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Genetic Analysis of Lung Cancer and the Germline Impact on Somatic Mutation Burden

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Abstract

**Background:** Germline genetic variation contributes to lung cancer (LC) susceptibility. Previous genome-wide association studies (GWAS) have implicated susceptibility loci involved in smoking behaviors and DNA repair genes, but further work is required to identify susceptibility variants. **Methods:** To identify LC susceptibility loci, a family history-based genome-wide association by proxy (GWAX) of LC (48 843 European proxy LC patients, 195 387 controls) was combined with a previous LC GWAS (29 266 patients, 56 450 controls) by meta-analysis. Colocalization was used to explore candidate genes and overlap with existing traits at discovered susceptibility loci. Polygenic risk scores (PRS) were tested within an independent validation cohort (1 666 LC patients vs 6 664 controls) using variants selected from the LC susceptibility loci and a novel selection approach using published GWAS summary statistics. Finally, the effects of the LC PRS on somatic mutational burden were explored in patients whose tumor resections have been profiled by exome (n = 685) and genome sequencing (n = 61). Statistical tests were 2-sided. **Results:** The GWAX–GWAS meta-analysis identified 8 novel LC loci. Colocalization implicated DNA repair genes (CHEK1), metabolic genes (CYP1A1), and smoking propensity genes (CHRNA4 and CHRNA5). PRS analysis demonstrated that these variants, as well as subgroup-wide significant variants related to expression quantitative trait loci and/or smoking propensity, assisted in LC genetic risk prediction (odds ratio = 1.37, 95% confidence interval = 1.29 to 1.45; P < .001).

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Lung cancer (LC) is the most common cause of cancer-related deaths worldwide. Although most LC risk is attributable to exposure to tobacco smoke, a genetic basis for LC susceptibility was initially identified from familial aggregation studies after accounting for personal smoking habits (1–3), segregation-based analyses (4), and twin studies (5). Genome-wide association studies (GWAS) have subsequently identified multiple LC susceptibility loci in genes related to smoking behaviors (CHRNA5, CHRNA3, CHRNA4, CYP2A6) (6–8), DNA repair (CHEK2, BRCA2, ATM) (9–11), and genes related to telomere regulation (TERT, RTEL, OBF1) (12,13) as well as many loci where the target genes are less obvious (13).

Although traditional GWAS approaches continue to expand in size, novel analytical approaches can leverage existing data from large, genotyped cohorts to identify additional susceptibility loci and explore candidate genes and potential mechanisms by which the susceptibility is mediated. In this current study, we undertook a genome-wide association by proxy (GWAx) of LC. This approach considers unaffected individuals with a first-degree relative diagnosed with the given trait as proxy patients and unaffected individuals without relatives diagnosed with the given trait as proxy controls in large genotyped biobanks (14,15). After meta-analyzing the results with existing LC GWAS, we explored potential candidate genes and functional mechanisms at the newly identified susceptibility loci by considering how the variants in candidate loci overlap (colocalize) with variants associated with other traits, such as tobacco-consumption, lung function, and gene expression quantitative trait loci (eQTL). We then performed polygenic risk scores (PRS) analyses based on the genome-wide significant variants from this expanded GWAS, as well as a novel variant method that selected variants (including subgenome-wide significant variants) that shared association with LC-related traits. Finally, we investigated how these LC PRSs affect the somatic mutation environment in patients whose tumors have been characterized by exome or whole genome sequencing (WGS).

**Methods**

**Statistical Analysis**

All statistical tests described below were 2-sided. A family history GWAx analysis of LC was undertaken using the method described by Liu et al. (14). After applying genotyping quality control metrics to exclude suboptimal genotypes and samples and limit genetic ancestry analysis to European descent (Supplementary Table 1 and Supplementary Materials, available online), 48,843 individuals with a family history of LC and 195,387 controls with no reported cancer diagnosed or reported family members with cancer were identified from the UK Biobank. A GWAx was performed using unconditional logistic regression model (with adjustment for age, sex, array type, number of siblings, and principal components from genetic-inferred ancestry). Association statistics were corrected to account for the genetic dilution related to the GWAx by doubling beta coefficients and standard error as described previously (14). Adjusted statistics from the UK Biobank GWAx and a previous LC GWAS (Transdisciplinary Research In Cancer of the Lung) cohort—29,266 patients and 56,450 controls—(Supplementary Materials, available online) were meta-analyzed using METASOFT under a fixed-effects assumption based on the inverse-variance-weighted effect size (16). Statistical significance (genome-wide significance) was defined as a P value less than 5 x 10^-8. Independent genetic variants at a given locus were defined using linkage disequilibrium (LD) clumping (with a LD threshold of R^2 < 0.1 and a window size of 10,000 kb). Genetic correlation (r_g) between the GWAx and the GWAS was estimated using LD score regression, which was performed using the LDSC package (17).

**Colocalization Analysis**

Colocalization of genetic associations between LC, gene expression, and related traits was calculated using the COLOC package (https://github.com/anthony-aylward/coloco) (18) using default thresholds and a window size of 75 kb. eQTLs within lung tissues or brain regions related to addiction (substantia nigra, nucleus accumbens, frontal cortex, putamen, caudate) were obtained from the Genotype-Tissue Expression (GTex) project (19). GWAS summary statistics were obtained from the GWAS & Sequencing Consortium of Alcohol and Nicotine use (GSCAN) for smoking behaviors, and summary statistics from additional related traits (forced vital capacity [FVC] and coffee consumption) were retrieved from OpenGWAS (20,21) (Supplementary Materials, available online). For this manuscript, we report the posterior probability of colocalization for a single shared variant responsible for the associations in both traits (posterior probability for hypothesis 4 [PP4]).

**PRS Analysis**

The PRSs were computed as the sum of the individuals weighted genotypes using PLINK and PRSice-2 software with the PRS subsequently scaled to a normalized distribution (Supplementary Materials, available online) (22,23). Genotyping weighting was derived from the estimates (log odd ratios) from the LC GWAx-GWAS meta-analysis. Variants selected for PRS inclusion were chosen by different criteria. First, we selected variants that achieved genome-wide significance (GWS) in the GWAx-GWAS meta-analysis (gwPRS). Second, a partial least squares (PLS) method was used to select variants involved with both LC and smoking (smPRS) or gene expression (eQTLPRS) based on previously published GWAS summary statistics and the GTEx summary statistics (Supplementary Materials, available online). Finally, a combined PRS of the unique variants from gwPRS, smPRS, and eQTLPRS was also considered (Supplementary Materials, available online).

The PRS construction considered LD between variants (any variant with R^2 > 0.1 with a sentinel variant was excluded). The PRSs were assessed in a validation cohort of 1,666 LC patients and 6,664 controls from the UK Biobank that were not included in the GWAx described above. Unconditional logistic regression was used to test the association between PRSs and LC, with sex, array type, age of recruitment, and the first 5 principal
components from genetic-inferred ancestry as covariates. Regression results are reported as an odds ratios unit change per a standard deviation in the PRS distribution.

**Somatic Mutation Analysis**

The association between the PRSs and somatic mutational burden was tested in The Cancer Genome Atlas (TCGA) cohorts of 685 LC samples of European ancestry and in an independent cohort of 61 LC patients, identified from central and eastern Europe (13). Germline genotypes were derived from Affymetrix arrays which was accessed via the database of Genotypes and Phenotypes (dbGaP) (application Project #2731) using single nucleotide polymorphism (SNP) imputation to the 1000 Genome phase 3 reference panel (Supplementary Materials, available online). TCGA somatic mutations (derived from WGS) were retrieved from the study of Elliott et al. (24). In the replication cohort, germline genotypes and somatic mutations were derived from WGS undertaken in the patients paired normal tumor samples sequenced on a IlluminaX5 DNA sequencer (Supplementary Materials, available online). Total number of mutations was computed as a mutational feature and reported smoking tobacco-related signatures (cosmic signature annotation Single Base Substitution 4 (SBS4), Doublet Base Substitutions 2 (DBS2), and Indel signature 83 [A and B] (ID83A, ID83B) were generated using the SigProfiler pipeline (Supplementary Materials, available online). To test the association between the PRS and the tumor somatic mutational burden, the PRS were regressed against tumor somatic mutational burden as a continuous trait using Quasi-Poisson regression to account for overdispersed and left skewed distributions of the DNA mutational features. Covariates added in all PRS models included age, sex, the first 5 principal components from genetic inferred ancestry, tumor purity, and a categorical variable indicating the cohort type as appropriate. Effect estimates are given as incidence rate ratios (IRR). A P value less than .05 was considered as level of significance for PRS analyses. All statistical tests performed to calculate P values were 2-sided tests.

**Results**

The 8 Novel Susceptibility Loci

The family history GWAS (GWAx) on 48 843 self-reported family history LC patients and 195 387 controls from the UK Biobank (Supplementary Table 1, available online) identified 5 loci (5p15.33, 6p21.32, 12p13.33, 13q13.1, and 15q25.1) that had previously been discovered from the traditional GWAS previously performed on the Transdisciplinary Research In Cancer of the Lung cohort (Supplementary Table 2 and Supplementary Figure 1, available online). Genetic correlation using LD score regression confirmed a strong relationship between GWAx and the GWAS (r² = 1, SE = 0.066, P < .001) supporting the utility of the GWAx approach to detect susceptibility loci.

Meta-analysis between the GWAx and the traditional LC GWAS identified 65 variants that achieved a P value less than 5 x 10⁻⁸ across 23 distinct genomic loci defined by cytoband (Figure 1) after LD clumping genetic variants (Supplementary Table 2, available online). At previously described LC susceptibility loci, the meta-analysis also identified independent (R² < 0.1) low-frequency (minor allele frequency [MAF] < 0.05) variants associated with LC at 5p15.33 (rs35812074), 19q13.2 (rs1801272), 15q25.1 (rs2229961, rs8192479, rs151118057), and 12p13.33 (rs7487683) in addition to the previously described common genetic variants (Supplementary Table 2, available online). At 13q13.1, where a rare LC susceptibility allele has been described (rs11571833, K326X BRCA2; MAF = 0.01), an independent common susceptibility allele was also noted (rs11571734; MAF = 0.28).

Eleven LC susceptibility variants at 8 loci have previously not been associated with LC at genome-wide (GW) significance (Figure 1). We explored these loci using colocalization with traits related to LC; smoking behavior; gene expression, particularly in the lung epithelium and brain (addiction); and lung function. Using the GSCAN summary statistics, we observed that the LC susceptibility variants at 1q21.3-rs78062588, 6p22.2-rs766641, and 20q13.33-rs11697662 were also associated at GW significance with traits related to propensity to smoke tobacco (Supplementary Table 2, available online). The sentinel variants at 1q21.3-rs78062588 and 20q13.33-rs11697662 are also eQTLs for the nicotinic acetylcholine receptors (nAChRs) subunits CHRN2 and CHRNA4 (Figure 2, A and B; Supplementary Tables 2 and 3, available online). At 6p22.2, LC susceptibility loci were noted (Supplementary Table 2, available online), typified by 2 sentinel variants: rs6913550 and rs7766641. rs7766641 was also associated with propensity to smoke (colocalization between GSCAN cigarettes per day [CPD] and LC: PP4 = 99%), whereas, curiously, rs6913550 was not (colocalization between CPD and LC: PP4 = 0%) (Supplementary Table 2, available online).

At 1q31.2, 11p11.2, 11q24.2, and 15q24, the sentinel variants (rs4252707, rs72905558, rs61612408, rs12441817, respectively) were not associated with smoking behaviors (colocalization between CPD and LC for all 4 variants: PP4 = 0%). 11q24.2-rs61612408 was associated with the expression of the CHEK1 gene in multiple tissues including lung epithelia. Further, there was evidence for colocalization between these associations (colocalization between CHEK1 lung eQTL and LC: PP4 = 91.7%), with the allele associated with increased expression correlating with decreased risk of LC (Figure 2, C). The 15q24-CYP1A1 locus has been associated with multiple traits, including coffee consumption (20) and FVC (21). In this study, there was evidence for colocalization with LC but only for FVC (colocalization for rs12441817 between coffee consumption and LC: PP4 = 0.00%; colocalization between FVC and LC: PP4 = 97.05%) (Supplementary Figure 2, available online). There was also colocalization for rs12441817 and CYP1A1 expression in the nucleus accumbens (colocalization: PP4 = 93.52%) (Supplementary Figure 3, available online) and an eQTL effect with the processed pseudogene RP11-10017.1 in lung tissue (colocalization between eQTL RP11-10017.1 and LC: PP4 = 99.02%) (Figure 2, D). At 4q13.2-rs18566783, the candidate genes remain ambiguous.

**PRS Evaluation**

Next, we constructed PRSs from variants selected from our meta-analysis and tested their ability to predict LC risk in a validation cohort of 1 666 LC patients and 6 664 matched controls from the UK Biobank. First, we selected 65 independent variants that reached a GWS threshold from our meta-analysis. This PRS was associated with LC (gwPRS: odds ratio [OR] per standard deviation increase in PRS = 1.27, 95% confidence interval [CI] = 1.20 to 1.35; P < .001) (Figure 3). We also sought to select relevant variants that did not pass the GWS threshold. As many of LC GWS variants identified by our meta-analysis also tended to be associated with smoking behaviors and/or eQTLs (Supplementary Table 2, available online), we...
used PLS of published GWAS summary statistics (GSCAN and GTEx) to identify variants associated with LC as well as smoking behaviors and/or eQTL features (see Supplementary Materials). There appeared to be an excess of evidence for association with LC in an important number of the variants selected by this approach (Figure 3, A). Two polygenic risk scores, smPRS (80 variants) and eQTLPRS (961 variants), were constructed using the non-GWS variants selected by this PLS approach, with number of variants selected guided by the degree of enrichment observed (see Supplementary Materials, available online; Figure 3, A and B). Both PRSs were associated with LC in the validation LC patient-control cohort implying these non-GWS genetic variants are enriched for susceptibility alleles and add value to risk prediction (Figure 3, C). Lastly, we constructed a combined PRS (1049 variants) from the GWS variants, eQTL, and the smoking propensity variants, which improved LC risk prediction in this independent series (OR = 1.37, 95% CI = 1.29 to 1.45; P < .001).

PRS Germline Influences on Mutational Burden and Mutational Signatures

Finally, we evaluated the association of the GWS PRS (gwPRS), smoking propensity PRS (smPRS), eQTLPRS, and the combined PRS with somatic mutational burden. In the analysis of 685 lung tumors from TCGA, there was no evidence for association involving the GWS PRS (IRR = 1.03, 95% CI = 0.96 to 1.10; P = .44), eQTLPRS (IRR = 1.05, 95% CI = 0.9 to 1.13; P = .14) and the combined PRS (IRR = 1.03, 95% CI = 0.96 to 1.10; P = .37) on mutation burden (Supplementary Figure 5, available online), however smPRS was associated with somatic mutation load (IRR = 1.12, 95% CI = 1.04 to 1.19; P = .001) (Figure 4, A). The smPRS was similarly associated with burden of mutational signatures attributed to tobacco smoke (SB54 : IRR = 1.18, 95% CI = 1.09 to 1.29; P < .001) (Figure 4, B) and was also observed in somatic insertions and deletions mutation signatures related to tobacco smoke: DBS2 (IRR = 1.17, 95% CI = 1.05 to 1.29; P = .003), ID83A (IRR = 1.21, 95% CI = 1.07 to 1.37; P = .002), and ID83B (IRR = 1.13, 95% CI = 1.01 to 1.27; P = .04), respectively (Supplementary Figure 6, available online). These associations were observed more prominently in patients with Lung Adenocarcinoma (IRR = 1.18, 95% CI = 1.06 to 1.31; P = .002) (Figure 4, A). The 15q25 CHRNA5 LC sentinel variant rs55781567 had the most striking effect (average total mutation count of 327 for homozygous risk carriers of the G allele compared to 283 in homozygous nonrisk carriers of the C allele) (Supplementary Figure 7, available online), but the associations remained significant after excluding GW variants for LC (Figure 4, A). The association between the smPRS and somatic mutation burden was replicated in 61 patients who have undergone WGS (IRR = 1.39, 95% CI = 1.03 to 1.89; P = .04) (Supplementary Figure 8, available online).

Discussion

This study identified 23 LC susceptibility loci, including 8 novel loci, by combining large, genotyped biobank data and traditional GWAS. Of the 8 novel loci, 3 were also associated with propensity to smoke. This included brain eQTLs variants for both subunits of the neuronal nAChRs α4β2 receptor. Variants in LD with the α4 subunit (rs2373500) have been described in nicotine dependency and LC risk, albeit not at GW significance for LC (25), whereas the β2 receptor association with LC risk has not been previously described. The neuronal nAChRs α4β2 receptor is the
Figure 2. Brain and lung eQTLs discovered within the 8 novel loci. Colocalization plots between lung cancer (x-axis) and CHRNA4 putamen expression (1q21.3). A) CHRNA4 putamen expression (20q13.33); B) CHEK1 lung expression (11q24.2); C) RP11-10017.1 lung gene expression (15q24); D) (y-axis). Each variant and eQTL status were compared using COLOC for colocalization to confirm that the lung cancer SNP was the same SNP driving the eQTL effect in both brain and lung tissues, the Bayesian posterior probability (PP4) of each gene was tested. Stars indicate the variant of interest and shading scaled representing the level of LD shared between other markers with sentinel variant ($r^2 > 0.8$; $r^2 > 0.4$; $r^2 > 0.1$). eQTL = expression quantitative trait loci; LC = lung cancer; LD = linkage disequilibrium; SNP = single nucleotide polymorphism.

Figure 3. Germline polygenic risk score construction using smoking and eQTL related SNPs and performance testing within the UK Biobank lung cancer cohort. A) The mean lung cancer association statistics calculated by variant bins (100 variants per bin) ranked by partial least squares (PLS) components. Variants (clumped on LD based on lung cancer P values) were ranked based on PLS components for smoking propensity (Component1_smoking, top) and eQTLs (Component1_eQTL, [B] (x-axis) and plotted against the mean lung cancer Z statistics calculated across variants in each bin (y-axis). Bin values that exceed 3 SDs from the mean are noted, with the excess observed (number of bins smoking propensity = 9, number of bins eQTL = 37) implying that the variants within these bins are enriched for LC-susceptibility alleles. C) A forest plot of the performance of the constructed PRSs in comparison to the PRS based on the 65 GWAS independent loci as a baseline which included array type, sex, age of recruitment and the first 5 principal components from genetic-inferred ancestry). CI = confidence interval; eQTL = expression quantitative trait loci; LC = lung cancer; LD = linkage disequilibrium; GWS = genome-wide significant; OR = odds ratio; PRS = polygenic risk scores; SNP = single nucleotide polymorphism.
most abundant nAChR subtype within the human brain and important within the dopaminergic signaling pathway. The α4/2 receptor has a key role in nicotine dependence behaviors (26) and is a major target in nicotine addiction intervention (27,28). The third novel locus related to LC and propensity to smoke is telomeric to the major histocompatibility complex (MHC) region, where the target candidate gene(s) is less obvious. The MHC region was among the first susceptibility loci to be associated with LC (6,29–31). However, rs7766641 is not in LD with any previously described variants (R² < 0.001) and associated with the number of CPD, implying that these are distinct associations.

This meta-analysis also identified additional LC susceptibility loci that appear to be independent of smoking propensity. These included variants at 15q24 near CYP1A1, CYP1A2, and CYP11A1 that participate in the metabolism of many different xenobiotics and some endogenous substrates. Variants at the 15q24 CYP1A1 and CYP1A2 locus have been linked with multiple traits, notably other functions of propensiby coffee consumption (20) and FVC (21), although these variants associated to each trait appear to be distinct. Colocalization appears to implicate FVC as more likely to be involved in the LC association, and the etiological link also seems more plausible considering aspects of lung function and LC risk. For tissue expression, rs12441817 colocalized with lung tissue expression of the processed pseu-
domorphism.

An additional novel LC susceptibility variant, rs61612408, was a lung tissue eQTL for the DNA repair gene CHEK1 (Figure 2, C). We additionally noted the variant rs4252707 impacting the MDM4 gene, which is an important p53 regulator. This variant was previously associated with nonglioblastoma tumors (32) and more recently squamous cell carcinomas of the lung and head and neck (33). At 11p11.2, despite evidence for an eQTL effect, colocalization analysis showed little evidence for involvement with genes CIQTNF4 (lung) and MTCH2 (brain-cortex), suggesting that these signals are unlikely to explain the LC association. At 4q13.2, the finding remains ambiguous, but from histological subtypes analysis performed from the previous reported GWAS study, it appears that this signal is mostly found in lung adenocarcinoma.

We additionally sought to use the shared genetic etiology between LC susceptibility, smoking-related traits, and gene expression annotations (eQTL) to explore variants that did not achieve GW significance. We used the PLS method to select variants related to these traits for the PRS analyses and demonstrated that such variants are indeed enriched for susceptibility alleles. Although the role of these individual variants remains to be confirmed, these sub-GWS variants were located near relevant candidate genes (propensity to smoke candidates like CHNRA6 and DBH, and eQTLs for ERCC2, RAD51C, XRCC3, and CASP8). Combining both sub-GWS PRS lists (smPRS and eQTL PRS) with GW-significant results reached an odds ratio of 1.37 per standard deviation unit increase in score improving on previous PRS predictions (OR = 1.17 and 1.26, respectively) (34,35), despite the conservative clumping approach (R² < 0.1) employed. This suggests that integrating functional annotations may be of interest for PRS analysis.

Lastly, the analysis of the smPRS demonstrated an association between a person’s genetic risk load and mutation burden...
and/or burden of tobacco-related somatic mutational signatures, within 2 independent patient cohorts and using different sequencing methods (exome sequencing and WGS). These associations appear consistent with the notion that genetic variants influence individuals’ smoking behavior, which in turn influences their carcinogenic exposure, and consequently, their somatic mutation burden (36).

In conclusion, this work has increased the number of variants associated with LC susceptibility, with the identification of novel susceptibility loci.PRS analysis highlighted that many additional variants remain to be discovered and provided insights into the carcinogenic mechanisms.

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**Notes**

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**Author contributions:** AG: Conceptualization, Formal analysis, Data curation, Methodology, Investigation, Software, Validation, Writing—Draft, review and editing, Visualization. JA: Conceptualization, Formal analysis, Data curation, Methodology, Investigation, Software, Validation, Writing—Draft, review and editing, Visualization. RP: Formal analysis, Data curation, Validation, Investigation, Writing-Review & Editing. KSB: Formal analysis, Writing-Review & Editing. VG: Formal analysis, Data curation. CV: Formal analysis, Writing-Review & Editing. JF: Formal analysis, Data curation. DF: Formal analysis, Data curation. DD: Formal analysis, Data curation. AB: Formal analysis, Data curation. AM: Resources. BS: Resources. VJ: Resources. MS: Resources. DM: Resources. JS: Resources. MO: Resources. ILCCO consortium: Resources, Funding acquisition JW: Data curation. SK: Data curation. SR: Data curation. AV: Data curation. MF: Data curation. MJ: Writing—Review and Editing. CA: Conceptualization, Writing—Review and Editing, Funding acquisition, Resources. MF: Conceptualization, Supervision, Methodology, Writing—Draft, review and editing. JM: Conceptualization, Supervision, Data curation, Methodology, Writing—Draft, review and editing, Funding acquisition.

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**Data Availability**

Additional information, including summary statistics,PRS lists, code used in this study can be found on the project webpage here: https://iarc-genetics.github.io/GWAX_lung/. Oncoarray data and summary statistics can be accessed by the database of Genotypes and Phenotypes (dbGaP) under accession phs000876.v1.p1. TCGA data was accessed by dbGaP through Project #2731: Investigation of tobacco-related cancer susceptibility genes following a 2-hit carcinogenic model.

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