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Association of rs401681 (C > T) and rs402710 (C > T) polymorphisms in the CLPTM1L region with risk of lung cancer: a systematic review and meta-analysis

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Although many genome-wide association studies (GWAS) have confirmed the negative associations between rs401681[T] / rs402710[T] in the Cleft lip and cleft palate transmembrane protein 1 (CLPTM1L) region and lung cancer (LC) susceptibility in Caucasian and Asian populations, some other studies haven't found these negative associations. The purpose of this study is to clarify the associations between them and LC, as well as the differences in these associations between patients of different ethnicities (Caucasians and Asians), LC subtypes and smoking status. Relevant literatures published before July 7, 2023 in PubMed, EMbase, Web of Science, MEDLINE were searched through the Internet. Statistical analysis of data was performed in Revman 5.3, including drawing forest plots, funnel plots and so on. Sensitivity and publication bias were performed in Stata 14.0. TSA software was performed for the trial sequential analysis (TSA) tests to assess the stability of the results. Registration number: CRD42023407890. A total of 41 literatures (containing 44 studies: 16 studies in Caucasians and 28 studies in Asians) were included in this meta-analysis, including 126476 LC patients and 191648 healthy controls. The results showed that the T allele variants of rs401681 and rs402710 were negatively associated with the risk of LC (rs401681[T]: [OR] = 0.87, 95% CI [0.86, 0.88]; rs402710[T]: [OR] = 0.88, 95% CI [0.86, 0.89]), and the negative associations were stronger in Caucasians than in Asians (Subgroup differences: $I^2 > 50\%$). In LC subtypes, the rs401681[T] was negatively associated with the risk of Non-small-cell lung carcinoma (NSCLC), Lung adenocarcinoma (LUAD) and Lung squamous cell carcinoma (LUSC) ($P < 0.05$), and these negative associations were stronger in Caucasians than in Asians (Subgroup differences: $I^2 > 50\%$). The rs402710[T] was negatively associated with the risk of NSCLC, LUAD and LUSC ($P < 0.05$), and these negative associations in Caucasians were the same as in Asians (Subgroup differences: $I^2 < 50\%$). The rs401681[T] was negatively associated with the risk of LC in both smokers and non-smokers ($P < 0.05$), and the negative association for smokers equals to that of non-smokers (Subgroup differences: $P = 0.25$, $I^2 = 24.2\%$). In LC subtypes, the rs401681[T] was negatively associated with the risks of NSCLC and LUAD in both Caucasian smokers and Asian non-smokers ($P < 0.05$). The rs402710[T] was negatively associated with the risk of LC in both smokers and non-smokers ($P < 0.05$), and there was no difference in the strength of this negative risk association between them in Caucasians (Subgroup differences: $I^2 = 0\%$). In Asians, this negative association was found to be predominantly among smokers ([OR] = 0.80, 95%CI [0.65, 0.99]). In LC subtypes, the rs402710[T]was negatively associated with the risk of NSCLC in non-smokers, and this negative association was found to be predominantly among non-smokers in Asians ([OR] = 0.75, 95%CI [0.60, 0.94]). The T allele variants of rs401681 and rs402710 are both negatively associated with the risk of developing LC, NSCLC (LUAD, LUSC) in the Caucasian and Asian populations, and the negative associations with the risk of LC are higher in Caucasians. Smoking is an important risk factor for inducing the rs401681 and rs402710 variants and causes LC development in both populations. Other factors like non-smoking are mainly responsible for inducing the development of NSCLC in Asians, and is concentrated in LUAD among Asian non-smoking women.

Keywords CLPTM1L, Polymorphism, Lung cancer, Meta-analysis

Abbreviations

LC	Lung cancer
GWAS	Genome-wide association studies
TERT	Telomerase reverse transcriptase
CLPTM1L	Cleft lip and cleft palate transmembrane protein 1
OR	Odds ratio
95% CI	95% Confidence interval
HWE	Hardy–Weinberg equilibrium
NOS	Newcastle Ottawa scale
TSA	Trial sequential analysis
NSCLC	Non-small-cell lung carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
SCLC	Small cell lung carcinoma
LD	Linkage disequilibrium
BMI	Body mass index
COPD	Chronic obstructive pulmonary disease

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Lung cancer (LC) is one of the cancers with a high mortality rate in the world, accounting for approximately one quarter of all cancer deaths[1]. And smoking was currently considered to be a major risk factor for it[2]. However, it's not only smoking that contributes to LC susceptibility, but also environmental factors, genetic differences, and so on. Over the past two decades, multi-population Genome-wide association studies (GWAS) has identified dozens of risk loci for LC, and most of these loci are concentrated in Telomerase reverse transcriptase—Cleft lip and palate transmembrane protein 1 (TERT-CLPTM1L) region[3–11].

Located in the chromosome 5p15.33 region, CLPTM1L (alias CRR9) is responsible for encoding the transmembrane 1-like protein associated with cleft lip and palate. Overexpression of CLPTM1L has been observed in LC cells[12,13], and its function and role in the development of LC are remained unclear. Previous studies have reported that CLPTM1L, a common overexpressed anti-apoptotic factor in LC, protects cells from genotoxic stress-induced apoptosis by modulating Bcl-xL, suggesting that it has inhibitory effects in genotoxic stress-induced apoptosis[13]. Later studies have also reported that CLPTM1L is overexpressed and resistant to cisplatin in human ovarian tumor cell lines, and that overexpression in LC cells prevents genotoxic stress-induced apoptosis[14–16]. These evidences suggest that it has anti-apoptotic properties.

In previous large-scale GWAS, several loci in CLPTM1L has been found to be associated with the risk of developing LC, the most frequently reported of which are rs401681 and rs402710[5,6,17–22]. These studies have shown that their minor alleles (rs401681[T], rs402710[T]) are negatively associated with LC risk, meaning that these minor alleles reduce the risk of LC. In contrast, their major alleles (rs401681[C], rs402710[C]) are positively associated with LC risk and are risk alleles for LC. These variants have been reported to be able to influence telomere length and lead to cancer risk[23]. Although many GWAS have confirmed the negative risk associations between rs401681[T]/rs402710[T] and LC susceptibility, most of these GWAS were conducted in the same ethnicity (either just in Caucasians[5,17,21] or just in Asians[6,18–20]), and negative risk associations were not found between rs401681[T] and rs402710[T] in some previous GWAS of Asian populations: Dong J 2017[10]: rs401681 (T vs.C): OR = 0.77, 95%CI[0.58, 1.03]; Shiraishi K 2012[24]: rs401681 (T vs.C): OR = 1.04, 95%CI[0.92, 1.17], rs402710 (T vs.C): OR = 0.99, 95%CI[0.91, 1.08]. In addition, although the negative risk associations between rs401681[T]/rs402710[T] and LC were found in some case–control studies of Caucasian[11,25–27] or Asian[28–31] populations, there was no such negative risk associations between them and LC were found in some other case–control studies in Caucasian[32–34] or Asian[35–40] populations: Caucasians: Zienolddiny S 2009[33]: rs401681(T vs.C): OR = 0.84, 95%CI[0.69, 1.03], rs402710 (T vs.C): OR = 0.82 [0.67, 1.01]; Tseng TS 2014[34]: rs402710 (T vs.C): OR = 0.99, 95%CI[0.85, 1.14] / Asians: Bae EY 2012[37]: rs401681(T vs.C): OR = 0.93, 95%CI[0.82, 1.06], rs402710 (T vs.C): OR = 0.91, 95%CI[0.80, 1.04]; Liang Y 2014[38]: rs401681(T vs.C): OR = 0.86, 95%CI[0.68, 1.10], rs402710 (T vs.C): OR = 0.83, 95%CI[0.65, 1.06]; Zhao Z 2013[39]: rs402710 (T vs.C): OR = 1.00, 95%CI[0.86, 1.16]; Sun Y 2013[40]: rs401681(T vs.C): OR = 0.95, 95%CI[0.71, 1.29].The reasons for these different results may still be related to different ethnicities, countries, study methods, sample sizes, LC subtypes, patterns of linkage imbalance and smoking/non-smoking. Therefore, there is a lack of unified conclusions about the associations of these risk loci with LC in Caucasian and Asian populations. However, meta-analysis is sufficient to address these issues[41]. Although a number of meta-analyses[42,43] have been conducted, they haven't included comprehensive literatures due to the length of time that has elapsed. In addition, Tian et al[44] recently conducted a large-scale meta-analysis on the risk association of various cancers and confirmed that eight loci in the TERT-CLPTM1L region, including rs401681 and rs402710, were associated with the risk of LC, but they didn't conduct an in-depth analysis of various LC subtypes. In addition, they didn't seem to report the effect of environmental factors such as smoking on LC which is a major risk factor for the disease[2]. Therefore, an updated, more comprehensive, in-depth and targeted meta-analysis is very necessary.

This study included data from GWAS and case–control studies that have so far reported the associations of CLPTM1L rs401681, rs402710 with LC in Caucasian or Asian populations, with the aim of clarifying the associations between them and LC and the differences in these associations between patients of different ethnicities (Caucasian and Asian populations), LC subtypes and smoking status. We performed systematic

review and meta-analysis through the processes of literature searching, screening, cross-checking and quality assessment, data extraction, and statistical analysis.

Data and methods

This study has been registered in PROSPERO (https://www.crd.york.ac.uk/prospero/), registration number: CRD42023407890.

Inclusion and exclusion criteria

Inclusion criteria

① The type of studies should be GWAS or case–control studies on CLPTM1L rs401681 (C > T) or rs402710 (C > T) polymorphisms and susceptibility to LC as these types of studies contained the available data for the current study; ② The language of these studies should be English because most high quality studies were published in English; The ethnicity of the population should be Caucasians or Asians as these two populations have been studied the most, and it could affect the accuracy of the results of this study if the number of studies was too small; The method of genetic testing should be accurately described because the method of genetic testing were credible only if it is described in detail; ③ Genotypic data should be used to calculate the Odds ratios (OR) and 95% confidence intervals (95% CI) of allele as it's necessary for the statistics of the data in this study; ④ The distribution of genotype frequency of all controls conforms to Hardy–Weinberg (HWE)[45], as only this criterion could be met to demonstrate that the selection of controls is representative of the population and that the sample is in a state of equilibrium with random assignment and in a broader population; ⑤The score of Newcastle Ottawa scale (NOS)[46] should be no less than 7 (≥7), as less than 7 (<7) is classified as a low quality study, which could affect the overall quality of this study.

Exclusion criteria

① Studies with incomplete allele data, as these studies did not have usable data; ② Studies of the types of reviews, meta-analyses, conference reports and case reports, as these studies didn't have usable data; ③ Studies with pedigree as the reporting object, as the data from these studies may bias the results of the current study; ④ Same studies have published for multiple times, only the one with the most complete data should be included, as only the results of the study with the most complete data are representative.

Outcomes

The pre-specified primary outcomes were to investigate whether the rs401681[T], rs402710[T] polymorphisms decreased the risk of LC in the overall populations (Caucasians and Asians).

The secondary outcomes were to confirm whether there were differences in the strength of the negative associations of the rs401681[T], rs402710[T] polymorphisms with LC (including various LC subtypes) between Caucasian and Asian populations or between smokers and non-smokers.

Retrieval strategy

Relevant literatures on CLPTM1L rs401681 (C > T), rs402710 (C > T) polymorphisms and LC susceptibility in PubMed, EMbase, Web of Science, MEDLINE databases published before July 7, 2023 were searched by theme words and keywords. The language was limited to English. Search terms (Table S1 in supplemental content): "rs401681" OR "rs402710" AND "polymorphism" AND "Lung cancer" OR "LC". Manual retrieval and literature tracing methods were also used to expand the searching scope at the same time.

Literature screening and data extraction

Two relatively independent researchers (X-ZW and WL) completed literature searching and screening according to the inclusion criteria, and they cross checked and discussed them afterwards. For the literature with different opinions, the third party (Y-ZC) made the decision. For some literatures with incomplete data, they tried to contact the author by e-mail to obtain the complete data. Finally, data extraction was carried out for the literature being chosen after the final decision. These data include: author, year of publication, country, participants' ethnicity, gender, age, and smoking distribution, type of LC, number of cases in LC and control groups, frequency of each genotype in LC and control groups, and the OR and 95% CI of each genotype.

Literature quality assessment

The quality of the included literatures was assessed in the NOS[46] (X-ZW and WL), and those with a score of no less than 7 (≥7) were considered as literatures with high-quality.

Statistical methods

The HWE of the genotypes of the controls was detected by Pearson's chi-square test in SPSS 24.0 software. Statistics and analysis were carried out on the genetic model data of all polymorphisms (allele, additive, heterozygous, dominant, recessive) in Revman5.3 software, including drawing forest map and funnel plot. When there was no heterogeneity among all studies ($P > 0.1$ or $I^2 < 50\%$), the fixed-effects model was used for statistical analysis; otherwise, the random-effects model was used for statistical analysis. The effect size and effect value of the statistical results were presented by OR value and 95% CI. Begg's Test and Egger's Test were performed in Stata 14.0 software to assess publication bias among studies, and sensitivity analysis was performed to assess the results of statistical analysis with greater heterogeneity. TSA 0.9.5.10 software was performed for the trial sequential analysis (TSA) tests to evaluate the stability of the results ([Type I error] probability = 5%, statistical test power = 80%, relative risk reduction = 20%).

Results

Literature search results

A total of 787 literatures were initially detected in 4 databases, and 41 literatures were finally included after screening[5,6,10,11,17–22,24–31,33,34,37–40,47–63], and a flow diagram was prepared based on the PRISMA statement[64] (Fig. 1). A total of 44 studies (12 GWAS and 32 case–control studies containing 126476 LC patients and 191648 healthy controls) were included, including 16 studies of Caucasians (containing 87780 LC patients) and 28 studies of Asians (containing 38696 LC patients) (Table S2 in supplemental content). More detailed data of the 2 polymorphisms were shown in Table S3-S4 in supplemental content.

Quality evaluation

All 44 studies had high NOS[46] assessment scores (≥7), indicating that they were all at low risk of bias (Table S5 in supplemental content).

Meta-analysis

There was a significant negative association between the T allele variant (allele model) in rs401681 and LC risk ([OR] = 0.87, 95% CI [0.86, 0.88]), also in Caucasians ([OR] = 0.86, 95% CI [0.85, 0.88]) and Asians ([OR] = 0.90, 95% CI [0.87, 0.92]). Comparing the two ethnicities, it was found that the negative association of LC in Caucasians was more than in Asians (Caucasians: [OR] = 0.86 /Asians: [OR] = 0.90; Subgroup differences: P = 0.01, I^2 = 84.8%) (Table 1, Fig. 2). Further analysis found that the other four genetic models (additive, heterozygous, dominant, recessive) of rs401681[T] were negatively associated with the risk of LC (P < 0.05), and these negative associations existed in both Caucasians and Asians (P < 0.05) (Table S6 and Figures S1–S4 in supplemental content).

In LC of different ethnicities/pathological subtypes, the T allele variant in rs401681 was negatively associated with the risk of NSCLC in the overall populations ([OR] = 0.86, 95%CI [0.85, 0.87]), and the same results were also shown in the Caucasians ([OR] = 0.85, 95%CI [0.84, 0.87]) and Asians ([OR] = 0.89, 95%CI [0.86, 0.92]). Comparison between subgroups found that the T allele variant was more negatively associated with NSCLC in Caucasians than in Asians (Caucasians: [OR] = 0.85 /Asians: [OR] = 0.89; Subgroup differences: P = 0.01, I^2 = 83.9%) (Table 1, Figure S5 in supplemental content). For SCLC, the T allele variant was not associated with the risk of SCLC in Asians ([OR] = 0.98, 95%CI [0.79, 1.20]) (Table 1, Figure S6 in supplemental content). In the two subtypes of NSCLC, the T allele variant was negatively associated with the risk of LUAD in the overall populations ([OR] = 0.87, 95%CI [0.85, 0.88]), and the same results were also shown in Caucasians ([OR] = 0.85, 95%CI [0.84, 0.87]) and Asians ([OR] = 0.89, 95%CI [0.86, 0.92]).Comparison between subgroups found that the T allele variant had a higher negative association with LUAD in Caucasians than in Asians (Caucasians: [OR] = 0.85 /Asians: [OR] = 0.89; Subgroup differences: P = 0.04, I^2 = 76%) (Table 1, Figure S7 in supplemental content). The T allele variant was negatively associated with the risk of LUSC in the overall

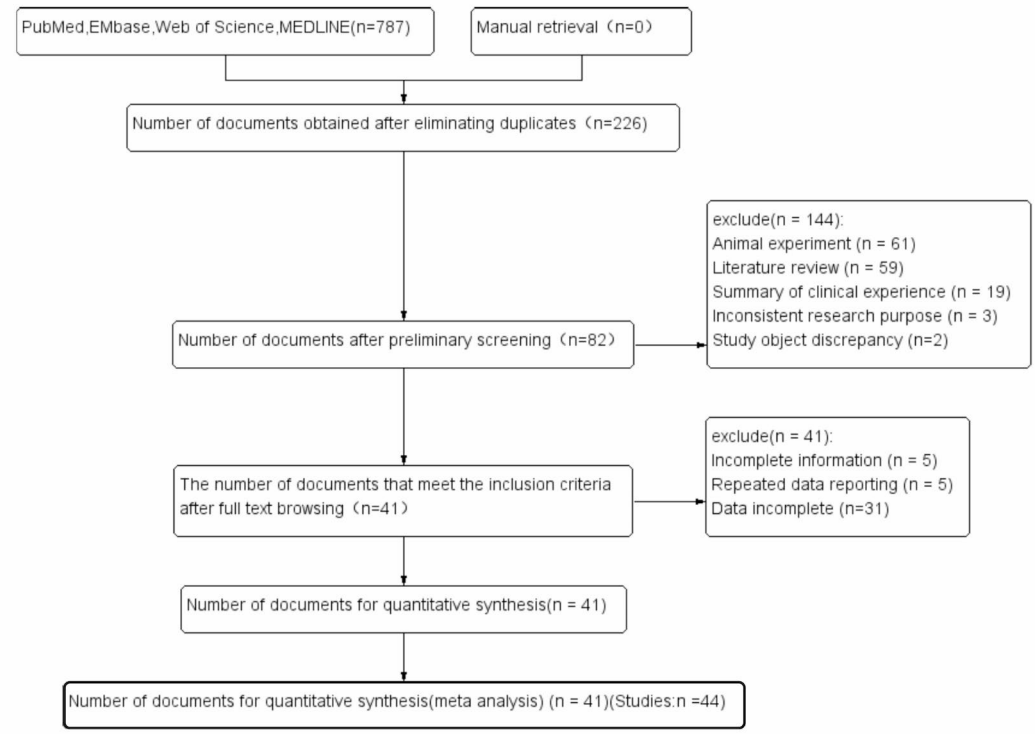

Fig. 1. The flow diagram of PRISMA literature screening.

LC subtypes	Subgroups	Studies (n)	Heterogeneity test		Sample		Model	OR[95% CI]	Effect p value	Subgroup differences		Publication bias	
			P values	I^2(%)	Cases (n)	Controls (n)				P values	I^2(%)	P_{Begg}	P_{Egger}
LC	Caucasians	12	0.67	0	1,51,738	2,67,494	Fixed	**0.86 [0.85, 0.88]**	**< 0.00001**	–	–	0.583	0.326
	Asians	22	0.03	39	62,844	75,042	Fixed	**0.90 [0.87, 0.92]**	**<0.00001**	–	–	0.324	0.531
	Overall	34	0.03	33	2,14,582	3,42,536	Fixed	**0.87 [0.86, 0.88]**	**<0.00001**	**0.01**	**84.8**	0.262	0.226
NSCLC[a]	Caucasians	9	0.71	0	76,812	3,31,746	Fixed	**0.85 [0.84, 0.87]**	**<0.00001**	–	–	0.835	0.765
	Asians	15	0.13	30	36,438	65,266	Fixed	**0.89 [0.86, 0.92]**	**<0.00001**	–	–	0.216	0.371
	Overall	24	0.11	28	113,250	3,97,012	Fixed	**0.86 [0.85, 0.87]**	**<0.00001**	**0.01**	**83.9**	0.227	0.398
SCLC	Asians	2	0.38	0	476	2880	Fixed	0.98 [0.79, 1.20]	0.82	–	–	–	–
LUAD[b]	Caucasians	5	0.64	0	48,776	1,72,996	Fixed	**0.85 [0.84, 0.87]**	**<0.00001**	–	–	0.327	0.05
	Asians	9	0.14	35	29,828	47,894	Fixed	**0.89 [0.86, 0.92]**	**<0.00001**	–	–	1.000	0.84
	Overall	14	0.12	31	78,604	2,20,890	Fixed	**0.87 [0.85, 0.88]**	**<0.00001**	**0.04**	**76**	0.701	0.003
LUSC	Caucasians	2	0.74	0	27,066	1,57,600	Fixed	**0.84 [0.82, 0.87]**	**<0.00001**	–	–	0.317	–
	Asians	6	0.31	16	4458	20,874	Fixed	**0.91 [0.85, 0.98]**	**0.01**	–	–	0.348	0.327
	Overall	8	0.2	29	31,524	1,78,474	Fixed	**0.85 [0.83, 0.87]**	**<0.00001**	**0.05**	**73.3**	0.268	0.264

Table 1. The results of meta-analysis and publication bias (rs401681: Allele genetic model, T vs.C). LC:Lung cancer;NSCLC:Non-small-cell lung carcinoma;SCLC:Small-cell lung carcinoma;LUAD:Lung adenocarcinoma;LUSC:Lung squamous cell carcinoma. [a]NSCLC(Overall) vs. SCLC(Asians): Test for subgroup differences: $Chi^2 = 1.45$, df = 1 (P = 0.23), $I^2 = 31.2\%$; NSCLC(Asians) vs. SCLC(Asians): Test for subgroup differences: $Chi^2 = 0.79$, df = 1 (P = 0.37), $I^2 = 0\%$. [b]LUAD(Overall) vs. LUSC(Overall): Test for subgroup differences: $Chi^2 = 1.18$, df = 1 (P = 0.28), $I^2 = 15.1\%$; LUAD(Caucasians) vs. LUSC(Caucasians): Test for subgroup differences: $Chi^2 = 0.66$, df = 1 (P = 0.42), $I^2 = 0\%$;. LUAD(Asians) vs. LUSC(Asians): Test for subgroup differences: $Chi^2 = 0.25$, df = 1 (P = 0.62), $I^2 = 0\%$. Significant are in value [bold].

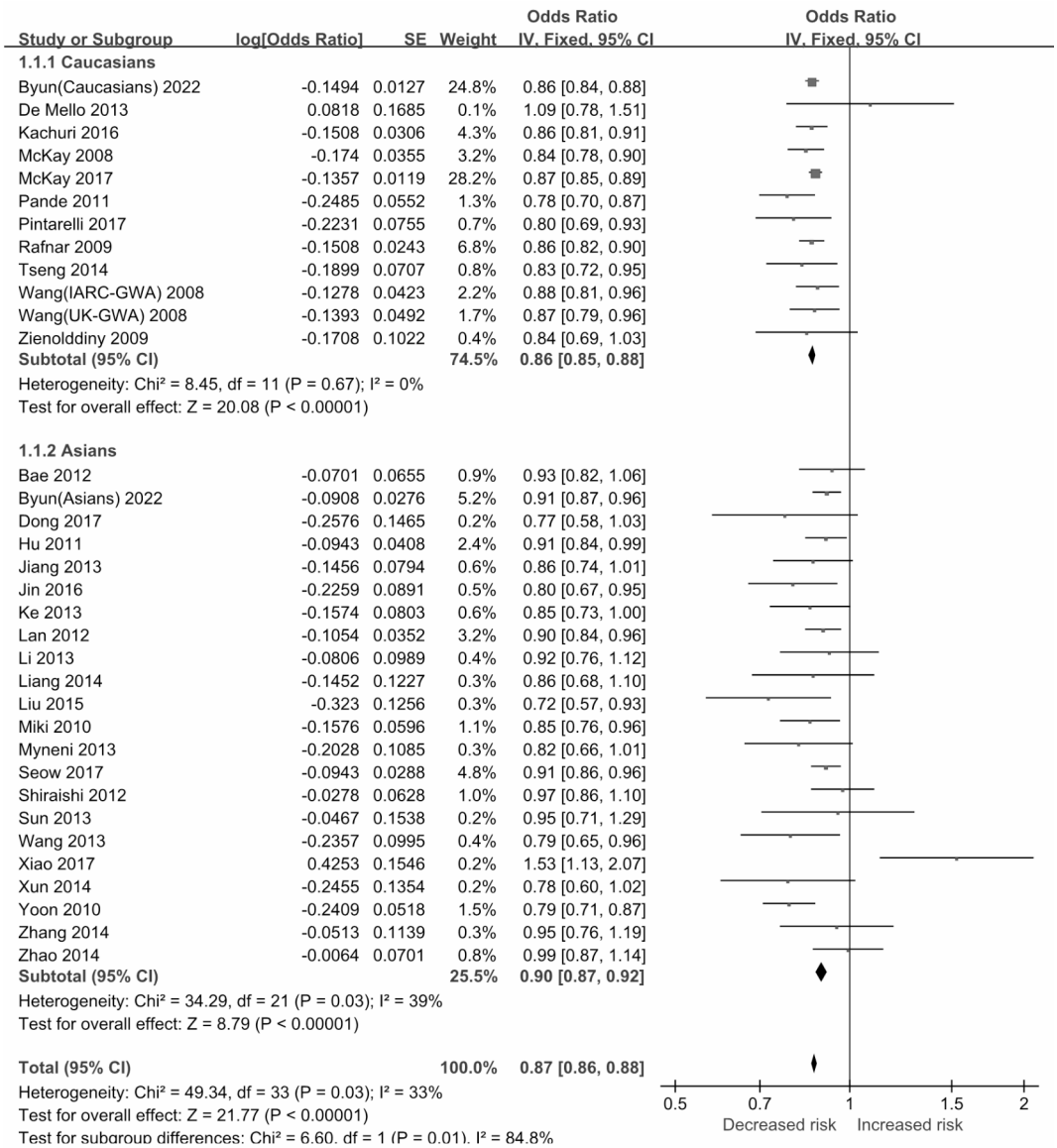

Fig. 2. Forest plot of the association between rs401681 (T vs.C) and LC.

populations ([OR] = 0.85, 95%CI [0.83, 0.87]), also in Caucasians ([OR] = 0.84, 95% CI [0.82, 0.87]) and Asians ([OR] = 0.91, 95% CI [0.85, 0.98]). Comparing the two ethnicities, it was found that the negative association of LC in Caucasians was more than in Asians (Caucasians: [OR] = 0.84 /Asians: [OR] = 0.91; Subgroup differences: P = 0.05, I^2 = 73.3%) (Table 1, Figure S8 in supplemental content). Comparing the strength of the association between LUAD and LUSC, the negative association between the T allele variant and LUAD was the same as that of LUSC in the overall populations (LUAD: [OR] = 0.87 /LUSC: [OR] = 0.85; Subgroup differences: P = 0.28, I^2 = 15.1%), also in Caucasians (LUAD: [OR] = 0.85 /LUSC: [OR] = 0.84; Subgroup differences: P = 0.42, I^2 = 0%) and Asians (LUAD: [OR] = 0.89 /LUSC: [OR] = 0.91; Subgroup differences: P = 0.62, I^2 = 0%) (Table 1).

There was a significant negative association between the T allele variant (allele model) in rs402710 and LC risk ([OR] = 0.88, 95% CI [0.86, 0.89]), and this negative association occurred in both Caucasians ([OR] = 0.87, 95% CI [0.85, 0.88]) and Asians ([OR] = 0.90, 95% CI [0.87, 0.92]). Comparing the two ethnicities, it was found that the negative association of LC in Caucasians was more than in Asians (Caucasians: [OR] = 0.87 /Asians: [OR] = 0.90; Subgroup differences: P = 0.07, I^2 = 69.8%) (Table 2, Fig. 3). Further analysis found that the other four genetic models (additive, heterozygous, dominant, recessive) of rs402710[T] had the negative associations with the risk of LC (P < 0.05), and these negative associations existed in both Caucasians and Asians (P < 0.05) (Table S7 and Figures S9–S12 in supplemental content).

LC subtypes	Subgroups	Studies (*n*)	Heterogeneity test		Sample		Model	OR[95% Cl]	Effect *p* value	Subgroup differences		Publication bias	
			P values	I^2(%)	Cases (*n*)	Controls (*n*)				*P* values	I^2(%)	P_{Begg}	P_{Egger}
LC	Caucasians	11	0.27	18	103,636	198,750	Fixed	**0.87 [0.85, 0.88]**	**<0.00001**	–	–	0.697	0.524
	Asians	14	0.08	37	39,834	50,446	Fixed	**0.90 [0.87, 0.92]**	**<0.00001**	–	–	0.014	0.066
	Overall	25	0.05	34	143,470	249,196	Fixed	**0.88 [0.86, 0.89]**	**<0.00001**	**0.07**	**69.8**	0.073	0.079
NSCLC[a]	Caucasians	9	0.42	2	60,872	261,754	Random	**0.87 [0.85, 0.89]**	**<0.00001**	–	–	0.835	0.9
	Asians	9	0.04	51	23,988	55,660	Random	**0.89 [0.84, 0.94]**	**<0.0001**	–	–	0.677	0.582
	Overall	18	0.07	35	84,860	317,414	Random	**0.88 [0.85, 0.90]**	**<0.00001**	0.48	0	0.883	0.7
SCLC	Overall	2	0.9	0	2422	21,376	Random	1.00 [0.92, 1.10]	0.96	–	–		
LUAD[b]	Caucasians	3	0.11	55	28,070	67,144	Random	**0.89 [0.84, 0.94]**	**<0.0001**	–	–	0.602	0.823
	Asians	5	0.02	67	17,932	34,324	Random	**0.88 [0.81, 0.96]**	**0.002**	–	–	0.624	0.63
	Overall	8	0.02	58	46,002	101,468	Random	**0.89 [0.85, 0.93]**	**<0.00001**	0.87	0	0.824	0.813
LUSC	Caucasians	3	0.82	0	31,074	175,996	Random	**0.86 [0.83, 0.88]**	**<0.00001**	–	–	0.602	0.767
	Asians	4	0.61	0	4656	23,658	Random	**0.90 [0.84, 0.97]**	**0.008**	–	–	0.497	0.117
	Overall	7	0.69	0	35,730	199,654	Random	**0.86 [0.84, 0.88]**	**<0.00001**	0.19	41.3	0.393	0.017

Table 2. The results of meta-analysis and publication bias (rs402710: Allele genetic model, T vs.C). LC: Lung cancer; NSCLC: Non-small-cell lung carcinoma; SCLC: Small-cell lung carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma. [a]NSCLC(Overall) vs. SCLC(Overall): Test for subgroup differences: $Chi^2 = 7.97$, df = 1 (**P = 0.005**),**I^2= 87.4%**. [b]LUAD(Overall) vs. LUSC(Overall): Test for subgroup differences: $Chi^2 = 1.11$, df = 1 ($P = 0.29$), II^2= 10.0%; LUAD(Caucasians) vs. LUSC(Caucasians): Test for subgroup differences: $Chi^2 = 1.13$, df = 1 ($P = 0.29$), I^2= 11.7%; LUAD(Asians) vs. LUSC(Asians): Test for subgroup differences: $Ch^2 = 0.21$, df = 1 ($P = 0.65$), I^2= 0%. Significant are in value [bold].

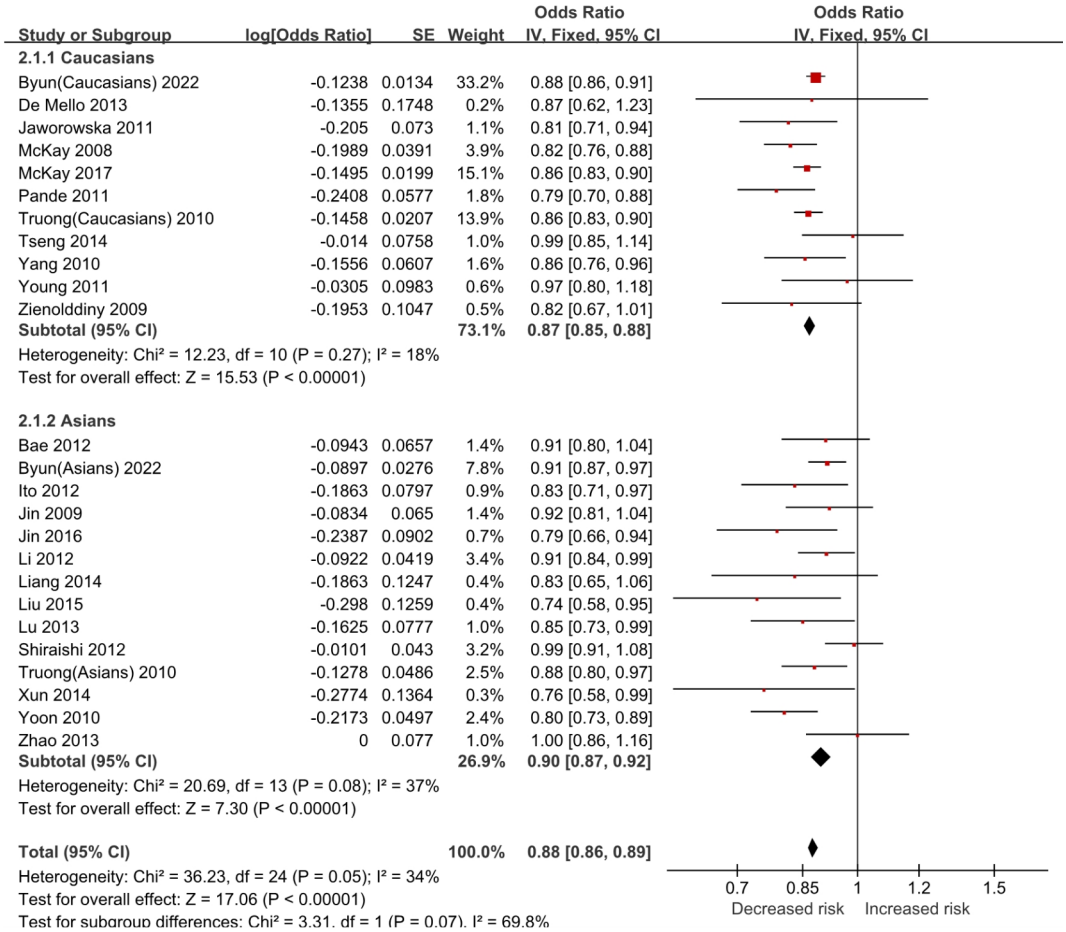

Fig. 3. Forest plot of the association between rs402710 (T vs.C) and LC.

In LC of different ethnicities/pathological subtypes, the T allele variant (allele model) in rs402710 was negatively associated with the risk of NSCLC in the overall populations ([OR] = 0.88, 95%CI [0.85, 0.90]), and the same results were also shown in the Caucasians ([OR] = 0.87, 95%CI [0.85, 0.89]) and Asians ([OR] = 0.89, 95%CI [0.84, 0.94]). Comparison between subgroups found that the negative association of NSCLC in Caucasians was the same as in Asians (Caucasians: [OR] = 0.87 /Asians: [OR] = 0.89; Subgroup differences: P = 0.48, I^2 = 0%) (Table 2, Figure S13 in supplemental content). For SCLC, the T allele variant was not associated with the risk of SCLC in the overall populations ([OR] = 1.00, 95%CI [0.92, 1.10]) (Table 2, Figure S14 in supplemental content). In the two subtypes of NSCLC, the T allele variant was negatively associated with the risk of LUAD in the overall populations ([OR] = 0.89, 95%CI [0.85, 0.93]), and the same results were also shown in Caucasians ([OR] = 0.89, 95%CI [0.84, 0.94]) and Asians ([OR] = 0.88, 95%CI [0.81, 0.96]). Comparison between subgroups found that the negative association of LUAD in Caucasians was the same as in Asians (Caucasians: [OR] = 0.89/Asians: [OR] = 0.88; Subgroup differences: P = 0.87, I^2 = 0%) (Table 2, Figure S15 in supplemental content). The T allele variant was negatively associated with the risk of LUSC in the overall populations ([OR] = 0.86, 95%CI [0.84, 0.88]), also in Caucasians ([OR] = 0.86, 95% CI [0.83, 0.88]) and Asians ([OR] = 0.90, 95% CI [0.84, 0.97]). Comparing the two ethnicities, it was found that the negative association of LUSC in Caucasians was the same as in Asians (Caucasians: [OR] = 0.86 /Asians: [OR] = 0.90; Subgroup differences: P = 0.19, I^2 = 41.3%) (Table 2, Figure S16 in supplemental content). Comparing the strength of the association between LUAD and LUSC, the negative association between the T allele variant and LUAD was the same as that of LUSC in the overall populations (LUAD: [OR] = 0.89 /LUSC: [OR] = 0.86; Subgroup differences: P = 0.29, I^2 = 10%), also in Caucasians (LUAD: [OR] = 0.89 /LUSC: [OR] = 0.86; Subgroup differences: P = 0.29, I^2 = 11.7%) and Asians (LUAD: [OR] = 0.88 /LUSC: [OR] = 0.90; Subgroup differences: P = 0.65, I^2 = 0%) (Table 2).

Analysis of smoking status

The results showed that the T allele variant in rs401681 was negatively associated with the risk of LC in both smokers ([OR] = 0.83, 95%CI [0.75, 0.91]) and non-smokers ([OR] = 0.89, 95%CI [0.83, 0.95]). Comparing smokers and non-smokers, it was found that the negative association for smokers equals to that of non-smokers (Smoking: [OR] = 0.83/Non-smoking: [OR] = 0.89; Subgroup differences: P = 0.25, I^2 = 24.2%) (Table 3, Fig. 4). In Caucasians, this negative association was found in both smokers ([OR] = 0.86, 95%CI [0.81, 0.92]) and non-smokers ([OR] = 0.71, 95%CI [0.59, 0.86]), and the negative association was stronger for non-smokers than for smokers (Smoking: [OR] = 0.86/Non-smoking: [OR] = 0.71; Subgroup differences: p = 0.06, I^2 = 71.3%) (Table 3, Figure S17 in supplemental content). In Asians, this negative association was found in both smokers ([OR] = 0.58, 95%CI [0.40, 0.85]) and non-smokers ([OR] = 0.91, 95%CI [0.87, 0.95]), and the negative association was stronger for smokers than for non-smokers (Smoking: [OR] = 0.58/Non-smoking: [OR] = 0.91; Subgroup differences: p = 0.02, I^2 = 80.3%) (Table 3, Figure S18 in supplemental content). Comparison between the two populations revealed a stronger negative association for Asian smokers than for Caucasian smokers (Smoking Caucasians: [OR] = 0.86/Smoking Asians: [OR] = 0.58; Subgroup differences: P = 0.05, I^2 = 74.3%), and the negative association was higher among Caucasian non-smokers than Asian non-smokers (Non-smoking Caucasians: [OR] = 0.71/Non-smoking Asians: [OR] = 0.91; Subgroup differences: P = 0.01, I^2 = 83.4%) (Table 3).

Stratified analysis was performed according to the pathological types of LC, and the result showed that the T allele variant in rs401681 was negatively associated with the risk of NSCLC in both Caucasian smokers ([OR] = 0.83, 95%CI [0.72, 0.95]) and Asian non-smokers ([OR] = 0.91, 95%CI [0.86, 0.96]), and there was no difference in the strength of this negative risk association between them (Smoking Caucasians: [OR] = 0.83/Non-smoking Asians: [OR] = 0.91; Subgroup differences: P = 0.22, I^2 = 33.2%) (Table 3, Figure S19 in supplemental content). In the subtypes of NSCLC, the T allele variant was negatively associated with the risk of LUAD in both Caucasian smokers ([OR] = 0.83, 95%CI [0.72, 0.95]) and Asian non-smokers ([OR] = 0.91, 95%CI [0.87, 0.96]), and there was no difference in the strength of this negative risk association between them (Smoking Caucasians: [OR] = 0.83/Non-smoking Asians: [OR] = 0.91; Subgroup differences: P = 0.19, I^2 = 42.5%) (Table 3, Figure S20 in supplemental content). In addition, the T allele variant was not negatively associated with the risk of LUSC in Asian non-smokers ([OR] = 0.72, 95%CI [0.46, 1.11]) (Table 3, Figure S21 in supplemental content).

The results showed that the T allele variant in rs402710 was negatively associated with the risk of LC in both smokers ([OR] = 0.87, 95%CI [0.80, 0.94]) and non-smokers ([OR] = 0.88, 95%CI [0.81, 0.95]), and there was no difference in the strength of this negative risk association between them (Smoking: [OR] = 0.87/

Non-smoking: [OR] = 0.88; Subgroup differences: P = 0.88, I^2 = 0%) (Table 4, Fig. 5). In Caucasians, this negative association was found to be present in both smokers ([OR] = 0.89, 95%CI [0.81, 0.97]) and non-smokers ([OR] = 0.87, 95%CI [0.76, 1.00]), and there was no difference in the strength of this negative risk association between them (Smoking: [OR] = 0.89/Non-smoking: [OR] = 0.87; Subgroup differences: P = 0.8, I^2 = 0%) (Table 4, Figure S22 in supplemental content). In Asians, this negative association was found to be predominantly among smokers ([OR] = 0.80, 95%CI [0.65, 0.99]) rather than non-smokers ([OR] = 0.89, 95%CI [0.79, 1.01]) (Table 4, Figure S23 in supplemental content). The two populations were compared and this negative association was found to be equal for Caucasian smokers to Asian smokers (Smoking Caucasians: [OR] = 0.89/Smoking Asians: [OR] = 0.80; Subgroup differences: P = 0.37, I^2 = 0%), and also for Caucasian non-smokers to Asian non-smokers (Non-smoking Caucasians: [OR] = 0.87/Non-smoking Asians: [OR] = 0.89; Subgroup differences: P = 0.76, I^2 = 0%) (Table 4).

Stratified analysis was performed according to the pathological types of LC, and the result showed that the T allele variant in rs402710 was negatively associated with the risk of NSCLC in non-smokers ([OR] = 0.75, 95%CI [0.60, 0.94]) but not in smokers ([OR] = 0.93, 95%CI [0.83, 1.05]) (Table 4, Figure S24 in supplemental content). In Caucasians, this negative association was found to be absent among smokers ([OR] = 0.99, 95%CI [0.85, 1.14]) (Table 4, Figure S25 in supplemental content). In Asians, this negative association was found to be predominantly among non-smokers ([OR] = 0.75, 95%CI [0.60, 0.94]) but not among smokers ([OR] = 0.82, 95%CI [0.66, 1.02]) (Table 4, Figure S26 in supplemental content).

Heterogeneity analysis

In terms of LC, the results of rs401681 and rs402710 had no obvious heterogeneity (I^2 < 50%) (Tables 1, 2, Table S6-S7 in supplemental content). Among LC subtypes, heterogeneity was predominantly seen in the NSCLC and LUAD of rs402710 (T vs. C) and was mainly concentrated in Asian populations (I^2 > 50%) (Table 2). The reason may be related to the fact that these Asian population studies were conducted in different countries, and there were different research methods and different genetic testing methods. In terms of smoking status, heterogeneity (I^2 > 50%) was mainly presented in the LC results for rs401681 (T vs. C) and rs402710 (T vs. C), which may be related to the presence of different ethnic groups as well as the small number of studies (Tables 3, 4).

Publication bias

Most funnel plots appeared to be symmetric (Figure S27–S30 in supplemental content). In the overall populations, the Egger's test results of LUAD in rs401681 (T vs.C) and LUSC in rs402710 (T vs.C) were biased to a certain extent (P_{Egger} = 0.003 and 0.017), but Begg's test results were not biased (P_{Begg} = 0.701 and 0.393) (Tables 1, 2). In the Asian population, the Begg's test result of LC in rs402710 (T vs.C) was biased to a certain extent (P_{Begg} = 0.014), but not for the Egger's test (P_{Egger} = 0.066) (Table 2). In terms of smoking status, the Egger's test results of LC Smokers in rs401681 (T vs.C) was biased to a certain extent (P_{Egger} = 0.028), but not for the Begg's test (P_{Begg} = 0.174) (Table S8 in supplemental content). All other genetic models were not significantly biased (P_{Begg} > 0.05, P_{Egger} > 0.05) (Tables 1, 2, Table S9 in supplemental content).

LC subtypes	Subgroups	Smoking status	Studies (n)	Heterogeneity test		Sample		Model	OR[95% CI]	Effect p value	Subgroup differences	
				P values	I^2(%)	Cases (n)	Controls (n)				P values	I^2(%)
LC[a]	Overall	Smoking	4	0.06	60	50,536	38,258	Random	**0.83 [0.75, 0.91]**	**0.0002**	–	–
		Non-smoking	5	0.18	36	24,040	23,946	Random	**0.89 [0.83, 0.95]**	**0.0002**	–	–
		Total	9	0.07	44	74,576	62,204	Random	**0.87 [0.83, 0.91]**	**<0.00001**	0.25	24.2
	Caucasians	Smoking	3	0.22	33	50,206	38,076	Random	**0.86 [0.81, 0.92]**	**<0.00001**	–	–
		Non-smoking	1	–	–	924	1016	Random	**0.71 [0.59, 0.86]**	**0.0004**	–	–
		Total	4	0.05	61	51,130	39,092	Random	**0.83 [0.76, 0.90]**	**<0.0001**	**0.06**	**71.3**
	Asians	Smoking	1	–	–	330	182	Random	**0.58 [0.40, 0.85]**	**0.006**	–	–
		Non-smoking	4	0.98	0	23,116	22,930	Random	**0.91 [0.87, 0.95]**	**<0.00001**	–	–
		Total	5	0.26	24	23,446	23,112	Random	**0.90 [0.85, 0.95]**	**0.0004**	**0.02**	**80.3**
NSCLC	Overall	Smoking (Caucasians)	1	–	–	1322	2694	Fixed	**0.83 [0.72, 0.95]**	**0.007**	–	–
		Non-smoking (Asians)	4	0.18	39	12,196	15,098	Fixed	**0.91 [0.86, 0.96]**	**0.0002**	–	–
		Total	5	0.17	37	13,518	17,792	Fixed	**0.90 [0.85, 0.94]**	**<0.0001**	0.22	33.2
LUAD[b]	Overall	Smoking (Caucasians)	1	–	–	1322	2694	Random	**0.83 [0.72, 0.95]**	**0.007**	–	–
		Non-smoking (Asians)	3	0.79	0	11,944	14,026	Random	**0.91 [0.87, 0.96]**	**0.0008**	–	–
		Total	4	0.53	0	13,266	16,720	Random	**0.90 [0.86, 0.95]**	**<0.0001**	0.19	42.5
LUSC	Asians	Non-smoking	2	0.16	49	252	1472	Random	0.72 [0.46, 1.11]	0.14	–	–

Table 3. Meta-analysis results of smoking status (rs401681: Allele genetic model, T vs.C). LC:Lung cancer;NSCLC:Non-small-cell lung carcinoma;SCLC:Small-cell lung carcinoma;LUAD:Lung adenocarcinoma;LUSC:Lung squamous cell carcinoma. [a]LC(Smoking Caucasians) vs. LC(Smoking Asians): Test for subgroup differences: $Chi^2 = 3.90$, df = 1 **($P = 0.05$), $I^2 = 74.3\%$**; LC(Non-smoking Caucasians) vs. LC(Non-smoking Asians): Test for subgroup differences: $Chi^2 = 6.02$, df = 1 **($P = 0.01$), $I^2 = 83.4\%$**. [b]LUAD(Non-smoking Asians) vs. LUSC(Non-smoking Asians): Test for subgroup differences: $Chi^2 = 1.18$, df = 1 ($P = 0.28$), $I^2 = 14.9\%$. Significant are in value [bold].

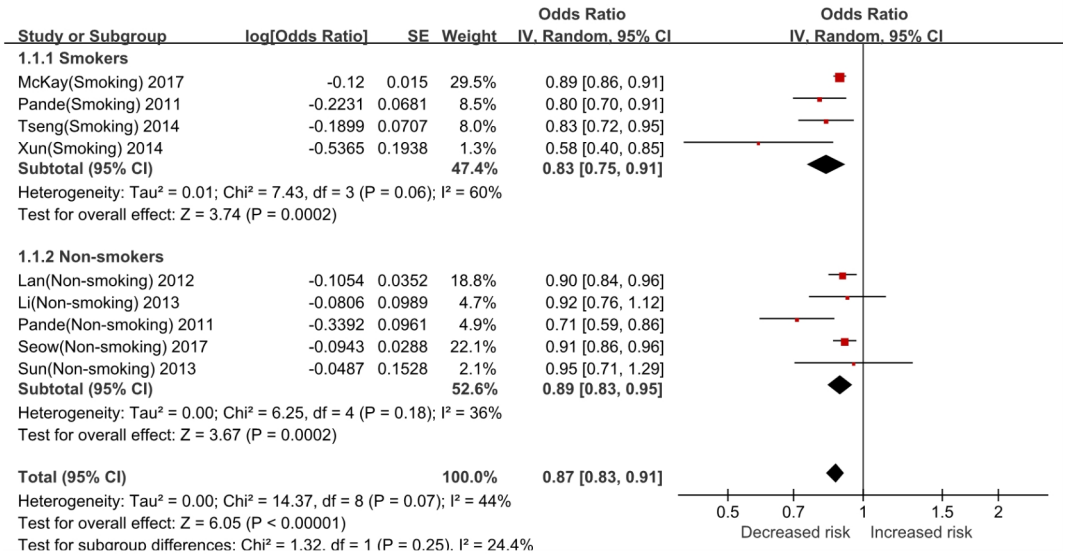

Fig. 4. The result of smoking status of the association between rs401681 (T vs.C) and LC in the overall populations.

Sensitivity analysis

The sensitivity analysis results of all genetic models showed no apparent sensitivity in any of the studies, indicating that there was no significant difference in the result of the meta-analysis after removing any study (Figures S31–S34, Tables S10–S27 in supplemental content).

Trial sequential analysis (TSA)

The TSA analysis of rs401681 and rs402710 showed that the Z-curve (blue line) crossed both the traditional boundary (green dashed line) and the TSA boundary (red line), proving that the results of LC, NSCLC and LC smoking subgroups were stable and credible (Figure S35–S38 in supplemental content).

Discussion

CLPTM1L is located on chromosome 5p15.33, which encodes a cleft lip and palate-associated transmembrane 1-like protein. Overexpression of CLPTM1L has been observed in LC cells[12,13], and it's been confirmed that the occurrence of LC is closely related to the anti-apoptotic function of CLPTM1L[14–16]. Multiple genetic polymorphisms associated with LC risk (specifically rs401681 and rs402710) has been identified in CLPTM1L[5,65,66] and can affect telomere length[23]. However, in some studies, no associations between these sites and susceptibility to LC have been found. Therefore, these results leading to the associations between CLPTM1L rs401681, rs402710 polymorphisms and LC currently lack a unified conclusion. This study included data from GWAS and case–control studies that have so far reported the associations of CLPTM1L rs401681, rs402710 polymorphisms with LC, with the aim of clarifying the associations between them and LC, and the differences in these associations between patients of different ethnicities (Caucasians and Asians), LC subtypes and smoking status.

The T allele variant of rs401681 is negatively associated with LC, as well as in Caucasians and Asians, and these results are similar to previous GWAS results[5,10,11,25,31]. It's been reported that rs401681[C] may affect transcriptional regulation, leading to overexpression of the CLPTM1L gene and increasing the risk of LC[67], and rs401681[C] is associated with shorter telomeres[62]. These evidences suggest that the T allele variation in rs401681 reduce the risk of LC in Caucasians and Asians by regulating CLPTM1L gene expression and telomere length. In different LC subtypes, our study found that the T allele variant primarily reduced the risk of NSCLC (LUAD, LUSC) in Caucasians and Asians, and it didn't appear to reduce the risk of SCLC in Asians. In previous studies, rs401681[T] has been reported to reduce the risk of NSCLC, LUAD, and LUSC in Caucasians[15,34,63] and Asians[18,31,54], but it didn't reduce the risk of SCLC in Chinese populations[68]. Thus, the T allele variant is mainly able to reduce the risk of NSCLC (LUAD, LUSC) but not SCLC in both populations. And from our results, the negative associations between T allele variant and LUAD in Caucasians and Asians were the same as that of LUSC. Therefore, both subtypes of NSCLC (LUAD, LUSC) have high risks of prevalence in Caucasian and Asian populations.

LC subtypes	Subgroups	Smoking status	Studies (n)	Heterogeneity test		Sample		Model	OR[95% CI]	Effect p value	Subgroup differences	
				P values	I^2(%)	Cases (n)	Controls (n)				P values	I^2(%)
LC[a]	Overall	Smoking	9	0.06	46	19,178	13,776	Random	**0.87 [0.80, 0.94]**	**0.0006**	–	–
		Non-smoking	6	0.39	4	6018	13,620	Random	**0.88 [0.81, 0.95]**	**0.0007**	–	–
		Total	15	0.13	30	25,196	27,396	Random	**0.87 [0.83, 0.92]**	**<0.00001**	0.88	0
	Caucasians	Smoking	5	0.09	51	15,912	11,040	Random	**0.89 [0.81, 0.97]**	**0.006**	–	–
		Non-smoking	3	0.14	50	3272	8404	Random	**0.87 [0.76, 1.00]**	**0.04**	–	–
		Total	8	0.09	43	19,184	19,444	Random	**0.88 [0.82, 0.94]**	**0.0002**	0.8	0
	Asians	Smoking	4	0.11	50	3266	2736	Random	**0.80 [0.65, 0.99]**	**0.04**	–	–
		Non-smoking	3	0.58	0	2746	5216	Random	0.89 [0.79, 1.01]	0.07	–	–
		Total	7	0.27	21	6012	7952	Random	**0.86 [0.77, 0.95]**	**0.005**	0.37	0
NSCLC[b]	Overall	Smoking	3	0.33	11	3028	4634	Fixed	0.93 [0.83, 1.05]	0.25	–	–
		Non-smoking (Asians)	2	0.26	21	1244	2690	Fixed	**0.75 [0.60, 0.94]**	**0.01**	–	–
		Total	5	0.18	37	4272	7324	Fixed	**0.89 [0.80, 0.99]**	**0.03**	**0.09**	**64.4**
	Caucasians	Smoking	1	–	–	1322	2694	Fixed	0.99 [0.85, 1.14]	0.86	–	–
	Asians	Smoking	2	0.58	0	1706	1940	Fixed	0.82 [0.66, 1.02]	0.07	–	–
		Non-smoking	2	0.26	21	1244	2690	Fixed	**0.75 [0.60, 0.94]**	**0.01**	–	–
		Total	4	0.59	0	2950	4630	Fixed	**0.79 [0.67, 0.92]**	**0.002**	0.56	0

Table 4. Meta-analysis results of smoking status (rs402710: Allele genetic model, T vs.C). LC:Lung cancer;NSCLC:Non-small-cell lung carcinoma. [a]LC(Smoking Caucasians) vs. LC(Smoking Asians): Test for subgroup differences: $Chi^2 = 0.79$, df = 1 (P = 0.37), $I^2 = 0\%$; LC(Non-smoking Caucasians) vs. LC(Non-smoking Asians): Test for subgroup differences: $Chi^2 = 0.09$, df = 1 (P = 0.76), $I^2 = 0\%$. [b]NSCLC(Smoking Caucasians) vs. NSCLC(Smoking Asians): Test for subgroup differences: $Chi^2 = 1.94$, df = 1 (P = 0.16), $I^2 = 48.4\%$. Significant are in value [bold].

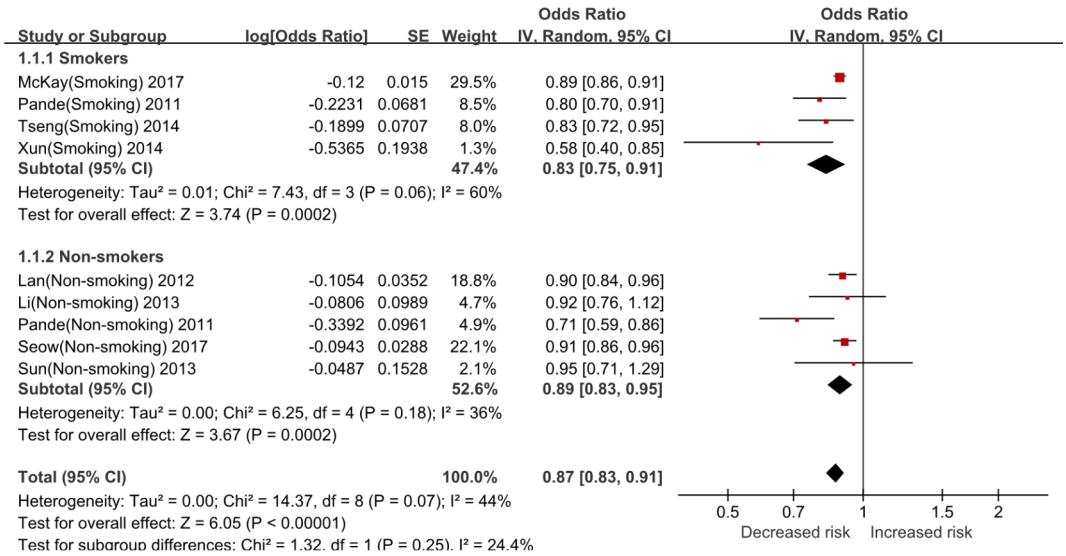

Fig. 5. The result of smoking status of the association between rs402710 (T vs. C) and LC in the overall populations.

From the perspective of pathogenesis association strength, it's found that the negative associations of LC, NSCLC (LUAD, LUSC) in Caucasians were more than in Asians, suggesting that the T allele variation in rs401681 is more strongly negatively associated with the risk of developing LC in the Caucasian population.

Evidence from Phase 3 of the 1000 Genomes Project[69] and the latest literature[44] showed that rs401681 was in strong linkage disequilibrium (LD) with rs465498 both in Europeans ($r^2 > 0.809$) while was in moderate LD in East Asians ($r^2 = 0.4839$), and rs401681 was in strong LD with rs31489 in Europeans ($r^2 = 0.8145$) while was in moderate LD in East Asians ($r^2 = 0.4785$). This evidence, combined with our results, provides further evidence that the Caucasian populations may be more at risk for LC, NSCLC (LUAD, LUSC) due to the variant in rs401681.

The T allele variant in rs402710 is also negatively associated with LC, as well as in both populations, and previous case–control studies have also reported the decrease in the frequency of rs402710[T] in LC patients in Caucasians and Asians[26]. It's been reported that rs402710 may block DNA damage-induced apoptosis by enhancing the accumulation of Bcl-xL, a member of the anti-apoptotic Bcl2 family, thereby affecting lung tissue tumorigenesis in vitro[70]. The rs402710 also maintain the telomere length, which may reduce the risk of LC in non-smokers because the protective effects of rs2736100 are offset in patients with bladder cancer who currently smoke[26]. In addition, Zienolddiny et al.[33] found that rs402710 was associated with increased formation of DNA adducts in the lung, which may be a precursor to LC. These evidences suggest that the T allele variation in rs402710 reduce the risk of LC in Caucasians and Asians by regulating apoptosis and telomere length. In different LC subtypes, our findings showed that rs402710 [T] reduced the risk of NSCLC (LUAD, LUSC) in Caucasians and Asians. Previous studies have also reported that rs402710[T] reduced the risk of NSCLC(LUAD,LUSC) in Caucasians[22,50] and Asians[22]. Thus, the T allele variant is mainly able to reduce the risk of NSCLC (LUAD, LUSC) in both populations. And from our results, the negative association between T allele variant and LUAD in Caucasians and Asians were the same as that of LUSC. Therefore, both subtypes of NSCLC (LUAD, LUSC) have the high risks of disease in Caucasian and Asian populations. In addition, we didn't find the association between rs402710 [T] and the development of SCLC, the result is the same as that of a previous study[50], therefore, the variant of rs402710 may not cause SCLC.

Comparing the two ethnicities, it was found that the negative association of LC in Caucasians was more than in Asians, suggesting that the T allele variation in rs402710 is more strongly negatively associated with the risk of developing LC in the Caucasian population. However, in different subtypes of LC, the risk negative associations of NSCLC (LUAD, LUSC) in Caucasians were the same as in Asians, which suggests that the negative risk associations between the T allele variant of rs402710 and NSCLC (LUAD, LUSC) are strong in both populations. Previous study[44] have confirmed that rs402710 was in moderate LD with rs31489 in Europeans ($r^2 = 0.6624$) and East Asians ($r^2 = 0.4634$), and it appeared that LD seemed to be a bit stronger in Caucasian populations. Therefore, Caucasian populations seems to have a higher risk of LC due to the variation of rs402710. However, further studies are needed to confirm exactly which LC subtype is involved.

Although smoking was currently recognized as a major risk factor for LC[2], there were many other non-smoking factors. Therefore, we further compared this difference in genetic susceptibility to LC between smokers and non-smokers in both populations. The results confirmed that the T allele variation of rs401681was

negatively associated with the risk of LC in both smokers and non-smokers, and this negative association was found among Caucasian smokers or non-smokers and Asian smokers or non-smokers. Previous studies have found that rs401681[T] was negatively associated with the risk of LC in Caucasian smokers[21,25,34] and Caucasian non-smokers[25], and this negative association have also been found in Asian smokers[57] and in non-smoking Asian women[19,31]. Our results also found that the strength of the negative association of rs401681[T] with LC in smokers was the same as in non-smokers. These evidences suggest that regardless of whether Caucasian and Asian populations smoke or not, they may be at risk of developing LC due to the rs401681 variant. Smoking remains an important risk factor for the induction of the rs401681 variant leading to the development of LC. Other factors like non-smoking are also important contributors to the development of LC. Evidence showed that education level, body mass index (BMI), prior diagnosis of Chronic obstructive pulmonary disease (COPD), occupational exposure to pesticides, duration of smoking, exposure to a large number of cooking emissions, dietary factors (including less fish and shrimp, vegetables, soy products and nuts) and the excessive intake of meat in LC patients were all related to the development of LC[71]. Therefore, the effect of smoking on the risk of LC is multi-directional, and it's not the only factor that contributes to the risk of LC. LC is a multiaetiologic disease caused by a combination of genetic and lifestyle factors. Our results also found that this negative risk association appeared to be stronger in Caucasian non-smokers than in Caucasian smokers and appeared to be stronger than in Asian non-smokers. In Asian populations, this negative risk association appeared to be stronger for smokers than for non-smokers and appeared to be stronger for Caucasian smokers. These results imply that the risk factors for LC development are different in the two populations. In Asian populations, smoking seems to be the main risk factor for inducing the rs401681 variation that leads to LC. Whereas in Caucasian populations, it is other factors such as non-smoking that appear to be the major risk factor for inducing the rs401681 variation and thus causing LC. However, these conclusions are inconclusive because we included fewer studies of Caucasian non-smokers (n = 1) and Asian smokers (n = 1), and more studies need to be included to confirm these conclusions.

Among different LC subtypes, our study found that rs401681[T] was negatively associated with the risk of NSCLC (LUAD) development primarily in Caucasian smokers and Asian non-smokers, and the strength of this negative risk association was approximately the same in both populations. These results were identical to those of previous studies[31,34]. It confirmed that in Caucasian populations, smoking is a major risk factor for the induction of the rs401681 variant and thus the development of NSCLC (LUAD). In Asian populations, other factors like non-smoking may be the main cause. However, due to the limitations of the included samples, we didn't search for evidence of the association between rs401681[T] and the risk of NSCLC (LUAD) development in Caucasian non-smokers and Asian smokers. In addition, rs401681 [T] wasn't associated with the risk of developing LUSC in Asian non-smokers, which was the same as the results of a study in Chinese non-smokers[40]. It's confirmed that the variant of rs401681 mainly induced the risk of developing LUAD in Asian non-smokers. Further analysis revealed that these samples included in the LUAD subgroup analysis were predominantly Asian female non-smokers (n = 12640/12985), confirming that Asian female non-smokers are more likely to have a variant in rs401681 and thus the development of LUAD. Previous studies have also confirmed that LUAD was more common in women[72,73]. Patel et al. showed that among the never-smoking LC patients, the number of women exceeded that of men[74]. There was evidence confirmed that the common genetic variation of TERT-CLPTM1L was associated with the risk of LUAD in non-smoking Asian women[75]. This can be explained by the following assumptions: women are more likely to be in the exposure of second-hand smoking, household cooking emissions and hormone replacement therapy. These causes lead to variations in the TERT-CLPTM1L gene that evade apoptosis and ultimately lead to cancer[76].

The results confirmed that the T allele variant of rs402710 was negatively associated with LC in smokers and non-smokers, and this negative association was predominantly found among Caucasian smokers or non-smokers and Asian smokers. These results were the same as those of previous studies in smokers in China[57] and the United States[25,27], and in non-smokers in the United States and Europe[50]. Comparisons between smokers and non-smokers revealed that the strength of the negative association for risk of developing LC was the same between them and in the Caucasian population. This confirms that regardless of whether Caucasians are smokers or not, they are likely to have the rs402710 variant that contributes to the risk of developing LC. Our results also showed that the T allele variation was negatively associated with the risk of LC incidence in Asian smokers, but did not seem to be associated with the risk of LC incidence in Asian non-smokers, as found in several previous studies[47,49,50]. This implies that the T allele variant of rs402710 may not be associated with the risk of LC development in Asian non-smokers. This evidence suggests that smoking is an important risk factor for inducing the rs402710 variant and thus the development of LC in both populations, but it's not the only factor, so genetic factors are also important for LC risk.

Among different LC subtypes, our study found that rs402710[T] was negatively associated with the risk of NSCLC incidence mainly in non-smokers, and this negative association was mainly found in Asian non-smokers. Previous findings also showed that rs402710[T] was negatively associated with the risk of NSCLC development in Chinese non-smokers[47]. These results confirmed that in Asian populations, other factors like non-smoking may be the main reason for inducing rs402710 variants and thus the development of NSCLC. This point seems to be in contradiction with the above conclusions. However, it's worth noting that other subtypes such as SCLC are included in LC. Therefore, rs402710[T] may not be associated with the risk of SCLC in Asian non-smokers. Our study also found that rs402710 [T] wasn't associated with the risk of NSCLC development in smokers and was also found in Caucasian and Asian populations. These results were similar to those of previous studies[34,47,49]. However, due to the small number of included studies, this conclusion needs to be further validated by increasing the number of studies. In addition, we didn't search for evidence of the association between rs402710[T] and the risk of developing NSCLC in Caucasian non-smokers due to the limitations of the

included samples. We also didn't search for evidence of association of rs402710[T] with the risk of developing LUAD and LUSC. They need to be further studied.

Overall, this is a more comprehensive meta-analysis to date, and it's an in-depth exploration of the associations between the rs401681, rs402710 variants and the risk of LC from the perspective of different populations (Caucasians and Asians), LC subtypes and smoking status. And more satisfactory results were obtained through the in-depth analysis of these aspects. The results of our study intuitively confirmed that the associations between the variants of rs401681, rs402710 and the risk of LC were different due to the existence of different population groups, LC subtypes and smoking status. These results not only provided a valuable genetic diagnosis basis for the clinical diagnosis of LC (including various LC subtypes) but also provided a rich theoretical reference basis for the clinical prediction of the risk of LC, the development of interventions in advance, and the change of lifestyle habits in advance. In addition, since the variants of rs401681 and rs402710 caused telomere length changes[23,62] as mentioned earlier, the results of the study reflected that there may be differences in telomere length between Caucasian and Asian populations. It also indicated that the induction of smoking[26] or other risk factors such as non-smoking may cause the telomere length to be altered, which may lead to the risk of developing different subtypes of LC in these two populations. Of course, these inferences and conclusions need to be confirmed by further experimental studies.

Limitations: ① This meta-analysis was based on the research reports of two ethnic groups and different types of LC, which will inevitably produce some heterogeneity and publication bias; ② Most studies used different methods of genetic testing and genotyping, which also may lead to bias in the analyses; ③ The sample size of the study is generally sufficient, but after subgroup analyses according to different LC subtypes, ethnic groups, and smoking status of the patients, the results showed relatively small sample sizes for LUAD, LUSC, SCLC, and smoking subgroups. Therefore, this inevitably produces some false negatives, heterogeneity, sensitivity, and publication bias in their results, especially in the smoking subgroups; ④ Due to the insufficient sample size of smoking cases in the included studies and the lack of separate reports on male or female studies, this study could not further discuss the impact of factors such as smoking, gender, etc. on LC subtypes; ⑤ Although some other LC risk loci have been reported in the CLPTM1L gene, such as rs31489 and rs451360, etc., we didn't include them in the current study due to the small amount of available data (especially various subgroup analyses) collected for these loci. These risk loci will be further studied in depth in the future; ⑥ Since other ethnicities such as African (n = 1[22]) and mixed (n = 1[77]) populations have been reported less frequently, the results of these ethnic groups were not analyzed in this study.

Conclusion

The T allele variant of rs401681 is mainly negatively associated with the risk of LC, NSCLC (LUAD, LUSC) in Caucasian and Asian populations, and the negative association with the risk of LC, NSCLC (LUAD, LUSC) is higher in Caucasian populations. In Caucasian populations, smoking is a major risk factor for the induction of the rs401681 variant leading to the development of LC and NSCLC (LUAD). In Asian populations, smoking is also an important risk factor for the induction of the rs401681 variant leading to the development of LC. However, other factors like non-smoking are also important causes of LC in Caucasians and LC, NSCLC (LUAD) in Asians. And in Asian populations, it is mainly concentrated in LUAD of non-smoking women.

The T allele variant in rs402710 is mainly negatively associated with the risk of LC, NSCLC (LUAD, LUSC) in Caucasian and Asian populations, and the negative association seems to be higher for LC in Caucasian populations. However, further studies are needed to confirm which LC subtype it is. In Caucasian populations, the presence of the rs402710 variant and thus the risk of LC may be present irrespective of whether they smoke or not. In Asian populations, smoking is also a major risk factor for inducing the rs402710 variant leading to LC. However, other factors like non-smoking are mainly responsible for the development of NSCLC in Asian populations and may not be associated with the risk of SCLC.

Data availability

Data supporting our findings are contained within the manuscript.

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Author contributions

This study is initiated by Xiaozheng Wu. Xiaozheng Wu will develop the search strategies, conduct data collection, and analyze independently. Wen Li and Yunzhi Chen will revise it. All authors have approved the fifinal manuscript. Conceptualization: Xiaozheng Wu. Methodology: Xiaozheng Wu, Wen Li. Software: Xiaozheng Wu. Supervision: Yunzhi Chen. Writing – original draft: Xiaozheng Wu. Writing – review & editing: Wen Li, Yunzhi Chen.

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Declarations

Ethics approval and consent to participate

The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethics and dissemination

This review does not require ethical approval because the included studies are published data and do not involve the patients' privacy. The results of this review will be reported in accordance with the PRISMA extension statement and disseminated to a peer-reviewed journal.

Competing interests

The authors declare no competing interests.

Additional information

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