Lysyl oxidase G473A (rs1800449) polymorphism influences the risk of cancer progression: a meta-analysis

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Abstract

The genetics of cancer progression is important for the design of optimal therapeutic strategies. Lysyl oxidase (LOX) is a cancer progression gene that has been studied enough to warrant a synthesis of its primary data from association studies. However, reported associations between the LOX G473A (rs1800449) gene polymorphism and cancer have been inconsistent, prompting a meta-analysis so that we could obtain more precise estimates. Database searches of the published literature yielded seven case-control studies. We calculated pooled odds ratios (ORs) and 95% confidence intervals (CIs) using four genetic models: homozygous (H), recessive (R), dominant (D) and codominant (C). Subgroup analysis was based on ethnicity and cancer type. Outlier analysis was used to examine sources of heterogeneity. The strength of evidence was based on the magnitude of effects, high associative significance, consistency and homogeneity. H/R outcomes exerted more associative effects than D/C results. Two reasons for this are as follows: (i) H/R analyses precluded outlier treatment; and (ii) the magnitude of H/R (ORs of > 2.0) was twice that of D/C (ORs > 1.0). All else being equal, significant pooled ORs in all genetic models had high significance (Pa < 10-5). Strong evidence for associations was found in the outcomes for Asian and gastrointestinal (GI) cancers. In summary, the LOX rs1800449 polymorphism confers significant overall susceptibility, particularly in GI cancers, and places Asians at risk.

Background

The multifactorial etiology of cancer metastasis involves the lysyl oxidase (LOX) protein, the role of which points to its participation in extracellular processes leading to increased extracellular matrix (ECM) deposition and subsequent tissue stiffness, which drives malignant progression [1]. Clinical studies have reported increased expression of ECM components (tenascins and collagens) in cancer patients [2-4]. Furthermore, increased expression of LOX has been found to significantly correlate with increased metastasis and reduced patient survival in a number of clinical cancer studies [1, 5]. Thus, the role of LOX in the metastatic milieu of various cancers [6-9] renders this protein a useful clinical target [10]. However, clinical differences in mRNA and protein expression attributed to tumor type and tissue specificity warrant caution in the interpretation of associations [10]. The *LOX* gene has seven exons that encode several functional domains of the LOX protein [11]. LOX undergoes a series of transformations with size changes expressed in kilo Daltons (kDa) from a preproenzyme (46 kDa) to a proenzyme (50 kDa) to a propeptide (18 kDa) and ends up as a functional protein (32 kDa) in the ECM [12]. The *LOX* gene has an important single-nucleotide polymorphism (SNP) located at exon 1 of chromosome 5q23.1–q23 (rs1800449). At this location, the open reading frame at position 473 contains the guanine (G)-adenine (A) bases [13]. A shift from 473G to 473A changes the amino acid arginine (Arg) at residue 158 to glutamine (Gln) (Arg158Gln) in the LOX propeptide [11]. Since it was discovered [11], *LOX* rs1800449 has been extensively studied for its relationship with carcinogenesis [14-18]. At the gene level, single-study reports of *LOX* rs1800449 associations with cancer have not been consistent. It is thus opportune to statistically synthesize the findings of these studies using meta-analysis. Here, we examine the role of the *LOX* rs1800449 SNP in the risk of cancer progression, which might guide potential future directions in cancer genetics. To obtain less ambiguous, clearer estimates of the role of SNPs in this investigation, we assessed the strength of evidence using statistical and meta-analytical criteria. This study aims to highlight the genetic role of *LOX* rs1800449 in cancer progression and to provide important information that could be useful in clinical decision making.

Methods

Selection of studies

We searched MEDLINE using PubMed, Google Scholar and Science Direct for association studies as of August 17, 2019. The terms used were "*Lysyl oxidase*", "*LOX*", "*polymorphism*" and "*cancer*" as medical subject headings and text. References cited in the retrieved articles were also screened manually to identify additional eligible studies. Inclusion criteria were (i) case-control studies evaluating the association between *LOX* polymorphisms and cancer risk and (ii) sufficient genotype frequency data presented to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). The exclusion criteria were as follows: (i) reviews; (ii) studies whose control frequencies deviated from the Hardy-Weinberg equilibrium (HWE); (iii) articles that were not case-control studies; and (iv) studies with unusable genotype data.

Data extraction

Two investigators (RM and NP) independently extracted data and arrived at consensus. The following information was obtained from each publication: first author's name, the year of publication, the country of origin, ethnicity, cancer type, study design, studies that matched their controls with cases and the criteria used, sample sizes and genotype frequencies.

Methodological quality of the studies

We used the Clark-Baudouin (CB) scale to evaluate the methodological quality of the included studies [19]. The CB scale is based mainly on statistical (*P*-values, statistical power, and corrections for multiplicity) and genetic (genotyping methods and the HWE) criteria. In this scale, low, moderate and high quality have CB scores of < 5, 5-6 and ³ 7, respectively.

Data distribution and power calculations

Data distribution was assessed with the Shapiro-Wilks test using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Gaussian (normal) distribution ($P > 0.05$) warranted the use of the mean ± standard deviation (SD). Otherwise, the median (with interquartile range) was used. Using the G*Power program [20], we evaluated statistical power as its adequacy bolsters the level of associative evidence. Assuming an OR of 1.5 at a genotypic risk level of $\alpha = 0.05$ (two-sided), power was considered adequate at ≥ 80%.

HWE

HWE was assessed with the application in https://ihg.gsf.de/cgi-bin/hw/hwa1.pl. A *P*-value of < 0.05 indicated deviation from the HWE. Deviations were found in six studies [14-18, 21] and were thus excluded from the analysis (Table S1). Table S2 includes a column that details the nonsignificance ($P > 0.05$) of the HWE-compliant studies.

Data synthesis

Cancer risks (ORs and 95% CIs) were estimated for each study using the following genetic models: (i) homozygous [H], (ii) recessive [R], (iii) dominant [D], and (iv) codominant [C]. Comparing the effects on the same baseline, we calculated pooled ORs and 95% CIs. In addition to the overall analysis, we examined two subgroups: Asians (3,834 cases/4,061 controls) and cancer type. The latter was stratified into gastrointestinal (GI) (1,453 cases/1,546 controls) and breast (935 cases/923 controls) cancer. The strength of evidence was assessed with four indicators: First, the magnitude of effects are higher or lower when the pooled ORs are farther from or closer to the OR value of 1.0 (null effect), respectively [22]. Second, associative *P*-values (P^a) with more zero decimals (e.g., 0.00001) are considered to be stronger than those closer to 0.05. Third, statistical significance found across the comparisons

and genetic models indicates the consistency of effects. Fourth, homogeneity is preferred to heterogeneity, but heterogeneity is unavoidable [23]. This is because conclusions in the milieu of homogeneity (or at least non-heterogeneity) have greater evidential strength than those that are heterogeneously derived. Nevertheless, the presence of heterogeneity between studies was estimated with the c^2-based Q test [24] at a threshold of significance set at $P^b < 0.10$. Heterogeneity was quantified with the I^2 statistic, which measures variability between studies [25]. I^2 values of > 50% indicate more variability than those £ 50%, with 0% indicating zero heterogeneity (homogeneity). Similarities in population features of the studies warranted using the fixed-effects (Fe) model [26]; otherwise, the random-effects (Re) model [27] was used. Sensitivity analysis, which involves the omission of one study at a time and the recalculation of the pooled OR, was used to test the robustness of the summary effects. Publication bias was not assessed because none of the comparisons had ≥ 10 studies. Less than this number presents low sensitivity of the publication bias tests [28]. Except for heterogeneity estimation [24], two-sided *P*-values of £ 0.05 were considered significant. All associative outcomes were Bonferroni corrected. Data for the meta-analysis were analyzed using Review Manager 5.3 (Cochrane Collaboration, Oxford, England), SIGMASTAT 2.03, and SIGMAPLOT 11.0 (Systat Software, San Jose, CA).

Results

Characteristics of the included studies

Figure 1 outlines the selection process in a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)-sanctioned flowchart [29]. A total of 107 citations were identified from the initial search, the screening of which yielded 20 full-text articles. Of the 20, 13 were excluded for not conforming to our inclusion criteria. A manual search of the references from the publications yielded no additional articles. Table 1 lists the seven articles included in this meta-analysis [30-36]. One article [35] covered two cancer types (lung and colorectal), which were treated as two studies. Subjects were all Asians except in two publications [30, 32]. Three articles focused on breast cancer [30, 32, 33]. Four articles [31, 34-36] examined GI cancers (oral, gastric and colorectal). The normally distributed (Shapiro-Wilks: $P = 0.139$) CB scores showed a mean ± SD of 6.00 ± 1.16, indicating that the methodological quality of the component studies was moderate. Table S2 shows the quantitative traits of the eight included studies. Sample sizes ranged from 299 to 1,273. One study [31] was statistically adequate (94.4%). The PRISMA checklist provides a detailed description of this meta-analysis (Table S3).

Overall and subgroup analysis

Tables 2 and 3 present 15 significant ($P^a < 0.05$) outcomes, 14 (93.3%) of which had P^a-values that reached $< 10^{-5}$, indicating both consistency and strong effects. Table 2 shows all the Fe results with three important features: (i) initial Fe precluded outlier treatment; (ii) H/R outcomes (ORs 2.3-2.5) had twice the magnitude of effect over the D/C results (ORs 1.4-1.6) and (iii) a strong case of *LOX* rs1800449 associations with cancer based on the core outcomes. Judged on the basis of high significance ($P^a < 10^{-5}$) and homogeneity ($I^2 = 0\%$), we identified four core outcomes (é), which were ranked based on the number of studies (n), with elevated n indicating a higher rank. Thus, the Asian outcome (n = 6) in the C model was ranked first (OR 1.53, 95% CI 1.38-1.70, $P^a < 10^{-5}$). This core finding (Table 2) is the same as the overall outcome seen in the PSO panel for the C model (Table 3), with all Asian subjects. Table 2 shows the subordinate-in-rank results with a collective outcome that identified significant associations in GI cancers (R/D/C: ORs 1.45-2.34, 95% CIs 1.24-3.15, $P^a < 10^{-5}$). The P^a-values in these outcomes

showed that in Bayesian Factor terms [37], this indicates strong evidence of association, underpinned by study compatibility. Of note, sensitivity treatment rendered robustness to all the highly significant outcomes (Table 4).

Impact and mechanism of outlier treatment

The impact of outlier treatment is best seen in Table 3, which is bisected into left and right panels, indicating PRO and PSO, respectively. A salient feature of this table is that all the PRO outcomes were Re derived (analysis model column), concomitant with I^2 values of 65%-94%. On the other hand, all PSO results were Fe derived (I^2 = 0%-38%). The mechanism of outlier treatment for the C model in the overall analysis is visualized in Figs 2-4. Fig. 2 shows the PRO forest plot, where the pooled outcome indicated significantly increased risk (OR 1.60, 95% CI 1.27-2.02, $P^a < 10^{-4}$) and heterogeneity ($P^b < 10^{-5}$, I^2 = 81%). The Galbraith plot identified two studies [30, 32] as outliers located above and below the +2 and -2 confidence limits (Fig. 3). In Fig. 4, the PSO outcome (outliers omitted) shows eliminated heterogeneity (P^b = 0.43, I^2 = 0%), modulated increased risk (OR 1.53, 95% CI 1.38-1.70) and retained significance ($P^a < 10^{-5}$). This operation is numerically summarized in Table 3.

Discussion

Summary of findings

The main findings of this study presented layers of evidence pointing to strong associations between *LOX* rs1800449 and a risk of cancer progression. These layers included a consistency of effects (the significant ORs were > 1.0 across all comparisons) and high associative significance (93.3% of the comparisons had $P^a < 10^{-5}$). This level of significance enabled the outcomes to survive the Bonferroni correction, attesting to their stability (an added layer) and minimizing the risk of type 1 error (false positives). The outcomes that met the criteria for strong evidence ($P^a < 10^{-5}$ and I^2 = 0%) were found in the C model (Asian) and the R/D/C models (GI cancers), which comprised our core findings. These Fe-derived core outcomes were initially homogeneous without the necessity of outlier treatment. One purpose of biomedical meta-analysis is to subject the overall summary effects to meta-analytical procedures (outlier and sensitivity treatments, subgrouping) to test for their stability, consistency and robustness (another added layer). These features have, for the most part, characterized our findings. Furthermore, ethnicity and subgrouping by cancer type did not materially alter the overall results, both in terms of the direction of association and statistical significance. The lack of material differences (all the significant outcomes indicated increased risks) across comparisons suggests the consistency of our results. Our GI cancer findings, in particular, were noteworthy for their high significance and homogeneity in most genetic models. These significant findings underpin the importance of subgrouping in the evaluation of the risk of disease polymorphisms. Primary study findings about the roles of genetic variants in cancer are not only conflicting but also difficult to replicate. These differences may be due to limited statistical power, heterogeneous subjects, the presence of confounders and population stratification [19].

Comparison with a previous meta-analysis

The previous meta-analysis [31] and ours each comprised seven papers, but only two [33, 36] were common in both. These differences in the five included studies between the two meta-analyses impacted the study design with differential subgroupings (ethnicity and cancer type in our study versus Asian ethnicity in the study of Gao *et al* [31]). The eventual findings also differed despite the use of standard genetic models in both meta-analyses. The present meta-analysis involved the following protocols that were not performed previously. First, we formally

evaluated the methodological quality of the included studies using CB. This facilitated a better understanding of the quality of the source materials. Second, we used the I^2 metric in assessing the variability of the studies, providing a clearer picture of the magnitude of heterogeneity of the summary outcomes or the lack of heterogeneity. Third, we confined our analysis to HWE-compliant studies, which minimized issues of genotyping error. Last, we performed subgrouping by cancer type, which unmasked specific tumor sites associated with *LOX* rs1800449. In the overall analysis, associative outcomes between the previous model and ours by a genetic model are as follows: (i) H: 1.5-fold versus 2.3-fold; (ii) R: 2.1-fold versus 2.3-fold; (iii) D: both at 1.4-fold; and (iv) C: 1.3-fold versus 1.6-fold. These numbers indicate modulated effects in the previous meta-analysis compared to ours in the H/R/C models but not in the D model. The heterogeneity of these values did not differ between the two meta-analyses. The two insights that we offer from our meta-analysis are (i) attention to study quality and (ii) differential findings from subgroup analysis by cancer type.

The *LOX* gene and LOX protein in cancer

Metastasis is the last stage of cancer progression that warrants a good understanding of its genetic etiology. First, the role of the *LOX* gene as a metastatic promoter contrasts with its ability to suppress tumors [38]. This dual role was based on the detection of both the upregulation and the downregulation of LOX in tumor tissues and cancer cell lines [38, 39]. An indication of *LOX* gene promoter activity was shown in a lung cancer clinical study [17], where non-small-cell lung carcinoma (NSCLC) patients with the 473AA genotype had a significantly ($P < 0.01$) shorter survival time (20 months) than patients with the 473G allele (28.0 months). This shows an association of *LOX* rs1800449 with an increased risk of NSCLC, rendering a prognostic role of this polymorphism for this disease. On the other hand, evidence for *LOX* gene suppressor activity involves cell and population studies. A previous study has shown that the rs1800449 SNP in the coding region of the highly conserved LOX propeptide domain inhibits tumor formation of NF639 cells in a xenograft model [32]. However, the A allele of rs1800449, which is transcribed to the Gln amino acid, has been shown to impair the ability of *LOX* to inhibit growth and function as a tumor suppressor [40]. Overall, Gln seems to be influential in constraining the ability of various LOX proteins (LOX-PP, Pro-LOX) to suppress tumor formation [32]. A population study of *LOX* rs1800449 found that AA homozygous carriers are more susceptible to various cancers than GG carriers [35]. Moreover, *LOX* studies on Chinese women suggest that homozygous and heterozygous genotypes containing the A allele of rs1800449 are associated with increased risks for cancers of the cervix [14], ovaries [18, 21] and breast [33]. Given the evidence for both *LOX* gene activities, both promoter and suppressor functions may well be complementary because they enable the formulation of therapeutic strategies to ameliorate the patient condition.

LOX expression seems to be tumor specific for increased metastasis [7, 9, 10, 32, 41, 42] These differential clinical outcomes underpin the complex role of LOX in cancer progression. This complexity led some to conclude that the role of LOX is relevant only in the progression stage of metastasis but not when it is already established [30]. Despite the complex role of *LOX* in cancer metastasis, this gene remains an appealing therapeutic target [43]. Barker *et al* [1] have reported that therapeutic targeting of LOX to prevent metastasis in the preclinical milieu involving inhibitors and function-blocking antibodies has been effective.

Strengths and limitations

Interpreting our meta-analysis findings is better contextualized in terms of their strengths and limitations. The limitation is that the majority (84%) of the studies had Asian subjects, indicating an underrepresentation of other ethnic groups. We had one study each for non-Hispanic Caucasians [30] and African-Americans [32]; these

ethnicities warrant inclusion in future studies. Despite this limitation, the following strengths increase confidence in our findings. (i) Our criteria of including only HWE-compliant studies effectively controlled for genotyping error and thus minimized methodological weaknesses in our study [44]. (ii) Most controls were uniformly defined (healthy or cancer-free); (iii) most tissue sources were blood. (iv) In most of the articles (five [30-33, 35] of seven or 71%), controls were matched with cases, with 80% (four [31-33, 35] of five) based on age. (v) Six [30-32, 35, 36] of the eight studies (75%) had sample sizes of ≥ 500. (vi) Of the 20 comparisons, 15 (75%) were statistically significant, 14 (93.3%) of which had extreme *P*-values ($< 10^{-5}$). (vii) Outlier treatment proved effective in erasing heterogeneity in the C model of the core finding, and (viii) sensitivity analysis rendered robustness to all significant outcomes.

Conclusion

In conclusion, this study presents meta-analytic evidence of the significant association between *LOX* rs1800449 and increased cancer risk. The observed associations here suggest that this polymorphism might be a useful susceptibility cancer marker. This marker potential may apply more for GI cancers than for breast cancer and more for Asians than for other ethnic groups. However, a single locus effect on cancer will likely be small given the involvement of other factors, such as gene-gene and gene-environment interactions. All seven publications focused only on *LOX*. Four [31, 33, 35, 36] of the seven articles (57.1%) mentioned gene-environment interactions but did not provide data that could have been useful in further analysis. More studies that explore other cancer types and based on sample sizes commensurate with the detection of small genotypic risks should allow more definitive conclusions about the association of the *LOX* rs1800449 polymorphism and cancer.

Abbreviations

AA	African-American
A	adenine
AM	analysis model
Arg	arginine
C	codominant
CB	Clark-Baudouin
CI	confidence interval
D	dominant
EH	eliminated heterogeneity
Fe	fixed effects
Glu	glutamine
G	guanine
H	homozygous

HB	hospital-based
Het	heterogeneity
HWE	Hardy-Weinberg equilibrium
I^2	measure of variability
in	increased risk
LOX	*Lysyl oxidase* gene
LOX	Lysyl oxidase protein
n	number of studies
NM	not mentioned
OR	odds ratio
P^a	*P*-value for association
P^b	*P*-value for heterogeneity
PB	population-based
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRO	preoutlier
PSO	postoutlier
R	recessive
Re	random effects
RH	reduced heterogeneity
RNS	retained nonsignificance
RS	retained significance
Sig	significance
SNP	single-nucleotide polymorphism
USA	United States of America

Declarations

-Ethics approval and consent to participate not applicable

-Consent for publication	not applicable
-Availability of data and material	in supporting information
-Competing interests	the authors declare that they have no competing interests
-Funding	this study was unfunded
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Methodology	PT, NP
Software	NP, AB
Writing - original draft	RM, NP, PT
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Tables

Table 1 Characteristics of the included articles that examined *lysyl oxidase* (rs1800449) associations with cancer

	First author	[R]	Year	Country	Ethnic group	Cancer type	Study design	Matching based on	CB
1	Friesenhengst	[30]	2014	Austria	Caucasian	Breast	HB	Residence	6
2	Gao	[31]	2014	China	Asian	Colorectal	HB	Age, sex	6
3	Min	[32]	2009	USA	AA	Breast	PB	Age, geography	7
4	Ren	[33]	2011	China	Asian	Breast	HB	Age	7
5	Shieh	[34]	2009	Taiwan	Asian	Oral	NM	NM	4
6	Wang	[35]	2016	China	Asian	Lung, colorectal	PB	Age, sex, residence	7
7	Yoon	[36]	2011	South Korea	Asian	Gastric	PB	NM	5

USA: United States of America; AA: African-American; HB: hospital based; NM: not mentioned; PB: population-based; CB: Clark-Baudouin.

Table 2 Summary associations of all fixed-effects outcomes between *lysyl oxidase*

(rs1800449) and cancer

		Test of association			Test of heterogeneity	
Comparison	n	OR	95% CI	P^a	P^b	I^2 (%)
All						
H	8	**2.28**	**1.79-2.90**	**10^{-5}**	0.19	30
R	8	**2.26**	**1.79-2.86**	**10^{-5}**	0.13	37
Asian						
H	6	**2.47**	**1.90-3.20**	**10^{-5}**	0.27	22
R	6	**2.43**	**1.89-3.14**	**10^{-5}**	0.17	36
Cé	6	**1.53**	**1.38-1.70**	**10^{-5}**	0.43	0
GI cancers*						
H	4	**2.32**	**1.71-3.15**	**10^{-5}**	0.27	23
Ré	4	**2.34**	**1.74-3.15**	**10^{-5}**	0.58	0
Dé	4	**1.45**	**1.24-1.68**	**10^{-5}**	0.52	0
Cé	4	**1.48**	**1.31-1.68**	**10^{-5}**	0.61	0
Breast cancer						
H	3	1.57	0.95-2.58	0.08	0.52	0
R	3	1.37	0.84-2.24	0.21	0.59	0

H: homozygous; R: recessive; D: dominant; C: codominant; GI: gastrointestinal; é core findings (judged on the basis of high associative significance ($P^a < 10^{-5}$ and homogeneity: $I^2 = 0\%$); n: number of studies; OR: odds ratio; CI: confidence interval; P^a: *P*-value for association; P^b: *P*-value for heterogeneity; I^2 is a measure of variability. All comparisons are fixed effects with their ORs indicating increased risk. * Gastrointestinal cancers (colorectal, oral, gastric). Values in bold indicate significant associations.

Table 3 Outlier outcomes of *lysyl oxidase* (rs1800449) and cancer

		Test of association				Test of heterogeneity				Test of association				Test of heterogeneity			Effect of outlier treatment	
n	OR	95% CI	Risk	P^a	P^b	I^2 (%)	AM	n	OR	95% CI	Risk	P^a	P^b	I^2 (%)	AM	Sig	Het	
				PRO								PSO						
All																		
D	8	**1.37**	**1.12-1.69**	in	**0.003** û	0.003	68	Re	7	**1.28**	**1.14-1.44**	in	10^{-4}	0.14	38	Fe	RS	RH
C	8	**1.60**	**1.27-2.02**	in	10^{-4}	10^{-5}	81	Re	6é	**1.53**	**1.38-1.70**	in	10^{-5}	0.43	0	Fe	RS	EH
Asian																		
D	6	**1.51**	**1.21-1.90**	in	10^{-5}	0.01	65	Re	5	**1.38**	**1.20-1.58**	in	10^{-5}	0.32	15	Fe	RS	RH
Breast cancer																		
D	3	1.38	0.77-2.50	in	0.28	0.002	88	Re	2	1.03	0.81-1.31	null	0.80	0.45	0	Fe	RNS	EH
C	3	1.92	0.89-4.14	in	0.10	10^{-5}	94	Re	--	---	--------	---	-----	---	--	---	---	---

D: dominant; C: codominant; n: number of studies; OR: odds ratio; null: ORs 0.97-1.03; CI: confidence interval; P^a: *P*-value for association; P^b: *P*-value for heterogeneity; I^2 is a measure of variability; in: increased risk; Re: random effects; Fe: fixed effects; RS: retained significance; RNS: retained nonsignificance; RH: reduced heterogeneity; EH: eliminated heterogeneity. Values in bold indicate significant associations; û Did not survive the Bonferroni correction. éCore finding. (–) outlier treatment did not reveal any outlier nor alter the heterogeneity.

Table 4 Sensitivity analysis of significant outcomes

Comparison genetic model	Outlier status	P^a-value	Sensitivity outcome
All			
H	—	$< 10^{-5}$	Robust
R	—	$< 10^{-5}$	Robust
D	PRO	0.003	Robust
C	PRO	$< 10^{-4}$	Robust
D	PSO	$< 10^{-4}$	Robust
C	PSO	$< 10^{-5}$	Robust
Asian			
H	—	$< 10^{-5}$	Robust
R	—	$< 10^{-5}$	Robust
D	PRO	$< 10^{-5}$	Robust
D	PSO	$< 10^{-5}$	Robust
C é	—	$< 10^{-5}$	Robust
GI cancers*			
H	—	$< 10^{-5}$	Robust
R é	—	$< 10^{-5}$	Robust
D é	—	$< 10^{-5}$	Robust
C é	—	$< 10^{-5}$	Robust

H: homozygous; R: recessive; D: dominant; C: codominant; GI: gastrointestinal;

écore findings; *colorectal, oral, gastric cancers; PRO: preoutlier; PSO: postoutlier. (–) OS initially fixed effects, thus not part of the PRO /PSO scheme.

Figures

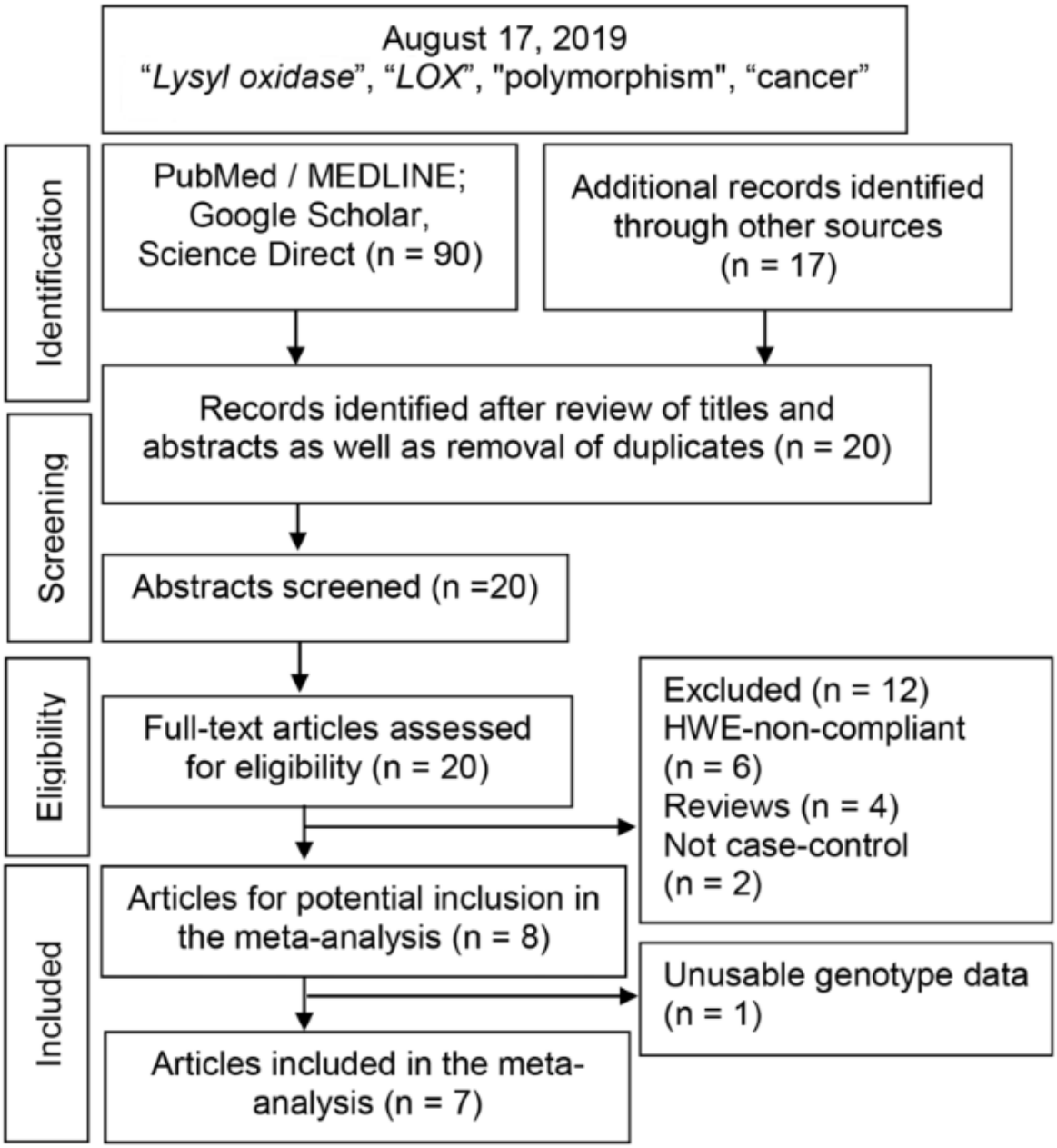

Figure 1

Summary flowchart of literature search HWE: Hardy-Weinberg Equilibrium

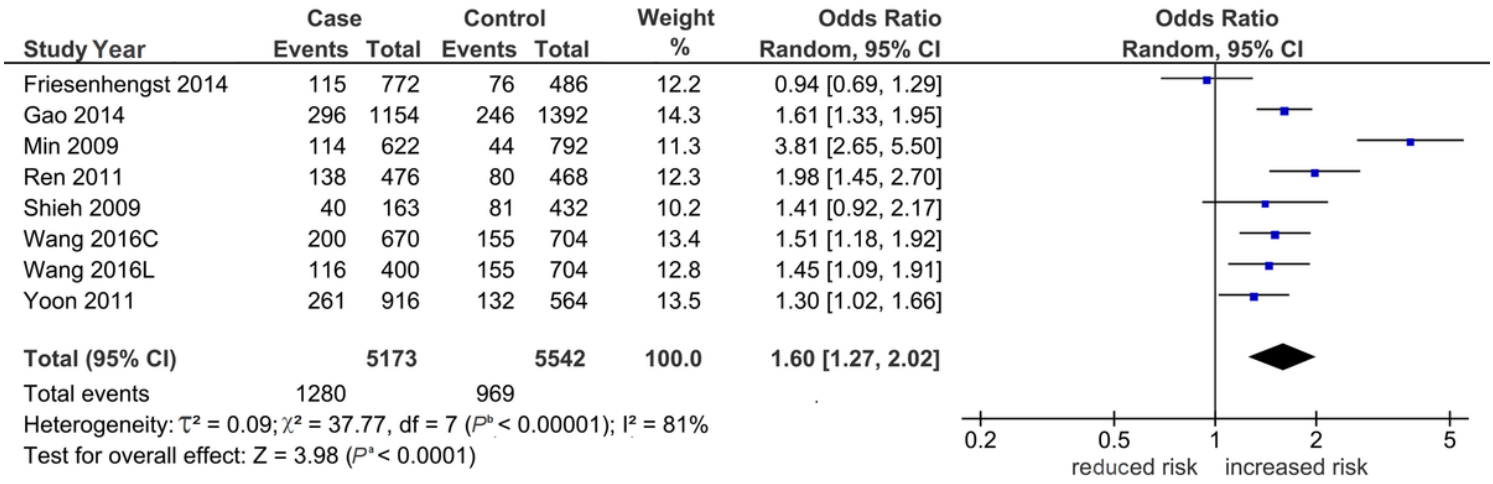

Figure 2

Forest plot outcome in the overall analysis for the codominant model Diamond denotes the pooled odds ratio (OR), here indicating increased risk (OR 1.60). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z test for overall effect is highly significant (Pa < 10-4). The 2 test shows the presence of heterogeneity (Pb < 10-5; I2 = 81%); I2: a measure of variability expressed in %

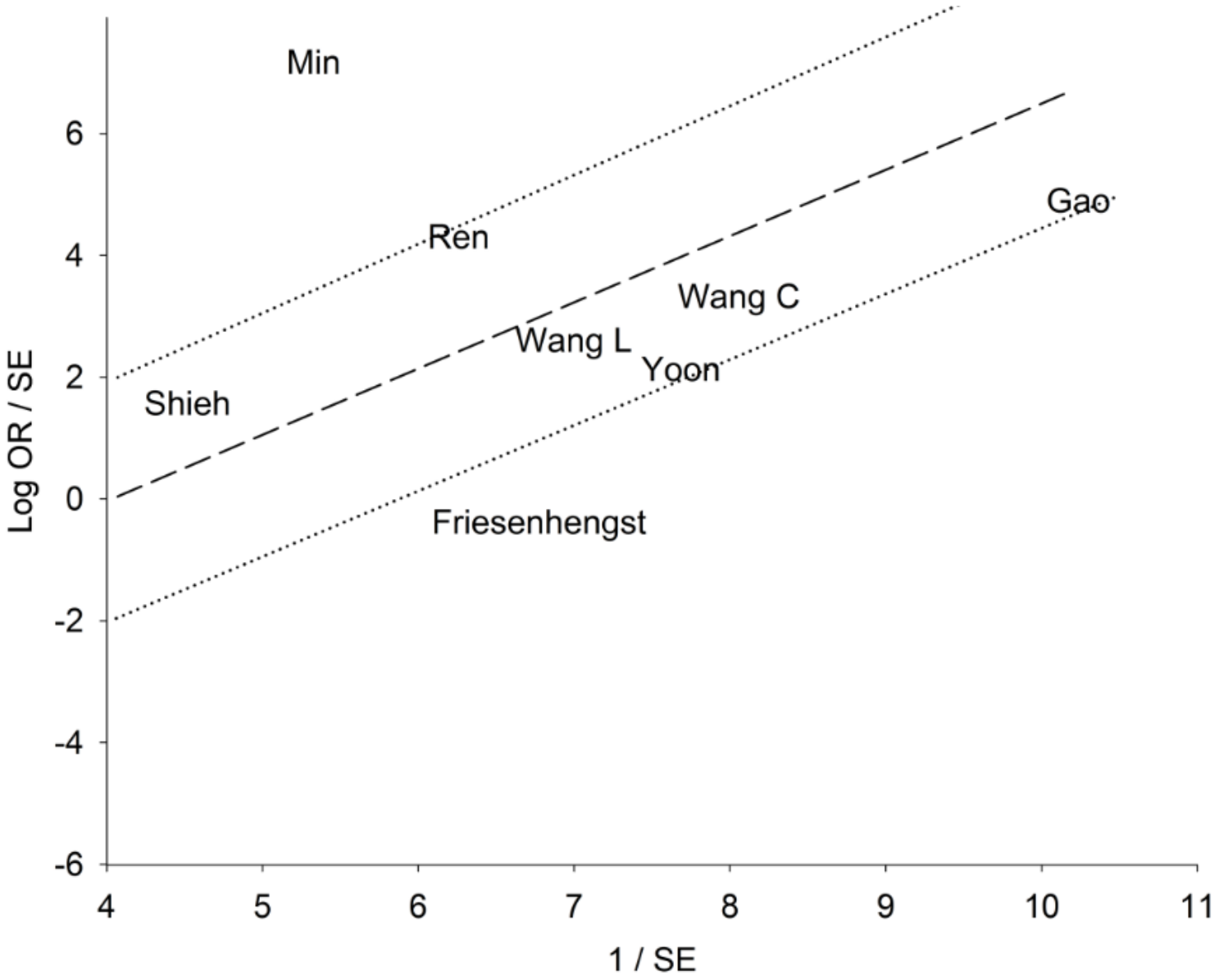

Figure 3

Galbraith plot analysis in the overall analysis for the codominant model Log OR: logarithm of standardized odds ratio; SE: standard error. The two studies above and below the +2 and -2 confidence limits are the outliers

Study Year	Case Events	Case Total	Control Events	Control Total	Weight %	Odds Ratio Fixed, 95% CI
Gao 2014	296	1154	246	1392	29.6	1.61 [1.33, 1.95]
Ren 2011	138	476	80	468	10.2	1.98 [1.45, 2.70]
Shieh 2009	40	163	81	432	6.0	1.41 [0.92, 2.17]
Wang 2016C	200	670	155	704	19.0	1.51 [1.18, 1.92]
Wang 2016L	116	400	155	704	14.3	1.45 [1.09, 1.91]
Yoon 2011	261	916	132	564	20.9	1.30 [1.02, 1.66]
Total (95% CI)		**3779**		**4264**	**100.0**	**1.53 [1.38, 1.70]**
Total events	1051		849			

Heterogeneity: $\chi^2 = 4.87$, df = 5 ($P^b = 0.43$); $I^2 = 0\%$

Test for overall effect: Z = 7.89 ($P^a < 0.00001$)

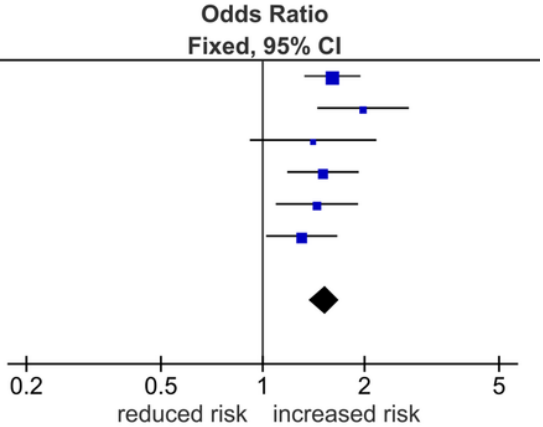

Figure 4

Forest plot outcome of outlier treatment in the overall analysis for the codominant model Diamond denotes the pooled odds ratio (OR) indicating increased risk (OR 1.53). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z test for overall effect shows high significance (Pa < 10-5).The 2 test indicates eliminated heterogeneity (Pb = 0.43; I2 = 0%); I2: a measure of variability expressed in %

Supplementary Files

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- SupplementaryFile.docx