subgroup analyses by ethnicity further identified the possible association above (Table 1).

Publication bias

Funnel plots' shape of all contrasts did not reveal obvious evidence of asymmetry, suggesting publication bias was not obvious in this meta-analysis.

Discussion

This present analysis investigating the relationship between CNND1 A870G polymorphism and risk of lung cancer is based on the large amount of published data giving greater information to detect significant differences. Sensitivity analyses and the cumulative meta-analyses were also performed. Ten case-control studies with a total of 10, 548 subjects were eligible. At the overall analysis the CCND1 870A allele seemed to be associated with elevated lung cancer risk (for allele model, pooled OR = 1.24, 95% CI: 1.08-1.44, P = 0.004; for homozygous model, pooled OR = 1.45, 95% CI: 1.14-1.84, P = 0.003; for recessive model, pooled OR = 1.29, 95% CI: 1.06-1.58,

P = 0.013; for dominant model, pooled OR = 1.33, 95% CI: 1.08-1.65, P = 0.009). Both subgroup analyses by ethnicity and sensitivity analysis further identified the possible association above. Thus, this meta-analysis suggests that the A allele of the CCND1 G870A polymorphism may confer additional lung cancer risk.

In the literature, several studies showed that the genotypes of CNND1 A870G were associated with lung cancer risk and clinical outcome (Betticher et al., 1996; Gautschi et al., 2006). The CCND1 gene is located at 11q13 and can act as an oncogene (Hinds et al., 1994). CCND1 plays a critical role in cell cycle control, and there is evidence that reduced DNA repair capacity is associated with increased risk of lung cancer (Hinds et al., 1994). A commonly occurring G-to-A polymorphism at nucleotide 870 (G870A) of CCND1 has been subject of many case-control association studies of lung cancer in different ethnic populations. However, the results were different or even contradictory. We pooled the results of the eleven eligible case–control studies in this meta-analysis and found significant association between CCND1 G870A polymorphism and lung cancer. These findings were consistent with most of the related studies as summarized in our meta-analysis.

Molecular analysis of CCND1 polymorphism has demonstrated that CCND1 G870A polymorphism leads to an alternative splice site in DNA, which creates two kinds of functional transcripts (Solomon et al., 2003). Allele G tends to produce the full transcript (transcript a), whereas allele A is more closely associated with the truncated transcript (tran-script b) (Solomon et al., 2003). The full functioning transcript a can interact with and activates the G1 cyclin-dependent kinases (CDK), CDK4 and CDK6, and then the complex phosphorylates/ inactivates the retinoblastoma (RB) tumor suppressor, which is required for the transition into S-phase (Solomon et al., 2003). However, transcript b encodes the protein short of the PEST region in the C-terminal domain, but this protein has a decreased ability to phosphorylate RB (Lu et al., 2003; Knudsen et al., 2006). On the other hand, the truncated transcript has a longer half-life than the full transcript, leading to an over expression of CCND1, which subsequently results in deregulation of cell proliferation, and increases the risk of cancer (Lu et al., 2003; Solomon et al., 2003). Thus, the results of our meta-analysis were consistent with the consequence of functional studies mentioned above.

Several limitations need to be addressed. First, our results were based on unadjusted estimates, lacking of the original data of the eligible studies limited the evaluation of the effects of the gene-gene, gene-environment interactions in lung cancer development. Second, CCND1 has several common single nucleotide polymorphism identified, because we could not obtain more detailed individual information on genotypes of the other polymorphisms, we did not perform linkage disequilibrium and haplotype analysis.

To sum up, th present meta-analysis provided evidence that the A allele of the CCND1 G870A polymorphism confers additional lung cancer risk, particularly in Asian populations.

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