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

Rungrawee Mongkolrob
Thammasat University

Phuntila Tharabenjasin
Thammasat University

Apom Bualuang
Thammasat University

Noel Pabalan (noelpabalan@mail.com)
Angeles University Foundation  https://orcid.org/0000-0003-2069-5535

Research article

Keywords: Lysyl oxidase, LOX, polymorphisms, cancer, meta-analysis

DOI: https://doi.org/10.21203/rs.2.18129/v3

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
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 invade 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) 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 $\alpha = 0.05$ (two-sided) and 5% minor allele frequency (maf), power was considered adequate at $\geq 80\%$. 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 $\geq 10$ studies warranted assessment for publication bias. Except for heterogeneity estimation [26] two-sided $P$-values of $\leq 0.05$ 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 var473G/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 (CIs 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 \textit{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 \textit{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 \textit{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 \textit{LOX}. Functional studies have shown that other genes such as \textit{hypoxia-inhibiting factor 1 (HIF-1)} transforming growth factor -beta (\textit{TGFβ}), and \textit{interferon-gamma (IFN)} interact with \textit{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 \textit{LOX} polymorphisms and cancer.

**Abbreviations**

A: adenine; ASP: aggregate statistical power; CI: confidence interval; C: cytosine; G: guanine; GIC: GYC: HWE: Hardy-Weinberg equilibrium; $\hat{P}$: measure of variability; \textit{LOX}: \textit{Lysyl oxidase} gene; \textit{LOX}: Lysyl oxidase protein; N: number of participants; n: number of studies; NOS: Newcastle-Ottawa Scale; OR: odds ratio; $P_a$: \textit{P}-value for association; $P_{HET}$: \textit{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

-Funding

This study was unfunded

-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

-Acknowledgments

not applicable

References

1. Dillekas H, Rogers MS, Straume O: Are 90% of deaths from cancer caused by metastases? Cancer medicine 2019, 8(12):5574-5576.

2. Gupta GP, Massague J: Cancer metastasis: building a framework. Cell 2006, 127(4):679-695.

3. Fidler IJ: The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nature reviews Cancer 2003, 3(6):453-458.

4. Chambers AF, Groom AC, MacDonald IC: Dissemination and growth of cancer cells in metastatic sites. Nature reviews Cancer 2002, 2(8):563-572.

5. Cox TR, Garland A, Erler JT: Lysyl Oxidase, a Targetable Secreted Molecule Involved in Cancer Metastasis. Cancer research 2016, 76(2):188-192.

6. Siddikuzzaman, Grace VM, Guruvayoorappan C: Lysyl oxidase: a potential target for cancer therapy. Inflammopharmacology 2011, 19(3):117-129.

7. Steeg PS: Tumor metastasis: mechanistic insights and clinical challenges. Nature medicine 2006, 12(8):895-904.

8. Barker HE, Cox TR, Erler JT: The rationale for targeting the LOX family in cancer. Nature reviews Cancer 2012, 12(8):540-552.

9. Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, Le QT, Giaccia AJ: Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. Cancer cell 2009, 15(1):35-44.

10. Perryman L, Erler JT: Lysyl oxidase in cancer research. Future oncology 2014, 10(9):1709-1717.
11. Bais MV, Ozdener GB, Sonenshein GE, Trackman PC: Effects of tumor-suppressor lysyl oxidase propeptide on prostate cancer xenograft growth and its direct interactions with DNA repair pathways. Oncogene 2015, 34(15):1928-1937.

12. Baker AM, Cox TR, Bird D, Lang G, Murray GI, Sun XF, Southall SM, Wilson JR, Erler JT: The role of lysyl oxidase in SRC-dependent proliferation and metastasis of colorectal cancer. Journal of the National Cancer Institute 2011, 103(5):407-424.

13. Barker HE, Chang J, Cox TR, Lang G, Bird D, Nicolau M, Evans HR, Gartland A, Erler JT: LOXL2-mediated matrix remodeling in metastasis and mammary gland involution. Cancer research 2011, 71(5):1561-1572.

14. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, Ferrari M, Egevad L, Rayford W, Bergerheim U et al.: Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proceedings of the National Academy of Sciences of the United States of America 2004, 101(3):811-816.

15. Erler JT, Giaccia AJ: Lysyl oxidase mediates hypoxic control of metastasis. Cancer research 2006, 66(21):10238-10241.

16. Csiszar K, Mariani TJ, Gosin JS, Deak SB, Boyd CD: A restriction fragment length polymorphism results in a nonconservative amino acid substitution encoded within the first exon of the human lysyl oxidase gene. Genomics 1993, 16(2):401-406.

17. Kagan HM, Li W: Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. Journal of cellular biochemistry 2003, 88(4):660-672.

18. Lysyl Oxidase. LOX Lysyl Oxidase (Homo Sapiens (Human)) Gene ID: 4015, updated on 5-Jan-2020 (accessed on 23 January 2020). [http://www.ncbi.nlm.nih.gov/gene/4015]

19. Han S, Feng S, Yuan G, Dong T, Gao D, Liang G, Wei X: Lysyl oxidase genetic variants and the prognosis of glioma. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 2014, 122(3):200-205.

20. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non randomised studies in meta-analyses [: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp]

21. Faul F, Erdfelder E, Lang AG, Buchner A: G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods 2007, 39(2):175-191.

22. Peng S, Lu B, Ruan W, Zhu Y, Sheng H, Lai M: Genetic polymorphisms and breast cancer risk: evidence from meta-analyses, pooled analyses, and genome-wide association studies. Breast cancer research and treatment 2011, 127(2):309-324.

23. Tharabenjasin P, Pabalan N, Jarjanazi H: Association of the ACTN3 R577X (rs1815739) polymorphism with elite power sports: A meta-analysis. PloS one 2019, 14(5):e0217390.

24. Higgins JP, Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med 2002, 21(11):1539-1558.

25. Higgins JP: Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. International journal of epidemiology 2008, 37(5):1158-1160.

26. Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. Bmj 2003, 327(7414):557-560.

27. DerSimonian R, Laird N: Meta-analysis in clinical trials. Control Clin Trials 1986, 7(3):177-188.

28. Mantel N, Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. Journal of the National Cancer Institute 1959, 22(4):719-748.

29. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Journal of clinical epidemiology 2009, 62(10):1006-1012.
30. Friesenhengst A, Pribitzer-Winner T, Schreiber M: Association of the G473A polymorphism and expression of lysyl oxidase with breast cancer risk and survival in European women: a hospital-based case-control study. *PloS one* 2014, 9(8):e105579.

31. Min C, Yu Z, Kirsch KH, Zhao Y, Vora SR, Trackman PC, Spicer DB, Rosenberg L, Palmer JR, Sonenshein GE: A loss-of-function polymorphism in the propeptide domain of the LOX gene and breast cancer. *Cancer research* 2009, 69(16):6685-6693.

32. Ren J, Wu X, He W, Shao J, Cheng B, Huang T: Lysyl oxidase 473 G>A polymorphism and breast cancer susceptibility in Chinese Han population. *DNA and cell biology* 2011, 30(2):111-116.

33. Jia J, Tian Q, Liu Y, Shao ZW, Yang SH: Interactive effect of bisphenol A (BPA) exposure with -22G/C polymorphism in LOX gene on the risk of osteosarcoma. *Asian Pacific journal of cancer prevention : APJCP* 2013, 14(6):3805-3808.

34. Liu Y, Lv B, He Z, Zhou Y, Han C, Shi G, Gao R, Wang C, Yang L, Song H et al: Lysyl oxidase polymorphisms and susceptibility to osteosarcoma. *PloS one* 2012, 7(7):e41610.

35. Shi W, Yang B, Li X, Sun S, Wang L, Jiao S: The effect of lysyl oxidase polymorphism on susceptibility and prognosis of nonsmall cell lung cancer. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2012, 33(6):2379-2383.

36. Wang G, Shen Y, Cheng G, Bo H, Lin J, Zheng M, Li J, Zhao Y, Li W: Lysyl Oxidase Gene G473A Polymorphism and Cigarette Smoking in Association with a High Risk of Lung and Colorectal Cancers in a North Chinese Population. *International journal of environmental research and public health* 2016, 13(7).

37. Gao X, Zhang S, Zhu Z: Lysyl oxidase rs1800449 polymorphism and cancer risk among Asians: evidence from a meta-analysis and a case-control study of colorectal cancer. *Molecular genetics and genomics : MGG* 2014, 290(1):23-28.

38. Shieh TM, Lin SC, Liu CJ, Chang SS, Ku TH, Chang KW: Association of expression aberrances and genetic polymorphisms of lysyl oxidase with areca-associated oral tumorigenesis. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007, 13(15 Pt 1):4378-4385.

39. Yoon JH, Park JK, Kang YH, Park YK, Nam SW, Lee JY, Park WS: Lysyl oxidase G473A polymorphism is closely associated with susceptibility to gastric cancer in a South Korean population. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica* 2011, 119(11):762-768.

40. Bu M, Li L, Zhang Y, Xu Y, An S, Hou F, Jie X: Lysyl oxidase genetic variants affect gene expression in cervical cancer. *DNA and cell biology* 2014, 33(11):787-792.

41. Wang X, Cong JL, Qu LY, Jiang L, Wang Y: Association between lysyl oxidase G473A polymorphism and ovarian cancer in the Han Chinese population. *The Journal of international medical research* 2012, 40(3):917-923.

42. Wu J, Cai C, Tong D, Hou H: Lysyl oxidase G473A polymorphism is associated with increased risk of ovarian cancer. *Genetic testing and molecular biomarkers* 2012, 16(8):915-919.

43. Yang Y, Liang A, Cui F, Li N, Cong J, Qu L, Jiang H, Li Q, Liu P, Chen Y et al: Lysyl oxidase single-nucleotide polymorphism (SNP) (G473A) is negatively associated with ovarian cancer prognosis. *Int J Clin Exp Med* 2017, 10(12):16595-16602.

44. Shen Y: *Studies on correlation between lysyl oxidase gene polymorphism and lung cancer and colorectal cancer*. Tangshan, PRC: North China University of Science and Technology; 2015.

45. Shen Y, Lin J, Chen G, Wang G, Li W: Correlation between lysyl oxidase gene polymorphism and the risk of colorectal cancer. *Chin J Dig* 2016, 26(1):35-40.
46. Pabalan N, Bapat B, Sung L, Jarjanazi H, Francisco-Pabalan O, Ozcelik H: Cyclin D1 Pro241Pro (CCND1-G870A) polymorphism is associated with increased cancer risk in human populations: a meta-analysis. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2008, 17(10):2773-2781.

47. Albinger-Hegyi A, Stoeckli SJ, Schmid S, Storz M, Iotzova G, Probst-Hensch NM, Rehrauer H, Tinguely M, Moch H, Hegyi I: Lysyl oxidase expression is an independent marker of prognosis and a predictor of lymph node metastasis in oral and oropharyngeal squamous cell carcinoma (OSCC). International journal of cancer 2010, 126(11):2653-2662.

48. Lee YS, Park Y, Kwon M, Roh JL, Choi SH, Nam SY, Kim SY: Expression of Lysyl Oxidase Predictive of Distant Metastasis of Laryngeal Cancer. Otolaryngology-head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery 2017, 156(3):489-497.

49. Umezaki N, Nakagawa S, Yamashita YI, Kitano Y, Arima K, Miyata T, Hiyoshi Y, Okabe H, Nitta H, Hayashi H et al: Lysyl oxidase induces epithelial-mesenchymal transition and predicts intrahepatic metastasis of hepatocellular carcinoma. Cancer science 2019, 110(6):2033-2043.

50. Liu JL, Wei W, Tang W, Jiang Y, Yang HW, Li JT, Zhou X: Silencing of lysyl oxidase gene expression by RNA interference suppresses metastasis of breast cancer. Asian Pacific journal of cancer prevention : APJCP 2012, 13(7):3507-3511.

51. Miller BW, Morton JP, Pinese M, Saturno G, Jamieson NB, McGhee E, Timpson P, Leach J, McGarry L, Shanks E et al: Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. EMBO molecular medicine 2015, 7(8):1063-1076.

52. Osawa T, Ohga N, Akiyama K, Hida Y, Kitayama K, Kawamoto T, Yamamoto K, Maishi N, Kondoh M, Onodera Y et al: Lysyl oxidase secreted by tumour endothelial cells promotes angiogenesis and metastasis. British journal of cancer 2013, 109(8):2237-2247.

53. Zhang J, Zhang L, Li C, Yang C, Li L, Song S, Wu H, Liu F, Wang L, Gu J: LOX-1 is a poor prognostic indicator and induces epithelial-mesenchymal transition and metastasis in pancreatic cancer patients. Cellular oncology 2018, 41(1):73-84.

54. Fraga A, Ribeiro R, Coelho A, Vizcaino JR, Coutinho H, Lopes JM, Principe P, Lobato C, Lopes C, Medeiros R: Genetic polymorphisms in key hypoxia-regulated downstream molecules and phenotypic correlation in prostate cancer. BMC urology 2017, 17(1):12.

55. Taylor MA, Amin JD, Kirschmann DA, Schiemann WP: Lysyl oxidase contributes to mechanotransduction-mediated regulation of transforming growth factor-beta signaling in breast cancer cells. Neoplasia 2011, 13(5):406-418.

Tables

Table 1 Characteristics of the included articles that examined lysyl oxidase polymorphism associations with cancer
| In Gao | Cancer group | [R] | Year | Country | Ethnic Group | Patients age * (years) | LOXSNP | Primary tumor site | Study-specific outcome for var genotypte OR (95% CI) | 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 | 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 10 | 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 11 | Yoon | [39] | 2011 | South Korea | Asian | 60 (22-91) | 473G/A | Stomach | 1.47 (1.09-1.98) < 0.05 AA | Healthy | RFLP | NM | 5 |
| GYC (4) | | | | | | | | | | | | | | |
| No 12 | 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 13 | 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 14 | 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 15 | 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*: 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

| Cancer group | Genetic model | n | OR  | 95% CI  | CID  | P*  | P_HET | I² (%) | 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*: P-value for association; P_HET: P-value for heterogeneity; I² 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 | 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 | 0.0001 | 0 | Robust |
| Codominant* | 4 | 1.6 | 0.49 | 0.0001 | 0 | Robust |
| Homozygous | 4 | 2.7 | 1.78 | 0.0001 | 0 | Robust |
| Recessive | 4 | 2.5 | 1.63 | 0.0001 | 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 [I^2 = 0%].

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

|                      | This study | Geo 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 |

I oxidase; G: guanine; A: adenine; C: cytosine; CNKI: China National Knowledge Infrastructure; I^2: measure of variability; NOS: Newcastle-Ottawa Scale; HWE: Hardy-Weinberg Equilibrium