Genome-wide interaction analysis identified low-frequency variants with sex disparity in lung cancer risk

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Abstract

There is growing evidence showing that sex disparity in lung cancer risk cannot be fully explained by sex differences in smoking behavior, implying existence of genetic and molecular basis for sex disparity in lung cancer development. However, the information about sex dimorphism in lung cancer risk is quite limited despite the great success in lung cancer association studies. By adopting a stringent two-stage analysis strategy, we performed a genome-wide gene-sex interaction analysis using genotype from a lung cancer cohort including ~ 47,000 individuals with European ancestry. Three low-frequency variants (minor allele frequency < 0.05), rs17662871 (OR=0.71, $p=4.29 \times 10^{-8}$); rs79942605 (OR=2.17, $p=2.81 \times 10^{-8}$); and rs208908 (OR=0.70, $p=4.54 \times 10^{-8}$), were identified with different risk effect of lung cancer between men and women. Further eQTL and functional annotation analysis suggested rs208908 affect lung cancer risk through differential regulation of CXADR (Coxsackie Virus And Adenovirus Receptor) gene expression in lung tissues between men and women. Our study is one of the first studies to provide novel insights about the genetic and molecular basis for sex disparity in lung cancer development.

Introduction

Lung cancer is the leading cause of cancer death for both men and women worldwide with a complex genetic and molecular mechanism. Differences by sex in lung cancer incidence and mortality have been reported¹⁻². Historically, the sex difference in lung cancer risk development was explained by sex-differences in smoking behavior as women were less likely to smoke cigarettes, initiated smoking at older ages, and smoked fewer cigarettes per day³⁻⁵. However, as smoking behavior has become increasingly similar between men and women, there is growing evidence that sex differences in lung cancer risk cannot be fully explained by differences in smoking behavior, implying sex-based variations in the genetic and molecular basis for lung cancer⁶. However, the information for sex differences in lung cancer risk remains poorly understood despite the extensive efforts spent in lung cancer research and great success in identifying genetic factors through lung cancer association studies.

Genome-wide association studies (GWAS) have been used to identify sexual dimorphism in genetic susceptibility to lung cancer. Sex-specific GWAS identified *VTI1A*, *ACVR1B* and *FOXP4-AS1* genes in women that influence lung cancer development⁷⁻⁸. However, prior female sex-specific GWAS could not test for statistical significance in males or for gene-sex interactions. Gene-sex interaction analysis, on the other hand, will evaluate the information from both male and female group systematically and can identify the variants with significant difference between men and women, although those variants may not achieve genome-wide significance in stratified analysis. But genome-wide genetic interaction (GWGI) studies still remain challenging as most genome-wide association studies were designed for main effect association analysis and have had limited power for interaction analysis. A two-step analysis has been proposed and utilized in GWGI: step 1, a case-only analysis to test the association between SNPs and environmental/or biological factors; step 2, candidate SNPs from step 1 are further submitted to standard case-control logistic interaction analysis⁹. This two-step study design benefits from robustness from case-only analysis and stringency from case-control analysis and has been applied in a number of GWGI studies¹⁰.

Functional inference of genetic variants is important for gaining insights about the molecular mechanism of the disease and clinical application of GWAS findings. Over 90% of GWAS variants are located within non-coding regions and they may affect disease risk through regulating expression of nearby genes¹¹⁻¹². Expression quantitative trait loci (eQTL) analysis, an allelic association analysis with gene expression, provides a straightforward method to identify susceptibility genes associated with the GWAS hits and it has been widely used in GWAS to investigate the regulatory effect of variants¹³⁻¹⁵. However, there are few or no reports about the application of eQTL in gene-sex interaction analysis in human diseases because of the limited studies in sexual dimorphism in disease risk. Another important approach for assessment of genetic variants is functional annotation analysis. Researchers developed various tools to infer the functional roles for the variants such as CADD (Combined Annotation Dependent Depletion) and RegulomeDB¹⁶⁻¹⁸. These tools provide insightful information about the functional inference and are very helpful for fine mapping and pinning down the true causal allele.

In this report, we performed a genome-wide gene-sex interaction analysis using genotype from a large lung cancer cohort including ~ 47,000 individuals with European ancestry. Adopting the two-step analysis strategy, we identified novel variants with different risk effects between men and women that were missed by main-effect association analysis. eQTL and functional annotation analysis provided further information about the functional role for the identified genetic variants and supplied multiple lines of evidence for the sexual disparity in lung cancer development. Our study is one of the first studies for sexual difference in risk of cancer development between men and women. It is also the largest scanning for gene-sex interaction in lung cancer and explored the genetic and molecular basis for sexual differences in risk of this deadly disease.

Material And Methods

Materials

The imputed genotypes (reference panel: HRC r1.1) from 46,783 individuals from European population, with lung cancer phenotype, smoking (ever- vs never-smokers) and sex information, in the INTEGRAL (Integrative Analysis of Lung Cancer Etiology and Risk)-ILCCO (International Lung Cancer Consortium) lung cancer consortium were analyzed in this study¹⁹. The subjects came from 9 independent studies: the OncoArray Consortium Lung Study (OncoArray), including 16,845 lung cancer cases and 13,057 controls, was used as the discovery dataset²⁰⁻²¹. Individuals from another 8 smaller independent studies: Affymetrix Axiom Array Study (AFFY), the Genetic Epidemiology of Lung Cancer (GELCC) Consortium, the Environment and Genetics in Lung cancer Etiology study (EAGLE), the International Agency for Research on Cancer (IARC), MD Anderson Cancer Center Study (MDACC), NCI Lung Cancer and Smoking Phenotypes in African-American Cases and Controls (NCI), the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), and Samuel Lunenfeld Research Institute Study (SLRI), were combined together and used as replication dataset including 7,378 lung cancer cases and 9,503 controls (Table 1, Supplementary Table S1)²²⁻²⁵. There were 12,084 women and 17,818 men in discovery dataset; and 6,366 women and 10,515 men in replication dataset. 60.4% of the lung cancer patients were male in the discovery study compared with 64.0% in replication study. 81.6% of individuals in both discovery and

validation studies were ever-smokers. Stringent quality controls were applied on SNPs and SNPs selected for the analysis had to qualify by two criteria: 1, had imputation information score ≥ 0.7 in discovery data; 2, had information score > 0.2 in each of the eight studies and sample-size weighted information score ≥ 0.7 in replication data. About ~ 20,000,000, out of 39,000,000 imputed SNPs, with information score ≥ 0.7 were analyzed in discovery study; and ~ 10,000,000 SNPs were analyzed in replication study (Fig. 1). 193,050 markers, common to the 9 studies and with linkage disequilibrium r^2 value less than or equal to 0.5, were selected to calculate principal components in PLINK. Detailed information about data collection, genotype imputation and quality control procedures can be found from our earlier publication¹⁹.

Table 1
Characteristics of participants that were studied during the Discovery and Replication phases.

	Discovery ¹			Replication ²		
	Male (%)	Female (%)	Total	Male (%)	Female (%)	Total
Controls	7646 (58.6)	5411 (41.4)	13057	5796 (61.0)	3707 (39.0)	9503
Cases	10172 (60.4)	6673 (39.6)	16845	4719 (64.0)	2659 (36.0)	7378
Ever-smokers	15584 (63.9)	8813 (36.1)	24397	9230 (67.0)	4553 (33.0)	13783
Never-smokers	2234 (40.6)	3271 (59.4)	5505	1285 (41.5)	1813 (58.5)	3098
Samples with European ancestry in INTEGRAL-ILCCO consortium were analyzed in the study. Number of overall lung cancer cases was provided in the table. 1, genotype data from Oncoarray study was used in Discovery study; 2, genotype from the other 8 studies were used in replication study.						

Statistical Methods For Gwgi

Following the two-step analysis strategy, case-only analysis was first performed between SNP dosage (additive model) and sex (male and female were coded as 1 and 2, respectively) phenotype using lung cancer patients from discovery study (N = 16,845) and replication study (N = 7,378) (Case-only model, S denotes smoking status). Fixed-effect meta-analysis was conducted to combine information from both studies. Variants selected for further case-control analysis had to satisfy all three criteria: 1, with case-only joint p-value $< 5 \times 10^{-8}$; 2, case-only $p < 0.1$ in replication study; and 3, same direction of effect in both studies. All the samples in discovery and replication study including 24,223 lung cancer patients and 22,560 controls were applied in case-control analysis (Case-control model, D denotes disease status). The SNPs with case-control gene-sex interaction p-value < 0.05 and same direction of interaction effect in both discovery and replication datasets were reported as significant findings. For the significant variants with low allele frequency (MAF < 0.05), we further validated the signals using firth logistic regression, a method designed for rare variants association test to reduce small-sample bias in regular logistic regression (Fig. 1)²⁶. The statistical analysis was adjusted for smoking status (ever- and never-smokers) and the first 5 principal components. The analysis was conducted in overall lung cancer as well as adenocarcinoma (No. Cases = 9,791) and squamous (No. Cases = 6,107) lung cancer.

$$\text{logit}(D) = \beta_0 + \beta_1 \times \text{SNP} + \beta_2 \times \text{sex} + \beta_3 \times \text{SNP} \times \text{sex} + \gamma \text{ (case - control model)}$$

$$\text{logit}(S) = \beta_0 + \beta_1 \text{SNP} + \gamma \text{ (case - only model)}$$

Eqtl Analysis

Genotype dosage and gene expression rpkm (Reads Per Kilobase Million) data from lung tissue were downloaded from GTEx (phs000424.GTEx.v7.p2). There were 377 individuals with European ancestry available for the analysis, including 244 men and 133 women. Average rpkm for the gene was used if there were duplicated samples. Individuals with rpkm < 0.25 were removed from the analysis. For imputed variants, we applied a best guess algorithm to assign most likely genotypes to each individual to transform the dosage into genotype. For variants with minor allele frequency (MAF) ≥ 0.1 , we adopted an additive model: dosage values ≤ 0.2 were coded as 0; dosage between 0.4-0.6 were coded as 1; and dosage ≥ 0.8 was coded as 1. For variants with MAF < 0.1 , we adopted a dominant model: dosage values ≤ 0.4 were coded as 0 (non-carrier group with no risk alleles) and dosage ≥ 0.6 was coded as 1 (carrier group with at least 1 risk allele). For each candidate SNP, the boxplot of $\log_2(\text{rpkm})$ across different genotype were displayed for all the individuals, men and women groups. Student's test was performed to compare the mean $\log_2(\text{rpkm})$ across different genotype groups and general linear regression was conducted to test SNP-sex interaction in gene expression prediction.

$$\log_2(\text{rpkm}) \sim \beta_0 + \beta_1 \times \text{SNP} + \beta_2 \times \text{sex} + \beta_3 \times \text{SNP} \times \text{sex} + \gamma \text{ (general linear model)}$$

Functional annotation analysis

Two public functional annotation tools, CADD and RegulomeDB were applied for functional inference. CADD was designed to predict functional variants by integrating diverse information from wide range of function categories¹⁶. It computed a score inferring the functional ranking of the variants which is helpful for fine mapping. RegulomeDB is a tool designed to predict regulatory DNA elements in the Human genome based on information from GEO, ENCODE and public literature¹⁷. It adopted SURF (Score of Unified Regulatory Features) model, trained on data from massively parallel reporter assays, to predict the

functional variants in enhancer and promoter elements. RegulomeDB provides a probability score for the variant being transcription factor binding site (TFBS), promotor or DNase hypersensitivity, etc. PROMO, an online tool to perform computational-based searches for gene expression regulatory sequence motif based on TFBS database, was used to search for the functional motif with sequence containing the candidate variants as input¹⁸.

Table 3
Functional annotation of candidate SNPs from significant regions.

SNP	rsID	Case-only	CADD	RegulomeDB	
POS	SNP	P	PHRED ¹	Function Annotation	Probability ²
17:49639139:G:A	rs17662871	4.29x10 ⁻⁸	2.534	TF binding or DNase peak	0.49
20:15642704:G:A	rs76314075	1.33x10 ⁻⁷	1.445	Other	0.18
20:15644218:T:C	rs79942605	2.81x10 ⁻⁸	8.228	TF binding or DNase peak	0.04
21:18776825:C:T	rs208900	5.87x10 ⁻⁶	0.668	TF binding or DNase peak	0.13
21:18779654:G:A	rs184089	5.79x10 ⁻⁶	1.066	Motif hit	0.48
21:18783833:G:A	rs11088636	4.24x10 ⁻⁶	0.039	Other	0.18
21:18784296:T:C	rs423598	4.78x10 ⁻⁶	2.243	TF binding or DNase peak	0.13
21:18785818:G:A	rs208908	4.54x10⁻⁸	5.773	TF binding or DNase peak	0.92
21:18787462:T:C	rs208914	5.01x10 ⁻⁶	2.944	TF binding + DNase peak	0.61
21:18787572:T:C	rs9637031	1.34x10 ⁻⁷	4.018	TF binding + DNase peak	0.61
21:18787948:A:G	rs6517771	1.76x10 ⁻⁶	5.095	Motif hit	0.34
21:18788445:A:C	rs1389157	1.30x10 ⁻⁶	6.257	TF binding or DNase peak	0.03
SNPs with case-only joint p-value < 1x10 ⁻⁵ were selected for annotation analysis. eQTL displayed the information from GTEx Portal. The annotation information for rs208908 was bolded. 1, scaled CADD score by expressing the rank in order of magnitude terms; 2, RegulomeDB probability score is ranging from 0 to 1, with 1 being most likely to be a regulatory variant.					

Results

Novel signals from genome-wide gene-sex interaction analysis

In the case-only analysis stage, a total of 19,943,415 and 10,359,674 SNPs were tested in the discovery and replication studies, respectively. Figure 2a displays the Manhattan plot of joint case-only analysis of gene-sex interactions in lung cancer. No inflated type I error rate was detected ($\lambda = 0.98$). There were 22 SNPs with a joint case-only p-value < 5x10⁻⁸ in meta-analysis and three of them, rs17662871, rs79942605 and rs208908 had case-only p-value < 0.1 in the replication study. Those SNPs were submitted to case-control interaction analysis using pooled discovery and replication data including 24,223 lung cancer patients and 22,560 controls. All of these SNPs had interaction p-value < 0.05 in case-control analysis (Supplementary Table S2). All three candidate variants had MAF < 0.05 and thus were further validated using Firth logistic regression method.

The results from Firth test for the three significant variants are reported in Table 2. SNP rs17662871, located near the *CA10* (Carbonic Anhydrase 10) gene, had an OR of 0.69 and case-only p-value of 1.21x10⁻⁷ in discovery study, and OR of 0.80 and p-value of 7.03x10⁻² in the replication study. Further case-control validation using Firth test detected an interaction OR of 0.78 and p-value of 4.06x10⁻³. SNP rs208908, located near *CXADR* (Coxsackie Virus And Adenovirus Receptor) gene, had an interaction p-value of 2.84x10⁻⁶ (OR = 0.71) and 4.49x10⁻³ (OR = 0.67) in case-only discovery and replication studies, respectively. It has a joint interaction p-value of 4.54x10⁻⁸ (OR = 0.70) and p-value of 1.74x10⁻² (OR = 0.80) in case-control analysis. rs79942605 had an OR of 2.17 (p = 2.81x10⁻⁸) in joint case-only analysis. In addition, case-control analysis identified an interaction OR of 1.68 (p = 1.64x10⁻²) in lung cancer cohort. We performed the same test in other lung cancer subtypes, such as lung adenocarcinoma and squamous lung cancer, etc., but did not identify any significant interactions.

Table 2
Significant signals identified and replicated in gene-sex interaction analysis in overall lung cancer.

Case-only				Case-control									
				Discovery		Replication		Meta-analysis					
SNP	rsID	MAF	GENE	OR	P	OR	P	P	OR	Q	I	OR	P
17:49639139:G:A	rs17662871	0.049	CA10 distal	0.69	1.21×10^{-7}	0.80	7.03×10^{-2}	4.29×10^{-8}	0.71	0.27	17.10	0.78	4.06×10^{-3}
20:15644218:T:C	rs79942605	0.012	MACROD2	2.37	9.05×10^{-8}	1.67	6.34×10^{-2}	2.81×10^{-8}	2.17	0.28	14.83	1.68	1.64×10^{-2}
21:18785818:G:A	rs208908	0.039	CXADR distal	0.71	2.84×10^{-6}	0.67	4.49×10^{-3}	4.54×10^{-8}	0.70	0.76	0.00	0.80	1.74×10^{-2}

The results from analysis using Firth logistic regression method were reported. In case-control analysis, all the cases and controls from both discovery and replication study were pooled together for the study. The first 5 principal components and smoking status were adjusted in the analysis. Infor. Q indicated p-values for Cochran's Q statistic; I indicated I² heterogeneity index (0-100); Score indicated the imputation quality score for the variants.

Regional Plot At The Significant Regions

We examined the regional information around the significant findings. rs17662871, close to *CA10* gene, was the single variant achieving genome-wide significance of gene-sex interaction in the region and there were no other variants that were in strong LD with this variant. This variant had an imputation quality score of 0.72 (Fig. 2b Top). SNP rs79942605, located within *MACROD2* (Mono-ADP Ribosylhydrolase 2) gene, had an interaction p-value of 2.81×10^{-8} and OR 2.17 in joint analysis. Another variant rs76314075, in high LD with rs79942605 ($r^2 > 0.8$), had an OR of 2.11 and p-value of 1.33×10^{-7} in the joint analysis (Fig. 2b Middle). For the third novel variant, rs208908 (OR = 0.70, $p = 4.54 \times 10^{-8}$), located upstream of *CXADR* gene, we found 8 variants with joint case-only interaction p-value $< 1 \times 10^{-5}$ and the strongly associated variant rs9637031 had an OR of 0.70 and p-value of 1.34×10^{-7} in the joint analysis (Fig. 2b bottom).

Stratified Analysis Of Lung Cancer Risk At Significant Snps

To explore how sex influenced genetic risk in lung cancer, we conducted stratified lung cancer risk analysis in men and women using discovery and replication combined dataset. We observed very distinct lung cancer risk-patterns between men and women at the three identified variants. For example, rs208908, had a protective effect for lung cancer in women with OR of 0.81 ($p = 1.17 \times 10^{-3}$) but had no significant effect in males (OR = 1.03, $p = 0.59$) (Fig. 2c). Similarly, SNP rs17662871 had a protective effect for lung cancer in women with OR of 0.86 ($p = 1.44 \times 10^{-2}$). We further stratified the analysis by smoking status (ever- Vs. never-smokers) and the risk effect did not vary by smoking status for these two SNPs. rs79942605, on the other hand, had an increased risk for lung cancer in women (OR = 1.52, p-value = 4.76×10^{-3}). And this variant only had risk effect in ever-smoking women (OR = 2.03, $p = 4.47 \times 10^{-4}$) while not never-smoking women (OR = 0.60, $p = 1.79 \times 10^{-1}$).

Discussion

Sex is an important biological factor in human disease development and extensive studies have been conducted to demonstrate sex differences in incidence, prognosis, and treatment of various diseases including cancer¹⁻². In 2016, a comprehensive characterization of molecular differences between male and female patients in 13 cancer types using multidimensional genomic data in TCGA (The Cancer Genome Atlas) categorized lung cancer into the strong sex effect group with more extensive sex-biased genetic and molecular signatures²⁹. However, differences in risk based on sex are one of the least studied factors in cancer susceptibility. And study of sex disparity in lung cancer risk development is quite limited despite the success of GWAS in lung cancer research during the past decade. A comprehensive gene-sex interaction scanning combined with functional annotation analysis provided exploratory insights about molecular mechanism underlying difference in lung cancer risk between men and women.

Leveraging the rich resource from INTEGRAL-ILCCO lung cancer consortium, we conducted one of the largest gene-sex interaction analysis in overall lung cancer as well as lung cancer subtypes using imputed genotype from ~ 47,000 individuals with European ancestry. By adopting a robust and stringent two-stage analysis strategy, we identified three significant gene-sex interactions in overall lung cancer and all the three variants had small minor allele frequency between 0.01 and 0.05. Considering all the three variants had MAF < 0.05 and the standard regression method may not preserve the type I error rate for variants with low allele frequency, we further applied the Firth test and validated the signals at the three SNPs. We even retrieved ~ 200 SNPs from the three candidate regions and compared the results between regular logistic regression method and Firth logistic regression method. The beta estimation and p-values between these two methods were highly concordant with each other implying that our results from case-only analysis were reliable (Supplementary Figure S4). Among the three significant variants, rs17662871 and rs208908 displayed a protective effect for lung cancer in women and no effect in men. And the protective effect did not vary much by smoking status (ever- vs never-) in women. rs79942605, from *MACROD2* gene, displayed strong risk effect in only ever-smoking women (OR = 1.52, $p = 4.76 \times 10^{-3}$) while no effect in men or never-smoking women was observed. We also conducted the analysis in lung

adenocarcinoma and squamous lung cancer but didn't identify any significant interactions, possibly due to decreased sample sizes in these subgroups. This suggests that genome-wide scanning for interactions between genes and environmental/biological factors still remains a challenge requiring a more powerful analysis strategy.

eQTL analysis has been very useful in GWAS to provide additional functional evidence for the identified susceptibility loci. However, very few studies were reported about its application in interaction analysis between genes and environmental/biological factors probably because of the limited studies in interaction scanning, restricting the contribution of eQTL in genetic association studies. We explored the application of eQTL in gene-sex interaction study. Take variant rs208908 and *CXADR* as an example, we didn't identify significant association between rs208908 and *CXADR* gene in main-effect eQTL analysis ($p = 0.47$, Fig. 3a middle). However, we identified distinct gene expression patterns between men and women and we detected significant gene-sex interaction in *CXADR* gene expression prediction ($p = 3.85 \times 10^{-3}$). In addition, the signals from gene-sex interactions between lung cancer risk prediction and *CXADR* gene expression prediction are highly correlated among the SNPs in high LD with rs208908 ($r^2 > 0.8$) (Fig. 3a right). The eQTL analysis provided strong evidence suggesting rs208908 is a functional variant with difference in lung cancer risk by sex; and revealed great potential for application of eQTL in large-scale scanning for interactions between genes and environmental/biological factors.

Further functional annotation analysis inferred rs208908 as a transcription factor binding site with probability of 0.92. Based on information from existing TFBS, an 8-bp transcription factor binding site motif containing rs208908 was predicted and the minor allele "A" at rs208908 is predicted to increase the binding of transcription factor of GR- β and YY1 (Fig. 3b). Both of these transcription factors have been reported to be involved in cancer development and YY1 is a dual function transcription factor and has been implicated as a major driver of many cancers including lung cancer²⁷⁻²⁸. DNase-seq analysis also identified a cis-regulatory region including rs208908 variant in Human cells. These results, combined with eQTL analysis, suggested rs208908 regulated *CXADR* gene expression by interacting with cellular factors such as transcription factors that function through recognition of conserved sequence motif located upstream of gene coding sequence. In 2018, a large-scale GWAS including ~ 370,000 individuals from European population showed that *CXADR* gene was associated with lung function (FEV1/FVC ratio)³⁰. These multiple lines of evidence, from the results from our integrated study to that from previous reports, suggested a disease model for SNP rs208908 with sexual disparity in lung cancer risk. SNP rs208908 has different MAF between women (0.030) and men (0.025). This difference combined with differentiated gene expression regulation mechanism between men and women, leads to different gene expression patterns between these groups resulting in different risk between male and female groups (Fig. 3c). In summary, rs208908 affects lung cancer risk differentially in men and women through downstream CXADR gene expression regulation in lung tissues.

For the other two variants, rs17662871 (close to *CA10*) and rs79942605 (within *MACROD2*), very few supporting variants were identified nearby, which is not unusual for variants with low allele frequency. The low allele frequency also makes eQTL analysis challenging. rs79942605 was not available in GTEx and there were only a few samples with homogenous minor-allele genotype at rs17662871 in GTEx genotype data, which limited our ability to investigate the gene expression pattern across different genotype groups. Both *CA10* and *MACROD2* were reported to be associated with smoking behavior and age at menarche in Caucasian³¹⁻³². Controversial results have been reported for association between age at menarche and lung cancer risk³³⁻³⁵. Our study detected an effect for lung cancer in only women group in these two regions, especially rs79942650 from *MACROD2* gene which has a risk effect in only smoking women, suggesting that sex plays an important role in lung cancer susceptibility and may interact with smoking behavior in cancer risk.

In summary, we conducted a large-scale gene-sex interaction scanning in lung cancer and we identified three significant variants with different risk effects based on sex. Our study is one of the first studies to examine sex disparity in lung cancer development and our results provided insights about the genetic and molecular mechanism underlying the differences in lung cancer susceptibility between men and women. Our findings also suggested quite a few variants still remain undetected in lung cancer, including those with low allele frequency requiring a very large sample size. Variants affecting disease risk through interaction with environmental and biological factors require a more robust analysis strategy.

Declarations

Acknowledgment

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Author contribution

Yafang Li designed and planned the study, presented the results and wrote the manuscript. Xiangjun Xiao assisted the data preparation and genome-wide gene-sex interaction analysis; Jianrong Li and Chao Cheng assisted the motif analysis in functional annotation; Jinyoung Byun assisted the data preparation;

Yohan Bossé assisted the eQTL analysis. Christopher I. Amos conceived and supervised the study. The other authors contributed to data collection. All authors discussed the results and commented on the manuscript.

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Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Flow chart of analysis strategy in genome-wide gene-sex interaction analysis in lung cancer. In genetic association analysis, all the tested SNPs have information score ≥ 0.7 in discovery data; information score > 0.2 in each of the 8 studies and sample-size weighted information score ≥ 0.7 in replication data. Smoking status (ever- vs never-smokers) and first 5 principal components were adjusted in case-only, case-control and firth validation analysis.

a

b

c

Figure 3

Functional analysis of identified variants. a, eQTL analysis at candidate variants. Left and middle, box plot of gene expression from lung between individuals with none (0) and at least on risk allele (1) in female and male combined, female and male groups for CA10 and CXADR, respectively. The data from samples with Caucasian ancestry in GTEx were used in the analysis. Individuals with rpkm < 0.25 were removed from the analysis. P-values and number of samples were labeled above each plot. rs208908 was genotyped in GTEx; rs17662871 was and imputed SNP and SNP dosage ≤ 0.4 was coded as 0; SNP dosage ≥ 0.6 was coded as 1. Linear regression analysis of gene-sex interaction in predicting CXADR gene expression displayed $p=3.85 \times 10^{-3}$. Right, Scatter plot of z-scores from 229 SNPs, ranging from 18745702 to 18806105 (hg19) on chromosome 21, were displayed on the plot. X-axis represented gene-sex interaction analysis in lung cancer risk; and Y-axis represented the z-scores from gene-sex interaction analysis in CXADR gene expression prediction. LD r^2 was computed for each of the SNPs with rs208908 as reference. b, Functional inference of rs208908. The predicted binding motif at rs208908 (highlighted in red color) for transcription factor (TF) using PROMO (Upper). DNase-seq analysis in HEK293T (Human embryonic kidney) cells detected a peak covering rs208908 suggesting it was located within a cis-regulatory DNA sequence element (RegulomeDB bottom). c, Presumptive disease risk model at rs208908. This variant, located upstream of CXADR, may affect lung cancer risk through gene expression regulation.

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