RESEARCH ARTICLE

# Association between *RASSF1A* Ala133Ser Polymorphism and Cancer Susceptibility: A Meta-Analysis Involving 8,892 Subjects

**Suleyman Bayram**

## Abstract

**Background: Published studies on the association between the *Ras Association Domain Family 1 isoform A* (*RASSF1A*) Ala133Ser polymorphism and cancer susceptibility have yielded conflicting results. Thus, a meta-analysis was here performed to assess the possible association. Materials and Methods: All eligible case-control studies published up to November 2013 on the association between *RASSF1A* Ala133Ser polymorphism and cancer susceptibility were identified by searching PubMed, Web of Science, Science Direct and hand search. Bothfixed-effect and random-effect models were used to calculate pooled odds ratios (ORs) with 95% confidence intervals (CIs) by using the Comprehensive Meta-Analysis software version 2.2. Results: A total of 10 studies including 4,572 cancer cases and 4,320 controls were included in the meta-analysis. Overall, significantly increased cancer risk was associated with the variant Ser133 when all studies were pooled (Ser *vs* Ala: OR=1.51, 95% CI=1.08-2.12, $P_{heterogeneity}$≤0.001; Ser/Ser+Ala/Ser *vs* Ala/Ala: OR=1.55, 95% CI=1.08-2.22, $P_{heterogeneity}$≤0.001). Moreover, in subgroup analyses by cancer types, a significant association between RASSF1A Ala133Ser polymorphism and lung cancer risk was found (Ser vs Ala: OR=2.27, 95% CI=1.29-4.02, Pheterogeneity=0.61; Ser/Ser+Ala/Ser *vs* Ala/Ala: OR=2.42, 95% CI=1.33-4.42, $P_{heterogeneity}$=0.75). In addition, in subgroup analyses by ethnicity, it was found that the RASSF1A Ala133Ser polymorphism was associated with overall cancer risk in Asians (Ser *vs* Ala: OR=1.37, 95% CI=1.06-1.77, $P_{heterogeneity}$=0.06) and Caucasians (Ser/Ser+Ala/Ser *vs* Ala/Ala: OR=2.21, 95% CI=1.01-4.82, $P_{heterogeneity}$≤0.001). Conclusions: This meta-analysis suggests, for the first time, that RASSF1A Ala133Ser polymorphism may contribute to cancer susceptibility, especially for lung cancer. Besides, additional well-designed studies with larger sample size focusing on different ethnicities and cancer types are needed to confirm these findings.**

**Keywords:** *RASSF1A* - *RASSF1A* Ala133Ser polymorphism - cancer susceptibility - meta-analysis

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## Introduction

The *Ras-Association Domain Family (RASSF)* gene family members are tumor suppressor proteins, activators of cell death, cell cycle modulators, microtubule stabilizers and possibly inflammatory mediators linked to Nuclear Factor kappa B (NFκB) (Gordon and Baksh, 2011). RASSF gene family comprises 10 members, termed *RASSF1* to *RASSF10*. There are seven different RASSF1 isoforms (*RASSF1A* to RASSF1G) that are generated by differential usage of two promoters and through alternative splicing (van der Weyden and Adams, 2007). Tumor suppressor *RASSF1A* gene has been reported to play a role in diverse activities including cell cycle regulation, apoptosis and regulating microtubules dynamics as well as maintenance of genomic instability, and thus may serve as a node in the integration of signaling pathways controlling a range of critical cellular functions (Donninger et al., 2007; van der Weyden and Adams, 2007; Richter et al., 2009; Gordon and Baksh, 2011). Promoter methylation may be asscoated with cancer development (Liu et al., 2013; Vo et al., 2013).

A guanine (G)/thymine (T) common single nucleotide polymorphism (SNP) at first position of codon 133 in exon 3 of *RASSF1A* (dbSNP ID: rs2073498), resulting in the substitution of an alanine (Ala) residue (GCT) by serine (Ser) residue (TCT) (c.397G>T, also designated *RASSF1A* Ala133Ser) in the ATM phosphorylation site, has been demonstrated to affect *RASSF1A* function (Shivakumar et al., 2002; El-Kalla et al., 2010). To date, a few molecular epidemiological studies have investigated the association between the *RASSF1A* Ala133Ser polymorphism and the cancer risk including breast cancer (Schagdarsurengin et al., 2005; Gao et al., 2008; Bergqvist et al., 2010; Donninger et al., 2011), lung cancer (Kanzaki et al., 2006; Xiao et al. 2012), colorectal cancer (Kanzaki et al., 2006), head and neck cancer (Kanzaki et al., 2006), esophageal cancer (Kanzaki et al., 2006; Zhou et al., 2013), renal cell carcinoma (Kawai et al., 2012), hepatocellular carcinoma (Bayram, 2012), gastric cancer (Zhou et al.,

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2013), prostate cancer (Meyer et al., 2013). However, no consistent conclusion has been drawn. The frequency of the Ser133 allele of *RASSF1A* Ala133Ser polymorphism varies in different geographic areas and ethnic populations. Besides, genetic effects of the *RASSF1A* Ala133Ser polymorphism have been shown to vary form one type of cancer to other. Even at the same cancer type, the results are conflicting (Schagdarsurengin et al., 2005; Gao et al., 2008; Bergqvist et al., 2010; Donninger et al., 2011). As a result, the statistical power of an individual study could be very limited for efficient assessment of the *RASSF1A* Ala133Ser polymorphism. For these reason, integration of these data sets may ensure improved statistical power to detect any significant effects. As is known, meta-analysis could improve the statistical power and draw reliable conclusion. To date, no meta-analysis has been conducted to investigate the association between Ala133Ser polymorphism of *RASSF1A* and cancer risk. Therefore, a meta-analysis based on a total of ten independent case-control studies was performed to identify whether there was any evidence of relationship between the *RASSF1A* Ala133Ser polymorphism and cancer susceptibility.

## Materials and Methods

*Study identification and selection*

Publication search: in this meta-analysis, a comprehensive literature research of the US National Library of Medicine's PubMed database, ISI Web of Knowledge, and Science Direct was conducted using the search terms including "*RASSF1A*" or "tumor suppressor gene Ras Association Domain Family 1 isoform A", "Ala133Ser" or "rs2073498", "polymorphism" or "SNPs", "cancer" or "carcinoma", "tumor" or "neoplasm" and the combined phrases in order to obtain all genetic studies on the relationship of *RASSF1A* Ala133Ser polymorphism and cancer. Last search was updated on November 23, 2013.

The search was focused on studies that had been conducted in humans. Furthermore, citations in the original studies or reviewed articles on this topic were manually examined to identify additional studies.

*Inclusion and exclusion criteria*

The following criteria were used to select studies for this meta-analysis (a) published in peer reviewed journals, (b) articles about *RASSF1A* Ala133Ser polymorphism and risk of cancers, (c) case-control studies comparing cancer cases with healthy or non-cancerous controls (d) articles containing useful allele and genotype frequency. The exclusion criteria were (a) studies with case only (without control population), (b) animal studies, (c) pure cell studies, (d) not concerned with cancer risk, (f) meta-analysis or reviews and (f) duplication of previous publication.

*Data extraction*

I reviewed and extracted information from all eligible studies independently, according to the inclusion and exclusion criteria listed above. The following characteristics were collected from each study: name of the first author, year of publication, country where the study was conducted, genotyping method for the assessment of *RASSF1A* Ala133Ser polymorphism, ethnicity, cancer types, source of controls, total number of case and controls with Ala/Ala, Ala/Ser and Ser/Ser genotypes of *RASSF1A* Ala133Ser polymorphism, and Hardy-Weinberg equilibrium (HWE). Different ethnicities were classified as Caucasian, Asian, and Mixed. All eligible studies were defined as hospital-based (HB) or population-based (PB) according to the source of controls. When study included subjects of more than one cancer types, genotype data was extracted separately for subgroup analysis.

*Statistical analysis*

Observed genotype frequencies for *RASSF1A* Ala133Ser polymorphism in controls were examined for deviations form Hardy-Weinberg equilibrium (HWE) using a goodness-of-fit $\chi^2$-test with one degree of freedom and a $p<0.05$ was considered with a significant selective bias. The strength of the association between *RASSF1A* Ala133Ser polymorphism and cancer susceptibility was assessed by using crude ORs with 95% CIs. The significance of the summary OR was determined with a Z test and $p<0.05$ was considered as statistically significant. In this meta-analysis, the following comparisons for *RASSF1A* Ala133Ser polymorphism were evaluated: allele model (Ser *vs* Ala), homozygous model (Ser/Ser *vs* Ala/Ala), heterozygous model (Ser/Ser *vs* Ala/Ser), dominant genetic model (Ser/Ser+Ala/Ser *vs* Ala/Ala) and recessive genetic model (Ser/Ser *vs* Ala/Ser+Ala/Ala). The statistical heterogeneity among each study were estimated by $\chi^2$-based Q-test, and the heterogeneity was considered significant when $p<0.05$. I also quantified the effect of heterogeneity using the $I^2$ test (Higgins and Thompson, 2002; Higgins et al., 2003) with the value >50% as a statistically significant heterogeneity. $I^2$ statistics was used to quantify inter study variability that can be attributed to heterogeneity rather than chance. It ranges between 0% and 100%, where a value of 0% indicates no observed heterogeneity and larger values indicates an increasing degree heterogeneity ($I^2$=0-25%, no heterogeneity; $I^2$=25-50%, moderate heterogeneity; $I^2$=50-75%, large heterogeneity; $I^2$=75-100%, extreme heterogeneity). A p value greater than 0.05 for the Q test indicates a lack of heterogeneity between studies; so the pooled OR estimate of each study was calculated by fixed-effects model (the Mantel-Haenszel method) (Mantel and Haenszel, 1959). Otherwise, the random-effects model (the DerSimonian-Laird method) was used (DerSimonian and Laird, 1986).

Subgroup analyses were also performed to investigate the effects of confounding factors: cancer types, ethnicities, genotyping methods, study design, and HWE. Sensitivity analysis was performed by sequential omission of each study to assess the stability of the results. Funnel plots, which is the main graphical method of assessing publication bias, were used to assess publication bias by Begg's test (Begg and Mazumdar, 1994) and Egger's test (Egger et al., 1997). An asymmetric plot suggested possible publication bias ($p>0.05$ suggested no bias).

All statistical analysis for the current meta-analysis was performed by comprehensive meta-analysis version 2.2

software (Biostat, Englewood, New Jersey) (Borenstein et al., 2007). All p values were two-sided. Statistical tests performed in the present analysis were considered significant whenever the corresponding null-hypothesis probability was $p<0.05$.

## Results

*Characteristics of eligible studies*

After careful retrieve and selection, 10 articles listed in Table 1 were identified according to inclusion and exclusion criteria. The study selection process is shown in Figure 1. Kanzaki's and Zhou's studies sorted the data into four and two types of cancers respectively. Each group in these studies was considered separately. Thus, a total of 14 case-control studies including 4572 cases and 4320 controls were analyzed in this meta-analysis (Schagdarsurengin et al., 2005; Kanzaki et al., 2006; Gao et. al, 2008; Xiao et al. 2009; Bergqvist et al., 2010; Donninger et al., 2011; Bayram, 2012; Kawai et al. 2012; Meyer et al., 2013; Zhou et al., 2013). The characteristics of selected studies are summarized in Table 1. Genotype and allele distributions of *RASSF1A* Ala133Ser polymorphism among cancer cases and controls and p value of HWE in controls were shown in Table 2. The sample size in these case-control studies varied considerably (range 56-1972). All studies were case-control studies, including four breast cancer studies (Schagdarsurengin et al., 2005; Gao et al., 2008; Bergqvist et al., 2010; Donninger et al., 2011), two lung cancer studies (Kanzaki et al., 2006; Xiao et al. 2009), two esophageal squamous cell carcinoma (ESCC) studies (Kanzaki et al., 2006; Zhou et al., 2013), and the others including colorectal cancer (Kanzaki et al., 2006), head and neck cancer (Kanzaki et al., 2006), renal cell carcinoma (Kawai et al. 2012), hepatocellular carcinoma (Bayram, 2012), gastric cancer

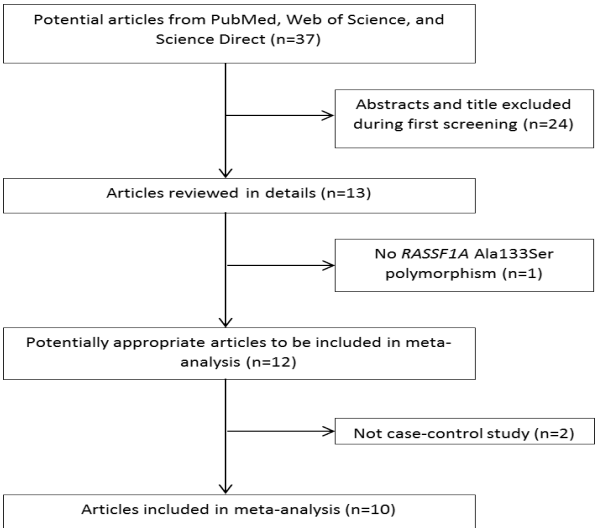


**Figure 1. Flow Diagram of Inclusion/Exclusion of the Individual Articles**

**Table 1. Main Characteristics of Included Studies in the Meta-Analysis**

| Author (year) | Cancer type | Country | Ethnicity | Genotyping | Source | Case N | Control N |
|---|---|---|---|---|---|---|---|
| Schagdarsurengin et al. (2005) | Breast | Germany | Caucasian | Sequencing | HB | 178 | 70 |
| Kanzaki et al. (2006)a | Lung | Japan | Asian | PCR-RFLP | PB | 101 | 110 |
| Kanzaki et al. (2006)b | Colorectal | Japan | Asian | PCR-RFLP | PB | 72 | 110 |
| Kanzaki et al. (2006)c | Head and neck | Japan | Asian | PCR-RFLP | PB | 63 | 110 |
| Kanzaki et al. (2006)d | Esophageal | Japan | Asian | PCR-RFLP | PB | 56 | 110 |
| Gao et al. (2008) | Breast | USA | Caucasian | TaqMan | PB | 653 | 190 |
| Xiao et al. (2009) | Lung | China | Asian | PCR-RFLP | HB | 100 | 100 |
| Bergqvist et al. (2010) | Breast | UK | Caucasian | TaqMan | PB | 209 | 331 |
| Donninger et al. (2011) | Breast | USA | Mixed | Sequencing | PB | 1972 | 1776 |
| Kawai et al. (2012) | Renal Cell | Japan | Asian | TaqMan | PB | 224 | 224 |
| Bayram (2012) | Hepatocellular | Turkey | Caucasian | PCR-RFLP | HB | 236 | 236 |
| Zhou et al. (2013)a | Esophageal | China | Asian | PCR-RFLP | HB | 112 | 235 |
| Zhou et al. (2013)b | Gastric | China | Asian | PCR-RFLP | HB | 116 | 235 |
| Meyer et al. (2013) | Prostate | Germany | Caucasian | TaqMan | HB | 480 | 483 |

*hospital-based; PB: population-based; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism

**Table 2. Distribution of the *RASSF1A* Ala133Ser Genotypes and Allele Frequencies, and p values of HWE**

| Author (year) | Distribution of *RASSF1A* Ala133Ser genotypes | | | | | | Distribution of *RASSF1A* Ala133Ser alleles | | | | HWE p value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Case (n) | | | Control (n) | | | Case (n) | | Control (n) | | |
| | Ala/Ala | Ala/Ser | Ser/ser | Ala/Ala | Ala/Ser | Ser/ser | Ala | Ser | Ala | Ser | |
| Schagdarsurengin et al. (2005) | 140 | 31 | 7 | 68 | 2 | 0 | 311 | 45 | 138 | 2 | 0.9 |
| Kanzaki et al. (2006)a | 81 | 19 | 1 | 99 | 10 | 1 | 181 | 21 | 208 | 12 | 0.21 |
| Kanzaki et al. (2006)b | 67 | 5 | 0 | 99 | 10 | 1 | 139 | 5 | 208 | 12 | 0.21 |
| Kanzaki et al. (2006)c | 56 | 7 | 0 | 99 | 10 | 1 | 119 | 7 | 208 | 12 | 0.21 |
| Kanzaki et al. (2006)d | 48 | 7 | 1 | 99 | 10 | 1 | 103 | 9 | 208 | 12 | 0.21 |
| Gao et al. (2008) | 504 | 138 | 11 | 162 | 27 | 1 | 1146 | 160 | 351 | 29 | 0.91 |
| Xiao et al. (2009) | 83 | 16 | 1 | 93 | 7 | 0 | 182 | 18 | 193 | 7 | 0.72 |
| Bergqvist et al. (2010) | 161 | 48 | 0 | 270 | 60 | 1 | 370 | 48 | 600 | 62 | 0.22 |
| Donninger et al. (2011) | 1665 | 287 | 20 | 1491 | 264 | 21 | 3617 | 327 | 3246 | 306 | 0.02 |
| Kawai et al. (2012) | 204 | 17 | 3 | 194 | 29 | 1 | 425 | 23 | 417 | 31 | 0.94 |
| Bayram (2012) | 100 | 118 | 18 | 189 | 45 | 2 | 318 | 154 | 423 | 49 | 0.7 |
| Zhou et al. (2013)a | 99 | 11 | 2 | 211 | 23 | 1 | 209 | 15 | 445 | 25 | 0.66 |
| Zhou et al. (2013)b | 94 | 18 | 4 | 211 | 23 | 1 | 206 | 26 | 445 | 25 | 0.66 |
| Meyer et al. (2013) | 388 | 90 | 2 | 377 | 99 | 7 | 866 | 94 | 853 | 113 | 0.86 |

*HWE: Hardy-Weinberg equilibrium

**Table 3. Results of Meta-Analysis for RASSF1A Ala133Ser Polymorphism and Cancer Risk**

| Study groups | | N | Cases/Controls | Allele (Ser *vs*. Ala) | | Homozygous (Ser/Ser *vs* . Ala/Ala) | | Heterozygous (Ser/Ser *vs* . Ala/Ser) | | Dominant (Ser/Ser+Ala/Ser *vs* . Ala/Ala) | | Recessive (Ser/Ser *vs* . Ala/Ser+Ala/Ala) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | OR (95% CI) | $p^a$ | OR (95% CI) | $p^a$ | OR (95% CI) | $p^a$ | OR (95% CI) | $p^a$ | OR (95% CI) | $p^a$ |
| Total | | 14 | 4572/4320 | 1.51 (1.08-2.12)* | <0.001 | 2.03 (0.94-4.40) | 0.03 | 1.13 (0.72-1.79)[c] | 0.62 | 1.55 (1.08-2.22)* | <0.001 | 1.36 (0.87-2.12)[c] | 0.1 |
| HWE[b] | | 13 | 2600/2544 | 1.59 (1.08-2.35)* | <0.001 | 2.55 (1.35-4.85)*[c] | 0.09 | 1.50 (0.77-2.89)[c] | 0.65 | 1.63 (1.07-2.49)* | <0.001 | 2.24 (1.18-4.25)*[c] | 0.23 |
| Cancer type | Breast | 4 | 3012/2367 | 1.49 (0.92-2.41) | 0.001 | 1.02 (0.58-1.80)[c] | 0.3 | 0.93 (0.52-1.67)[c] | 0.82 | 1.51 (0.92-2.46) | 0.002 | 1.01 (0.57-1.78)[c] | 0.36 |
| | Esophageal | 2 | 168/345 | 1.36 (0.80-2.31)[c] | 0.76 | 3.13 (0.50-19.4)[c] | 0.7 | 2.66 (0.4-17.88)[c] | 0.59 | 1.27 (0.71-2.25)[c] | 0.67 | 3.07 (0.50-19.03)[c] | 0.69 |
| | Lung | 2 | 201/210 | 2.27 (1.29-4.02)*[c] | 0.61 | 1.89 (0.23-15.49)[c] | 0.64 | 0.79 (0.09-6.95)[c] | 0.67 | 2.42 (1.33-4.42)*[c] | 0.75 | 1.69 (0.21-13.86)[c] | 0.64 |
| Etnicity | Asian | 8 | 844/1234 | 1.37 (1.06-1.77)*[c] | 0.06 | 2.49 (0.97-6.41)[c] | 0.83 | 2.01 (0.75-5.34)[c] | 0.81 | 1.31 (0.99-1.73)[c] | 0.06 | 2.42 (0.94-6.22)[c] | 0.84 |
| | Caucasian | 5 | 1756/1310 | 2.04 (1.00-4.17) | <0.001 | 2.42 (0.39-15.01) | 0.004 | 1.17 (0.48-2.89)[c] | 0.26 | 2.21 (1.01-4.82)* | <0.001 | 2.02 (0.41-9.95) | 0.02 |
| Study design | HB | 6 | 1222/1359 | 2.31 (1.09-4.88)* | <0.001 | 3.86 (0.83-17.98)[c] | 0.009 | 1.64 (0.70-2.84)* | 0.25 | 2.37 (1.04-5.36)* | <0.001 | 3.24 (0.82-12.72) | 0.03 |
| | PB | 8 | 3350/2961 | 1.06 (0.93-1.21)[c] | 0.07 | 1.01 (0.60-1.71)[c] | 0.86 | 0.98 (0.57-1.68)[c] | 0.85 | 1.07 (0.93-1.23)[c] | 0.05 | 1.00 (0.59-1.69)[c] | 0.88 |
| Genotyping | PCR-RFLP | 8 | 856/1246 | 1.82 (1.15-2.88)* | 0.001 | 4.61 (1.97-10.78)*[c] | 0.33 | 2.11 (0.88-5.07)[c] | 0.83 | 1.87 (1.10-3.20)* | <0.001 | 3.71 (1.59-8.68)*[c] | 0.55 |
| | TaqMan | 4 | 1566/1228 | 1.07 (0.74-1.55) | 0.02 | 0.93 (0.26-4.20)[c] | 0.18 | 0.93 (0.32-2.67)[c] | 0.2 | 1.07 (0.71-1.59) | 0.02 | 0.92 (0.33-2.60)[c] | 0.19 |
| | Sequencing | 2 | 2150/1846 | 2.76 (0.28-27.19) | 0.001 | not calculated | -- | 0.89 (0.48-1.65)[c] | 0.85 | 2.65 (0.29-23.9) | 0.002 | 0.93 (0.51-1.71)[c] | 0.19 |

*OR with statistical significance; [a]p value of $X^2$-based Q test for heterogeneity; [b]Conforming to Hardy-Weinberg equilibrium in controls; [c]OR estimates for fixed effects model; N: number of studies included; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PB: population-based; HB: hospital-based

(Zhou et al., 2013), prostate cancer (Meyer et al., 2013). 8 studies conducted in the Asian population, 5 in Caucasian population, and 1 in mixed population. Population-based controls were carried out in 8 studies, while hospital-based controls were carried in 6 studies (Table 1). The genotyping methods contained the classic polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan, and DNA sequencing (Table 1). The genotype frequencies of *RASSF1A* Ala133Ser polymorphism in the control subjects were in Hardy-Weinberg equilibrium except the one study (Schagdarsurengin et al., 2005).

*Quantitative synthesis*

The evaluations of the association of *RASSF1A* Ala133Ser polymorphism with cancer risk are shown Table 3 and Figure 2-6. Overall, the Ser133 allele was associated with a significantly increased cancer risk compared with the Ala133 allele (OR=1.51, 95% CI=1.08-2.12, Z=2.41, p=0.02) (Figure 2). Moreover, significantly increased risk was observed in dominant (Ser/Ser+Ala/Ser *vs* Ala/Ala) genetic model (OR=1.55, 95% CI=1.08-2.22, Z=2.36, p=0.02) (Figure 5). Homozygous (Ser/Ser *vs* Ala/Ala) genetic model showed a borderline association of *RASSF1A* Ala133Ser polymorphism with cancer risk (OR=2.03, 95% CI=0.94-4.40, Z=2.36, p=0.07) (Figure 3). No significant association were found in heterozygous (Ser/Ser *vs* Ala/Ser) and recessive (Ser/Ser *vs* Ala/Ser+Ala/Ala) genetic models respectively (OR=1.13, 95% CI=0.72-1.79, Z=0.54, p=0.59; OR=1.36, 95% CI=0.72-2.12, Z=1.35, p=0.18) (Figure 4 and 6).

Considering the influence of disequilibrium, i performed subgroup analysis by HWE. After excluding one study that is not conforming to HWE, significant associations were observed for four genetic models (allele, homozygous, dominant and recessive) (Table 3). When i performed subgroup analyses by cancer types, increased cancer risk was found in the allele and dominant genetic model comparisons for lung cancer. However, no significant association were found in breast and esophageal cancer types in any of the comparison models tested (Table 3). In the subgroup analysis by ethnicity, studies were




**Figure 2. Forest Plot of ORs with a Random Effect Model for Association *RASSF1A* Ala133Ser Polymorphism and Overall Cancer Risk Under Allele Model (Ser *vs* Ala)**

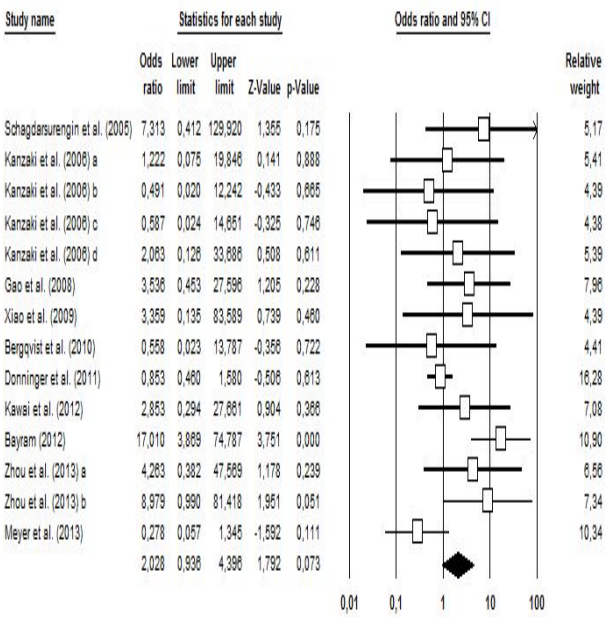


**Figure 3. Forest Plot of ORs with a Random Effect Model for Association *RASSF1A* Ala133Ser Polymorphism and Overall Cancer Risk Under Homozygous Model (Ser/Ser *vs* Ala/Ala)**

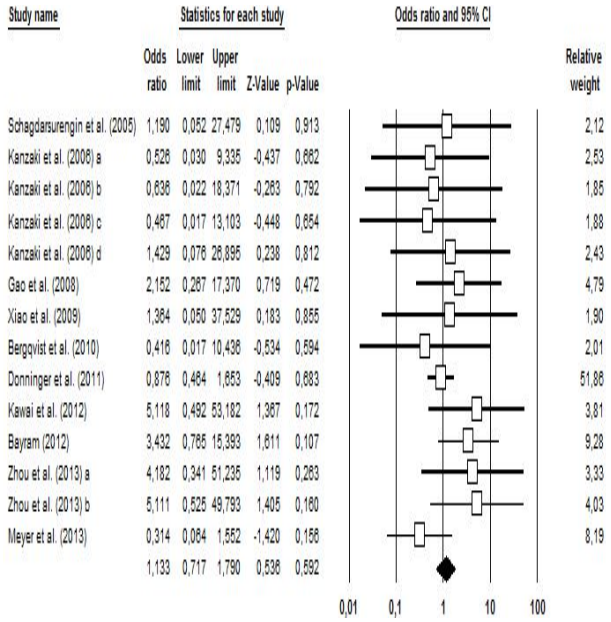


**Figure 4. Forest Plot of ORs with a Fixed Effect Model for Association *RASSF1A* Ala133Ser Polymorphism and Overall Cancer Risk Under Heterozygous Model (Ser/Ser *vs* Ala/Ser)**

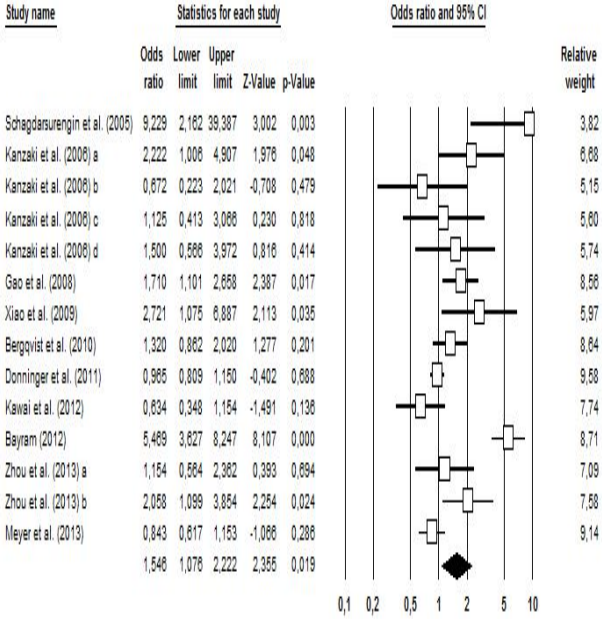


**Figure 5. Forest Plot of ORs with a Random Effect Model for Association *RASSF1A* Ala133Ser polymorphism and Overall Cancer Risk Under Dominant Model (Ser/Ser+Ala/Ser *vs* Ala/Ala)**

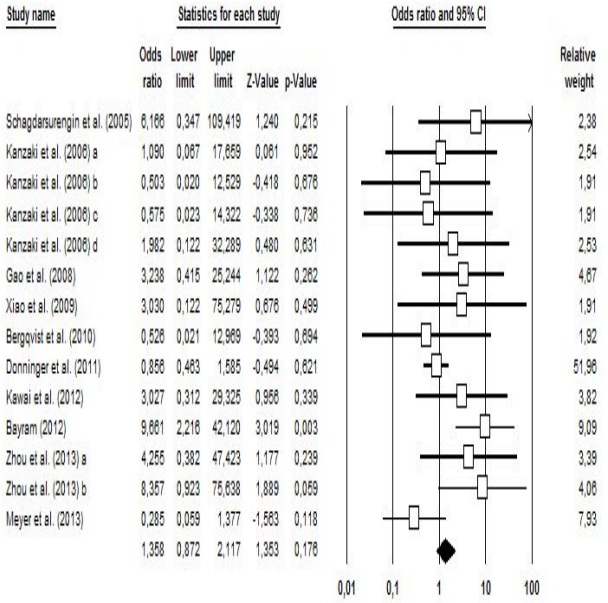


**Figure 6. Forest Plot of ORs with a Fixed Effect Model for Association *RASSF1A* Ala133Ser Polymorphism and Overall Cancer Risk Under Recessive Model (Ser/Ser *vs* Ala/Ser+Ala/Ala)**

categorized into two groups: Asians and Caucasians. In Asian population, significant association between the *RASSF1A* Ala133Ser polymorphism and the increased risk for cancer was observed in the allele comparison (Table 3). For the subgroup of Caucasian population, i found significant association in the dominant genetic model (Table 3).There was no significant association found in other genetic models. According to the source of controls, significant effects were observed in hospital-based studies (under allele, heterozygous and dominant models); while in population-based studies, significant association was not observed in any comparison (Table 3). When stratified separately by genotyping, i found that allele, homozygous, dominant and recessive genetic models increased cancer risk in the PCR-RFLP group (Table 3).

*Test of heterogeneity*

The heterogeneity was reckoned between each of the studies using the $\chi^2$-based Q-test. Significant heterogeneity existed in three genetic models (Ser *vs*

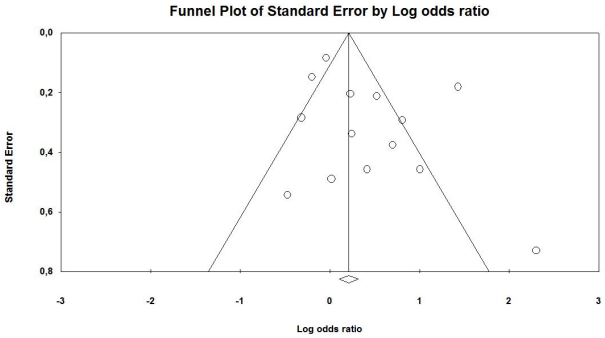


**Figure 7. Funnel Plot Analysis to Detect Publication Bias for the Allele (Ser *vs* Ala) Model**

Ala, Ser/Ser *vs* Ala/Ala, Ser/Ser+Ala/Ser *vs* Ala/Ala) of the *RASSF1A* Ala133Ser polymorphism (Table 3). However, stratification based on the cancer type reduced the heterogeneity in the breast (Ser/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.30), esophageal (Ser *vs* Ala: $P_{heterogeneity}$=0.76; Ser/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.70; Ser/Ser+Ala/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.67) and lung (Ser *vs* Ala: $P_{heterogeneity}$=0.61; Ser/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.64; Ser/Ser+Ala/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.75). When patients were stratified based on ethnicity, heterogeneity disappeared in the Asian (Ser *vs* Ala: $P_{heterogeneity}$=0.06; Ser/Ser *vs* Ala/Ala: Pheterogeneity=0.83; Ser/Ser+Ala/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.06). In stratified analyses by source of controls, i did not find heterogeneity in population-based subgroup (Ser *vs* Ala: $P_{heterogeneity}$=0.07; Ser/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.86; Ser/Ser+Ala/Ser *vs* Ala/Ala: $P_{heterogeneity}$=0.05)

*Sensitivity analysis*

Sensitivity analysis was conducted to evaluate the stability of the meta-analysis. Sensitivity analysis was carried out after sequential removal of each eligible study. When i investigated the *RASSF1A* Ala133Ser polymorphism and cancer susceptibility, the results suggested that the significance of the pooled ORs was not influenced by any single study in four genetic models (allele, homozygous, heterozygous, and dominant). Hence, results of the sensitivity analysis suggested that the data of four genetic models (allele, homozygous, heterozygous, and dominant) in this meta-analysis are relatively stable and credible. However, sensitivity analysis show that P value of Z test for statistical significance of summary OR (Ser/Ser *vs* Ala/Ser+Ala/Ala) is 0.01 when excluding one study conducted by Donninger et al., 2011.

*Publication bias*

Begg's funnel plot and Egger's test were performed to assess the publication bias. The shape of funnel plots did not reveal any evidence of obvious asymmetry in the overall meta-analysis. Then, the Egger's test was used to provide to statistical evidence of funnel plot symmetry. No publication bias was detected for *RASSF1A* Ala133Ser polymorphism (allele model: p=0.32 for Begg's test, p=0.17 for Egger's test; homozygous model: p=0.44 for Begg's test, P=0.24 for Egger's test; heterozygous model: p=0.32 for Begg's test, p=0.43 for Egger's test; dominant model: p=0.32 for Begg's test, p=0.18 for Egger's test; recessive model: p=0.44 for Begg's test, p=0.22 for Egger's test) (Figure 7).

## Discussion

Cancer has become one of the major public health problems in the world. However, cancer is a multifactorial disease and the precise etiology is still not exactly understood. SNP is the most common form of human genetic variation, and may contribute to susceptibility to cancer. It is therefore proper to investigate gene polymorphisms involved in human cancers (Akkız et al., 2011; Bayram et al., 2011). Many molecular epidemiological studies have been performed to evaluate the association between *RASSF1A* Ala133Ser polymorphism and cancer risk. However, the results were generally inconsistent (Schagdarsurengin et al., 2005; Kanzaki et al., 2006; Gao et. al, 2008; Xiao et al. 2009; Bergqvist et al., 2010; Donninger et al., 2011; Bayram, 2012; Kawai et al. 2012; Meyer et al., 2013; Zhou et al., 2013). These inconsistent results are possibly due to a small effect of the *RASSF1A* Ala133Ser polymorphism on cancer risk or the relatively low statistical power of the published studies. So, this meta-analysis was needed to show a quantitative approach for combining the different results. Meta-analysis is a statistic method with great statistical power and has been widely performed to epidemiological research, especially for evaluating genetic polymorphisms in cancer susceptibility. It is superior to single study potentially via augmenting sample size, improving statistical power, and subsequently drawing a more reliable conclusion (Qin et al., 2014).

To the best of my knowledge, this is the first meta-analysis of the association between the *RASSF1A* Ala133Ser polymorphism and cancer susceptibility. The present meta-analysis is based on 14 data sets extracted from 10 case-control studies including 4572 cases and 4320 controls. In the current meta-analysis, the *RASSF1A* Ala133Ser polymorphism was associated with a significantly increased cancer risk in allele comparison and dominant genetic model. In addition to these, borderline significant association between this polymorphism and cancer risk was observed in the homozygous genetic model. The distribution of genotypes in the control group was consistent with HWE in all studies except for one (Schagdarsurengin et al., 2005). When excluding this study, statistically significant associations were detected in allele, homozygous, dominant and recessive genetic models. In the subgroup analysis by cancer type, no statistically significant association was found except for allele and dominant model comparison of lung cancer. However, no evidence of association was observed in cancers of the breast or esophageal cancer. The discrepant results may be explained by the concept that different types of cancer may have different mechanisms of carcinogenesis. The discrepancy could also be interpreted partially by the influence of gene-environment interaction in multistep process of carcinogenesis. Another reason may be the limited sample size. Furthermore, in the subgroup analysis by ethnicity, significant association between the *RASSF1A* Ala133Ser polymorphism and cancer risk was observed

in both Asians (in allele comparison) and Caucasians (in dominant genetic model). In my meta-analysis, i also observed inconsistent results between hospital-based studies and population-based studies. Stratified analysis by the study design indicated that studies recruiting controls form hospital population are more included to acquire significant results in allele comparison, heterozygous and dominant genetic models. Different cancer risks were also found in the studies using different genotyping methods. I discovered that the association was significant among studies utilizing PCR-RFLP assay, but not for studies with TaqMan and sequencing genotyping assays. Because TaqMan and sequencing genotyping assays are more precise than the PCR-RFLP assay, and a limited number of studies were included in the TaqMan and sequencing genotyping assays, this results might reflect selection bias, and should be interpreted with caution.

Attention must be paid to the relatively large heterogeneity in my results. However, when stratified by cancer type, the subgroups of esophageal cancer and lung cancer failed to exhibit heterogeneity, suggesting that different cancer type might be a potential source of heterogeneity. Similarly, after stratifying by ethnicity, heterogeneity was absent in Asian population, suggesting that ethnicity could partly explain the heterogeneity. As these, when stratified by study design, my results showed that the heterogeneity was significantly reduced in subgroup of hospital-based study design. Therefore, it may be presumed that the heterogeneity exists mainly owing to differences of cancer types, ethnicity and study design.

A study concerning the function of *RASSF1A* Ala133Ser polymorphism found that Ser allele of *RASSF1A* Ala133Ser polymorphism cannot induce cell cycle arrest by blocking cyclin D1 accumulation (Shivakumar et al., 2002). Recently, El-Kalla et al. (2010) showed that *RASSF1A* Ala133Ser polymorphism also lost the ability to associate with α- and γ-tubulin and lost the ability to prevent tumor formation in a xenograft nude mouse model when compared with wild type *RASSF1A*. These results are consistent with my meta-analysis that *RASSF1A* Ala133Ser polymorphism is associated with risk of cancer.

Some limitations of this meta-analysis should be considered in interpreting the results. First, the number of some published studies was not sufficiently large, and some studies of small size may not have enough statistical power to explore the real association. Additionally, in the some subgroup analyses, the number of cases and controls was relatively small, where there was not enough statistical power to explore the true association. Second, my results were based on unadjusted estimates. In order to provide a more precise estimation on the basis of adjustment for confounders, well-designed studies are warranted by taking potential confounders such as smoking status, drinking status and environmental factors into account. Third, interactions of gene-gene or SNP-SNP or the possibility of linkage disequilibrium between polymorphisms or gene-environment that might have influence on gene-disease association were failed to address due to lack of relevant data. Fourth, because only published and English articles were included in the meta-analysis, publication and potential English language biases might have occurred, even though it was not determined by the use of statistical tests.

In conclusion, my meta-analysis suggested that *RASSF1A* Ala133Ser polymorphism is associated with an increased cancer risk. Further stratification by cancer type, study design and genotyping method also identified a significant association of this polymorphism with cancer risk, especially in lung cancer, hospital-based study design and the PCR-RFLP genotyping method groups. In the future, large-scale case-control studies are necessary to validate the risk and to investigate the potential gene-gene, and gene-environment interactions between *RASSF1A* Ala133Ser polymorphism and cancer susceptibility.

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