

# Genetically lowered concentrations of circulating sRAGE might cause an increased risk of cancer: Meta-analysis using Mendelian randomization

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## Abstract

**Objectives:** To undertake a systematic meta-analysis of all variants in the gene encoding receptor for advanced glycation end products (*RAGE*) to summarize their associations with cancer risk and changes in the levels of circulating soluble *RAGE* (s*RAGE*), with the aim of determining possible causality between circulating s*RAGE* and cancer risk.

**Methods:** Articles written in English were retrieved from MEDLINE<sup>®</sup> and EMBASE<sup>®</sup> databases. Two researchers independently identified eligible articles and extracted the data (analysed using STATA<sup>®</sup> software version 12.0).

**Results:** Fifteen articles qualified for inclusion in the meta-analysis of the *RAGE*–cancer association and three examined the *RAGE*–s*RAGE* relationship. The 82Ser/82Ser genotype was significantly associated with overall cancer risk compared with the 82Gly/Gly genotype (odds ratio 1.75, 95% confidence interval [CI] 1.46, 2.10). Carriers of the 82Ser/82Ser genotype had significantly reduced circulating s*RAGE* concentrations compared with the 82Gly/82Gly genotype. Mendelian randomization analysis demonstrated that a reduction of 100, 200 and 300 pg/ml in circulating s*RAGE* concentrations was associated with a 1.11-fold (95% CI 1.06, 1.25), 1.24-fold (95% CI 1.11, 1.57) and 1.38-fold (95% CI 1.18, 1.96) increased risk of developing cancer, respectively.

**Conclusions:** Genetically lowered concentrations of circulating s*RAGE* might cause an increased risk of cancer.

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## Keywords

Receptor for advanced glycation end products (RAGE), cancer, Mendelian randomization, meta-analysis

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## Introduction

The receptor for advanced glycation end products (RAGE) belongs to the immunoglobulin superfamily, and as a multiligand cell-surface receptor it can amplify immune and inflammatory responses.<sup>1</sup> It is widely recognized that the interactions between RAGE and its ligands can trigger the activation of key cell-signalling pathways, elicit oxidative stress generation and evoke proliferative, angiogenic and inflammatory reactions: these are the important steps in the development and progression of various types of cancer.<sup>1,2</sup> Evidence implicates *RAGE* genetic alterations and the soluble form of RAGE (sRAGE) in the pathogenesis of many malignancies.<sup>3,4</sup> For example, a recent meta-analysis of observational studies reported an inverse association between circulating sRAGE and overall cancer risk.<sup>3</sup> However, in observational studies, the association between a modifiable phenotype and a disease is sometimes confounded and vulnerable to reverse causation.<sup>5,6</sup> To allow an unbiased estimate, a new concept of horizontal randomized controlled trial, Mendelian randomization, has been introduced with wide applications. Mendelian randomization is an epidemiological methodology, which with the aid of genetic variation in genes of known function, aims to estimate a causal relationship between a modifiable risk factor and an outcome of interest.<sup>7</sup> This concept derives from the random assortment of genes from parents to offspring at conception, which is analogous to the random allocation of a treatment in a randomized controlled trial;<sup>8,9</sup> and it has been widely adopted to infer causality from observational data due to the

irrelevance of genetic variation to confounders.<sup>10,11</sup> To our knowledge, an exploration of causal relevance between circulating sRAGE and cancer has not been published. Therefore, this systematic meta-analysis of all published variants in the *RAGE* gene was undertaken, to summarize their associations with cancer risk and circulating sRAGE changes. To identify variants with simultaneous significant associations, the study employed Mendelian randomization to infer the causal relationship between circulating sRAGE and cancer risk.

## Materials and methods

### Article identification

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards. Only articles written in English and performed in human subjects were searched from the MEDLINE<sup>®</sup> and EMBASE<sup>®</sup> databases as of 27 January 2015, using the combination of the following keywords: 'receptor for advanced glycation end product' or 'receptor for advanced glycation endproduct' or 'RAGE' or 'AGER' in the 'Abstract' with 'cancer' or 'carcinoma' or 'neoplasia' or 'adenoma', 'neoplasm' or 'myeloma' or 'melanoma' or 'lymphoma' or 'leukaemia' or 'leiomyoma' in the 'Title'. In addition, a manual search of the bibliographies of reviews and major original investigations was conducted to track potentially missing citations. The search process was completed in duplicate by two authors (Q.H. and F.L.). Retrieved articles were managed and integrated with all duplicates being removed.

### Assessment of article eligibility

The eligibility of each retrieved article was independently appraised by two authors (Q.H. and F.L.) from the Title and Abstract only, to ascertain whether an association between *RAGE* genetic alterations or plasma/serum sRAGE and cancer risk was addressed. Any doubt over article eligibility was settled by resorting to the full text or an intragroup discussion with 100% agreement reached.

### Selection criteria

To further identify articles that qualified for inclusion, predefined standards were set, including both inclusion and exclusion criteria. Inclusion criteria were: (i) case-control studies; (ii) evaluation of *RAGE* genetic variants with either cancer risk or circulating sRAGE changes; (iii) adequate published data for estimating odds ratio (OR) or weighted mean difference (WMD) and its 95% confidence interval (95% CI). Exclusion criteria were: (i) meeting abstracts without information of interest; (ii) case reports; (iii) editorials or reviews including meta-analyses; (iv) articles published in languages other than English.

### Data extraction

From each article that qualified for inclusion, two authors (Q.H. and F.L.) were independently responsible for data extraction according to a uniform protocol developed by all contributing authors. The following data were extracted from eligible studies: surname of first author, year of publication, race, cancer type, study design, source of controls, matched status, sample size, age, sex, body mass index (BMI), smoking status and alcohol consumption status. Data were collected using an Excel spreadsheet, then compared using SAS<sup>®</sup> Proc Compare (SAS Institute, Inc., Cary,

NC, USA). Any disagreement was justified by consensus between the two authors.

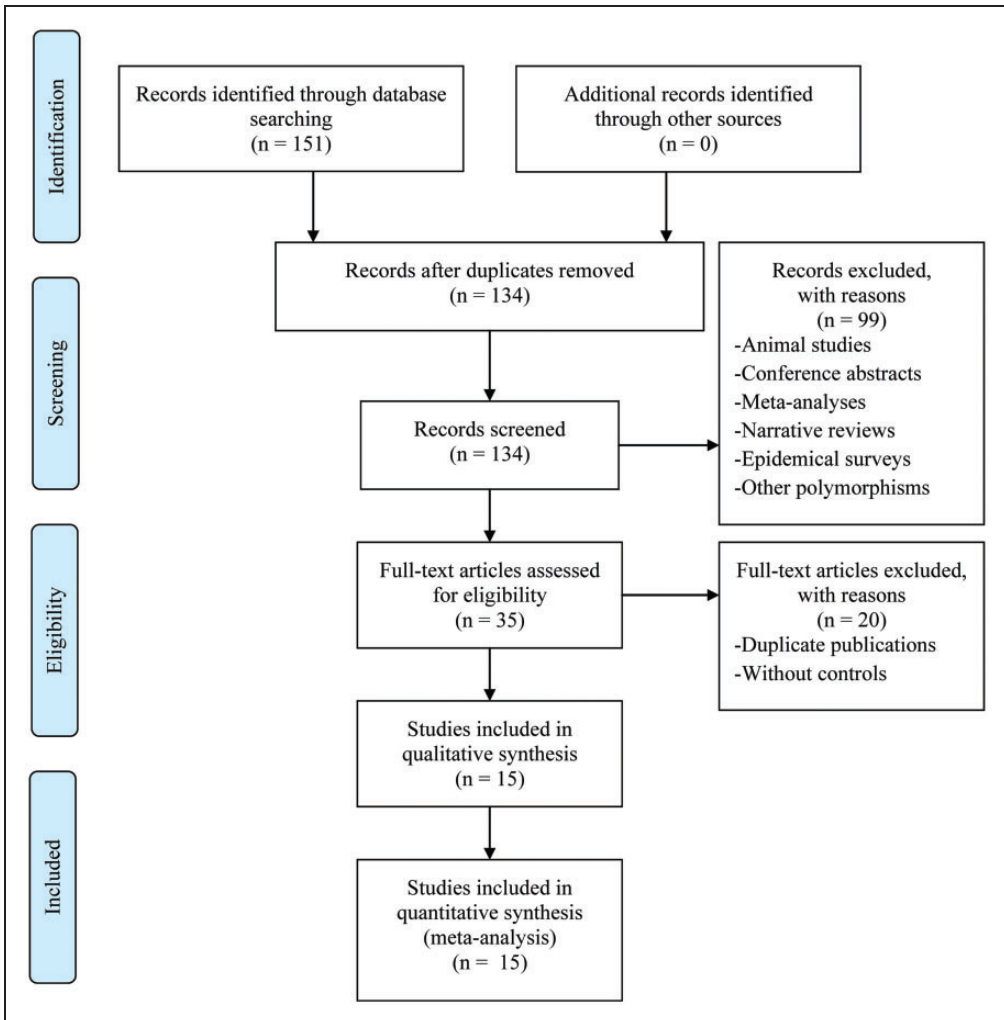
### Statistical analyses

The DerSimonian and Laird method was used to pool individual effect estimates together under a random-effects model.<sup>12</sup> The degree of heterogeneity was determined using the  $I^2$ -test, a statistic that estimates the percentage of variance in a meta-analysis that is attributable to between-study heterogeneity.<sup>13</sup> An  $I^2$ -value >50% was indicative of significant heterogeneity.<sup>14</sup> Subgroup analysis and meta-regression analysis were carried out to account for the potential sources of heterogeneity. Begg's funnel plot and the filled funnel plot, as well as Egger's test at a significance level of 10%, were used to assess bias stemming from selective publication. All statistical analyses were undertaken using STATA<sup>®</sup> software version 12.0 (StataCorp, College Station, TX, USA).

In the Mendelian randomization analysis, to quantify the potential causal relationship between circulating sRAGE and cancer, the variant in the *RAGE* gene that was simultaneously associated with cancer risk and circulating sRAGE changes was adopted as an instrument. This assumes that a mutant genotype (GG) increases cancer risk in comparison with its wild genotype (gg) as quantified by  $OR_{GG \text{ vs. } gg}$ , and that genotype GG causes a mean difference,  $\Delta P$ , in circulating sRAGE relative to genotype gg. After assuming linearity of the relationship between sRAGE variation and log OR for cancer,  $OR_{GG \text{ vs. } gg}^{1/\Delta P}$  is an unconfounded estimate of the OR of cancer resulting from a unit change in circulating sRAGE.<sup>15,16</sup>

### Results

The PRISMA flow diagram for the selection process of potentially eligible articles is presented in Figure 1. After assessing for



**Figure 1.** PRISMA flow diagram of the study selection process in a meta-analysis undertaken to determine the relationship between circulating soluble receptor for advanced glycation end products (sRAGE) and cancer risk.

eligibility and qualification, 15 studies that examined the association between *RAGE* genetic variants and cancer risk were identified,<sup>17–31</sup> totalling 4346 cancer patients and 4777 controls in the final analysis. Among them, three articles provided data on the relationship between *RAGE* genetic variants and circulating sRAGE changes.<sup>18,25,27</sup> In total, six variants were covered: rs1800625 (T–429C) ( $n = 11$ ),<sup>18,19,21–26,28,29,31</sup> rs1800

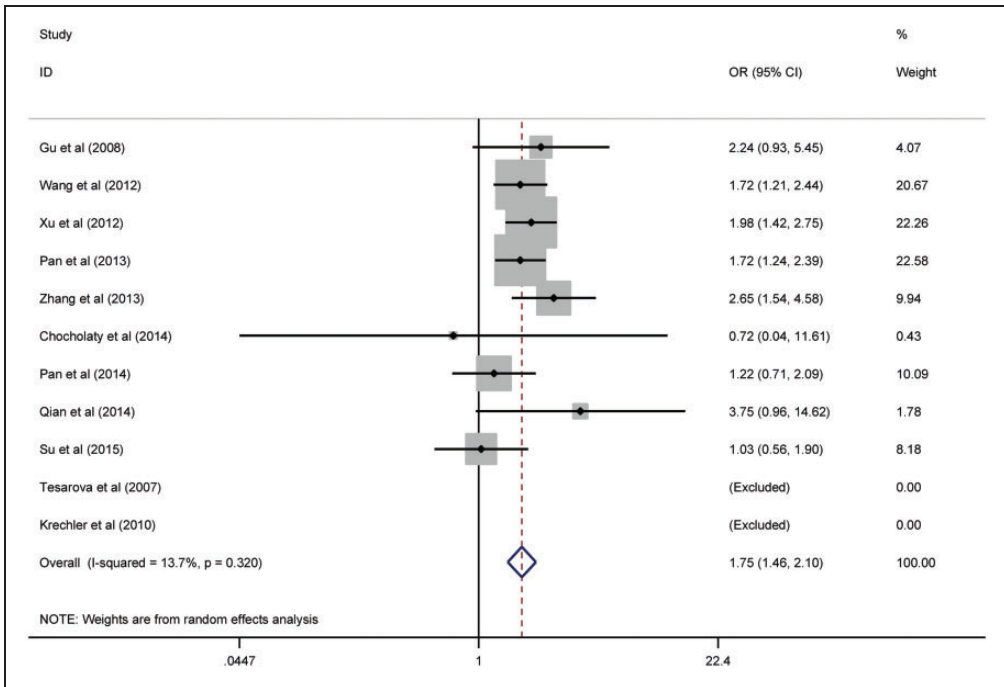
624 (T–374A) ( $n = 10$ ),<sup>17,18,22–26,28,29,31</sup> rs2070600 (Gly82Ser) ( $n = 11$ ),<sup>18,20,22,24–31</sup> rs184003 (G1704T) ( $n = 3$ ),<sup>25,29,31</sup> A2184G ( $n = 3$ ),<sup>18,22,28</sup> and 63 base pair (bp) insertion/deletion (I/D) ( $n = 2$ ).<sup>23,31</sup>

Extracted data are presented in Table 1.<sup>17–31</sup> Eight and seven studies involved study participants with Asian and Caucasian backgrounds, respectively. There were three studies on breast cancer, three on lung

**Table 1.** Baseline characteristics of eligible studies included in a meta-analysis undertaken to determine the relationship between circulating soluble receptor for advanced glycation end products (sRAGE) and cancer risk.<sup>17–31</sup>

Author, year	Race	Cancer	Design	Source	Matched	Sample size		Age, years		Male, %		BMI, kg/m <sup>2</sup>		Smoking, %		Drinking, %	
						Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Su, 2015 <sup>31</sup>	Asian	Oral	RS	HB	No	618	592	54.3	51.0	96.4	81.9	NR	NR	85.4	37.8	57.9	37.2
Qian, 2014 <sup>30</sup>	Asian	Colorectal	RS	HB	NR	90	72	58.5	NR	60	NR	NR	NR	NR	NR	NR	NR
Pan, 2014 <sup>29</sup>	Asian	Breast	RS	HB	Yes	509	504	55.6	56.3	0.0	0.0	23.6	23.5	NR	NR	NR	NR
Chocholaty, 2015 <sup>28</sup>	White	Renal	PS	PB	No	214	154	63	57	61.7	37.0	NR	NR	NR	NR	NR	NR
Zhang, 2013 <sup>27</sup>	Asian	Ovarian	RS	HB	Yes	190	210	53.6	53.5	0.0	0.0	NR	NR	82.1	72.9	NR	NR
Pan, 2013 <sup>26</sup>	Asian	Lung	RS	HB	Yes	819	803	57.4	57.0	64.8	64.8	NR	NR	36.3	8.0	16.9	8.1
Xu, 2012 <sup>25</sup>	Asian	Cervical	RS	HB	Yes	488	715	54.6	54.5	0	0	NR	NR	54.9	47.3	28.1	29.5
Wang, 2012 <sup>24</sup>	Asian	Lung	RS	PB	Yes	562	764	NR	NR	58.0	57.6	NR	NR	45.6	38.0	NR	NR
Hashemi, 2012 <sup>23</sup>	White	Breast	RS	HB	Yes	71	93	45.3	43.3	0	0	NR	NR	NR	NR	NR	NR
Krechler, 2010 <sup>22</sup>	White	Pancreas	RS	HB	NR	51	154	64.0	57.0	60.8	37.0	24.9	25.7	NR	NR	NR	NR
Kadar, 2008 <sup>21</sup>	White	Myeloma	RS	HB	Yes	94	141	68.0	68.9	29.8	57.5	NR	NR	NR	NR	NR	NR
Gu, 2008 <sup>20</sup>	Asian	Gastric	RS	HB	Yes	283	283	59.0	58.0	74.9	74.9	NR	NR	23.3	21.2	NR	NR
Toth, 2007 <sup>19</sup>	White	Colorectal	RS	HB	No	183	141	65.7	68.4	54.6	43.3	NR	NR	NR	NR	NR	NR
Tesarova, 2007 <sup>18</sup>	White	Breast	RS	HB	No	120	92	61.2	56.2	0	0	26.9	25.6	NR	NR	NR	NR
Schenk, 2001 <sup>17</sup>	White	Lung	RS	HB	No	54	59	62.7	69.6	79.6	32.2	NR	NR	NR	NR	NR	NR

RS, retrospective design; PS, prospective design; HB, hospital-based controls; PB, population-based controls; BMI, body mass index; NR, not reported.



**Figure 2.** Forest plot for the rs2070600 variant of the receptor for advanced glycation end products (*RAGE*) gene for cancer risk under the homozygous genotypic model.<sup>18,20,22,24–31</sup> OR, odds ratio; 95% CI, 95% confidence interval.

cancer, two on colorectal cancer, and one study each on cervical cancer, gastric cancer, myeloma, oral cancer, ovarian cancer, pancreatic cancer and renal cancer. Fourteen studies were designed retrospectively; 13 studies enrolled hospital-based controls. Cancer cases and controls were reported to be matched in eight studies, and not matched in five studies. No significance differences were noted between the two groups in the mean distributions of age and BMI, and the proportions of males, smokers and alcohol drinkers.

Overall comparisons of the six examined genetic variants with cancer were nonsignificant except for rs2070600, with the 82Ser allele corresponding to a 27% (OR 1.27; 95% CI 1.12, 1.44;  $P < 0.001$ ), 75% (OR 1.75; 95% CI 1.46, 2.10;  $P < 0.001$ ) (Figure 2) and 40% (OR 1.40; 95% CI 1.14,

1.73;  $P = 0.001$ ) increased risk under allelic, homozygous genotypic and dominant models, respectively. There was no evidence of heterogeneity for the homozygous genotypic model ( $I^2 = 13.7\%$ ) (Table 2).<sup>18,20,22,24–31</sup> For all variants, there were low probabilities of publication bias, as reflected by the Begg's funnel plots and filled funnel plots. Both visual funnel plots for variant rs2070600 (Egger's test:  $P = 0.735$ ) are provided in Figure 3.

As only the rs2070600 genetic variant was significantly associated with cancer risk, subgroup analyses were conducted for this variant under the best homozygous genotypic model (Table 3). Results were summarized if two or more studies were available for each subgroup. Carriers of the 82Ser/82Ser allele had an increased risk, compared with those patients who

**Table 2.** Overall comparisons of the six examined variants in the receptor for advanced glycation end products (*RAGE*) gene for cancer risk, under three genetic models using data from the 15 studies included in a meta-analysis undertaken to determine the relationship between circulating soluble *RAGE* and cancer risk.

Variants	Studies, <i>n</i>	Genetic models	OR	95% CI	Statistical significance <sup>a</sup>	<i>I</i> <sup>2</sup> , %
rs1800625	11	Allelic model	0.98	0.82, 1.18	NS	77.0
		Homozygous genotypic model	1.06	0.70, 1.63	NS	67.6
		Dominant model	0.93	0.76, 1.14	NS	65.2
rs1800624	10	Allelic model	1.03	0.91, 1.18	NS	59.3
		Homozygous genotypic model	1.18	0.90, 1.53	NS	42.5
		Dominant model	1.04	0.87, 1.25	NS	60.7
rs2070600	11	Allelic model	1.27	1.12, 1.44	<i>P</i> < 0.001	58.1
		Homozygous genotypic model	1.75	1.46, 2.10	<i>P</i> < 0.001	13.7
		Dominant model	1.40	1.14, 1.73	<i>P</i> = 0.001	70.5
rs184003	3	Allelic model	1.15	0.92, 1.43	NS	77.0
		Homozygous genotypic model	1.30	0.82, 2.04	NS	67.2
		Dominant model	1.17	0.84, 1.63	NS	80.3
A2184G	3	Allelic model	1.11	0.85, 1.46	NS	0.0
		Homozygous genotypic model	1.07	0.48, 2.37	NS	0.0
		Dominant model	1.15	0.84, 1.57	NS	0.0
63 bp I/D	2	Allelic model	1.19	0.79, 1.79	NS	0.0
		Dominant model	1.12	0.73, 1.71	NS	0.0

OR, odds ratio; 95% CI, 95% confidence interval; *I*<sup>2</sup>, inconsistency index; bp, base pair; I/D, insertion/deletion; NS, no significant between-group difference (*P* ≥ 0.05).

<sup>a</sup>Statistical significance determined by logistic regression analysis.

were homozygous for the 82Gly/82Gly genotype in Asians (OR 1.76; 95% CI 1.45, 2.13; *P* < 0.001), in lung cancer (OR 1.72; 95% CI 1.35, 2.18; *P* < 0.001) and in control-matched studies (OR 1.82; 95% CI 1.53, 2.15; *P* < 0.001), but not in unmatched studies. On stratification by total sample size, effect estimates were stronger in small studies (total sample size < 500; OR 2.67; 95% CI 1.62, 4.29; *P* < 0.001) than in large studies (total sample size ≥ 500; OR 1.70; 95% CI 1.40, 1.99; *P* < 0.001).

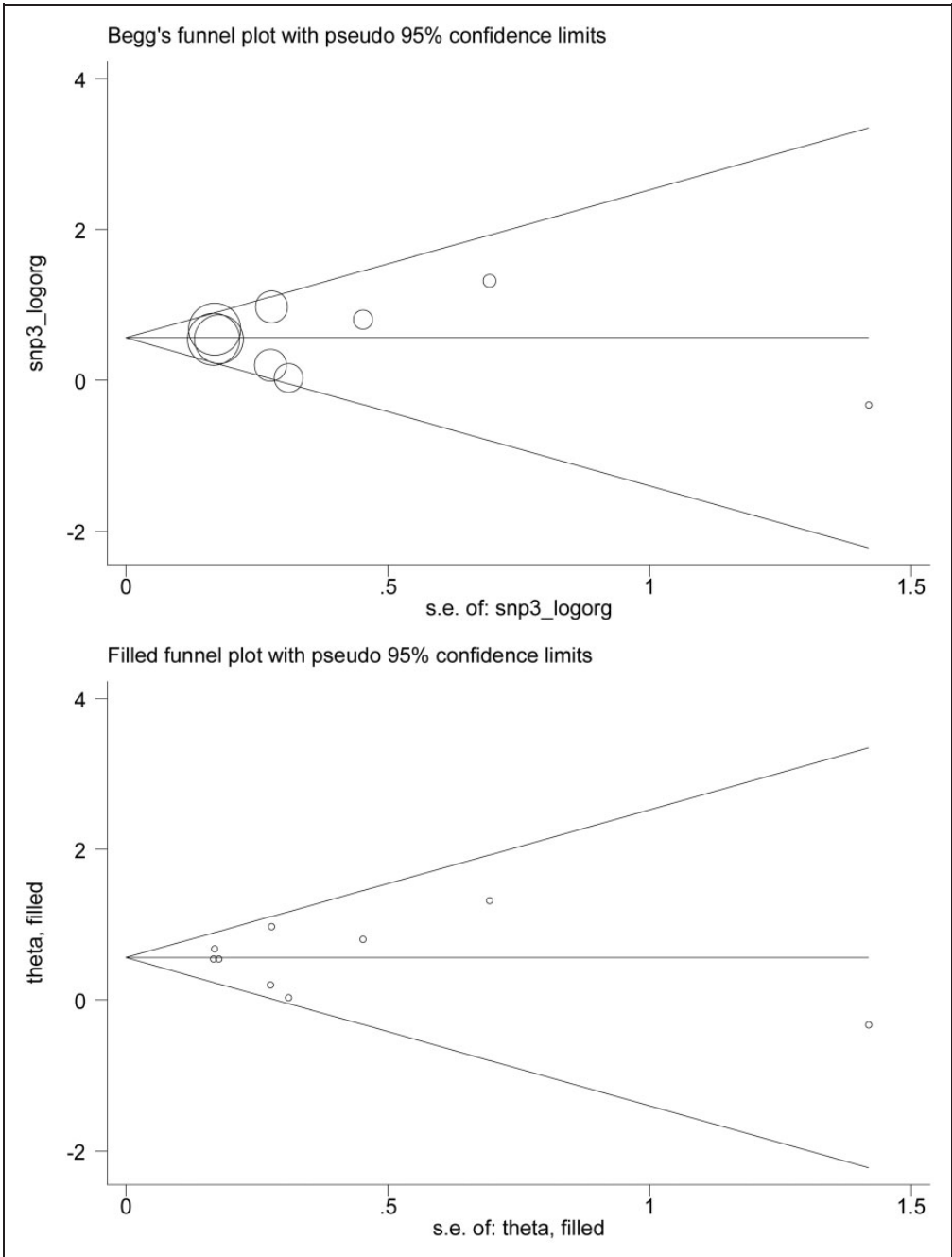
In view of the significant association between rs2070600 (Gly82Ser) and cancer risk, changes in circulating sRAGE between the Gly82Ser genotypes were summarized (Figure 4).<sup>18,25,27</sup> Carriers of 82Ser/82Ser and 82Gly/82Ser genotypes had significantly reduced circulating sRAGE concentrations than those carrying the 82Gly/82Gly

genotype by −515.75 pg/ml (95% CI −701.24, −330.26; *P* < 0.001) and −177.43 pg/ml (95% CI −311.54, −43.32; *P* = 0.010), respectively.

Under the homozygous genotypic model, a reduction of 100, 200 and 300 pg/ml in circulating sRAGE concentrations was associated with a 1.11-fold (estimated 95% CI 1.06, 1.25), 1.24-fold (estimated 95% CI 1.11, 1.57) and 1.38-fold (estimated 95% CI 1.18, 1.96) increased risk of developing cancer, respectively.

To detect whether other continuous variables including age, sex, BMI, smoking and drinking alcohol affected the association between *RAGE* genetic variants and cancer risk, these variables were modelled in a meta-regression analysis. It was of interest to note that differences in age (*P* = 0.038) and smoking (*P* = 0.025) were potentially





**Figure 3.** Begg's funnel and filled funnel plots for the rs2070600 variant of the receptor for advanced glycation end products (RAGE) gene for cancer risk under the homozygous genotypic model.<sup>20,24-31</sup> Only nine of the 11 studies are shown on the two funnel plots because two of the studies had the homozygous genotype of rs2070600 being 0,<sup>18,22</sup> thus under the homozygous genotypic model, the two studies were automatically removed from the Begg's and filled funnel plots.



**Table 3.** Subgroup analyses of the rs2070600 variant in the receptor for advanced glycation end products (*RAGE*) gene for cancer risk under the homozygous genotypic model.

Subgroups	Studies, n	OR	95% CI	Statistical significance <sup>a</sup>	I <sup>2</sup> , %
Ethnicity					
Asian	8	1.76	1.45, 2.13	P < 0.001	21.1
Cancer type					
Lung cancer	2	1.72	1.35, 2.18	P < 0.001	0.0
Source of controls					
Population-based	2	1.70	1.20, 2.40	P = 0.003	0.0
Hospital-based	7	1.77	1.39, 2.25	P < 0.001	32.2
Sample size					
Small studies	3	2.67	1.62, 4.39	P < 0.001	0.0
Large studies	6	1.70	1.40, 1.99	P < 0.001	3.3
Matched controls					
No	2	1.02	0.56, 1.84	NS	0.0
Yes	6	1.82	1.53, 2.15	P < 0.001	0.0

OR, odds ratio; 95% CI, 95% confidence interval; I<sup>2</sup>, inconsistency index; NS, no significant difference (P ≥ 0.05).

<sup>a</sup>Statistical significance determined by logistic regression analysis.

confounding factors for rs2070600. There was no detectable significance for the other confounders or the other genetic variants.

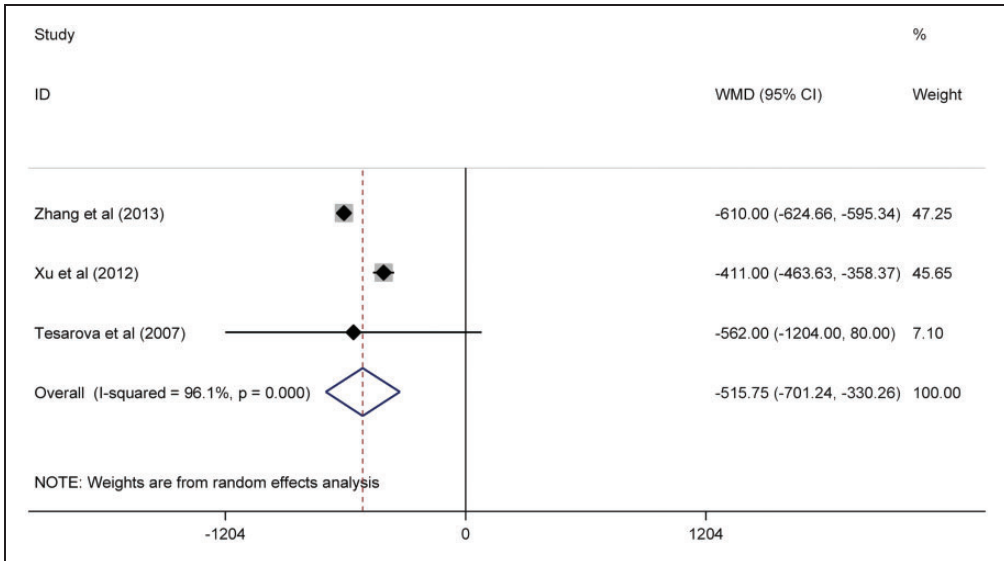
## Discussion

To the best of our knowledge, no study has reported on the causal relationship between circulating sRAGE and cancer risk. The validity of these current results was strengthened by the implementation of the Mendelian randomization concept in observational studies, which in this study relied on a nonsynonymous variant rs2070600 (Gly82Ser) that was simultaneously associated with cancer risk and circulating sRAGE changes as a surrogate marker to infer potential causality. The most noteworthy finding of this meta-analysis was that genetically lowered concentrations of

circulating sRAGE might cause an increased risk of cancer.

Circulating sRAGE is attracting the attention of scientists because of its ability to promote carcinogenesis and tumour cell growth.<sup>32,33</sup> Several observational studies, as well as a recent comprehensive meta-analysis,<sup>3</sup> have found that carriers of lower concentrations of circulating sRAGE were at an elevated risk of breast cancer,<sup>34</sup> lung cancer<sup>35</sup> and liver cancer.<sup>36</sup> In addition, there is evidence that circulating sRAGE is largely under genetic control.<sup>25,27</sup> It would be tempting to hypothesize that circulating sRAGE might be useful as a predictive marker for cancer occurrence. To test this hypothesis, this present meta-analysis investigated all possible variants in the *RAGE* gene; the most significant variant was then selected as an instrumental marker to assess the unbiased and unconfounded impact of long-term differences in circulating sRAGE on cancer risk, in a Mendelian randomization analysis.

In this present meta-analysis, a nonsynonymous variant rs2070600 in the third exon of the *RAGE* gene was observed to exhibit robust associations with cancer risk and circulating sRAGE changes simultaneously, leading to a marked causal estimate between lowered circulating sRAGE concentrations and increased cancer risk. The selection of the *RAGE* gene rs2070600 variant as an instrument in the Mendelian randomization analysis is biologically plausible as this variant itself appeared to be functional by altering the coding sequence of the *RAGE* gene, resulting in a change in amino acid (82Gly→82Ser). This change was reported to trigger proinflammatory induction and enhance molecular mechanisms underlying inflammatory diseases.<sup>37</sup> In addition, functional studies indicated that this variant can influence the degree by which RAGE is cleaved by certain proteases, with the 82Ser allele of this variant being associated with diminished



**Figure 4.** Forest plot for the rs2070600 variant of the receptor for advanced glycation end products (*RAGE*) gene for circulating serum RAGE changes under the homozygous genotypic model. WMD, weighted mean difference; 95% CI, 95% confidence interval<sup>18,25,27</sup>.

proteolysis;<sup>38</sup> this might explain why carriers of this allele had significantly lower concentrations of circulating sRAGE in the current study. The findings of this meta-analysis suggest that the genetic alteration of the rs2070600 variant is responsible for the circulating changes of sRAGE concentrations, further leading to altered susceptibility to cancer. Given the limited sample sizes at individual cancer sites, the possibility of tumour heterogeneity cannot be excluded. Different tumour cells can show distinct morphological and phenotypic profiles, therefore understanding tumour heterogeneity may be the next big quest in cancer research.

Generally, inferring causality from observational epidemiology is problematic; it is always difficult to determine which of the two associated variables is the cause and which is the effect.<sup>39</sup> Although Mendelian randomization provides an alternative method of addressing the problems of observational studies, a note of caution should be

sounded when interpreting these current findings, since the present meta-analysis was based on summary estimates of each retrieved article rather than individual participant data.<sup>40</sup> As such, it is impossible to rule out the pleiotropic effect of variant rs2070600 in this meta-analysis. Such effect may occur via mechanisms that have not been investigated in all retrieved studies. However, the utilization of a nonsynonymous variant rs2070600 at *RAGE* gene locus - robustly associated with circulating sRAGE changes - means the presented association is very unlikely to be attributable to a pleiotropic effect of this variant on another pathway.

These current findings must be interpreted in the light of several potential limitations. This meta-analysis was limited by the coverage of eligible articles in English language journals only, which might have led to selection bias. However, this bias is unlikely to affect the validity of the findings, as indicated by the Egger's test.

Another limitation centred on the repeatability of circulating sRAGE concentrations, which were measured only once, making it impossible to reflect on the effect of long-term levels in the development of cancer. A third limitation was that, as mentioned above, this meta-analysis was not based on individual participant data, which restricted further gene-to-environment interactions. A fourth limitation was that although the overall and subgroup analyses did not detect significant heterogeneity, this cannot be ruled out, given the limited number of studies involved. A fifth limitation was that the meta-analysis only tested the potential causal relationship between circulating sRAGE and all types of cancer as a whole, rather than for each individual cancer, as this meta-analysis examined six variants in the *RAGE* gene based on 15 eligible studies. This might limit the interpretation and extrapolation of these current findings.

In conclusion, these current findings extend prior work demonstrating that genetically lowered concentrations of circulating sRAGE might cause an increased risk of cancer. These current findings also suggest that circulating sRAGE might be useful as a predictive biomarker for the onset and progression of malignancies, and aid the development of personalized therapy and follow-up care in daily clinical practice.

### Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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