

Mitogen-Activated Protein Kinase Kinase 4 Gene Polymorphism and Cancer Risk

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Abstract: A number of epidemiological studies have assessed the association of $-1304T > G$ polymorphism in the *MKK4* gene and risk of cancer, but the results lack of statistical power due to the limited subjects used in these studies. This study was devised to identify the genetic effects of the $-1304T > G$ polymorphism on cancer risk in a large meta-analysis.

Eligible studies were identified by searching both Chinese and English databases. General as well as subgroup analyses were performed for 8 independent case-control publications with a total of 4623 cases and 5256 cancer-free controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the association.

Overall, this meta-analysis showed that the association between the $-1304T > G$ polymorphism and cancer risk was statistically significant (GG vs TT: OR = 0.63, 95% CI, 0.52–0.75; GG + TG vs TT: OR = 0.85, 95% CI, 0.79–0.91; GG vs TG + TT: OR = 0.67, 95% CI, 0.56–0.80; G vs T: OR = 0.82, 95% CI, 0.77–0.88; TG vs TT: OR = 0.86, 95% CI, 0.79–0.93).

Our meta-analysis reveals that the presence of the $-1304T > G$ polymorphism is likely to decrease risk of cancer. Future larger studies are necessary to validate the current finding.

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Abbreviations: CI = confidence interval, HWE = Hardy–Weinberg equilibrium, JNKs = Jun N-terminal kinases, MAP = mitogen-activated protein, MKK4 = Mitogen-activated protein kinase 4, OR = odds ratio, SNP = single-nucleotide polymorphism.

INTRODUCTION

Environmental carcinogens interacting with inherited factors is the result of cancer.^{1,2} The interaction is directly or indirectly involved in the activation of the mitogen-activated protein (MAP) kinase pathways that converge on c-Jun N-terminal kinases (JNKs) and p38 MAPKs and function as essential regulators of cellular senescence.³ Mitogen-activated

protein kinase 4 (*MKK4*, also known as MAP2K4, MEK4, JNKK1, and SEK1) is a member of the MAP kinase family, playing a key role in multiple physiologic and pathophysiologic processes such as inflammation and tumor suppression.⁴ *MKK4* is highly mutated and has a pro-oncogenic role in cancers of pancreatic, breast, colon, prostate,⁵ skin,⁶ and laryngeal squamous cell.⁷ Meanwhile, *MKK4* has also been proved as a suppressor gene in the metastasis of prostate and ovarian cancer cell lines.^{8,9} Its function in tumorigenesis remains highly controversial.

Mapped to chromosome 17p11.2, the *MKK4* gene encoding a 399-amino acid protein in humans spans over 120 kb and consists of 11 exons.^{4,10} A previous study reporting the association between $-1304T > G$ (rs3826392) polymorphism in the promoter of *MKK4* gene and the risk of sporadic colorectal cancer in a southern Chinese population showed a decreased risk correlated with the $-1304T > G$ polymorphism.¹¹ Identical results were also reported in a follow-up investigation focusing on colon cancer.¹² A number of genetic epidemiological studies looking at other cancers presented substantial evidence that the functional role of the $-1304T > G$ polymorphism in cancer risk differs depending on the type of cancer.^{13–18} Since the findings are on the ground of relatively small samples restricted to a specific population, thus they may have been underestimated and lack statistical power to elucidate the underlying mechanism of cancer onset associated with this polymorphism.

In an effort to identify the genetic effects of the $-1304T > G$ polymorphism on cancer risk, we performed a meta-analysis composed of the publications evaluating the association between the $-1304T > G$ polymorphism and risk of cancer.

METHODS

Identification and Eligibility of Relevant Studies

We searched both English (PubMed, Embase) and Chinese databases (CNKI) for all publications regarding the association between the $-1304T > G$ polymorphism and cancer risk by using the keywords: *MKK4*, $-1304T > G$ /rs3826392, polymorphism/polymorphisms/variant/genotype/SNP, and cancer. All references cited in the retrieved articles were also screened to identify the missing data eligible for the meta-analysis. Publications were included in the meta-analysis when satisfying the following criteria: (a) investigating the association between the $-1304T > G$ polymorphism and cancer risk in patients with cancer and control subjects; (b) having genotype data in full detail; (c) published as a full text, rather than a short summary or a comment letter before January 2014. The study was approved by the ethics committee of southwest hospital.

Data Extraction

Based on the inclusion criteria and a consensus reached on all items, 2 investigators independently extracted data including

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PG and JO are co-first authors.

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first author, year of publication, country, ethnicity, control source, sex proportion in cases and controls, cancer type, genotyping method, and genotype distributions in cases and controls. In addition, we merged colon cancer into the colorectal cancer when performing meta-analysis.

Statistical Analysis

STATA software, version 12.0 (Stata Corporation, College Station, TX) was used to analyze the data of the -1304T > G polymorphism. The fixed effects model or the random effects model was performed to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for each study. Heterogeneity between studies was measured by Chi-square-based *Q* test¹⁹ and *I*² statistic.²⁰ When no obvious inconsistency existed across the studies (*P* > 0.10 or *I*² < 50%), the fixed-effect model (Mantel-Haenszel) was used to pool the ORs; otherwise, the random effects model (DerSimonian and Laird) was selected.^{21,22} Sensitivity analysis was performed by sequentially excluding each study and rechecking whether the corresponding ORs were altered significantly. Funnel plots and Egger’s test²³ were used to determine the potential publication bias in the meta-analysis. All tests were 2-sided with *P* < 0.10 being statistically significant.

RESULTS

Study Characteristics

Through the comprehensive search, we yielded a total of 31 papers, whose eligibility was examined by reviewing the key words, titles, abstracts, and the full texts according to the predescribed criteria for inclusion. After excluding the unavailable publications, 8 studies with 4623 cases and 5256 cancer-free controls were included in this meta-analysis^{11–18} (Figure 1). Of these studies, 2 were published in Chinese^{12,15} and 6 were in English.^{11,13,14,16–18} All of the studies were conducted for the Asian populations. Table 1 lists the main characteristics of the eligible studies for this meta-analysis.

Quantitative Synthesis

As shown in Table 2, pooling all data on the association between the -1304T > G polymorphism and cancer risk into a large dataset revealed a significantly reduced risk of cancer in all genetic models (GG vs TT: OR = 0.63, 95% CI, 0.52–0.75, Figure 2; GG + TG vs TT: OR = 0.85, 95% CI, 0.79–0.91, Figure 3; GG vs TG + TT: OR = 0.67, 95% CI, 0.56–0.80; G vs

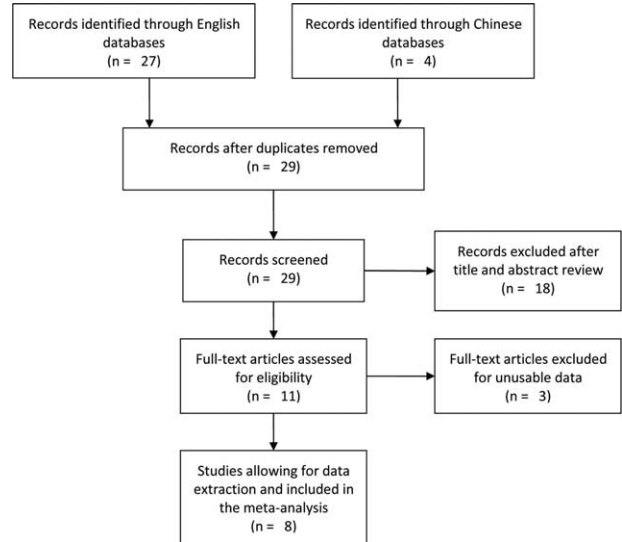


FIGURE 1. Flow diagram of the study selection process.

T: OR = 0.82, 95% CI, 0.77–0.88; TG vs TT: OR = 0.86, 95% CI, 0.79–0.93).

No significant between-study heterogeneity was suggested in the meta-analysis (Table 2). Further sensitivity analysis did not reveal any quantitative alternation occurring in the ORs (data not shown). In addition, the funnel plots were symmetrical (*P* = 0.902) and Egger’s test suggested no evidence of obvious publication bias among the studies (GG vs TT: *P* = 0.894) (Figure 4).

DISCUSSION

Based on 8 independent case–control publications with a total of 4623 cases and 5256 cancer-free controls, we performed a meta-analysis to comprehensively assess the relationship between the -1304T > G polymorphism and risk of different types of cancers. From the analysis results, we found statistically significant evidence for a reduction in cancer risk when combining all data together. Since the insufficient data on each cancer did not allow further stratified analysis, such as by type of cancer, thus we failed to estimate the associations of the studied polymorphism and various cancers.

TABLE 1. Distribution of *MKK4* -1304T>G Gene in Cancer Cases and Control Subjects

First Author	Ethnicity	Cancer Type	Sex Distribution (Case/Control, Male/Female)	Genotyping Method
Wei	Asian	Colorectal cancer	300/213, 300/223	PCR-RFLP
Liu	Asian	Lung cancer	746/310, 746/310	TaqMan
Jiang	Asian	AML	240/199, 336/264	PCR-RFLP
Feng	Asian	Esophageal cancer	503/68, 671/114	PCR-RFLP
Hu	Asian	Cervical cancer	Not report	PCR
Luo	Asian	Colon cancer	70/48, 70/48	PCR-RFLP
Shao	Asian	Prostate cancer	Not report	PCR-LDR
Zheng	Asian	NPC	763/474, 792/536	TaqMan

AML = acute myeloid leukemia, NPC = nasopharyngeal carcinoma, PCR = polymerase chain reaction, PCR-LDR = PCR-ligation detection reaction, PCR-RFLP = PCR-restriction fragment length polymorphism.

TABLE 2. Odds Ratio (OR) and Heterogeneity Results for *MKK4* -1304T>G Gene in Various Cancers

Genetic Models	Population	No. of Studies	Fixed Effects OR (95% CI)	I ² (%)	P-Value
GG vs TT	Total	8	0.63 (0.52, 0.75)	0	0.572
GG + TG vs TT	Total	8	0.85 (0.79, 0.91)	7.7	0.370
GG vs TG + TT	Total	8	0.67 (0.56, 0.80)	0	0.799
G vs T	Total	8	0.82 (0.77, 0.88)	36.9	0.134
TG vs TT	Total	8	0.86 (0.79, 0.93)	0	0.446

CI = confidence interval, P-value = P-value of between-study heterogeneity.

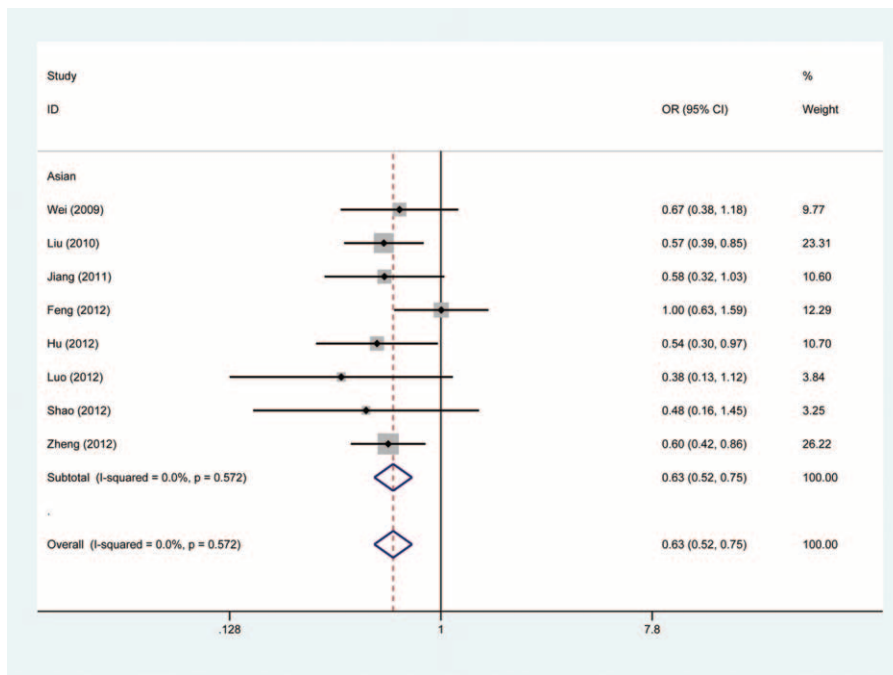


FIGURE 2. Forest plot of cancer susceptibility associated with *MKK4* -1304T>G polymorphism under GG vs TT with fixed-effects model.

MKK4 belongs to MAPK pathways known to have involvement in the regulation of apoptosis, inflammation, and tumorigenesis.¹¹ An increasing body of evidence has found that the molecular activity of *MKK4* is associated with the formation and initiation of cancers.^{24–26} Frequent mutations of *MKK4* have been reported in lung cancer and colorectal cancer.^{27,28} Also, loss-of-function mutations in the *MKK4* gene is shown in a portion of numerous human tissues, accounting for approximately 5%.^{24,29} Four common polymorphisms in the promoter region of the *MKK4* gene have been recorded in Genbank dbSNP database. The genetic variations in the promoter region could affect transcriptional activity and biological function of this gene¹⁴ resulting in tumorigenesis.

Since the discovery of a decreased risk associated with the -1304T > G polymorphism in the promoter region of *MKK4* was claimed in colorectal cancer,¹¹ a large number of replication studies on various cancers have been successively done in recent years. Most of the studies concluded that the -1304T > G polymorphism have protective effects on the development of cancer.^{12,13,16} Nevertheless, the susceptibility to esophageal cancer was not found to be associated with this polymorphism.¹⁵

Although our meta-analysis revealed an decreased risk in overall cancer, we cannot rule the possibility that the functional -1304T > G polymorphism decreases risk of some cancers by increasing the promoter activity, while it has no biological significance in other cancers, such as esophageal cancer.

Several potential factors must be concerned when interpreting the findings in this meta-analysis. First, the connections of various cancer risks associated with the -1304T > G polymorphism are on the basis of small samples, possibly leading to an underestimate of the true association, which should be further confirmed in a much larger study. Second, we only searched studies conducted among Asian subjects, thus the role of the studied polymorphism in cancer should be widely investigated in more ethnic populations. Third, cancer is known to be a multifactorial disease caused by complex interactions between environmental and genetic factors.³⁰ However, the estimate of such effects was not considered in the present meta-analysis, because the limited data did not allow us to do so.

In summary, this meta-analysis provides evidence that the -1304T > G polymorphism is strongly associated with a decreased risk of cancer. A large well-designed study in diverse

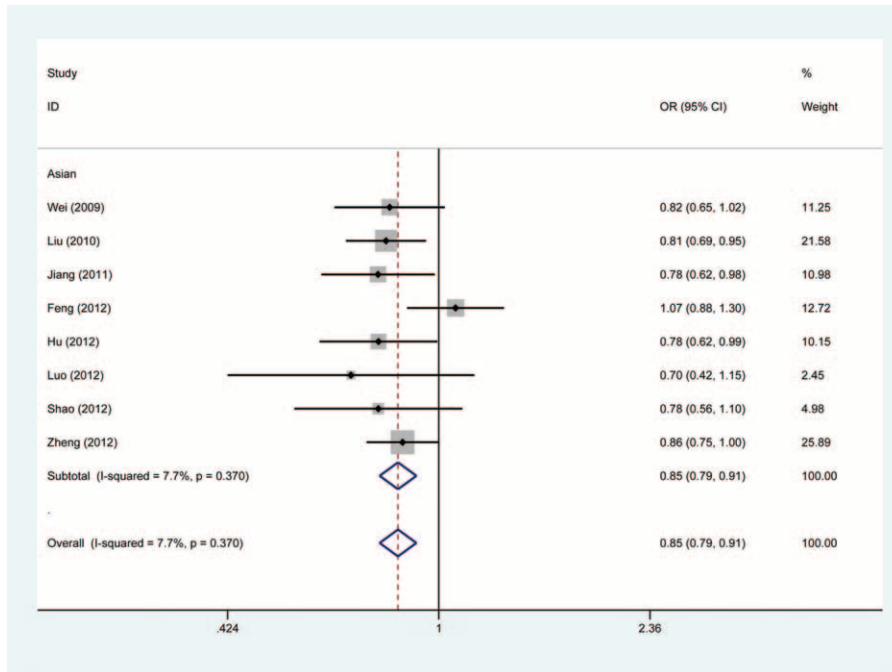


FIGURE 3. Forest plot of cancer susceptibility associated with *MKK4* -1304T>G polymorphism under GG + TG vs TT with fixed-effects model.

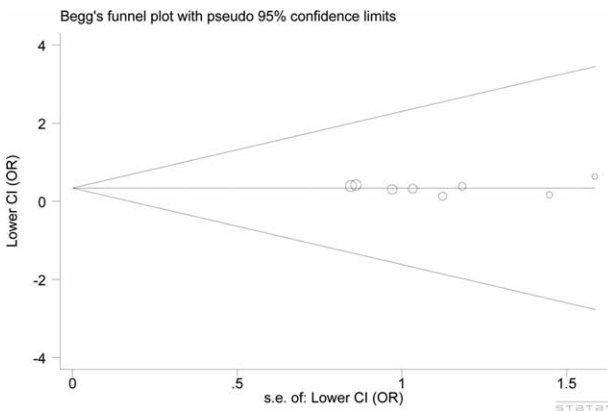


FIGURE 4. Funnel plots of *MKK4* -1304T>G polymorphism cancer risk. Model: GG vs TT, Egger's test: $P=0.894$.

ethnic populations is warranted to clarify the true association between the -1304T>G polymorphism and cancer.

REFERENCES

- Pharoah PD, Dunning AM, Ponder BA, et al. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer*. 2004;4:850–860.
- Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature*. 2001;411:366–374.
- Maruyama J, Naguro I, Takeda K, et al. Stress-activated MAP kinase cascades in cellular senescence. *Curr Med Chem*. 2009;16:1229–1235.
- Cuenda A. Mitogen-activated protein kinase kinase 4 (MKK4). *Int J Biochem Cell Biol*. 2000;32:581–587.
- Teng DH, Perry WL 3rd, Hogan JK, et al. Human mitogen-activated protein kinase kinase 4 as a candidate tumor suppressor. *Cancer Res*. 1997;57:4177–4182.
- Finegan KG, Tournier C. The mitogen-activated protein kinase kinase 4 has a pro-oncogenic role in skin cancer. *Cancer Res*. 2010;70:5797–5806.
- Huang C, Huang K, Wang C, et al. Overexpression of mitogen-activated protein kinase kinase 4 and nuclear factor-kappaB in laryngeal squamous cell carcinoma: a potential indicator for poor prognosis. *Oncol Rep*. 2009;22:89–95.
- Robinson VL, Shalhav O, Otto K, et al. Mitogen-activated protein kinase kinase 4/c-Jun NH2-terminal kinase kinase 1 protein expression is subject to translational regulation in prostate cancer cell lines. *Mol Cancer Res*. 2008;6:501–508.
- Spillman MA, Lacy J, Murphy SK, et al. Regulation of the metastasis suppressor gene MKK4 in ovarian cancer. *Gynecol Oncol*. 2007;105:312–320.
- Schaeffer HJ, Weber MJ. Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol Cell Biol*. 1999;19:2435–2444.
- Wei Y, Wang L, Lan P, et al. The association between -1304T>G polymorphism in the promoter of MKK4 gene and the risk of sporadic colorectal cancer in southern Chinese population. *Int J Cancer*. 2009;125:1876–1883.
- Luo HL, Huang J, Lai B. Effects of MKK4 promoter polymorphism on susceptibility of colon cancer. *Guangdong Med J*. 2011;32:3208–3211.
- Liu B, Chen D, Yang L, et al. A functional variant (-1304T>G) in the MKK4 promoter contributes to a decreased risk of lung cancer by increasing the promoter activity. *Carcinogenesis*. 2010;31:1405–1411.
- Jiang L, Zhou P, Sun A, et al. Functional variant (-1304T>G) in the MKK4 promoter is associated with decreased risk of acute myeloid leukemia in a southern Chinese population. *Cancer Sci*. 2011;102:1462–1468.

15. Feng F, Zheng J, Jiang L, et al. No association between the functional polymorphism (-1304T>G) in the MKK4 gene and the risk of esophageal cancer. *Chin J Cancer Prev Treat.* 2012;19:88–91.
16. Hu M, Zheng J, Zhang L, et al. The association between -1304T>G polymorphism in the promoter of mitogen-activated protein kinase kinase 4 gene and the risk of cervical cancer in Chinese population. *DNA Cell Biol.* 2012;31:1167–1173.
17. Shao N, Wang Y, Lu K, et al. Role of the functional MKK4 promoter variant (-1304T>G) in a decreased risk of prostate cancer: case-control study and meta-analysis. *J Cancer Res Clin Oncol.* 2012;138:1531–1539.
18. Zheng J, Liu B, Zhang L, et al. The protective role of polymorphism MKK4-1304 T>G in nasopharyngeal carcinoma is modulated by Epstein-Barr virus' infection status. *Int J Cancer.* 2012;130:1981–1990.
19. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med.* 1997;127:820–826.
20. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med.* 2002;21:1539–1558.
21. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst.* 1959;22:719–748.
22. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7:177–188.
23. Egger M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315:629–634.
24. Chae KS, Ryu BK, Lee MG, et al. Expression and mutation analyses of MKK4, a candidate tumour suppressor gene encoded by chromosome 17p, in human gastric adenocarcinoma. *Eur J Cancer.* 2002;38:2048–2057.
25. Ganiatsas S, Kwee L, Fujiwara Y, et al. SEK1 deficiency reveals mitogen-activated protein kinase cascade crossregulation and leads to abnormal hepatogenesis. *Proc Natl Acad Sci U S A.* 1998;95:6881–6886.
26. Cunningham SC, et al. MKK4 status predicts survival after resection of gastric adenocarcinoma. *Arch Surg.* 2006;141:1095–1099discussion 1100.
27. Davies H, et al. Somatic mutations of the protein kinase gene family in human lung cancer. *Cancer Res.* 2005;65:7591–7595.
28. Parsons DW, et al. Colorectal cancer: mutations in a signalling pathway. *Nature.* 2005;436:792.
29. Su GH, Song JJ, Repasky EA, et al. Mutation rate of MAP2K4/MKK4 in breast carcinoma. *Hum Mutat.* 2002;19:81.
30. Shih CM, Lee YL, Chiou HL, et al. Association of TNF-alpha polymorphism with susceptibility to and severity of non-small cell lung cancer. *Lung Cancer.* 2006;52:15–20.