Identification of lung cancer histology-specific variants applying Bayesian framework variant prioritization approaches within the TRICL and ILCCO consortia

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Abstract

Large-scale genome-wide association studies (GWAS) have likely uncovered all common variants at the GWAS significance level. Additional variants within the suggestive range (0.001 > P > 5 × 10⁻⁸) are, however, still of interest for identifying causal associations. This analysis aimed to apply novel variant prioritization approaches to identify additional lung cancer variants that may not reach the GWAS level. Effects were combined across studies with a total of 33456 controls and 6756 adenocarcinoma (AC; 13 studies), 5061 squamous cell carcinoma (SCC; 12 studies) and 2216 small cell lung cancer cases (9 studies). Based on prior information such as variant physical properties and functional significance, we applied stratified false discovery rates, hierarchical modeling and Bayesian false discovery probabilities for variant prioritization. We conducted a fine mapping analysis as validation of our methods by examining top-ranking novel variants in six independent populations with a total of 3128 cases and 2966 controls. Three novel loci in the suggestive range were identified based on our Bayesian framework analyses: KCNIP4 at 4p15.2 (rs6448050, P = 4.6 × 10⁻⁷) and MTMR2 at 11q21 (rs10501831, P = 3.1 × 10⁻⁷) with SCC, as well as GAREM at 18q12.1 (rs11662168, P = 3.4 × 10⁻⁷) with AC. Use of our prioritization methods validated two of the top three loci associated with SCC (P = 1.05 × 10⁻⁴ for KCNIP4, represented by rs9799795) and AC (P = 2.16 × 10⁻⁴ for GAREM, represented by rs3786309) in the independent fine mapping populations. This study highlights the utility of using prior functional data for sequence variants in prioritization analyses to search for robust signals in the suggestive range.

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide accounting for over 1 million deaths annually (1). While tobacco consumption remains unequivocally the main determinant of lung cancer incidence, evidence suggests a role of genetic variation in disease etiology (2). The main histological subtypes of lung cancer are adenocarcinoma (AC), squamous cell carcinoma (SCC) and small cell lung cancer
Genome-wide association studies (GWAS) of lung cancer have demonstrated in large-scale replicated analyses that germline genetic variation influences lung cancer risk in European populations 15q25.1 (CHRNA5–CHRNA3–CHRNA4) (5–7), with varying effects by tumor histology. Consistent associations have been observed for AC at Sp15.33 (TERT-CLPTM1L) (8,9) and 3q28 (10), and for SCC at 6p21.33 (BAT3-MSH5) (11), 9p21 (CDKN2A/p16INK4a/CDKN2B/p15INK4b), 12p13 (RAD52) (12), 13q13 (BRCA2) and 22q12 (CHEK2) (10,13). Single-nucleotide polymorphisms (SNPs) at 15q15.2 (TP53bp1) have also been consistently associated with lung cancer risk in candidate gene analyses (14).

Additional susceptibility regions at 13q12.12 and 22q12 (15) have been identified in Asian populations. These regions have not be associated with lung cancer risk in Europeans.

The Transdisciplinary Research In Cancer of the Lung (TRICL) research team and the International Lung Cancer Consortium (ILCCO) recently completed two comprehensive GWAS meta-analysis of lung cancer across multiple subgroups, including smoking, gender/sex, stage of disease, age at onset and histology (10,13,16). Both investigations employed a standard GWAS analytical approach, which relies on P-values from single marker analysis and stringent significance levels to adjust for multiple comparisons. This standard analytical approach is, however, limited in that (i) it does not take study power into account when comparing test statistics; (ii) it ignores biological function of the genes and variants and (iii) the GWAS-level significance is based on a global null hypothesis and true susceptibility loci may be left unidentified. Given the importance of differential response to therapy and subsequent outcomes by lung cancer histological subtypes, we aimed to conduct an in-depth GWAS investigation of lung cancer histological subtypes.

The main innovative aspects of this study include Bayesian framework prioritizations to incorporate prior biological information, five additional lung cancer studies genotyped based on Axiom array and two non-European populations (a Japanese and a Han Chinese population) for across ethnic generalizability. We conducted three Bayesian framework analyses including the stratified false discovery rate (SFDR) (17), the Bayesian false discovery probability (BFDP) (18) and hierarchical modeling (HM) methods (19). We used these methods to incorporate available functional and regulatory data for the genes and variants located on the 550K genotyping platform in order to identify additional genetic regions of interest.

Materials and methods

Study descriptions

Discovery set

The meta-analysis was based on summary data from 13 previously reported lung cancer GWAS conducted in European-descent populations providing genotype data on a total of 14049 lung cancer cases, including 5061 SCC, 6756 AC and 2216 SCLC with 33456 controls of European descent: the M.D. Anderson Cancer Center (MDACC) lung cancer study (6); the Liverpool Lung Project (LLP) (20); the UK lung cancer GWAS from the Institute for Cancer Research (ICR) (13); deCODE Genetics lung cancer study (21); the Helmholtz-Gemeinschaft Deutscher Forschungszentren lung cancer GWAS (22); the lung cancer study from Canada (Lunenfeld-Tanenbaum Research Institute (LTRI) and University of Toronto) (23); the Harvard lung cancer study (24); NCI lung cancer GWAS including the Environment and Genetics in Lung Cancer Etiology (EAGLE) GWAS (25), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) GWAS (26), the Prostate, Lung, Colon, Ovary Screening Trial (PLCO) (27), lung cancer GWAS and the Cancer Prevention Study II Nutrition Cohort (CPS-II) GWAS (28); the IARC lung cancer GWAS (29); the Memorial Sloan Kettering Lung Cancer GWAS (30) and the Mayo lung cancer GWAS (31). In each of these studies, SNP genotyping had been performed using Illumina HumanHap 300 BeadChips, HumanHap 550 or 610 Quad arrays. Further details about genotyping in each study are provided in Supplementary Table 2, available at Carcinogenesis Online. For those larger studies with 300K data (MDACC, IARC, LTRI, deCODE and LLP), we included imputed variants to match the genotyped variants on 550K.

IMPUTE 2, Mach or minimac using 1000 genome or HapMap2 were used to complete the imputation at the study level (Supplementary Table 2, available at Carcinogenesis Online). Conducting a meta-analysis based on imputed dosage from all studies without any genotyped data on those specific variants would create substantial uncertainty in the combined results. Therefore, for the discovery analysis, we restricted the baseline meta-analysis to those included on 550K array, instead of the maximum number of imputed variants possible. For those regions identified to be associated with lung cancer risk, we conducted further investigation based on regional imputation. To evaluate the association with lung cancer risk in the Japanese and Chinese populations, we analyzed the top regions in 594 lung cancer cases and 942 controls from the Aichi Lung Cancer Studies in Japanese (32) together with 2338 cases and 3077 controls of Han Chinese descent from the Nanjing and Beijing studies (15).

Fine mapping set as a validation approach of signals

For the susceptibility regions identified in the discovery set, we conducted dense genotyping for the purpose of replication and fine mapping based on 3128 cases and 2966 controls of European ancestry from 6 additional independent studies including Mount-Sinai Hospital-Princess Margaret (MSH-PMH) study (10), Multiethnic Cohort (MEC) study, Liverpool lung cancer study (LLP), Nurses’ Health Study (NHS), National Physicians Health Study (NPHS) and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Lung cancer. Details for each of the replication studies are shown at the bottom of Table 1. Genotyping for the replication set was done using the Axiom custom genotyping array from Affymetrix, Inc., Santa Clara, CA, USA.

Statistical methods

Study-specific analysis of GWAS data

For all studies, we used unconditional logistic regression models adjusted for sex, age (years or 5-year age intervals where available), country/study center where appropriate, smoking status (never/current/former or pack-years where available) and the top principal components of population structure (where available). Effects were estimated separately for SCC, AC and SCLC. Analyses among large cell lung cancers and other less frequent histology groups were not pursued, as the within-study numbers of cases were small. We therefore confined our analyses to the three main histology groups.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABF</td>
<td>approximate Bayes factors</td>
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<tr>
<td>AC</td>
<td>adenocarcinoma</td>
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<td>BFDP</td>
<td>Bayesian false discovery probability</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>DNase</td>
<td>deoxyribonuclease</td>
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<tr>
<td>eQTL</td>
<td>expression quantitative trait loci</td>
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<td>GWAS</td>
<td>genome-wide association studies</td>
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<tr>
<td>HM</td>
<td>hierarchical modeling</td>
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<tr>
<td>LD</td>
<td>linkage disequilibrium</td>
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<tr>
<td>MAF</td>
<td>minor allele frequency</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>P-het</td>
<td>P-heterogeneity</td>
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<tr>
<td>SCC</td>
<td>squamous cell carcinoma</td>
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<tr>
<td>SCLC</td>
<td>small cell lung cancer</td>
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<tr>
<td>SFDR</td>
<td>stratified false discovery rate</td>
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<tr>
<td>SNPs</td>
<td>single-nucleotide polymorphisms</td>
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Prior to undertaking the meta-analysis of all GWAS data sets, we searched for potential errors and biases in data from each case–control series. Lambda inflation factors ($\lambda$) were calculated for each study. With the exception of Memorial Sloan Kettering Cancer Center (MSKCC) study that has a smaller sample size ($n = 1.11$), quantile–quantile (Q–Q) plots showed no evidence of inflation of the test statistics indicating that substantial cryptic population substructure or differential genotype calling between cases and controls was unlikely in each of the individual GWAS (Supplementary Table 3, available at Carcinogenesis Online) (33).

Combined analysis
The combined analyses were conducted based on fixed-effects models for each of the three main histology groups. As for the individual studies, we examined for overdispersion of $P$-values in the meta-analysis by generating Q–Q plots and deriving an inflation factor. Cochran’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. $P$ values $>75\%$ are considered characteristic of large heterogeneity (34).

All calculations for the pooling of the effect estimates were performed using PLINK (35) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Q–Q and Manhattan plots were created using an R program. We used LocusZoom for regional visualization of results (36).

Prioritization approaches
We utilized three Bayesian framework analytical approaches, including the SFDR described in Sun et al. (17), the BFDP described in Wakefield (37) and HM described in Chen and Witte (19), in order to identify additional novel variants by incorporating prior knowledge. The three methods, although differing greatly in approach, are all based on the premise of using additional information to improve the identification of variants for prioritization. All methods used estimates from the fixed-effects meta-analyses as input data for prioritization.

Variant information
In order to inform the prioritization analyses, we utilized biological data on the genes and the variants including (i) functional annotations: whether a variant was in a coding region, non-synonymous or intergenic based on illumina annotation files (38); (ii) conservation scores across mammals [PhastCons (39)]; (iii) prediction of deleterious nature of a variant [SIFT Scores (40) and (iv) regulatory elements [whether a variant was in an area of a transcription factor (TF) binding or DNase hypersensitivity site (www.genome.ucsc.edu) for several cell lines including normal human lung fibroblasts] and (v) linkage disequilibrium (LD) tagging: as the variants on the platforms used were chosen as ‘tagSNPs’ for their tagging ability, we included information for those variants in LD (defined as $r^2 > 0.8$ within a 500kb window) with the directly measured variants. In order to determine variants in LD with tagSNPs, we used the SNAP tool from the broad institute (41). This process included functional variants (whether a variant was in LD with a variant at a splice site, a variant in a coding region, a non-synonymous variant, non-sense or missense variant, whether a variant was in a 3′ untranslated region or a frameshift variant).

To account for the aspects of pooled analysis and heterogeneity across the study-specific results, we also included the $P$-values for heterogeneity in the prior data for each variant for each histology analyses. This is motivated by the notion that ‘true’ effects will be consistent across studies of similar genetic ancestry and should be given additional weight in prioritization analyses. Each of the measures/categories of information was parameterized to have non-negative values in a similar range across the information columns. Examples and further detail of the variant information are provided in Supplementary Table 4, available at Carcinogenesis Online. We employed the functional information differently in each of the methods as described below. Specific details for each of the approaches have been published (17–19) previously; hence, only a brief description of the specific parameters in each application is described below.

Stratified false discovery rate
The SFDR is based on the premise of using stratification in the number of hypotheses tested based on prior knowledge as a stratum indicator for the calculation of false discovery rates (17). After stratification, provided that the stratum indicator is informative of association with the phenotype of interest, the false discovery rate for a given threshold should be lower in the strata with higher prior evidence: Within the strata, $Q$-values
Bayesian false discovery probability

The BFDP utilizes Bayes factors, the ratio of the probability of the data under the null and alternative hypotheses, as an alternative to the P-value for assessing the consistency of a set of data with a null hypothesis (18). We applied an asymptotic closed-form approximation of the Bayes factor that is based on results of the logistic regression. The sum of all the information categories was used as the prior odds of association in the calculation of the approximate Bayes factors (ABF). This is based on the assumption that the functional categories for exonic variants and regulatory regions (DNase hypersensitive sites and TF-binding sites) are given higher weighting in our prior distribution/stratum indicator that reflected the findings of Hindorff et al. (43) that showed these types of variants to be augmented in the replicated GWAS findings observed to date in the NHGRI catalogue. For each histology group, we determined 3-strata based on two sets of the prior probability distribution: at the 50% and 90% percentile cut-offs and the 75% and 95% percentile. P-values from fixed-effects pooling were used as input data for each SNP across histology groups into the software provided by Sun et al. (http://www.ustat.toronto.edu/sun/Software/SFDR/index.html).

Hierarchical modeling

We applied a HM method developed by Chen and Witte (19) for GWAS data to optimize variant prioritization based on prior biological information. This method takes prior information into account in a weighted two-stage regression model with the aim of improving effect and variance estimation. Their method was shown to be informative in deviations in the prior information used, provided that the altered prior distributions remain informative. The measures of function described in the variant information for the Z-matrix were used for the Z-matrix for this analysis, a key component of this particular HM approach. The input data for this model were the variant-specific regression coefficients and standard error estimates for each variant across histology groups. Based on our previous investigations (46), we used the P-value for heterogeneity across studies as a weighting column for the second-stage analyses. We used τ of 0.05 and ρ of 0.01 as variance parameters required for model estimation based on previous investigations (13) where τ represents the range of residual effects after adjustment for all first- and second-stage covariates and ρ constrains the minimum standard deviation across all variants. The model provides a HM estimate (B’ =) which can be considered a posterior estimate of the association after consideration of the prior (Z) matrix and weighting between first- and second-stage estimates based on the model parameters. Although B’ are not asymptotically unbiased estimators, previous theoretical and simulation work shows these are consistent estimators and that Wald procedures perform well in typical finite samples (47). We therefore present P-values from HM based on this approach.

Comparison of results and methods

We applied a straightforward ranking procedure for each of the additional prioritization techniques whereby those with low BFDP values, small P-values from HM or small Q-values from SFDR were given top rankings. These were compared with the results of the P-values and their respective rankings from fixed-effects pooled maximum likelihood estimates. The number of variants ranked in the top 100 that was not included in the P-value cut-offs of interest was investigated further since those were the variants that would have been missed if one only considered P-values as the selection criteria.

Regional imputation based on HapMap2 and 1000 genomes

When available, data from 1000 genomes or HapMap2 was used for the top regions of interest from the discovery set results. A protocol was circulated with the flanking positions for the four regions based on human genome build 18 and/or 19 depending on the imputation that was done in each of the centers. Centers used the software of preference for imputation including Minimac, MACH, IMPUTE or BEAGLE (Supplementary Table 2, available at Carcinogenesis Online). The analytical model was the same as in the initial first-stage analyses. Study-level results were then pooled for each of the regions to examine for additional variants of interest within the regions using GWAMA (48).

Fine mapping set analyses

In order to validate the regions identified through the prioritization techniques, a conventional analysis of regions of interests were conducted. We employed unconditional logistic regression models adjusted for sex, age (years or 5-year age intervals where available), country/study center where appropriate, smoking status (never/current/former or pack-years where available) and the top principal components of population structure (where available).

Results

In general, the Bayesian framework analysis in each histological subtype with a denser baseline of meta-analysis after imputation led to identification of additional susceptibility regions of lung cancer histology groups. Table 2 displays the number of novel variants that reached a level of significance at 10^-7 to 10^-3 as well as the number of variants ranked in the top 100 from the prioritization techniques for each histology group that had P-values > 0.001 in the pooled analysis. From the additional pooledfixed-effects, we identified regions of interest in the P-value range of P < 10^-7 where relative ranking persisted after incorporation of prior functional knowledge with a Q-value from SFDR < 0.05, ABF < 0.05 and P-value from HM < 0.001 (Figure 1). Our results included the previously observed variants from GWAS of lung cancer examining histology-specific effects (16). Following the application of prioritization methods, these variants remained top ranked, suggesting that true effects will not be altered or missed when using these alternative approaches (Supplementary Table 1, available at Carcinogenesis Online).

Squamous cell carcinoma

When estimates from the 12 studies including SCC cases were combined (n = 5061 cases, without MSKCC that has too few SCC), P-values were not overly inflated (κ = 1.01). We observed significant associations with the previously reported regions at 15q25, 5p15, 6p21 and 12p13 (Figure 2). In addition, a locus (rs6448050) in KCNIP4 gene at 4p15.2 (Supplementary Figure 1A, available at Carcinogenesis Online) was found across the studies to be associated with the risk of lung SCC with odds ratio (OR) of 1.18 [95% confidence interval (CI) = 1.10-1.26, P-heterogeneity (P-het) = 0.09] and P-value of 4.6 x 10^-7 (ABF = 1.01 x 10^-3, SFDR = 0.017, HM P-value = 3.0 x 10^-7) (Figures 1 and 2 A-D). When examining the ABF and P-value from HM, the relative significance of the variant persisted. The combined prior weight across all the columns of the prior matrix for this variant was in the 75th percentile, which placed it in an elevated stratum of prior probability of association for the SFDR analysis. This variant was not present on the 317K array therefore was not identified in Timofeeva et al. (16). By imputation, we were able to combine the genotyped data in Germany, NCI, Harvard, ICR and Mayo, with imputed data from the MDACC, Toronto, deCODE and Central Europe.
data sets. In the Japanese GWAS, we observed the association in the same direction which when combined provided a $P$-value of $7.07 \times 10^{-7}$. The results did not appear to be generalizable in the Han Chinese population in the Nanjing study.

In our fine mapping set, we increased the density of this region and genotyped 1304 markers (positions 20391615–23274625bps625bps in Build 37 coordinates) with an Axiom array in 3128 cases and 2966 controls. The overall signal in $KCNIP4$ was validated with $P$-value of $1.4 \times 10^{-6}$ for lung SCC, but interestingly the dense genotyping refined the location of the signal within $KCNIP4$. The lead variant in the fine mapping and validation sets was rs9799795 with an OR of 0.74 (95% CI = 0.64–0.86, $P = 1.05 \times 10^{-4}$, $P$-het = 0.23) (Supplementary Figure 1B, available at Carcinogenesis Online).

Another locus (represented by rs10501831) in the $MTMR2$ gene located on chromosome 11q21.1 was associated with SCC risk in the discovery set (Supplementary Figure 2A, available at Carcinogenesis Online) with an OR of 1.41 (95% CI = 1.22–1.63, $P = 3.1 \times 10^{-6}$, $P$-het = 0.95, SFDR = 0.0248, ABF = $1.44 \times 10^{-4}$). When examined among the Japanese population, consistent effects were observed with a combined $P$-value of $9.99 \times 10^{-7}$. SNP rs10501831 had a high prior weight (>90th percentile), which contributed to the higher-ranking HM and ABF and placed it in the third strata for SFDR (Figure 2A–D). From the regional imputation examination, the region of interest on chromosome 11 showed a large region of LD across the $MTMR2$ and $CEP57$ genes including two additional SNPs of interest, rs2155436 and rs10501832. A dense panel of 711 markers was genotyped with Axiom array for this region (between position 95472391 and 96125436 based on Build 37) in our validation and fine mapping set, but variant (rs10501831) was not significant (Supplementary Figure 2B, available at Carcinogenesis Online) for lung SCC. Although

### Table 2. Number of variants and regions included through each prioritization method

<table>
<thead>
<tr>
<th></th>
<th>SCC</th>
<th></th>
<th>AC</th>
<th></th>
<th>SCLC</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Variants</td>
<td>Regions</td>
<td>Variants</td>
<td>Regions</td>
<td>Variants</td>
<td>Regions</td>
</tr>
<tr>
<td>Novel variants $P = 10^{-6}$ to $10^{-3}$</td>
<td>15</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>$P &lt; 0.0001$ first stage</td>
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<td>75</td>
<td>93</td>
<td>62</td>
<td>92</td>
<td>68</td>
</tr>
<tr>
<td>$P &gt; 0.0001$ but SFDR rank in top 100</td>
<td>26</td>
<td>20</td>
<td>21</td>
<td>19</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>$P &gt; 0.0001$ but ABF rank in top 100</td>
<td>20</td>
<td>19</td>
<td>35</td>
<td>29</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>$P &gt; 0.0001$ but HM rank in top 100</td>
<td>12</td>
<td>12</td>
<td>24</td>
<td>24</td>
<td>47</td>
<td>39</td>
</tr>
</tbody>
</table>

Region corresponds to an area of LD $R^2 > 0.8$ or with 500 kbp.
the adjacent variants (represented by rs74663383) in the same region showed a suggestive association with \( P \)-value of 0.004, the overall evidence of an association based on the regional plot was not strong.

**Adenocarcinoma**

When estimates from the 13 studies examining AC were combined \( (n = 6756 \) cases including studies from Japan and China), significant values were not overly inflated \( (\lambda = 1.01) \). We observed significant associations with the previously reported regions of 15q25 and 5p15 (Figure 3A–D). In addition, we observed a novel locus (represented by rs11662168) at 18q12.1 in the GAREM (also known as FAM59A) gene with an OR of 1.34 and \( P \)-value of \( 3.44 \times 10^{-7} \) (95% CI = 1.19–1.49, \( P \)-het = 0.28, Q-SFDR = 0.0108, ABF = 2.0 \times 10^{-5}, \( P \)-value from HM = 5.30 \times 10^{-5} \) (Supplementary Figure 3A, available at Carcinogenesis Online).
The effect was observed in the same direction among the Japanese population, which provided a combined $P$-value of $2.95 \times 10^{-7}$ (Figure 1). The results did not appear to be generalizable in the Han Chinese population based on the Nanjing study. When examining the $Q$-values from the SFDR and ABF, the ranking of the variant was suggested to be higher than using fixed-effect $P$-values. Examining the regional imputation results using HapMap2, the results on chromosome 18 show an area of elevated significance around the lead SNP rs11662168 with rs11662168 remaining the most significant variant (Supplementary Figure 3A, available at Carcinogenesis Online). These variants map directly to the GAREM gene. The region also showed a significant association in the validation and fine mapping population. A dense panel of 1786 markers was genotyped with Axiom array (between positions 28571083 and 30434298 based on Build 37) in our replication and fine
mapping set. The lead variant in the replication set was rs3786309 (OR = 1.22, 95% CI = 1.10–1.35, P-value = 0.000192, P-het = 0.07) ([Supplementary Figure 3B, available at Carcinogenesis Online]) for lung AC. The original hit rs11662168 also remained significant in the replication set with P-value of 0.04.

Small cell lung cancer

When estimates from the nine studies with genotyped cases of SCLC were combined (n = 2216, without the Mayo and MSKCC studies as the sample sizes were too small to obtain reliable estimates), significant values were not overly inflated (I² = 1.00). We observed significant associations with the previously reported region of 15q25 ([Supplementary Figure 4A–D, available at Carcinogenesis Online]). When applying additional prioritization techniques, we did not identify additional regions of interest for SCLC. Two loci (DAOA and LTBI) warranted additional investigation; however, results did not appear to be consistent across studies and were being driven by single-study estimates. A lack of LD with other associated SNPs in regional plots was also suggestive of a chance finding at a single SNP. A full list of top results is included in [Supplementary Table 1, available at Carcinogenesis Online].

Discussion

We uncovered novel associations in the genome-wide suggestive range for both AC and SCC of the lung using additional prioritization approaches. We used fine mapping as validation for our methods that confirmed significant effects in two of the three susceptibility regions in independent data sets of five additional lung cancer studies using a different genotyping platform. We consider this result as an evidence to support the biological importance of this gene for lung cancer etiology in general. Our findings, in conjunction with previous lung cancer GWAS ([16] and recent histology-specific whole-genome sequencing initiatives ([49–51]), provided additional evidence of histology-specific genetic etiology of lung cancer. The previous combined analyses conducted within TRICL/LCCCO were well powered to detect common risk loci (MAF > 5%) of a moderate effect size (Relative Risk (RR) = 1.20) at power of over 90%. Therefore, this analysis demonstrated the potential for the application of additional statistical techniques under the Bayesian framework to identify associations in the suggestive regions that appeared to be robust to adjustment for additional measures of prior information.

Novel lung cancer susceptibility regions

For SCC and AC, we identified novel variants using a Bayesian framework analysis approach. We identified an association with variations in KCNIP4 gene in the suggestive range specifically in squamous cell lung cancer as indicated based on this analysis, and this was robust to several prioritization techniques. To our knowledge, this gene has not been implicated in lung cancer risk to date. This gene is believed to play a role in the regulation of Wnt-mediated beta catenin signaling and target gene transcription pathways ([52]). It is also implicated in the carcinogenic mechanism of kidney cancer ([53]) and pancreatic cancer ([54]) through candidate pathways analysis. KCNIP4 has also been implicated in the development of asthma ([55]). Asthma has been implicated in lung cancer risk ([56]) and may present a shared causal genetic contribution between the two diseases.

For the loci in GAREM (Grb2 associated and regulator of Erk/MAPK) (rs11662168/rs3786309), the effect was observed in the consistent direction in one of the study populations consisting entirely of never smokers (MSKCC and Mayo). This at least partially alleviates the possibility of the variant acting as a marker of tobacco exposure. GAREM, also referred to as FAM59A, has been identified as a protein that contributes to cellular transformation through the activation of extracellular signaling-regulated kinase signaling ([57]).

The possibility of the variants representing chance findings cannot be excluded despite the large amount of data included in these analyses. The validation of our prioritization results in an independent data set, the reliability of the relative rankings of the variants after adjustment for prioritization techniques and the homogeneity of results across all participating studies would, however, collectively argue against chance findings. Winner’s curse ([58]) cannot be precluded particularly for those variants with low MAFs. The lead variants of the two validated regions highlighted in this work all have MAF above 5% in the study populations (4p15 rs6448050 T allele 28%, rs9799795 A allele 45%, 18q12: rs11662168 C allele 8% and rs3786309 T allele 47%). Despite a standard protocol of covariate adjustment and quality control threshold, variations may be present in the results of imputed variants as they were conducted at each study level. However, the minimal heterogeneity across the study results for these two regions is most reassuring. Our analysis has included all lung cancer GWAS data that are available for European descendants to date, and it represents maximum statistical power for the analysis for each histological subtype.

Functional variation

As the validated variants in 4p15.2 and 18q12.1 representing the two novel loci are located in intronic regions (non-coding variants), we undertook additional investigation into probable function of the variants or those with which they are in LD. Non-coding variants in these regions were investigated in the RegulomeDB database ([59]) and using HaploReg ([60]). RegulomeDB and Haploreg synthesize data from multiple sources including protein-binding data (ChIP-Seq), expression quantitative trait loci (eQTL), location of TF binding sites, TF motif data (position weight matrices, PWM), DNase sensitivity (DNase-Seq), histone modification (Chip-Seq) data, manually curated regions and validated functional SNPs and provide a score for all non-protein coding variants in dbSNP132 ([regulome.stanford.edu]) as obtained from the ENCODE database. Application of this approach has shown that in 80% of validated GWAS findings taken from the NHGRI database the associated ‘lead SNP’ is in LD with a variant in the RegulomeDB with a substantially higher functional score than the lead SNP itself ([61]). Regions of LD where r² > 0.8 and within the 500-kb distance from the lead SNP were investigated. The top-ranked SNP for each variant using RegulomeDB and HaploReg is shown in Table 3. RegulomeDB provides a score from 1 to 7 with subcategories for 1–3 (1a being the highest level of evidence, e.g. variant is an eQTL + TF-binding site + matched TF motif + matched DNase Footprint + DNase peak). For example, the variant rs923673 that is in linkage with our lead SNP (rs9799795) and located in the KCNIP4 gene was scored as 1f in RegulomeDB as it was located in an eQTL in a TF-binding site from Chip-Seq data and the variant is likely to affect protein binding through histone modifications. HaploReg provides similar information about functionality of variants, including those in LD with the primary variant of interest. This tool suggests that variants in LD with our novel loci may alter regulatory motifs for genes with known relationships with lung cancer. Table 3 highlights the variants that may impact the function of genes known to be relevant in lung cancer development and/or progression. For
example, the variant rs11727733 that is in linkage with our lead SNP (rs9799795) and located in the KCNIP4 gene alters a Foxp3 regulatory motif. Furthermore, the variant rs10468834 that is in linkage with our lead SNP (rs11662168) and located in the GAREM gene alters a p53 regulatory motif.

Using novel prioritization approaches to replicate known variants

Our results included the previously observed variants from GWAS of lung cancer examining histology-specific effects (16). After calibration with the techniques employed in this investigation, these known loci remained top ranked, suggesting that true effects will not be altered or missed when using these alternative prioritization approaches (Supplementary Table 1, available at Carcinogenesis Online).

The observed association in 5p15 region appears to be different across SCC and AC cases as previously observed with the association localized to CLPTM1L (rs401681) for SCC and localized to TERT for (rs2736100) AC. The previously observed associations in RADS2 (12) and BAT3-MSH5 (11) specific to SCC are consistent with the premise that altered DNA-repair capacity is essential to lung cancer (62) development and for this subtype where tobacco smoking is an extremely strong risk factor.

As expected, we also observed the associations within the 15q25.1 region. We observed evidence consistent with two distinct signals in the region having independent effects on lung cancer (6,7). As identifying additional variants in novel regions using Bayesian prioritization approach was the main goal of this study, and conditional analyses (adjusting for the effects of the regions) were already conducted for a previous study based on similar data (16), we did not include conditional analyses for these known regions in this project.

Motivation for using prioritization approaches

The approaches employed in the prioritization analyses are based on the presumption that ‘functional’ SNPs or those in LD with such SNPs are more likely to be associated causally with risk of cancer development. A summary of enrichment/depletion of SNP types from published GWAS (43) suggested that non-synonymous sites were significantly enriched in addition to regulatory elements and intergenic with a depletion of effect among intergenic regions when compared with SNPs randomly selected from genotyping arrays. A recent analysis suggested that in validated GWAS associations, among those SNPs that are not in coding regions, a higher proportion of DNase hypersensitive sites are observed suggesting a great importance of variants in non-coding regulatory regions (63).

With additional tissue-specific database becoming available [e.g. GTEx and TCGA data portal (64,65)], it is possible that the prior data can be improved over time. The present study was also limited to the genetic variants tagged by the genotyping arrays used and the quality of the imputation data for those regions. Our approach did not consider a polygenic model to account for the joint effect of many variants with small effects. However, the novel variants identified in our study will contribute to future work on establishing polygenic model for lung cancer as the statistical methods continue to develop to adequately analyze polygenic effects in GWAS of complex diseases (66).

The results of these analyses and others (67–69) employing prioritization approaches to GWAS data utilizing the wealth of information available through online collaborative and data banking efforts suggest that these techniques are indeed useful as complimentary approaches to the traditional methods. Our results suggest that in this particular application and using this prior distribution for the variants, the Q-values from the SFDR stratified the ranking most clearly between those variants suggested for additional prioritization compared with the other two methods. This is based on the relative difference between those top values for the top-ranked SNPs and the rest of variants as observed in the Manhattan plots.
In this study, we identified two novel lung cancer susceptibility loci using multiple variant prioritization techniques with a total of 16,460 cases and 36,422 controls in a two-stage design. Moving forward, these variants are included on the OncoArray, a cancer-specific chip, being developed in partnership across the major cancer consortia at which point associations for multiple cancer sites can be investigated. Our results can also contribute to future work on establishing polygenic model for lung cancer.

Supplementary material

Supplementary Tables 1–4 and Figures 1–4 can be found at http://carcin.oxfordjournals.org/

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References