Lysyl oxidase polymorphisms influence the risk of cancer: an update meta-analysis

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Abstract

The genetics of cancer metastasis is important for designing optimal therapeutic strategies. The *lysyl oxidase* (*LOX*) gene has been found important in the metastatic process, with roles in setting the microenvironment for future metastatic sites. Associations between the *LOX* polymorphisms (473G/A and -22G/C) have been examined in several studies, however, results were inconsistent, prompting a meta-analysis in order to obtain more precise estimates.

Searches of six databases yielded 14 articles (15 studies) that examined associations of 473G/A and -22G/C with cancer. We examined five cancer groups: breast, lung, bone (osteosarcoma), GIC (gastrointestinal cancers) and GYC (gynecological cancers). For each cancer group, we calculated pooled odds ratios (ORs) and 95% confidence intervals (CIs) using standard genetic models. High significance (P^a < 0.00001), homogeneity (I^2 = 0%) and high precision of effects (CI difference < 1.0 [upper CI-lower CI]) comprised the three criteria for strength of evidence (SOE). Multiple comparisons were Bonferroni-corrected. Sensitivity analysis assessed robustness of the outcomes.

Thirteen significant associations indicating increased risk (OR > 1.00) were found in all cancer groups except breast (P^a = 0.10-0.91). Of the 13, two were in osteosarcoma where the -22G/C effects (ORs 4.05-4.07, 95% CIs 1.30-12.70, P^a = 0.02) were homogeneous (I^2 = 0%) but imprecise (CIDs 11.4) and did not survive the Bonferroni correction. In contrast, the Bonferroni-surviving dominant/codominant outcomes in lung cancer (OR 1.44, 95% CI 1.19-1.74) and GYC (ORs 1.52-1.62, 95% CIs 1.26-1.88) met all three SOE criteria (P^a = 0.00001, I^2 = 0%, CIDs 0.49-0.56).

In summary, associations of *LOX* 473G/A with lung, ovarian and cervical cancers indicate 1.4-1.6-fold increased risks. These outcomes were underpinned by robustness and high statistical power at the aggregate level.

Introduction

Between 70-90% of cancer deaths result from metastasis, whereby the cancer has spread through the body [1]. In metastasis, cancer cells form new tumors far from the location where cancer was first detected (primary tumor) [2]. Metastasis occurs when cancer cells from the primary tumor invades the surrounding tissue, use the lymph and/or blood to travel through the body, then enter a distant organ (extravasate), settle in the new microenvironment and proliferate to form a secondary tumor [3]. Ability of the extravasated cancer cells to grow depends on features that are inherent to both the cancer cells and target organ and the active interplay between these two [4]. These interactions underpin the complexity of metastasis, given that this systemic process involves nonmalignant host cells in both primary and secondary sites [5]. Metastatic transformation is a driving factor in cancer research because treatments are more successful before metastasis has occurred than after. Thus, the pivotal role of metastasis in determining the success of cancer treatments depends on thorough understanding of this cancer phenomenon [6]. Metastasis results from genetic and epigenetic alterations in pathways involving proteins that mediate cell invasion, survival outside of the primary tumor microenvironment, and colonization at a distant organ site [7]. Lysyl oxidase (LOX) is a protein that is involved in the etiology of cancer metastasis because of its functional role affecting signaling, transcription and translation, which alters cell adhesion, motility and proliferation resulting from increased extracellular matrix (ECM) deposition [8]. Elevated expression of LOX was found to significantly correlate with increased metastasis and reduced patient survival [9]. Thus, involvement of LOX in multiple stages of metastasis [10] and its role the metastatic milieu of various cancers [11-14] renders this protein a useful clinical target [15]. Furthermore, LOX accumulation in future metastatic sites [9] renders the gene for this protein important in understanding its emissary role in metastasis.

The *LOX* gene has seven exons that encode several functional domains of the LOX protein [16]. 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 [17]. 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 [18]. A shift from 473G to 473A changes the amino acid arginine (Arg) at residue 158 to glutamine (Gln) (Arg158Gln) in the LOX propeptide [16]. Since it was discovered [16], *LOX* polymorphisms (473G/C and -22G/C) have been closely studied for their relationship with carcinogenesis [5, 10, 19]. At the gene level, single-study reports of *LOX* SNP 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* SNPs in the risk of cancer metastasis, 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 (SOE) using statistical and meta-analytical criteria. This study aims to highlight the genetic role of *LOX* polymorphisms in cancer metastasis and to provide information that could be useful in clinical decision making.

Methods

Selection of studies

We searched MEDLINE using PubMed, Google Scholar, Scopus, Mednar, Wanfang and CNKI (China National Knowledge Infrastructure) databases for association studies as of August 11, 2020. The terms used were "*Lysyl oxidase*", "protein-lysine 6-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. In case of duplicates, the article with the most recent date was selected. Inclusion criteria were (i) case-control studies evaluating the association between the *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); (ii) articles that were not case-control studies; and (iii) studies with genotype data that could not be used to calculate ORs and 95% CIs.

Data extraction

Two investigators (RM and NP) independently extracted data and arrived at consensus. The following information was obtained from each publication: cancer group, family name of the first author, year of publication, the country of origin, ethnicity, *LOX* SNP, primary tumor site, study-specific association of the *LOX* SNP with cancer from each publication with their respective 95% CIs and *P*-values, status of the controls, genotyping platform, basis for matching the controls with cases, and study features needed to tally scores for the Newcastle-Ottawa Scale (NOS).

LOX polymorphisms and cancer groups

We examined two *LOX* polymorphisms in five cancer groups: -22G/C in (i) osteosarcoma (bone cancer) and 473G/A in the other four cancer groups that included (ii) breast, (iii) lung, (iv) gastrointestinal cancers (GIC) and (v) gynecological cancers (GYC). Three and two cancer types comprised GIC (oral, gastric and colorectal) and GYC (cervical and ovarian), respectively.

Quality of the studies

The NOS [20] was used to assess quality of the included studies. NOS scoring is based on three broad perspectives: selection, comparability, and exposure in case–control studies. The star rating system has scores ranging from zero (worst) to 9 (best). Scores of 5–6 and ≥7 stars indicate moderate and high quality, respectively.

Statistical power and Hardy-Weinberg equilibrium (HWE)

Using the G*Power program [21], we evaluated statistical power. Meta-analyses in cancer genetics have used the ORs of 1.2 and 1.5 to assess statistical power [22]. Thus, at these OR levels with a genotypic risk level of α = 0.05 (two-sided) and 5% minor allele frequency (maf), power was considered adequate at ≥ 80%. 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.

Data synthesis

Examining two *LOX* polymorphisms (473G/A and -22G/C) warranted the use of a common notation indicating *var* and wild-type (*wt*) alleles. Supplementary Table S2 includes a column for the minor (*var*) allele in both polymorphisms. After estimating cancer risk (OR) for each study, pooled ORs with 95% CIs were calculated for each of the five cancer groups in the following genetic models: (i) homozygous: (*var*–*var* and *wt*–*wt*) genotypes compared with *wt*–*wt*, (ii) recessive: (*var*–*var* versus wt–*var* + *wt*–*wt*), (iii) dominant: (*wt*–*wt* versus *wt*–*var* + *var*–*var*), and (iv) codominant: (*var* versus *wt*). Three indicators were used for strength of evidence (SOE): First, highly significant *P*-values (P^a = 0.00001) most likely to survive the Bonferroni correction, which was performed with Microsoft Excel (Microsoft, Redmond, WA, USA). Second, highly precise effects were assessed with the confidence interval difference (CID = upper CI-lower CI). High (> 1.0) and low (< 1.0) CID values indicate low and high precision, respectively [23]. Third, homogeneity was assessed with the I^2 metric, expressed as 0% [24]. In meta-analysis, however, studies differ from each other [25]. This heterogeneity was estimated with the c^2-based Q test [26] where significance was set at P_{HET} < 0.10. The random-effects model (DerSimonian–Laird) [27] was used in the presence of heterogeneity [24] and the fixed-effects model (Mantel–Haenszel) [28] in its absence. Summary effects that met the SOE criteria were tested for robustness, with use of sensitivity analysis, which involves serial omission of the studies followed by recalculation of the pooled OR. Significant outcomes (P^a < 0.05) with ≥ 10 studies warranted assessment for publication bias. Except for heterogeneity estimation [26] two-sided *P*-values of £ 0.05 were considered significant. 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 flowchart based on guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [29] with a checklist detailing the description of this meta-analysis (Supplementary Table S3). A total of 504 citations were identified from the initial search, the screening of which yielded 22 full-text articles. Of the 22, eight were excluded for not conforming to the inclusion criteria. Table 1 lists the 14 articles [30-43] included in this study , seven [30, 31, 33, 36, 38, 40, 43] of which were new additions to the meta-analysis literature on *LOX*-cancer. Two Chinese language publications [44, 45] were duplicates (excluded from this study) of the English language article [36] included in this study. This article [36] examined two cancer types (lung and colorectal), which were treated as two studies. Subjects were all Asians except in two publications in breast cancer. Three, two and two articles focused on breast, lung and bone, respectively. Four articles each examined GIC and GYC. Age (mean ± standard deviation years) of the patients were predominantly 50s to 60s in all cancers except two studies in GYC (38.2 ± 9.2y) and osteosarcoma (16.1 ± 2.8y). NOS scores (median interquartile range: 5 [5.3-6.8]) indicated that quality of the component studies was moderate. Supplementary Table S1 shows the quantitative traits of the included studies. Sample sizes ranged from 98 to 1,273 and those for cases and controls in each cancer group were as follows: breast (935/923), GIC (1,575/1,546), lung (538/748), osteosarcoma (369/488) and GYC (991/1,071).

Aggregate statistical power (ASP) in the five cancer groups were adequate at an OR = 1.5 (82.5%-99.9%), but not at an OR = 1.2, where only GIC was adequately powered (91.8%). Five studies were HW-non-compliant covering lung and bone cancers and all but one in GYC (Supplementary Table S2).

Overall and subgroup analysis

This meta-analysis yielded 28 comparisons (Tables 2 and Supplementary Table S4), of which 18 were non-significant ($P^a > 0.05$), found in breast, bone and lung cancers. Thirteen outcomes were significant ($P^a < 0.05$), all indicating increased risk (ORs 1.36-4.07). The low number of studies precluded assessment of publication bias.

Breast cancer

Three articles [30-32] in the breast cancer group were ethnically heterogeneous (Asians, Caucasians and African-Americans). Table 2 shows that associations were non-significant in all genetic models (ORs 0.98-1.92, 95% CIs 0.62-4.14, $P^a = 0.10$-0.91), not even when stratified by ER status (ORs 1.28-1.54, 95% CIs 0.67-2.91, $P^a = 0.12$-0.45) (Supplementary Table S4).

Lung cancer and osteosarcoma

The lung cancer (473G/A) and osteosarcoma (-22G/C) comparisons were each based on two studies collectively yielding eight outcomes (Table 2). Of the eight, five were significant ($P^a < 0.05$), three of which survived the Bonferroni correction, all in lung cancer ($P^a < 0.0001$). Of the three, only the codominant result was homogeneous ($I^2 = 0\%$) which, with high precision (CID 0.55), met all three SOE criteria. In osteosarcoma, two significant outcomes ($P^a = 0.02$) in the homozygous/recessive models had high magnitude (ORs 4.05-4.07). However, their imprecise effects (CIDs 11.35-11.39) and failure to survive the Bonferroni correction warrant caution in interpreting the risk that -22G/C poses for bone cancer.

GIC and GYC

Of the eight GIC and GYC significant outcomes, seven survived the Bonferroni correction (Table 2). These highly significant ($P^a < 0.0001$) pooled ORs presented a dichotomy of precision effects, low in homozygous/recessive (CIDs of 1.63-3.14), high in dominant/codominant (CIDs 0.40-0.56). Figure 2 visualizes of the difference between low and high precision studies in GYC. The diamond was broader and horizontal lines from each study in the homozygous plot were longer (CID: 1.78, low precision) compared to the shorter lines (CID: 0.49, high precision) and narrower diamond in the codominant plot.

Core outcomes

Table 2 and 3 show that lung cancer and GYC outcomes in the dominant/codominant models met all three SOE criteria: (i) high significance [$P^a < 0.00001$]; (ii) high precision [CIDs 0.49-0.55]; (iii) zero heterogeneity [$I^2 = 0\%$], underpinned by robustness and high ASP (94.2-99.5% at OR = 1.5).

Discussion

Summary of findings

Given the different clinical manifestations, etiologies and progression in the five cancer groups, we conducted the meta-analysis by cancer group, which reduced the number of studies (n = 2-4). However, each study contributed to the

aggregate sample size that resulted in adequate to high ASP in all five cancer groups (82.5-99.9% at an OR = 1.5) (Supplementary Table S2). This OR level has been used in previous studies that explored associations of genetic polymorphisms with cancer [46]. Breast cancer was the only comparison to yield non-significance (P^a = 0.10-0.91) in all genetic models (Table 2 and Supplementary Table S4). Study-specific ORs from the three component studies were unsurprisingly non-significant for the *var* 473G/A genotype in this ethnically heterogeneous cancer group (Table 1). These three articles have examined the influence of ER status in breast cancer risk, where two reported significant outcomes in their expression studies. Min *et al* [31] showed significantly higher expression levels of LOX in ER- breast cancers compared to ER+ ones (P^a < 0.05). Friesenhengst *et al* [30] favored the greater prognostic role of LOX expression over that of the 473G/A genotype. In contrast to the breast cancer findings, GIC and GYC increased risk effects were significant in all genetic models (Table 2), which presented contrasts according the genetic model. Homozygous and recessive odds in GIC indicated 3.0 to 3.3-fold risks, more than double the odds in the dominant/codominant models (1.4-fold). In GYC, the homozygous/recessive odds were 2.7 and 2.5-fold, while that in the dominant/codominant models were 1.5-1.6-fold. Thus, for both GIC and GYC, homozygous/ recessive odds were higher than the dominant/codominant odds. Between these two cancer groups, GIC may pose greater increased risks (3.3-fold) than those in GYC (2.7-fold). However, other meta-analytical evidence need to be considered for a more complete picture of *LOX* genetic associations with cancer. Thus, two dichotomies delineated effects between the genetic models and cancer groups of GIC/GYC. (i) precision was low in the homozygous/recessive models but not in the dominant/codominant models; (ii) GYC outcomes were homogeneous (I^2 = 0%) but not in GIC (I^2 = 30-61%). Between the non-significant breast cancer and significant GIC/GYC outcomes in all genetic models were significance in some, not all genetic models of osteosarcoma and lung cancer. In the -22G/C polymorphism of osteosarcoma, the codominant null outcome agreed with the lack of significant association in glioma [19] but contrasted with our moderately significant homozygous/ recessive finding. In lung cancer, the homozygous/recessive outcomes were highly significant (P^a = 0.00001) but imprecise (CIDs 2.61-2.71). In contrast, the codominant pooled OR met all SOE criteria (high significance + high precision [(CID 0.55)] + zero heterogeneity). This centralized the codominant lung cancer and dominant/codominant GYC outcomes, with evidence of association between *LOX* 473G/C with risk of cancer. Scaffolds that underpinned the SOE were robustness and high statistical power.

Comparison with a previous meta-analysis

Table 4 details the differences between a previous meta-analysis [37] and ours. Table 1 identifies which articles were and were not in Gao *et al* [37]. Of note, the article on glioma [19] was in Gao *et al* [37] but not in ours on account of our cancer group study design. Differences in study design (overall analysis: cancer groups in our study versus pooled cancer types in Gao *et al* [37]) between the two meta-analyses precluded direct comparisons of the results.

Role of *LOX* gene and LOX protein in cancer metastasis

Metastasis is the last stage of cancer progression that warrants a good understanding of its genetic etiology. Literature on the association between *LOX* polymorphisms and cancer metastasis, particularly 473G/A are uncommon, with outcomes that may require more clarity. Of the 14 articles in this meta-analysis, three examined lymph node metastasis [30, 32, 43], where significant associations with the *LOX* genotypes (*P* = 0.02) were found in the ovarian cancer study of Yang *et al* [43] but not for breast cancer (*P* = 0.41). In the breast cancer study of Friesenhengst *et al* [30], however, their findings involving 473A-carriers among ER- patients showed that 473G/A may increase the risk for breast cancer, particularly in ER- women with weaker outcome that involved metastasis. In their osteosarcoma study, Liu *et al* [34] found that the AA genotype and A allele were higher in patients with metastasis than those without metastasis indicating a significant 1.5 to 2.4-fold increased risk (*P* = 0.02- 0.03) but failed the Bonferroni correction. In their ovarian cancer study, Wang *et al* [41] posited that 473G/A reinforces LOX signaling

which may affect metastasis. At the mRNA level, high LOX expression was reported to favor metastasis and disfavor patient survival [47-49]. These findings underpin the ability of LOX as a potent predictor of cancer metastasis. Moreover, interventions that involved silencing of LOX gene expression and targeting the hypoxia pathway have been reported to suppress [50], even reverse metastasis in breast and pancreatic cancers [51]. These differential clinical outcomes underpin the complex role of LOX in cancer metastasis. Despite the complex role of *LOX* in cancer metastasis, this gene remains an appealing therapeutic target [10, 47, 52, 53].

Strengths and limitations

We identified four limitations in our study: First, majority (12/14: 86%) of the studies had Asian subjects, indicating an underrepresentation of other ethnic groups. The two studies [30, 31] with non-Hispanic Caucasian and African-American ethnicities warrant more of these two ethnic groups in future studies. Second, imprecise effects and failure to survive the Bonferroni correction of the significant -22G/C outcomes in the homozygous/recessive models of osteosarcoma may have decommissioned this polymorphism as a genetic risk factor for cancer, but future studies might modify this conclusion. Third, we did not explore gene-environment interactions. Four [32, 36, 37, 39] articles mentioned gene-environment interactions but did not provide data for further analysis. However, four articles explored the *LOX* polymorphism associations with cigarette smoking and cancers of the lung [35, 36], bone [33] and cervix [40] as well as bisphenol A (an environmental estrogen) and osteosarcoma [33]. Fourth, the core GYC and lung cancer outcomes had HW-deviating studies [35, 40-42], which may have posed methodological and representation bias. On the other hand, the strengths of our study include: (i) combinability of the component studies where most (54%) of the comparisons (15/28) were fixed-effects and 60% (9/15) had zero heterogeneity (I^2 = 0%); (ii) most controls (13/14: 93%) were uniformly defined (healthy or cancer-free); (iii) most tissue sources were blood specimens (12/14: 86%); (iv) most (11/14: 79%) of the articles had controls that were matched with cases, with 80% (eight articles based on age); (v) all significant core outcomes were robust.

Conclusion

We have presented evidence for the role of the *LOX* polymorphisms in increasing cancer risk, GYC and lung cancer in particular, which suggest that 473G/A might be a useful susceptibility cancer marker. However, a single locus effect on cancer will likely be small given the involvement of other factors, such as gene-gene interactions. All 14 publications focused only on *LOX*. Functional studies have shown that other genes such as *hypoxia-inhibiting factor 1* (*HIF-1*) *transforming growth factor -beta* (*TGFβ*), and *interferon-gamma* (*IFNγ*) interact with *LOX* to regulate metastasis [10, 15, 54, 55]. More studies based on sample sizes commensurate with the detection of small genotypic risks should allow more definitive conclusions about the association of the *LOX* polymorphisms and cancer.

Abbreviations

A: adenine; ASP: aggregate statistical power; CI: confidence interval; C: cytosine; G: guanine; GIC: GYC: HWE: Hardy-Weinberg equilibrium; I^2: measure of variability; *LOX*: *Lysyl oxidase* gene; LOX: Lysyl oxidase protein; N: number of participants; n: number of studies; NOS: Newcastle-Ottawa Scale; OR: odds ratio; P^a: *P*-value for association; P_{HET}: *P*-value for heterogeneity; SNP: single-nucleotide polymorphism

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

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-Authors' contributions

Conceptualization	RM, NP, PT
Data extraction and analysis	RM, NP, PT, AB
Validation	RM, PT, NP, AB
Methodology	PT, NP
Software	NP, AB
Writing - original draft	RM, NP, PT
Writing - review & editing	RM, NP, PT

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Tables

Table 1 Characteristics of the included articles that examined *lysyl oxidase* polymorphism associations with cancer

In Gao	Cancer group First author (Number of articles)	[R]	Year	Country	Ethnic Group	Patients age * (years)	*LOX*SNP	Primary tumor site	Study-specific outcome for *var*genotype OR (95% CI) P^a value	Status of controls	Genotyping platform	Match	NOS
	Breast cancer (3)												
No	1 Friesenhengst	[30]	2014	Austria	Caucasian	60.5 ± 14.8	473G/A	Breast	0.99 (0.38-2.59) 0.98†	Healthy	Taqman	Residence	6
No	2 Min	[31]	2009	USA	African-American	(21-69)	473G/A	Breast	1.99 (0.86-4.61) > 0.05 AA	NM	PCR	Age Residence	7
Yes	3 Ren	[32]	2011	China	Asian	48.8 ± 8.9	473G/A	Breast	1.84 (0.81-4.20) 0.15 AA_{crude}	Healthy	RFLP	Age	7
	Osteosarcoma (2)												
No	4 Jia	[33]	2013	China	Asian	16.1 ± 2.8	22G/C	Bone	1.48 (1.06-7.37) 0.02 GC/CC Bisphenol > 7.0	Cancer-free	RFLP	Age Sex	6
Yes	5 Liu	[34]	2012	China	Asian	(10-67)	22G/C	Bone	5.09 (1.41-18.41) 0.006 CC	Healthy	RFLP	Age Sex Residence	6
	Lung cancer (2)												
Yes	6 Shi	[35]	2012	China	Asian	± 50	473G/A	Lung	2.35 (1.29-4.29) 0.0004 AA	Cancer-free	RFLP	Age Sex Residence	5
No	7 Wang	[36]	2016	China	Asian	58.3 ± 9.3	473G/A	Lung	3.84 (2.03-7.24) < 0.01 AA	Healthy	RFLP	Age Sex Residence	7
	GIC (4)												
---	8 Gao	[37]	2015	China	Asian	59.4 ± 9.7	473G/A	Colon/rectum	2.86 (1.78-4.59) < 0.001 AA	Cancer-free	RFLP	Age Sex	6
No	9 Shieh	[38]	2007	Taiwan	Asian	57.8 ± 9.8	473G/A	Mouth	1.46 (0.55-3.90) 0.50 AA	Areca chewers	NM	NM	5
No	Wang	[36]	2016	China	Asian	59.0 ± 10.8	473G/A	Colon/rectum	2.74 (1.47-5.12) < 0.01 AA	Healthy	RFLP	NM	6
Yes	10 Yoon	[39]	2011	South Korea	Asian	60 (22-91)	473G/A	Stomach	1.47 (1.09-1.98) < 0.05 AA_{crude}	Healthy	RFLP	NM	5
	GYC (4)												
No	11 Bu	[40]	2014	China	Asian	38.2 ± 9.2	473G/A	Cervix	2.50 (1.32-4.72) 0.004 AA	Healthy	RFLP	Age Sex Residence	7
Yes	12 Wang	[41]	2012	China	Asian	55.3 ± 10.9	473G/A	Ovaries	2.30 (1.36-3.87) < 0.01 AA	Healthy	RFLP	NM	5
Yes	13 Wu	[42]	2012	China	Asian	53.6 ± 12.7	473G/A	Ovaries	2.52 (1.28-4.96) 0.006 AA	Cancer-free	RFLP	Age	6
No	14 Yang	[43]	2017	China	Asian	54.6 ± 10.4	473G/A	Ovaries	2.64 (1.24-4.53) 0.006 AA	Healthy	Taqman	Age	6

Gao et al meta-analysis; GIC: gastrointestinal cancers (oral, gastric, colorectal); GYC: gynecological cancers (cervical, ovarian); [R] reference; SNP: single nucleotide polymorphism; G/A; guanine/ adenine; OR: odds ratio; CI: confidence interval; † recessive effect; P^a: *P*-value for association; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; NOS: Newcastle-Ottawa Scale; * age column: values expressed as mean ± standard deviation, values in parentheses are median (range).

Table 2 Summary associations of outcomes between *lysyl oxidase* polymorphisms and cancer

		Test of association				Test of heterogeneity		
Cancer group Genetic model	n	OR	95% CI	CID	P^a	P_{HET}	I^2(%)	Analysis model
Breast cancer								
Homozygous	3	1.15	0.72-1.83	1.11	0.56	0.33	9	Fixed
Recessive	3	0.98	0.62-1.53	0.91	0.91	0.11	54	Fixed
Dominant	3	1.29	0.81-2.04	1.23	0.28	0.005	81	Random
Codominant	3	1.92	0.89-4.14	3.25	0.10	0.00001	94	Random
Osteosarcoma (-22G/C)								
Homozygous	2	**4.07**	**1.31-12.70**	11.39	**0.02**	0.36	0	Fixed
Recessive	2	**4.05**	**1.30-12.65**	11.35	**0.02**	0.39	0	Fixed
Dominant	2	1.70	0.41-7.09	6.18	0.47	0.02	81	Random
Codominant	2	1.01	0.06-16.03	15.97	1.00	0.0001	93	Random
Lung cancer								
Homozygous	2	**2.96**	**1.91-4.57**	2.61	**0.00001***	0.27	17	Random
Recessive	2	**3.07**	**2.00-4.71**	2.71	**0.00001***	0.15	53	Random
Dominant	2	1.20	0.95-1.52	0.57	0.12	0.36	0	Fixed
Codominant	2	**1.44**	**1.19-1.74**	0.55	**0.0002***	0.98	0	Fixed
GIC								
Homozygous	4	**3.27**	**2.06-5.20**	3.14	**0.00001***	0.06	59	Random
Recessive	4	**2.98**	**1.95-4.57**	2.62	**0.00001***	0.09	54	Random
Dominant	4	**1.36**	**1.17-1.57**	0.40	**0.0001***	0.23	30	Fixed
Codominant	4	**1.36**	**1.11-1.66**	0.55	**0.003**	0.05	61	Random
GYC								
Homozygous	4	**2.65**	**1.91-3.69**	1.78	**0.00001***	0.56	0	Fixed
Recessive	4	**2.46**	**1.78-3.41**	1.63	**0.00001***	0.50	0	Fixed
Dominant	4	**1.52**	**1.26-1.82**	0.56	**0.00001***	0.99	0	Fixed
Codominant	4	**1.62**	**1.39-1.88**	0.49	**0.00001***	0.88	0	Fixed

GIC: gastrointestinal cancers (oral, gastric, colorectal); GYC: gynecological cancers (cervical, ovarian); all cancer groups examined 473G/A unless otherwise specified; n: number of studies; OR: odds ratio; CI: confidence interval; CID: confidence interval difference; P^a: *P*-value for association; P_{HET}: *P*-value for heterogeneity; I^2 is a measure of variability attributed to heterogeneity; values in bold indicate significant associations; *survived the Bonferroni correction.

Table 3 Main outcome summary of *lysyl oxidase* 473G/A and cancer

Cancer group N cases/N controls ASP at OR 1.2 / 1.5 Genetic model			n	Fold-increase in risk	CID	P^a	I^2(%)	Sensitivity treatment outcome
Lung cancer	538 / 748	56.4% / 94.2%						
Codominant*			2	1.4	0.55	0.0002	0	Robust
GYC 77.7% / 99.5%	991 / 1,079							
Dominant*			4	1.5	0.56	0.00001	0	Robust
Codominant*			4	1.6	0.49	0.00001	0	Robust
Homozygous			4	2.7	1.78	0.00001	0	Robust
Recessive			4	2.5	1.63	0.00001	0	Robust

GYC: gynecological cancers (cervical, ovarian); N: number of participants; n: number of studies; ASP: aggregate statistical power where 80% is powered; CID: confidence interval difference; I^2: measure of variability ; * met all three criteria for strength of evidence (high significance [P^a] + high precision [CID < 1.0] + zero heterogeneity [$I2$ = 0%].

Table 4 Meta-analysis comparisons of *lysyl oxidase* polymorphisms and cancer risk

	This study	**Gao et al** [37]
Year	2020	2014
Country	Thailand	China
n articles/studies	14/15	7
LOX polymorphisms	473G/A and -22G/C	473G/A only
Genetic model	Standard	Standard
Number of databases in the literature search	PubMed, Google Scholar, Scopus, Mednar, CNKI, Wanfang	PubMed
Overall analysis	Summary effect for each cancer group	Pooled the cancer types
Study variability	I^2	None
Ethnicity profile of subjects	Caucasians, African-Americans, Asians	Asians only
Methodological quality	NOS	None
Addressed HWE	Yes	No
Sensitivity	Yes	Yes
Publication bias	No	Yes
Precision analysis	Yes	No
Power analysis	Yes	No
Correction for multiple comparisons	Bonferroni	None

l oxidase; G: guanine; A: adenine; C: cytosine; CNKI: China National Knowledge Infrastructure;

ıre of variability; NOS: Newcastle-Ottawa Scale; HWE: Hardy-Weinberg Equilibrium

Figures

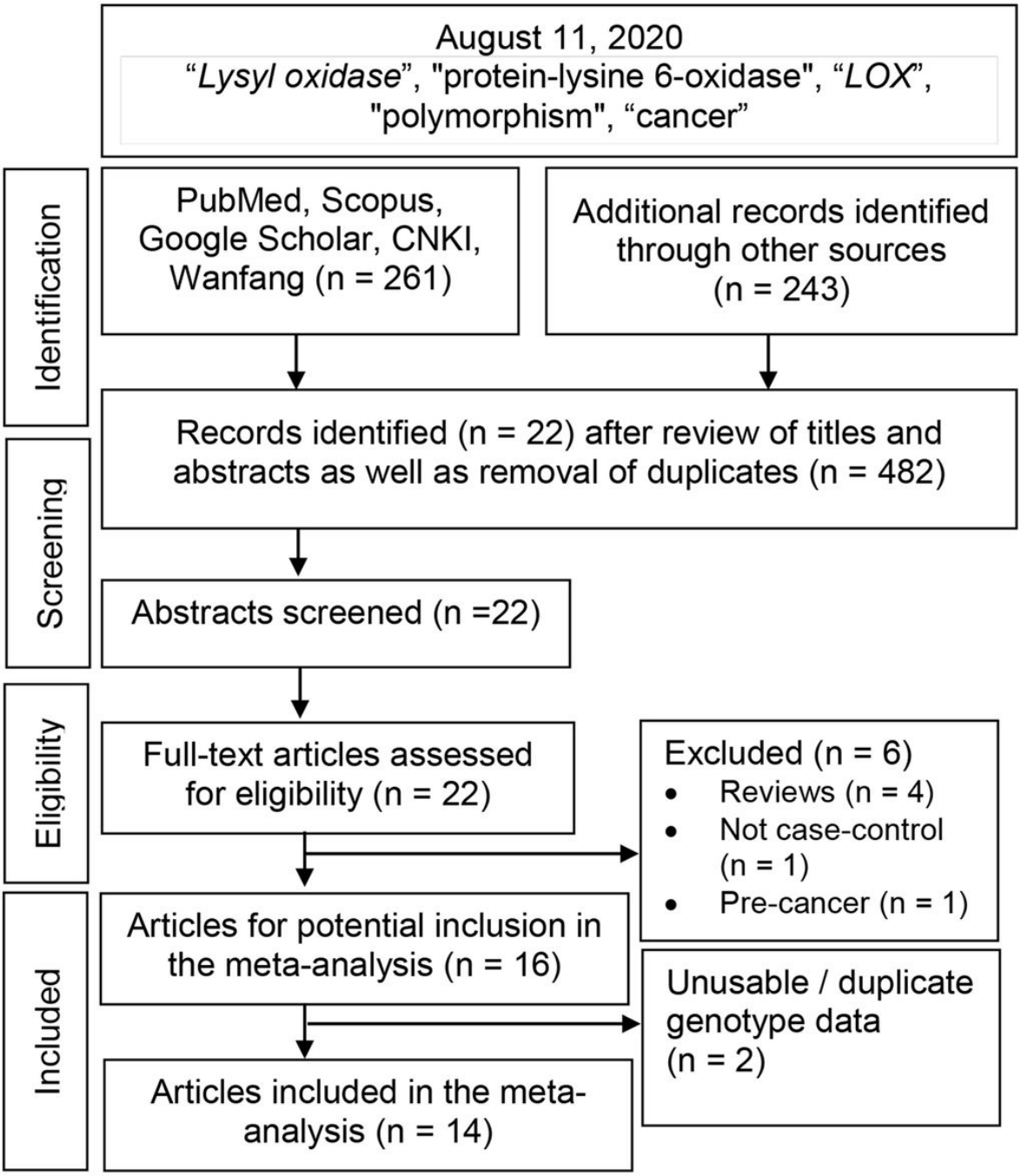

Figure 1

Summary flowchart of literature search

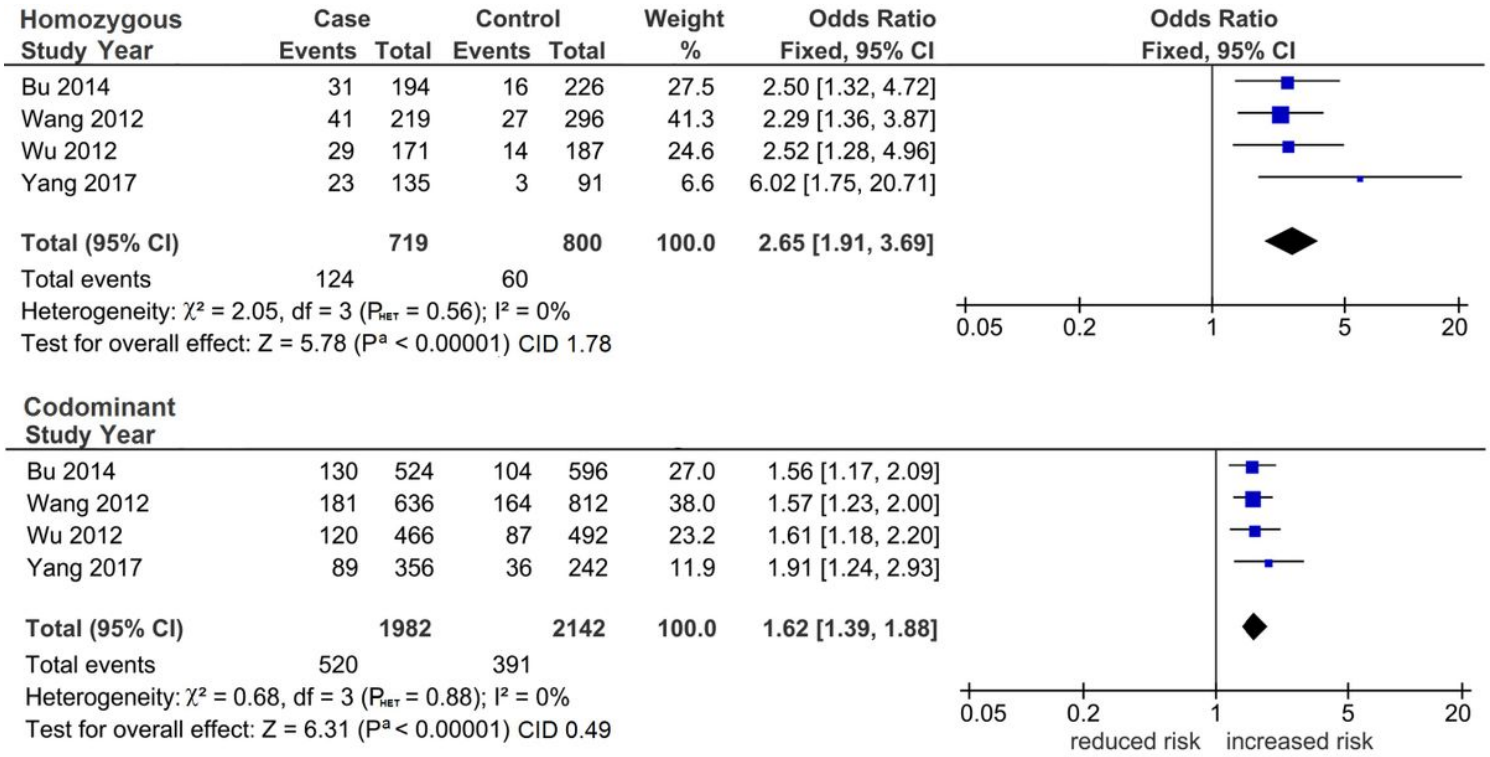

Figure 2

Forest plot of GYC outcomes in the overall analysis for the homozygous and codominant models Diamonds denote the pooled odds ratios (ORs) indicating increased risks (ORs 2.65 and 1.62). Squares indicate the OR in each study. Horizontal lines on either side of each square represent the 95% confidence intervals (CI). The Z tests for overall effect were highly significant (Pa < 0.00001). CID: confidence interval difference; The 2 tests show the absence of heterogeneity (I2 = 0%); I2: a measure of variability expressed in %

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