RESEARCH ARTICLE

Pathway-analysis of published genome-wide association studies of lung cancer: A potential role for the *CYP4F3* locus†

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Running title: *CYP4F3* locus and lung cancer

Key words: lung cancer, fatty acid metabolism, tumor markers, genome wide association study

Additional Supporting Information may be found in the online version of this article.

Received 8 January 2017; Accepted 30 January 2017
Molecular Carcinogenesis

DOI 10.1002/mc.22622

Abbreviations: Fatty acid, FA; genome-wide association study, GWAS; Transdisciplinary Research in Cancer of the Lung, TRICL; cytochrome P450, family 4, subfamily F, polypeptide 3, *CYP4F3*; single nucleotide polymorphisms, SNPs; false discovery rate, FDR; odds ratio, OR; confident interval, CI; expression quantitative trait loci, eQTL.

Acknowledgments

Jieyun Yin was sponsored by the China Scholarship Council for studying at Duke University.

As Duke Cancer Institute members, QW and KO acknowledge support from the Duke Cancer Institute as part of the P30 Cancer Center Support Grant (Grant ID: NIH CA014236). QW was also supported by a start-up fund from Duke Cancer Institute, Duke University Medical Center).

TRICL

This work was supported by the Transdisciplinary Research in Cancer of the Lung (TRICL) Study, U19-CA148127 on behalf of the Genetic Associations and Mechanisms in Oncology (GAME-ON) Network. The Toronto study was supported by Canadian Cancer Society Research Institute(020214), Ontario Institute of Cancer and Cancer Care Ontario Chair Award to RH The ICR study was supported by Cancer Research UK (C1298/A8780 and C1298/A8362—Bobby Moore Fund for Cancer Research UK) and NCRN,HEAL and Sanofi-Aventis. Additional funding was obtained from NIH grants (5R01CA055769, 5R01CA127219, 5R01CA133996, and 5R01CA121197). The Liverpool Lung Project (LLP) was supported by The Roy Castle Lung Cancer Foundation, UK. The ICR and LLP studies made use of genotyping data from the Wellcome Trust Case Control Consortium 2 (WTCCC2); a full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Sample collection for the Heidelberg lung cancer study was in part supported by a grant (70–2919) from the Deutsche Krebshilfe. The work was additionally supported by a Helmholtz-DAAD fellowship (A/07/97379 to MNT) and by the NIH (U19CA148127). The KORA Surveys were financed by the GSF, which is funded by the German Federal Ministry of Education, Science, Research and Technology and the State of Bavaria. The Lung Cancer in the Young study (LUCY) was funded in part by the National Genome Research Network (NGFN), the DFG (BI576/2-1; BI 576/2-2), the Helmholtzgemeinschaft (HGF) and the Federal office for Radiation Protection (BfS: STSch4454). Genotyping was performed in the Genome Analysis Center (GAC) of the Helmholtz Zentrum Muenchen. Support for the Central Europe, HUNT2/Tromsø and CARET genome-wide

studies was provided by Institut National du Cancer, France. Support for the HUNT2/Tromsø genome-wide study was also provided by the European Community (Integrated Project DNA repair, LSHG-CT- 2005–512113), the Norwegian Cancer Association and the Functional Genomics Programme of Research Council of Norway. Support for the Central Europe study, Czech Republic, was also provided by the European Regional Development Fund and the State Budget of the Czech Republic (RECAMO, CZ.1.05/2.1.00/03.0101). Support for the CARET genome-wide study was also provided by grants from the US National Cancer Institute, NIH (R01 CA111703 and UO1 CA63673), and by funds from the Fred Hutchinson Cancer Research Center. Additional funding for study coordination, genotyping of replication studies and statistical analysis was provided by the US National Cancer Institute (R01 CA092039). The lung cancer GWAS from Estonia was partly supported by a FP7 grant (REGPOT245536), by the Estonian Government (SF0180142s08), by EU RDF in the frame of Centre of Excellence in Genomics and Estoinian Research Infrastructure's Roadmap and by University of Tartu (SP1GVARENG). The work reported in this paper was partly undertaken during the tenure of a Postdoctoral Fellowship from the IARC (for MNT). The Environment and Genetics in Lung Cancer Etiology (EAGLE), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), and the Prostate, Lung, Colon, Ovary Screening Trial (PLCO) studies and the genotyping of ATBC, the Cancer Prevention Study II Nutrition Cohort (CPS-II) and part of PLCO were supported by the Intramural Research Program of NIH, NCI, Division of Cancer Epidemiology and Genetics. ATBC was also supported by US Public Health Service contracts (N01-CN-45165, N01-RC-45035 and N01-RC-37004) from the NCI. PLCO was also supported by individual contracts from the NCI to the University of Colorado Denver (NO1-CN-25514), Georgetown University (NO1-CN-25522), Pacific Health Research Institute (NO1-CN-25515),Henry Ford Health System (NO1-CN-25512), University of Minnesota(NO1-CN-25513), Washington University(NO1-CN-25516), University of Pittsburgh (NO1-CN-25511), University of Utah (NO1-CN-25524), Marshfield Clinic Research Foundation (NO1-CN-25518), University of Alabama at Birmingham (NO1-CN-75022, Westat, Inc. NO1-CN-25476), University of California, Los Angeles (NO1-CN-25404). The Cancer Prevention Study II Nutrition Cohort was supported by the American Cancer Society. The NIH Genes, Environment and Health Initiative (GEI) partly funded DNA extraction and statistical analyses (HG-06-033-NCI-01 andRO1HL091172-01), genotyping at the Johns Hopkins University Center for Inherited Disease Research (U01HG004438 and NIH HHSN268200782096C) and study coordination at the GENEVA Coordination Center (U01 HG004446) for EAGLE and part of PLCO studies. Funding for the MD Anderson Cancer Study was provided by NIH grants (P50 CA70907, R01CA121197, R01CA127219, U19 CA148127, R01 CA55769, K07CA160753) and CPRIT grant (RP100443). Genotyping services were provided

by the Center for Inherited Disease Research (CIDR). CIDR is funded through a federal contract from the NIH to The Johns Hopkins University (HHSN268200782096C). The Harvard Lung Cancer Study was supported by the NIH (National Cancer Institute) grants CA092824, CA090578, and CA074386.

Abstract

The Fatty acids (FAs) metabolism is suggested to play a pivotal role in the development of lung cancer, we explored that by conducting pathway-based analysis. We performed a meta-analysis of published datasets of six genome wide association studies (GWASs) from the Transdisciplinary Research in Cancer of the Lung (TRICL) consortium, which included 12,160 cases with lung cancer and 16,838 cancer-free controls. A total of 30,722 single-nucleotide polymorphisms (SNPs) from 317 genes relevant to FA metabolic pathways were identified. An additional dataset from the Harvard Lung Cancer Study with 984 cases and 970 healthy controls was also added to the final meta-analysis. In the initial meta-analysis, 26 of 28 SNPs that passed false discovery rate multiple tests were mapped to the *CYP4F3* gene. Among the 26 top ranked hits, was a proxy SNP, *CYP4F3* rs4646904 ($P = 8.65\times10^{-6}$, FDR = 0.018), which is suggested to change splicing pattern/efficiency and to be associated with gene expression levels. However, after adding data of rs4646904 from the Harvard GWAS, the significance in combined analysis was reduced to $P=3.52\times10^{-3}$ [odds ratio (OR)=1.07, 95% confidence interval (95%CI)=1.03-1.12]. Interestingly, the small Harvard dataset also pointed to the same direction of the association in subgroups of smokers (OR = 1.07) and contributed to a combined OR of 1.13 (95%CI = 1.06-1.20, $P=6.70\times10^{-5}$). The results suggest that a potentially functional SNP in *CYP4F3* (rs4646904) may contribute to the etiology of lung cancer, especially in smokers. Additional mechanistic studies are warranted to unravel the potential biological significance of the finding.

1. Introduction

Lung cancer is the leading cause of cancer deaths in the United States, with an estimated 224,210 new cancer cases and 159,260 deaths in 2014 [1]. Surgery is more effective for the early-stage lung cancer without metastasis, when the tumor can be completely removed. Unfortunately, the disease is typically asymptomatic, until it is in the late stages, and the best chance for surgical treatment is usually lost. Consequently, the mortality is high: the five-year survival rate for lung cancer is only 4.0% for patients with distant tumors, compared with 54.0% for patients with localized tumors [2]. Chest radiograph is a common approach for detecting lung cancer, but the annual screening with chest radiograph has not reduced lung cancer mortality, compared with usual care [3]. Although low-dose computed tomography (CT) screening is a promising method for detecting lung cancer in individuals at high risk [4,5], the treatment is expensive, and it exposes patients to some extra doses of radiation. Besides, the low-dose CT screening has been associated with a high rate of false positive results, with ~95% of benign lung nodules that are challenging for radiologists to identify [4]. Therefore, there is still a demand for new less-invasive but more efficient biomarkers in the risk assessment and early detection of lung cancer.

Cancer cells often share the attributes of metabolic abnormalities, such as perturbation in the energy metabolism of glucose, glutamine and lipids [6,7]. Fatty acids (FAs), an important subgroup of lipids, have emerged as a recent focus of cancer research. FAs are synthesized *de novo* to continually provide lipids for energy production, cell membrane regeneration and lipid modification of proteins to meet the excessive bioenergetics and structural demands of highly proliferating cancer cells. Additionally, FAs and their derivatives are also important signaling molecules that may affect many fundamental cell processes, including cellular survival, proliferation, migration, angiogenesis and therapy resistance [8]. Furthermore, the metabolites of FAs could augment survival [9-11] and enhance adhesion [12] in lung cancer cells, and thus promote progression and metastasis and induce angiogenesis in human lung cancer [13-16].

More recently, increasing evidence suggests that the FAs are potential biomarkers for monitoring development and progression of lung cancer [17-20]. For instance, in serum samples from 55 patients with lung cancer and 165 similar pulmonary patients without known cancer, Liu et al. found that free FAs and their metabolites demonstrated good sensitivity and specificity for the identification of adenocarcinoma of the lung [17]. Similarly, other studies demonstrated that FAs from erythrocyte total lipids might be used as diagnostic biomarkers of lung adenocarcinoma, squamous cell carcinoma, and small cell lung cancer [18,19]. Most recently, Zhang et al. revealed that serum unsaturated free FAs could be used as potential biomarkers for early

detection and disease progression of lung cancer [20]. Consequently, we hypothesized that genetic variants in genes involved in the FA synthesis, degradation, metabolism and transport were potential susceptibility factors for lung cancer.

The aim of the present study was to assess the associations between single-nucleotide polymorphisms (SNPs) in the FAs metabolic pathways and risk of lung cancer. We first conducted a meta-analysis of six lung cancer genome wide association studies (GWASs) within the Transdisciplinary Research in Cancer of the Lung (TRICL) consortium [21-23], and we added additional data from another GWAS from Harvard University [24] for the identified significant SNPs that were corrected by the false discovery rate (FDR) for multiple testing correction and with potential functions in bioinformatics analyses [25,26].

2. Methods and Materials

2.1 Study populations and genotyping

The detailed information about the study participants is presented in **Supplemental Table 1**, and all the participants were of European descendent. A written informed consent was obtained from each participant in the GWASs, and the present study followed the study protocols approved by the institutional review board for each of the participating institutions.

2.2 Initial meta-analysis

The present study started from the combined genotyping and imputation dataset of six previously published GWASs of lung cancer from the TRICL consortium. Within the TRICL consortium [21], 12,160 lung cancer cases and 16,838 controls from Europe and North America participated in the GWASs[22]. All the cases and controls were at least frequency-matched on age and sex. The participants were described in previous publications: The University of Texas MD Anderson Cancer Center (MDACC) GWAS [27], the Institute of Cancer Research (ICR) GWAS [28], the National Cancer Institute (NCI) GWAS [21], the International Agency for Research on Cancer (IARC) GWAS [27], Samuel Lunenfeld Research Institute study (SLRI) GWAS [22], Toronto, and The Helmholtz-Gemeinschaft Deutscher Forschungszentren Lung Cancer GWAS, Germany (GLC) [22].

For the TRICL GWASs, the overwhelming majority of patients had non-small cell lung cancer, leaving only a few with small cell lung cancer. Genotyping data were from combined datasets of different platforms of Illumina

HumanHap 317, 317+240S, 370Duo, 550, 610 or 1M arrays [21]. These datasets were imputed for all original scans for over 10 million SNPs using the 1000 Genomes Project (phase I integrated release 3, March 2012) as the reference by using IMPUTE2 v2.1.1, MaCH v1.0 or minimac (version 2012.10.3) software. The quality control process was detailed in previously published reports [21,22].

2.3 Additional data for meta-analysis

An additional independent GWAS dataset of a Caucasian population was provided by the Harvard Lung Cancer Susceptibility Study [24]. For the Harvard GWAS, for which details of participant recruitment have been described previously [24], genotyping data was derived from 1000 cases and 1000 controls using Illumina Humanhap610-Quad arrays. Cases were patients aged >18 years, with newly diagnosed, histologically confirmed primary non-small cell lung cancer. Controls were healthy non-blood-related family members and friends of patients with cancer or with cardiothoracic conditions undergoing surgery.

Unqualified samples were excluded, if they had (i) overall genotype completion rates <95%; (ii) gender discrepancies; (iii) unexpected duplicates or probable relatives (based on pairwise identity by state value, PI_HAT in PLINK>0.185); (iv) heterozygosity rates >6 times the standard deviation from the mean; or (v) individuals evaluated to be of non-Caucasians [using the HapMap release 23 including Japanese in Tokyo, Japan (JPT), Han Chinese in Bejing, China (CHB), Utah Residents (CEPH) with Northern and Western Ancestry (CEU) and Yoruba in Ibadan, Nigeria (YRI) populations as a reference]. Unqualified SNPs were excluded, when they (i) were not mapped on autosomes; (ii) had a call rate <95% in all GWAS samples; (iii) had minor allele frequency (MAF) <0.01; or (iv) had a genotype distribution deviated from those expected by Hardy-Weinberg equilibrium (P<1.0×10^{-6}). After applying pre-specified quality control, the Harvard genotype data were available for 984 cases and 970 controls.

2.4 Gene and variant selection

To identify relevant genes of interest, we used two electronic databases, Gene Cards [29] and MSigDB [30] to search for FA metabolism-related genes. Specifically, keyword searches using “fatty acid” in *pathway & interaction* section and “cancer” in *disorder* section in the Gene Cards and one heading “fatty acid” in the MSigDB. As a result, 317 genes located on autosomal chromosomes were identified from the FA biosynthesis, metabolism and degration pathways (**Supplementary Table 2 and Supplementary Figure 1**). Genotyped,

imputed common SNPs (minor allele frequency ≥ 0.05) within these genes or their ± 2kb flanking regions were selected for association analysis. Hence, 30,722 SNPs in the FA metabolic pathways had been extracted from the TRICL GWASs and used for further analysis.

2.5 Statistics

Meta-analysis was first performed among the six GWASs from the TRICL consortium. Briefly, the association between each SNP and lung cancer risk was assessed by unconditional logistic regression using an additive genetic model of the risk allele. The Cohran's Q statistic to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated [31]. Fixed-effects models were applied, when there was no heterogeneity among the datasets (based on the criteria $P > 0.10$ and $I^2 < 25\%$); otherwise, random-effects models were applied [32]. The Benjamini and Hochberg's false discovery rate (FDR) method was used for correction of the multiple comparisons[33]. Associations were considered significant, if an FDR value was less than 0.05. For the top-hit variants of interest, we then focused on those SNPs with potential functional as predicted by the online prediction tools: SNPinfo [25] and pfSNP [34]. Linear regression analysis was used to test expression quantitative trait loci (eQTL) associations with data obtained from the RNA sequencing project of the 1000 Genomes Project samples [conducted by Genetic European Variation in Health and Disease Consortium (GEUVADIS) [35]. Gene expression values that were more than three standard deviations from the mean were considered as outliers[36]. Finally, we tested the association between predicted functional SNP and lung susceptibility in the Harvard GWAS [24], and subgroup analyses stratified by histology types (squamous and adenocarcinoma) and smoking status (smoker and non-smoker) were also performed. LocusZoom was used to produce regional association plots [37]. All statistical analyses were carried out by SAS software (version 9.1.3; SAS Institute, Cary, NC, USA) or R (2.6.0) unless specified otherwise. The analysis flow chart is present in **Figure 1**.

3. Results

The six GWASs from the TRICL consortium consisted of 12,160 cases and 16,838 controls of European ancestry (**Supplementary Table 1**). The associations between SNPs of genes involved in the FA metabolic pathways and lung cancer risk in the TRICL consortium are shown in **Supplementary Figure 2** (the Manhattan plot). Among 30,722 SNPs, 1,609 were nominally associated with lung cancer susceptibility at $P < 0.05$. Of these, as detailed in **Table 1**, 28 SNPs in three genes reached the preset statistical thresholds (FDR < 0.05).

Among these 28 SNPs, two SNPs in *TNF* (rs1800628 G>A) and *GPX5* (rs116260720 C>A) were located in the previously identified and reported lung cancer susceptibility major histocompatibility complex (MHC) region; therefore, they were not further pursued in further analyses. The other 26 SNPs were mapped within the *CYP4F3* gene region on chromosome 19. **Supplementary Figure 3** shows the regional association plots of *CYP4F3*. All the top-hits (FDR < 0.05 and $P < 10^{-4}$) in the *CYP4F3* region were in high linkage disequilibrium (LD, $r^2 > 0.8$, **Figure 2**) based on the hg19/1000 Genome European populations.

Using the online tool SNPinfo and pfSNP, the *CYP4F3* rs4646904 G>A SNP was putatively functional, because it is predicted to influence exonic splicing efficiency of *CYP4F3.* We then performed an eQTL analysis to evaluate the mRNA expression levels of *CYP4F3* by the genotypes of rs4646904. The GEUVADIS RNA sequencing of the 1000 Genomes Project has only a combined normalized transcriptome and genome sequencing data by performing mRNA and small RNA sequencing on 465 lymphoblastoid cell lines derived from five populations: CEU, Finns (FIN), British (GBR), Toscani (TSI) and Yoruba (YRI). To keep populations consistent and comparable with the present study, we only used data of 373 samples from European descendants (i.e., TSI, GBR, FIN and CEU). Besides, six values for *CYP4F3* mRNA expression levels were considered an outlier and removed (**Supplementary Figure 4**). Gene expression differences among genotypes were examined using a regression model in which the association between gene expression and genotypes was considered additive, assuming that a trend by the number of variant alleles exists. As shown in **Figure 3**, the number of the variant A allele was shown to be associated with higher expression levels of *CYP4F3* ($P_{additive}$ = 0.001), compared with the common G allele.

Therefore, we chose rs4646904 as the tagSNP of the *CYP4F3* region. In the meta-analysis of the TRICL consortium, there was no heterogeneity observed among the six GWASs, with I^2 of 0 and the Q-test *P* value of 0.488. In combined analysis, we found that the per-unit increase of the variant A allele was associated with 1.09-fold increased risk of lung cancer [95% confidence interval (95% CI) = 1.05-1.13, $P = 8.65\times10^{-6}$**, Table 1**].

The genotyping data of the associated rs4646904 SNP was available in the Harvard GWAS (984 patients and 970 controls, minor allele frequency = 0.37, **Table 1**). When this dataset was added to the TRICL datasets, the summary effect estimate obtained from the expanded meta-analysis was 1.07 (95% CI =1.03-1.12, $P = 3.52\times10^{-3}$). For this analysis, we used in a random-effects model, because the effect estimate displayed a moderate degree of heterogeneity with I^2 = 30.9% and the Q test *P* = 0.192 (**Figure 4**). When stratified by lung tumor types, the combined effect was 1.10 (95% CI = 1.04-1.15, $P = 8.55\times10^{-4}$, I^2 = 1.5%, Q-test *P* = 0.413) in a

fixed-effects model (with a smaller I^2 value) for adenocarcinomas and 1.07 (95% CI = 0.97-1.19, P = 0.212, I^2 = 61.0%, Q-test P = 0.017) for squamous carcinomas in a random-effects model (with a larger I^2 value). We also examined the expected association of rs4646904 with risk of lung cancer in the 10,059 smokers (5078 cases and 4981 controls) from MDACC, IARC, SLRI, GLC and Harvard GWASs. Consistently, a significant effect of the rs4646904 A allele on lung cancer risk was observed in the combined meta-analysis (OR = 1.13, 95%CI= 1.06-1.20, $P = 6.7\times10^{-5}$, I^2 = 0.0%, Q-test P = 0.706). However, among 2232 non-smokers (381 cases and 1851 controls), the result failed to reach significance (OR = 0.84, 95%CI= 0.71-1.01, P = 0.063, I^2 = 0.0%, Q-test P = 0.911). Tests for heterogeneity indicated that the carcinogenesis effect of the rs4646904 A allele was predominantly limited to smokers (I^2 = 89.7%, Q-test P = 0.002).

4. Discussion

Deregulation of the FA metabolic pathways has been implicated in many cancers[38-40]. In the present study, we explored associations between genetic variants in the FA metabolic pathways and risk of lung cancer. We examined all typed and imputed SNPs of the genes involved in the FA metabolic pathways from six published GWASs of lung cancer within the TRICL consortium. We also tried to find additional support from the Harvard GWAS. In the meta-analyses of the TRICL GWASs, the predicted functional SNP, *CYP4F3* rs4646904G>A, showed a significant association with lung cancer risk, but this association was not significant in the Harvard Lung Cancer Susceptibility Study; however, this SNP remained significant in the final combined analysis as well as in subgroups of squamous cancer and smokers.

The *CYP4F3* gene contains 14 exons and 13 introns and encodes a member of the cytochrome P450 superfamily of enzymes. The *CYP4F3* pre-messenger RNA is spliced into two mature transcripts (i.e., *CYP4F3B* and *CYP4F3A*). Human CYP4F3s are the main catalysts in the oxidation of FAs: they can ω-hydroxylate a variety of long chains and very long chains as well as saturated, unsaturated and branched chain FAs, vitamins with long alkyl side chains, and the physiologically important prostaglandins and hydroeicosatetraenoic acids[41]. Additionally, the ability of CYP4F3 to ω-hydroxylate both pro- and anti-inflammatory leukotrienes (i.e., LTB4) indicates that they may function in both the activation and resolution phases of the inflammatory response[42]. The *CYP4F3* rs4646904G>A variant was a novel, potentially functional SNP that had the smallest P value in the present meta-analysis of the TRICL GWASs. Although *CYP4F3* rs4646904G>A is a synonymous

variant, it is located within an exon splicing enhancer (ESE)[25], which has discrete sequences within exons that promote both constitutive and regulated splicing[43]. Efficient splicing has limited tolerance of mutations in the ESEs, even if they have no effect on protein coding[44]. Additionally, ChIP-seq data indicate that rs4646904 is located in the enhancer region containing histone modification marks of H3k09me3, H3k27me3 and H3k09me3[45]. Cigarette smoking is the major cause of cancers of the respiratory tract, and *CYP4F3* was found to be up-regulated in human airway epithelium in healthy current smokers, compared with those of never smokers [46-48], which suggests that *CYP4F3* may be induced by smoking and thus contributes to carcinogenesis. The finding that the rs4646904 A variant allele was associated with significant higher gene expression levels than the G allele is consistent with the findings in other cancers, in which unregulated expression levels of *CYP4F3s* were found in pancreatic ductal adenocarcinoma, compared to benign lesions[49], as well as related with hepatocyte differentiation status [50] and progression of HCV-associated hepatocellular carcinoma [51].

Despite the underlying biological plausibility supporting this lung cancer-associated rs4646904 in the meta-analysis of the TRICL consortium with 12,160 cases and 16,838 controls, the relatively small Harvard GWAS dataset with 984 cases and 970 controls did not provide a further support for the association, but the combined meta-analysis revealed a slight effect with $P < 0.05$. Nevertheless, according to a PASS [52] power calculation, the Harvard GWAS had a mere power of 0.18 to detect an OR of 1.1. In contrast, the combined meta-analysis of the TRICL and Harvard GWASs with a total of 13,144 cases and 17,808 controls achieved a power of 0.98 for detecting an OR of 1.1, which suggest that rs4646904 may have a very small but genuine effect on lung cancer risk. Interestingly, the rs4646904-cancer risk association was significant in subgroups with squamous cancer and smokers but not adenocarcinoma and non-smokers, and there were high heterogeneity among the results of these subgroups, indicating that there might be an interaction between smoking and rs4646904 [53]. It may be because the exposure to cigarette smoke may negatively affect the synthesis of n−3 long-chain polyunsaturated fatty acid from the precursor in mammary gland cells [54]. Hence, rs4646904 might be biomarker for lung cancer risk in smokers.

It should be noted that there were limitations of the present study. First, none of the *CYP4F3* variants was identified in previous TRICL GWASs as significant at a GWAS threshold ($P < 10^{-7}$). Indeed, recent GWASs have identified at least 10 independent loci, and, as a result, only a small fraction of heritability could be explained by

these SNPs. The challenge remains to identify the many additional common risk loci that are expected to have smaller genetic effects [55]. The pathway-based analysis, such as in the present study, reduces the significance level of *P* values based on the number SNPs examined in genes in the studied pathway. Therefore, such a pathway analysis with integration of association results with gene expression should be considered a complementary approach [56,57] to the GWAS analyses. Second, we are aware that the observed effects may vary by body mass index or sex, but such data were not available from the TRICL consortium for us to further examine the risk modification. Thus, further analyses including body mass index and sex are warranted to improve our understanding of the apparent heterogeneity of effects. Third, because we only included non-Hispanic white populations, the generalizability to other ethnic populations needs further investigation.

In conclusion, this combined meta-analysis of six GWASs from the TRICL consortium and Harvard GWAS identified a potentially predictive functional marker (*CYP4F3* rs4646904) for lung cancer risk in Caucasian populations, especially in smokers.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Figures Legends

Figure 1. Analysis flow chart of the present study.

Figure 2. Gene structure of the *CYP4F3* gene and linkage disequilibrium plot of 26 SNPs mapped to *CYP4F3* and passed false discovery rate multiple tests.

Figure 3. Expression quantitative trait loci (eQTL) association of *CYP4F3* rs4646904. The eQTL analyses were preforemed in addtive model. We used RNA-seq data from 368 non-hispanic European individuals, which are part of the 1000 Genome Project.

Figure 4. Forest plot of A allele effect of *CYP4F3* rs4646904 in all cases (**Panel A**), adenocarcinoma (**Panel B**), Squamous cell carcinoma (**Panel C**), smokers (**Panel D**), non-smokers (**Panel E**) from GWASs [the Institute of Cancer Research (ICR) GWAS, the MD Anderson Cancer Center (MDACC) GWAS, the International Agency for Research on Cancer (IARC) GWAS, the National Cancer Institute (NCI) GWAS, the Samuel Lunenfeld Research Institute study (SLRI) GWAS, German Lung Cancer Study (GLC) and Harvard lung cancer study (Harvard)].

Table 1. Associations between SNPs in the fatty acid pathways and NSCLC risk with FDR-*P*<0.05

SNP	Gene	Chr	Position	Allele*	TRICL							Harvard		
					EAF	Q †	I^2	Effects ‡	OR (95%CI)	*P*	FDR	EAF	OR (95%CI)	*P*
rs1800628	*TNF*	6	31546850	G/A	0.11	0.541	0	++++++	1.18 (1.11-1.24)	5.46×10^{-9}	0.0002	0.09	1.14 (0.99-1.32)	0.071
rs4646521	*CYP4F3*	19	15769872	G/T	0.35	0.672	0	++++++	1.09 (1.05-1.13)	3.83×10^{-6}	0.018	0.37	0.99 (0.91-1.07)	0.774
rs10405638	*CYP4F3*	19	15764870	C/T	0.36	0.478	0	++++-+	1.09 (1.05-1.12)	7.59×10^{-6}	0.018	0.38	0.99 (0.92-1.08)	0.888
rs4646904§	*CYP4F3*	19	15763721	G/A	0.36	0.488	0	++++-+	1.09 (1.05-1.13)	8.65×10^{-6}	0.018	0.37	0.99 (0.92-1.08)	0.884
rs2102959	*CYP4F3*	19	15767392	A/G	0.36	0.573	0	++++-+	1.08 (1.05-1.12)	1.07×10^{-5}	0.018	0.38	0.99 (0.91-1.07)	0.779
rs10401516	*CYP4F3*	19	15760577	C/T	0.36	0.552	0	++++-+	1.08 (1.05-1.12)	1.17×10^{-5}	0.018	0.38	0.99 (0.92-1.08)	0.895
rs4646514	*CYP4F3*	19	15769270	A/C	0.36	0.562	0	++++-+	1.08 (1.04-1.12)	1.27×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.633
rs4646515	*CYP4F3*	19	15769379	G/C	0.36	0.562	0	++++-+	1.08 (1.04-1.12)	1.27×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.615
rs718258	*CYP4F3*	19	15766419	G/A	0.36	0.564	0	++++-+	1.08 (1.04-1.12)	1.28×10^{-5}	0.018	0.38	0.99 (0.91-1.07)	0.807
rs4646520	*CYP4F3*	19	15769775	A/G	0.36	0.558	0	++++-+	1.08 (1.04-1.12)	1.33×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.599
rs10404163	*CYP4F3*	19	15769805	A/T	0.36	0.558	0	++++-+	1.08 (1.04-1.12)	1.33×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.599
rs4646513	*CYP4F3*	19	15769253	C/A	0.36	0.561	0	++++-+	1.08 (1.04-1.12)	1.34×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.639
rs2073600	*CYP4F3*	19	15757643	C/T	0.36	0.475	0	++++-+	1.08 (1.04-1.12)	1.51×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.983
rs2072598	*CYP4F3*	19	15757528	A/G	0.36	0.472	0	++++-+	1.08 (1.04-1.12)	1.53×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs2280750	*CYP4F3*	19	15757428	T/A	0.36	0.473	0	++++-+	1.08 (1.04-1.12)	1.54×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.992
rs2283611	*CYP4F3*	19	15759828	A/G	0.36	0.461	0	++++-+	1.08 (1.04-1.12)	1.54×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs2283609	*CYP4F3*	19	15759764	C/T	0.36	0.462	0	++++-+	1.08 (1.04-1.12)	1.54×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs2077040	*CYP4F3*	19	15755840	T/C	0.36	0.474	0	++++-+	1.08 (1.04-1.12)	1.55×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs2077080	*CYP4F3*	19	15755392	G/C	0.36	0.474	0	++++-+	1.08 (1.04-1.12)	1.55×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs4808346	*CYP4F3*	19	15758931	T/C	0.36	0.462	0	++++-+	1.08 (1.04-1.12)	1.55×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs8107976	*CYP4F3*	19	15753683	A/G	0.36	0.481	0	++++-+	1.08 (1.04-1.12)	1.55×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.988
rs2203998	*CYP4F3*	19	15754893	A/G	0.36	0.477	0	++++-+	1.08 (1.04-1.12)	1.56×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990

rs8106799	*CYP4F3*	19	15753759	G/A	0.36	0.48	0	++++-+	1.08 (1.04-1.12)	1.56×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.990
rs2204000	*CYP4F3*	19	15755112	G/A	0.36	0.479	0	++++-+	1.08 (1.04-1.12)	1.56×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.986
rs2203999	*CYP4F3*	19	15754975	C/T	0.36	0.479	0	++++-+	1.08 (1.04-1.12)	1.56×10^{-5}	0.018	0.37	1.00 (0.92-1.08)	0.986
rs4646512	*CYP4F3*	19	15764220	C/T	0.36	0.454	0	++++-+	1.08 (1.04-1.12)	1.58×10^{-5}	0.018	0.38	1.00 (0.92-1.08)	0.948
rs116260720	*GPX5*	6	28502794	C/A	0.22	0.335	12.5	+++++-	1.10 (1.05-1.14)	1.65×10^{-5}	0.018	0.21	1.11 (1.00-1.22)	0.043
rs4646516	*CYP4F3*	19	15769449	A/G	0.36	0.568	0	++++-+	1.08 (1.04-1.12)	1.67×10^{-5}	0.018	0.38	0.98 (0.90-1.06)	0.639

NSCLC: non-small cell lung cancer; EAF, effect allele frequency; FDR, false discovery rate; TRICL, Transdisciplinary Research in Cancer of the Lung;

* reference allele / effect allele;

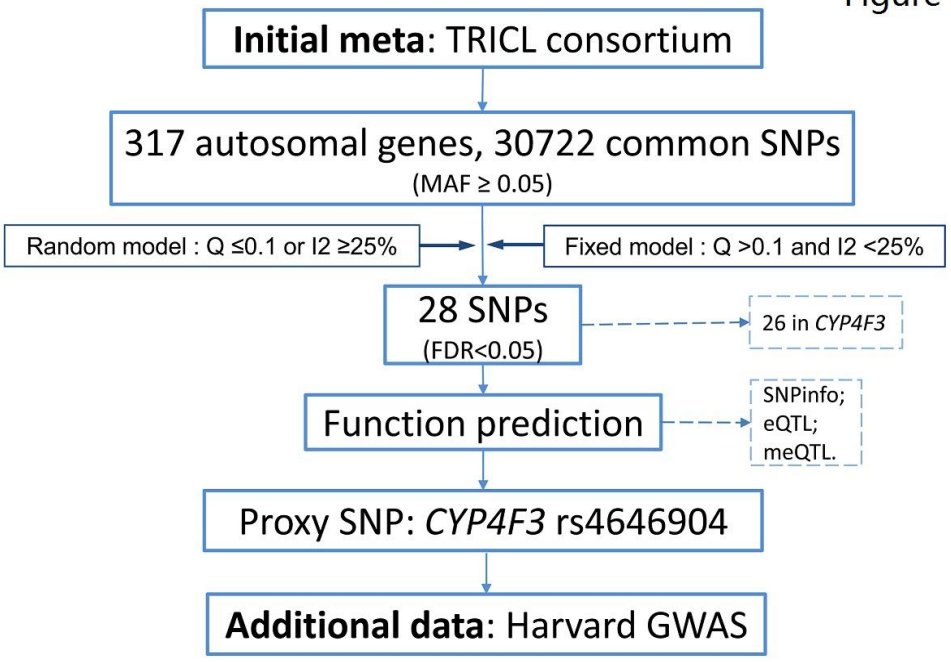
†fixed effect models were used when no heterogeneity was found between studies (Q>0.10 and I^2 <25.0%); otherwise, random effect models were used;

‡means positive association, and-means negative association;

§online function prediction tool, SNPinfo [25], found that rs4646904 might influence splicing efficiency of *CYP4F3*;

SNPs in bold were putative functional and were not located in the major histocompatibility complex region.

Figure 1



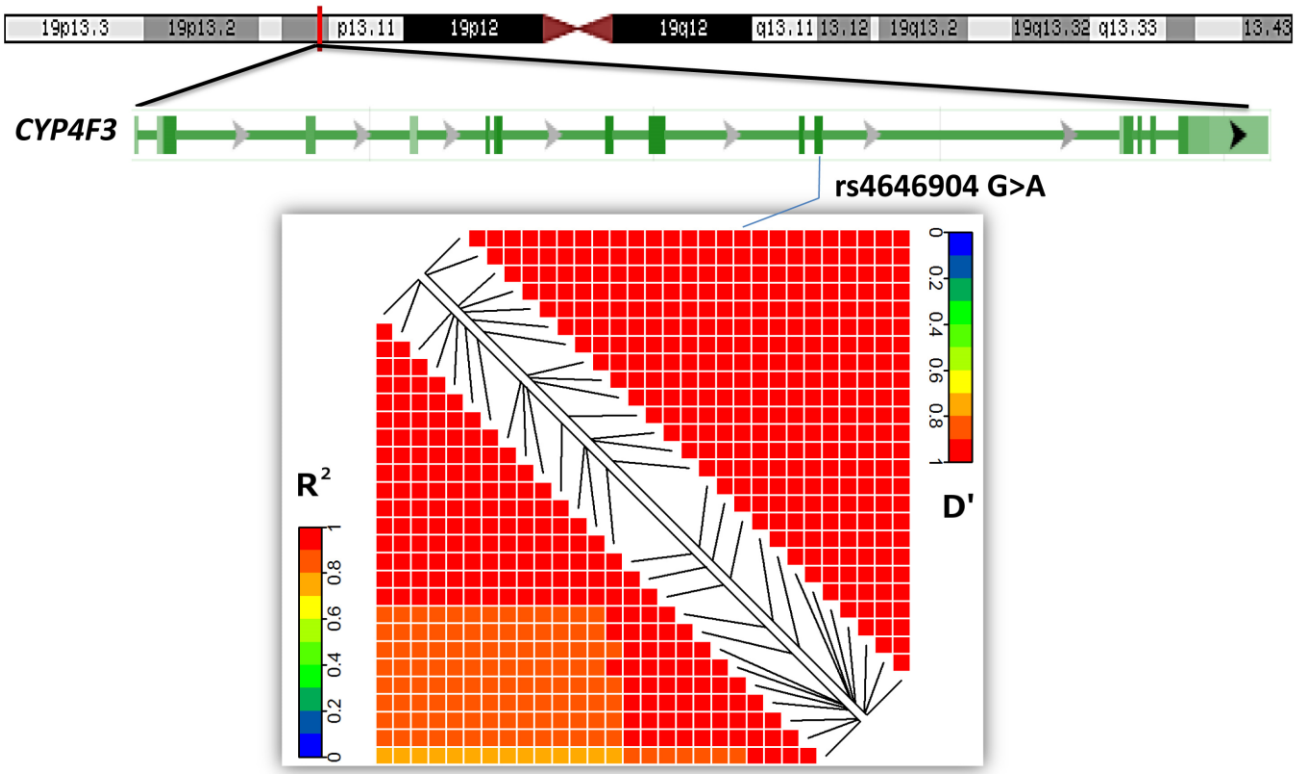


Figure 2

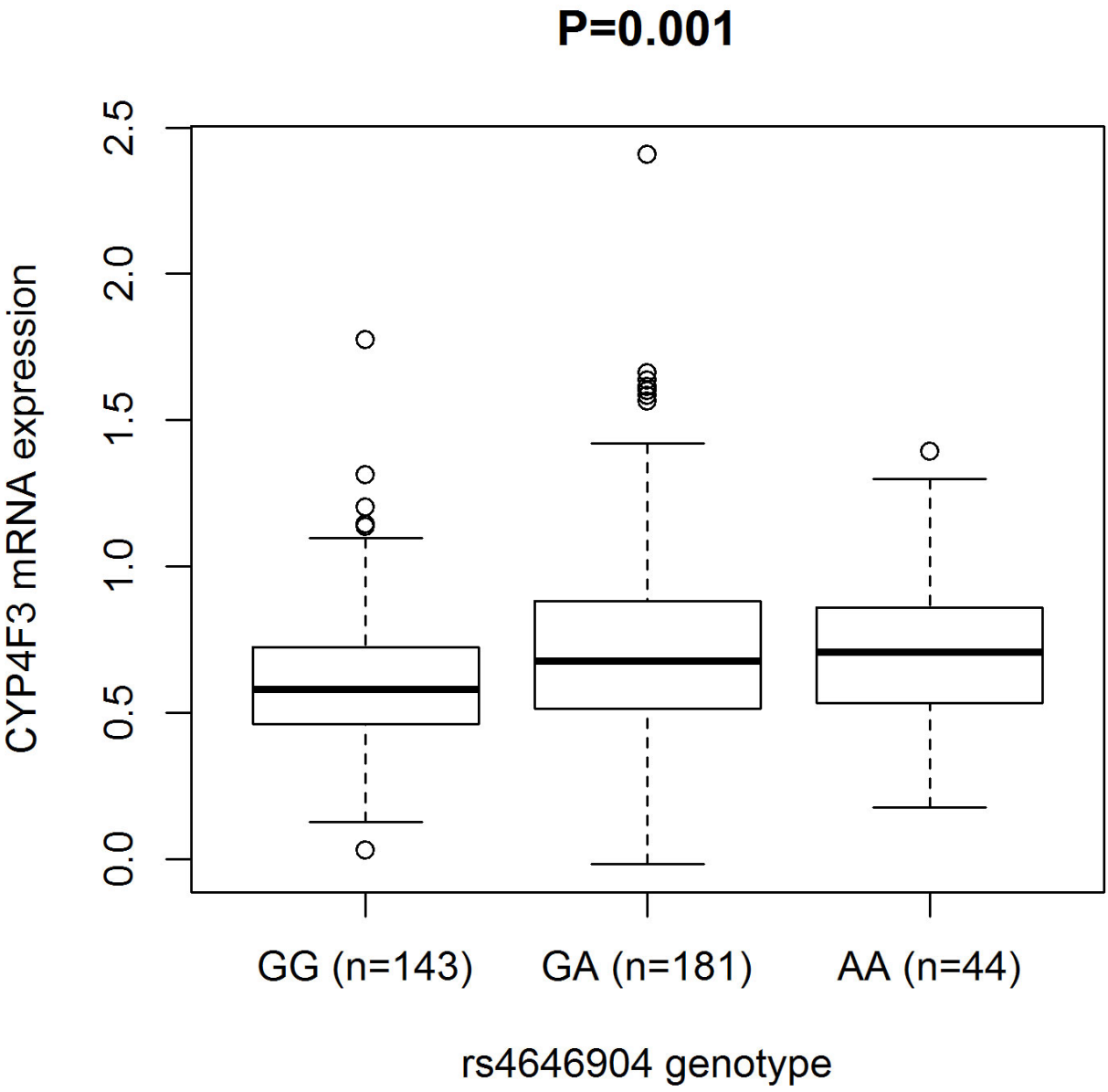
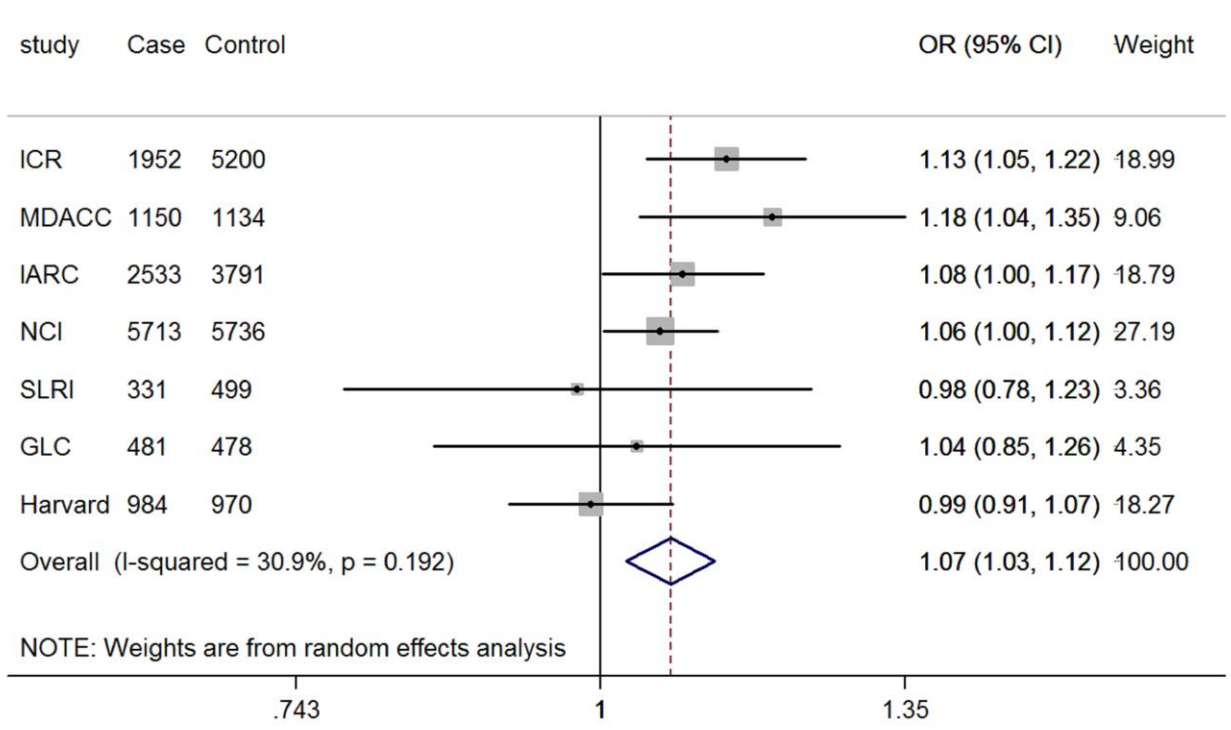


Figure 3



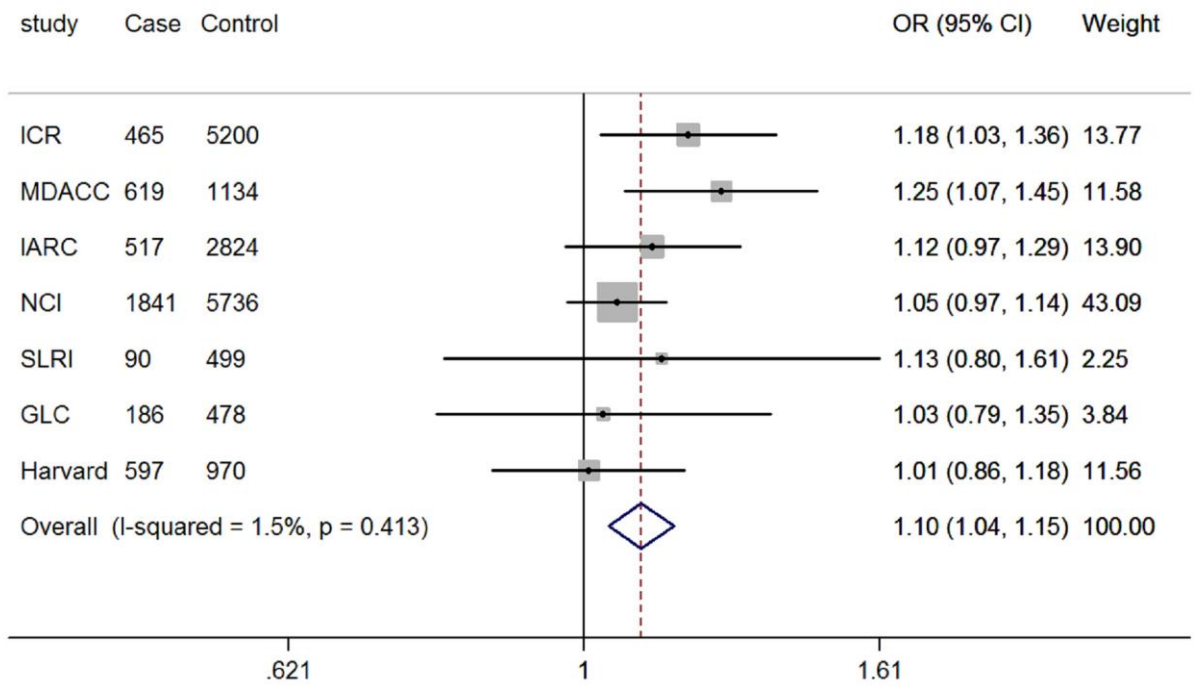
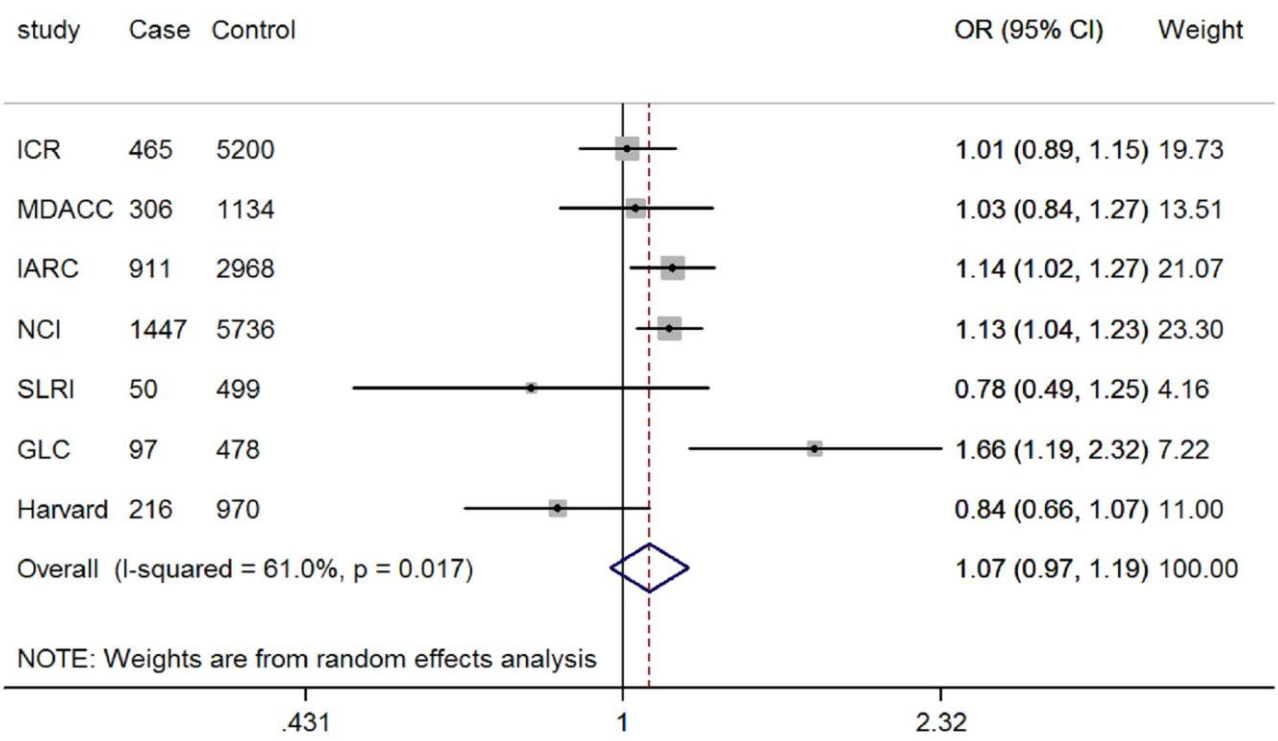


Figure 4



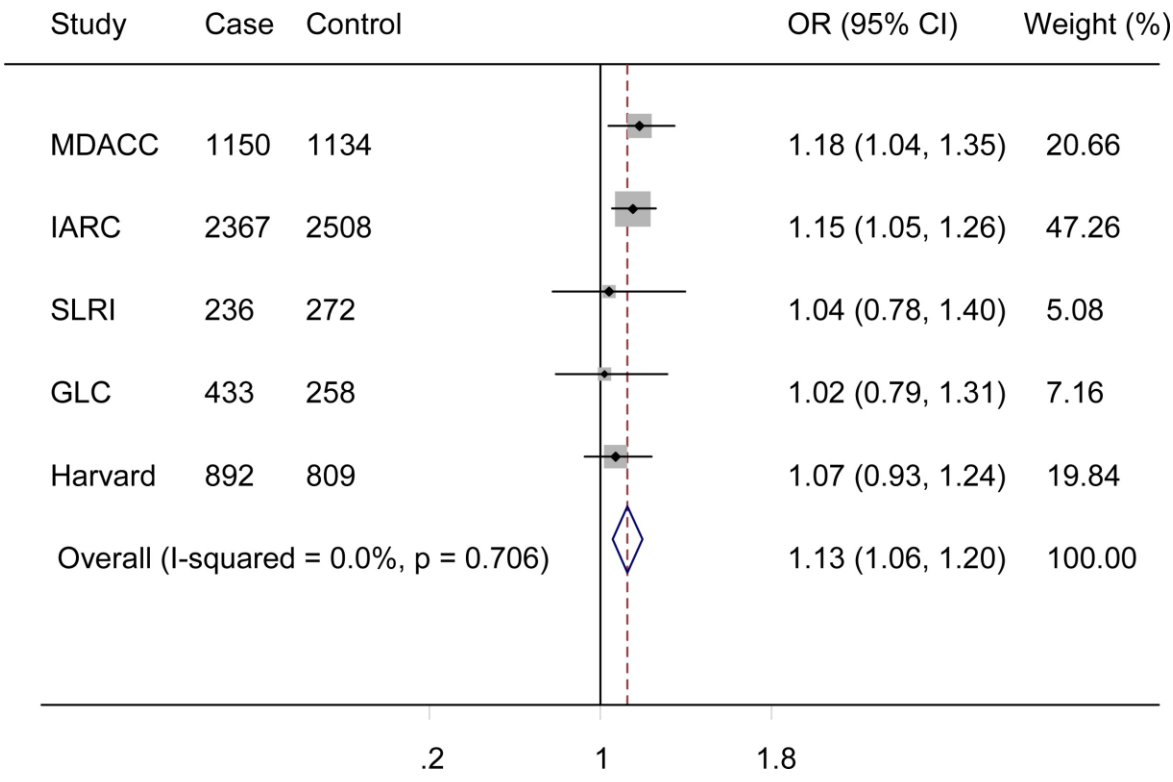


Figure 4

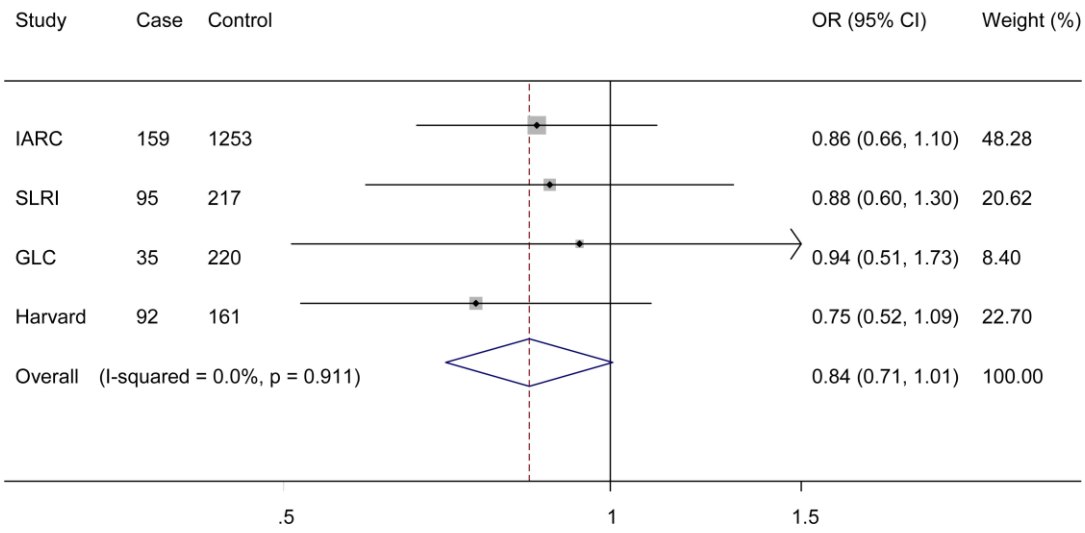


Figure 4