**Susceptibility loci of *CNOT6* in the general mRNA degradation pathway and lung cancer risk - a re-analysis of eight GWASs**

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**deCODE**

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**Abbreviations:** AD, Adenocarcinoma; CI, confidence interval; eQTL, expression quantitative trait loci; FDR, false discovery rate; GWAS, genome-wide association study; ILCCO, International Lung Cancer Consortium; LD, linkage disequilibrium; OR, odds ratio; SC, squamous cell carcinoma; SNP, single nucleotide polymorphisms; TCGA, The Cancer Genome Atlas; TRICL, Transdisciplinary Research in Cancer of the Lung.

**An abbreviated title:** Susceptibility loci in the general mRNA degradation pathway and lung cancer risk

**Key Words:** lung cancer risk, pathway analysis, molecular epidemiology.

**Abstract**

**Purpose:** mRNA degradation is an important regulatory step for controlling gene expression and cell functions. Genetic abnormalities involved in mRNA degradation genes have been found to be associated with cancer risk. Therefore, we systematically investigated the roles of genetic variants in the general mRNA degradation pathway in lung cancer risk.

**Experimental design:** We performed meta-analyses by using summary data from six lung cancer genome-wide association studies (GWASs) from the Transdisciplinary Research in Cancer of the Lung and additional two GWASs from Harvard University and deCODE in the International Lung Cancer Consortium. Expression quantitative trait loci analysis (eQTL) was used for *in silico* functional validation of the identified significant susceptibility loci.

**Results:** This pathway-based analysis included 6,816 single nucleotide polymorphisms (SNP) in 68 genes in 14,463 lung cancer cases and 44,188 controls. In the single-locus analysis, we found that 20 SNPs were associated with lung cancer risk with a false discovery rate threshold of <0.05. Among the 11 newly identified SNPs in *CNOT6*, which were in high linkage disequilibrium, the rs2453176 with a RegulomDB score "1f" was chosen as the tagSNP for further analysis. We found that the rs2453176 T allele was significantly associated with lung cancer risk (odds ratio=1.11, 95% confidence interval=1.04-1.18) in the eight GWASs. In the eQTL analysis, we found that levels of *CNOT6* mRNA expression were significantly correlated with the rs2453176 T allele, which provided additional biological basis for the observed positive association.

**Conclusion:** The *CNOT6* rs2453176 SNP may be a new functional susceptible locus for lung cancer risk.

**Introduction**

Lung cancer is one of the most frequently diagnosed cancers with about 1.8 million new lung cancer cases reported in 2012 worldwide, accounting for about 13% of total cancer diagnoses [1]. In the United States, 224,390 new lung cancer cases are estimated to occur in 2016 [2]. In addition to other factors, such as occupational and environmental carcinogens, cigarette smoking is the major risk factor for lung cancer [3,4], but not all smokers develop lung cancer, which suggests that genetic predisposition play an essential role in the lung carcinogenesis [5].

In recent years, some genome-wide association studies (GWASs) of lung cancer have been conducted, and a number of genetic variants, i.e., single nucleotide polymorphisms (SNPs), have been found to be associated with lung cancer risk. For example, the significant susceptibility loci associated with lung cancer risk include 5p15.3 (rs401681, rs4975616 and rs402710 in *CLPTM1L* and rs2736100 in *TERT*) [6-11], 6p21.3 (rs3117582 in *BAG6* or *APOM* and rs2395185 in *HLA-DRB5* or *HLA-DRB9*) [6,8,9,11], 6q22.1 (rs9387478 in *RAP1BP3* or *DCBLD1*) [11] and 15q25.1 (rs8034191 in *HYKK* and rs1051730 in *CHRNA3*) [6,8,9,12-15]. Among these SNPs, rs1051730, rs3117582 and rs2736100 were found to be specifically associated with risk of lung adenocarcinoma (AD) [9], whereas rs12296850 (mapped to 12q23.1) in *SLC17A8* or *NR1H4* was found to be a susceptibility locus for risk of squamous cell carcinoma (SC) [16]. Interestingly, the vast majority of the SNPs identified by GWASs are in introns or intergenic regions, and their functional evidence is limited. In the present study, we employed the pathway-based strategy that dramatically decreases the number of SNPs to be analyzed and thus significantly reduced multiple testing with the aim to identify possible lung cancer risk-associated functional SNPs that may have not been revealed by previous lung cancer GWASs.

The degradation of mRNA is an important regulatory step for controlling gene expression and cell functions [17]. The general cytoplasmic mRNA decay pathway usually begins with the deadenylation, which removes the poly(A) tail Ccr4-Not complex [18], followed by degradation of mRNA proceeding in two directions of 5'-3' or 3'-5'. The 5'-3' mRNA degradation initiates with decapping $N^7$-methylguanosine ($m^7G$) cap mainly by DCP1/DCP2 proteins and subsequently degraded by the exoribonuclease Xrn1, while the 3'-5' mRNA degradation is mainly catalyzed by 10-12 subunit exosome [19,20].

Some studies suggest that genetic abnormalities of genes involved in the general mRNA degradation pathway may be associated with lung cancer. For example, various genetic variants in *LSM*2-*LSM*8, which encode cofactors for mRNA decapping, were recently found in lung cancer cell lines [21]. Therefore, we hypothesize that genetic variants of the general mRNA degradation pathway are associated with lung cancer risk. To test the hypothesis, we conducted the comprehensive meta-analysis of the eight published lung cancer GWASs from the ILCCO (International Lung Cancer Consortium)-TRICL (Transdisciplinary Research in Cancer of the Lung) consortia, focusing on the SNPs of the genes in the general mRNA degradation pathway.

## Materials and Methods

### Study populations

The first part of the study populations came from the TRICL consortium, which included 12,160 lung cancer cases and 16,838 controls (all Europeans) of six previously published GWASs from: the MD Anderson Cancer Center (MDACC), the Institute of Cancer Research (ICR), the National Cancer Institute (NCI), the International Agency for Research on Cancer (IARC), Toronto study from Samuel Lunenfeld Research Institute study (Toronto), and the German Lung

Cancer Study (GLC) [22]. The second part of the study populations included GWASs of European ancestry from Harvard Lung Cancer Study (984 cases and 970 controls) [23] and Icelandic Lung Cancer Study (deCODE) (1,319 cases and 26,380 controls) [15] of the ILCCO. Written informed consents were achieved for all participants, and the present study was approved by each institutional review board of the participating institutions.

**GWAS genotyping and imputation**

Genotyping in the eight GWASs was performed by Illumina HumanHap 317, 317+240S, 370Duo, 550, 610 or 1M arrays. The imputation was conducted by IMPUTE2 v2.1.1 or MaCH v1.0 software using the reference panel from the 1000 Genomes Project (phase I integrated release 3, March 2012). Standard quality control on samples was performed on all scans in the analysis, excluding any participants with low call rate (< 90%), extremely high or low heterozygosity ($P < 1.0\times10^{-4}$), non-European (with the HapMap phase II CEU, JPT/CHB and YRI populations as a reference) and imputed SNPs with an information score < 0.40 in IMPUTE2 or $r^2 < 0.30$ in MaCH.

**Gene and SNP selection**

We first identified genes in the general mRNA degradation pathway from the Molecular Signatures Database [24] and the literature [18]. Overall, 75 genes located on autosomal chromosomes were selected, of which seven genes were pseudogenes or duplicates or withdrawn from updated NCBI. As a result, we then extracted genotype data of 68 genes (detailed in **Table 1**), including 2-kb of the flanking regions of each gene, from the GWAS datasets that also included those SNPs generated by imputation. The final meta-analysis contained 6,816 SNPs and covariates provided by the TRICL consortium in the summary data with the following standards:

genotyping rate $\geqslant$ 90%, minor allele frequency $\geqslant$ 1%, and Hardy Weinberg Equilibrium exact *P* value $\geqslant 10^{-5}$. The overall workflow is shown in **Figure 1**.

### *In silico* functional validation

Two *in silico* tools, SNPinfo (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) [25], RegulomeDB (http://regulomedb.org/) [26], were used to predict potential functions. Expression quantitative trait loci (eQTL) analysis was performed by using the expression data of lymphoblastoid cell lines from 373 Europeans available in the 1000 Genomes Project (http://www.1000genomes.org/category/frequently-asked-questions/gene-expression) [27] and The Cancer Genome Atlas (TCGA) ( https://tcga-data.nci.nih.gov/tcga/) [28]. In this TCGA dataset, 107 subjects had adjacent normal lung cancer samples used for the different expression testing, which were matched by 105 adjacent normal cancer tissue samples from the same individuals with the genotype data.

### Statistical analysis

Logistic regression model was used to calculate the odds ratios (ORs) and their 95% confidence intervals (CIs) in an additive genetic model with PLINK (v1.06) software. A meta-analysis with the inverse variance method was employed on the 6,816 SNPs with Stata software (v12, State College, Texas, US). Cochran's Q statistic was applied to test for heterogeneity and the $I^2$ statistic for the proportion of the total variation in the meta-analysis [29]. The fixed-effects model was used when there was no heterogeneity among GWASs (Q-test $P > 0.100$ and $I^2 <$ 50%); otherwise, the random-effects model was used. Multiple testing correction was conducted with false discovery rate (FDR) with a threshold < 0.050 [30]. A linear regression model was also performed to evaluate the correlation between SNPs and mRNA expression levels of the corresponding genes. A paired t-test was used to compare the mRNA expression levels of genes

in the lung cancer and normal adjacent tissue from the TCGA database. LocusZoom (http://locuszoom.sph.umich.edu/locuszoom/) was applied to construct regional association plots using Europeans from the 1000 Genomes Project as the reference (phase I integrated release 3, March 2012) [31]. Haploview v4.2 was used to generate the Manhattan plot and LD plots [32]. All analyses were conducted with SAS (version 9.4; SAS Institute, Cary, NC, USA) except for those specified otherwise.

## Results

### Associations of the SNPs with lung cancer risk

We first performed a meta-analysis in the TRICL database consisted of six previously published GWAS datasets with 12,160 cases and 16,838 controls. The basic information of these six studies is presented in **Supplemental Table S1**. A total of 6,816 SNPs in the pathway were extracted, of which 466 SNPs were associated with lung cancer risk at $P < 0.05$ in the additive model and 20 SNPs on *LSM2*, *SKIV2L* and *CNOT6* remained significantly associated with lung cancer risk with FDR < 0.05 after multiple testing corrections (**Figure 2A and Table 2**). Among these SNPs, we excluded those of *LSM2* and *SKIV2L*, because they were mapped to and in high LD with previously GWAS-reported locus at 6p21.33 [6,8]. As a result, 11 SNPs of *CNOT6* located at 5q35.3 were left for further analysis. In the LD analysis, these 11 SNPs shared moderate to high LD ($r^2 \geqslant 0.60$, **Figure 2B and 2C**). We finally chose rs2453176 as the tag SNP, because it was significantly associated with lung cancer risk (OR = 1.13, 95% CI = 1.06-1.19, $P = 4.33\times10^{-5}$) (**Table 2**) and potentially functional according to function prediction and its imputation quality was the best among the 11 SNPs (**Table 3**). We used the forest plot to illustrate the association between rs2453176 and lung cancer risk in the six GWASs (**Figure 3)**,

and the rs2453176 T allele was associated with an increased lung cancer risk in five GWASs, except for the GLC GWAS.

We expanded our analysis to include additional two independent lung cancer GWASs (**Supplemental Table S1**). The deCODE GWAS validated our result of the *CNOT6* rs2453176 tag SNP (OR = 1.14, 95% CI = 1.01-1.28, *P* = 0.032), while the GWAS from Harvard University displayed the same trend as the GLC GWAS (OR = 0.85, 95% CI = 0.68-1.05, *P* = 0.133) (**Figure 3 and Table 4)**.

As we combined the above results from the eight GWASs, the functional *CNOT6* rs2453176 tag SNP was found to be significantly associated with an increased risk of lung cancer (OR = 1.11, 95% CI = 1.04-1.18, *P* = 0.001) after the FDR correction (**Figure 3 and Table 4)**.

**Stratified analyses by lung cancer histology**

Since lung cancer has different histological types that could have distinct biological behaviors, we performed AD and SC subgroup analysis and found that the rs2453176 T allele was associated with a borderlinely increased risk in AD (OR = 1.13, 95% CI = 1.00-1.27, *P* = 0.050, **Table 4**), but it was significantly associated with SC risk (OR = 1.12, 95% CI = 1.03-1.22, *P* = 0.006, **Table 4**). Because smoking is a major risk factor for lung cancer, we further stratified the data into smokers and non-smokers and found that that the rs2453176 T allele was associated with a significantly increased risk in smokers (OR = 1.09, 95% CI = 1.02-1.17, *P* = 0.011, **Table 5**), while the allele was not statistically significant in non-smokers (OR = 1.10, 95% CI = 0.89-1.36, *P* = 0.363, **Table 5**). Homogeneity tests suggested that there was no heterogeneity between strata either in subgroups of histologic types or smoking status (**Table 4 and Table 5,** all *P* > 0.05).

## Functional validation by eQTL analysis

Because the *CNOT6* rs2453176 SNP was predicted with a score of "1f", suggesting the most confident functional annotation by regulomeDB [26], we further explored the underlying molecular mechanism by performing the eQTL analysis. With mRNA expression data of lymphoblastoid cell lines from 373 Europeans available from the 1000 Genomes Project, We found that expected mRNA expression levels of *CNOT6* were significantly decreased with an increased number of the rs2453176 T allele in both the additive ($P$ = 0.008) (**Figure 4A**) and dominant ($P$ = 0.007) (**Figure 4B**) models but not the recessive model (**Figure 4C)**. However, only 105 subjects had both DNA and RNA samples tested in this dataset. We also used the 105 normal adjacent tissue samples in the TCGA to further explore the correlation between the rs2453176 genotypes and their corresponding mRNA expression levels, but we did not observe a statistical significance ($P$ > 0.05) (**Supplemental Figure S1A-S1C**). We also compared the mRNA expression level of *CNOT6* in the 107 paired samples and did not find a statistically significant difference ($P$ > 0.05) (**Supplemental Figure S1D**).

## Discussion

In the present study, we found that a novel potentially functional susceptibility locus rs2453176 C>T of *CNOT6* in the general mRNA degradation pathway was associated with an increased lung cancer risk in 14,463 cases and 44,188 controls. This association was further supported by a significant correlation between a decreased mRNA expression level and an increasing number of the A allele in the eQTL analysis.

Gene expression disorder is one of cancer hallmarks, and instability of mRNA may result in altered transcript/protein levels of oncogenes and tumor repressor genes [33]. The degradation of mRNA is a key step in controlling the expression of genes related to cell proliferation. For example, the CCR4-Not complex consists of highly conserved exoribonucleases and adaptor proteins that hydrolyze and shorten the poly(A) tail, which starts the initial and the rate-limiting step of mRNA degradation [18,34-36]. Located at 5q35.3, *CNOT6* encodes a protein that has a 3'-5' RNase activity and acts as a catalytic subunit of the CCR4-Not deadenylation complex [37]. Although it remains unclear how the catalytic subunit works during the deadenylation process, some studies reported that its expression level was associated with carcinogenesis or prognosis. For example, one study of lung cancer found that the CNOT6 overexpression in lung SC predicted a significantly less metastasis [33]. Another study of acute leukemia discovered that CNOT6 had a significantly lower expression in patients than in controls [38]. These two studies suggest that high expression levels of CNOT6 may promote the degradation of mRNA of some oncogenes and the suppression of cell proliferation in carcinogenesis.

In the present study, we identified that the *CNOT6* rs2453176 T allele was associated with an increased risk of lung cancer, which was supported by the association of *CNOT6* rs2453176 T allele with a decreased mRNA expression level in lymphoblastoid cell lines from 373 Europeans. This finding is consistent with the role of CNOT6 in lung cancer prognosis as previously described [33]. The ENCODE project data from University of California Santa Cruz show that the *CNOT6* rs2453176 locus is located at the DNase I hypersensitive region (**Supplemental Figure S2**). Usually such an area has a loose chromatin structure and renders it a region with a high affinity for transcription factors (TFs). As a result, some TFs, including MAFK and MAFF, bond to this region in many cell types (**Supplemental Figure S2**). For example, MAFK and

MAFF were found to form heterodimers with a series of TFs and suppressed gene transcriptions [39,40]. Based on these, we speculate that the rs2453176 T allele may have a relatively high affinity with MAFK or MAFF and thus leads to the decreased mRNA expression of *CNOT6*. It is likely that a reduced quantity of CNOT6 may not be optimal in the mRNA degradation of some aberrant genes, which may in turn increases lung cancer risk, but these speculations need to be further investigated.

In the stratification analysis, rs2453176 was associated with lung cancer risk in both AD and SC subtypes, but it was significantly associated with cancer risk in the smokers but not in the non-smokers. Genetic susceptibility to smoking-related lung cancer risk may determine smoking behavior and tobacco metabolism [41]. Indeed, we found that the rs2453176 T allele was associated with a higher risk of lung cancer in smokers than in non-smokers. One study reported that smoking would enhance the activity of the GATA family [42], and another study reported that nicotine would increase the expression of EP300 and promote the lung cancer growth [43]. From the **Supplemental Figure S2**, GATA1, GATA2 and EP300 are the TFs that bind to the rs2453176 locus, possibly explaining why carriers of the rs2453176 T allele may have an increased risk of lung cancer in smokers than non-smokers.

There are some limitations in the present study. First, we employed the Molecular Signatures Database [24] to define the general mRNA degradation pathway to be investigated, but we may have missed some newly discovered genes in the pathway. However, we searched the literatures and added genes as many as possible. Second, due to the data limitation, we had no access to family history and others factors that may have an impact on lung cancer risk. Third, we used the eQTL analyses from lymphoblastoid cell lines and normal adjacent tissue in TCGA database to validate the risk association. Although the results from the cell lines support our identified

association, they may only reflect the baseline or genetically determined expression levels without exposure to smoking. The gene expressions in the normal adjacent lung tissues may be in some degree different from the normal lung tissue and did not support the association. Overall, the present study of eight published GWASs identified a novel *CNOT6* rs2453176 SNP in the general mRNA degradation pathway to be significantly associated with lung cancer risk in European populations, and the risk was more evident in smokers than in non-smokers. Although we used the publically available gene expression database from blood to confirm the biological significance of the variant, further functional evaluations in normal lung tissue are warranted to validate our findings.

**Conflict of interest:**

The authors disclose no potential conflicts of interest.

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## Figures and Tables

**Figure 1. Study workflow** SNP: single nucleotide polymorphism; FDR: false discovery rate; TRICL: Transdisciplinary Research in Cancer of the Lung; GWAS: genome-wide association study; eQTL: expression quantitative trait loci.

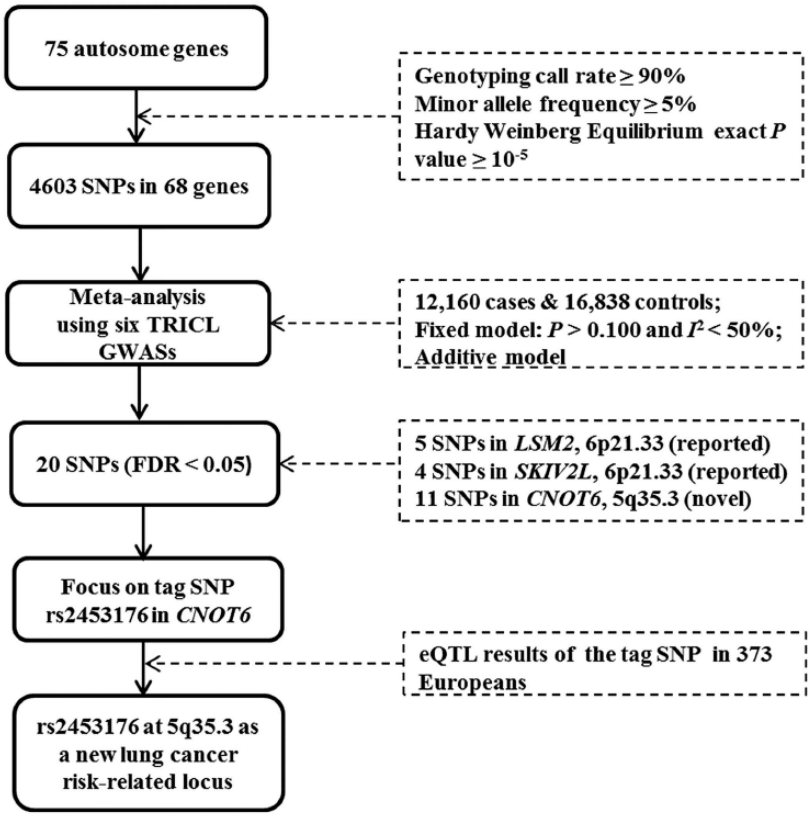


**Figure 2. Screening of SNPs in the general mRNA degradation pathway. A,** Manhattan Plot of genome-wide association results from the general mRNA degradation pathway in TRICL. The x-axis shows SNPs' positions on each chromosome. The y-axis shows the association *P* values with lung cancer risk (as –log10 *P* values). The FDR threshold of 0.05 was shown by a horizontal blue line. The *P* value of 0.05 was shown by a horizontal red line. **B**, Regional association plot for SNP rs2453176 in 500 kb up- and downstream region. The left-hand y-axis shows *P* values of the SNPs, which are transformed as −log10 (*P*) against chromosomal base pair positions. The right-hand y-axis shows the recombination rate estimated from HapMap Data Rel 22/phase II European population; **C,** The linkage disequilibrium plots of 11 SNPs in *CNOT6*. The value within each diamond represents the pairwise correlation between SNPs (measured as $r^2$) defined by the upper left and the upper right sides of the diamond.

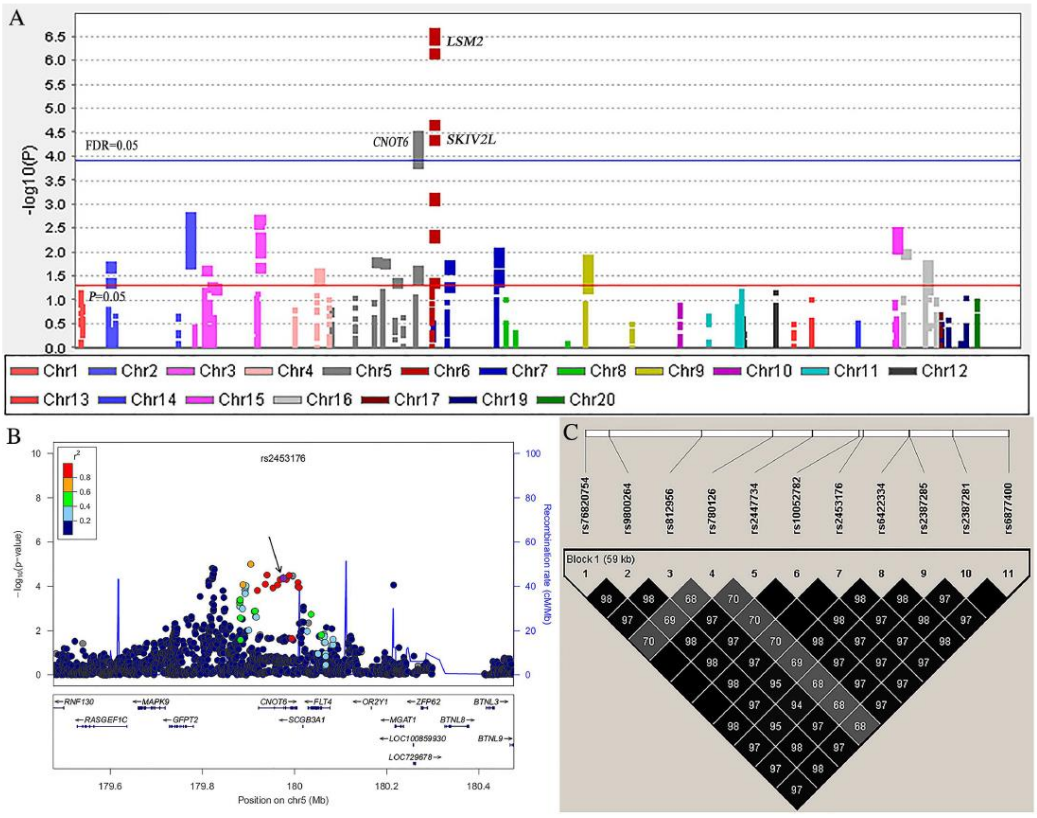


**Figure 3. Forest plots for associations between *CNOT6* rs2453176 and lung cancer risk for all participants** ($P$ = 0.0013).

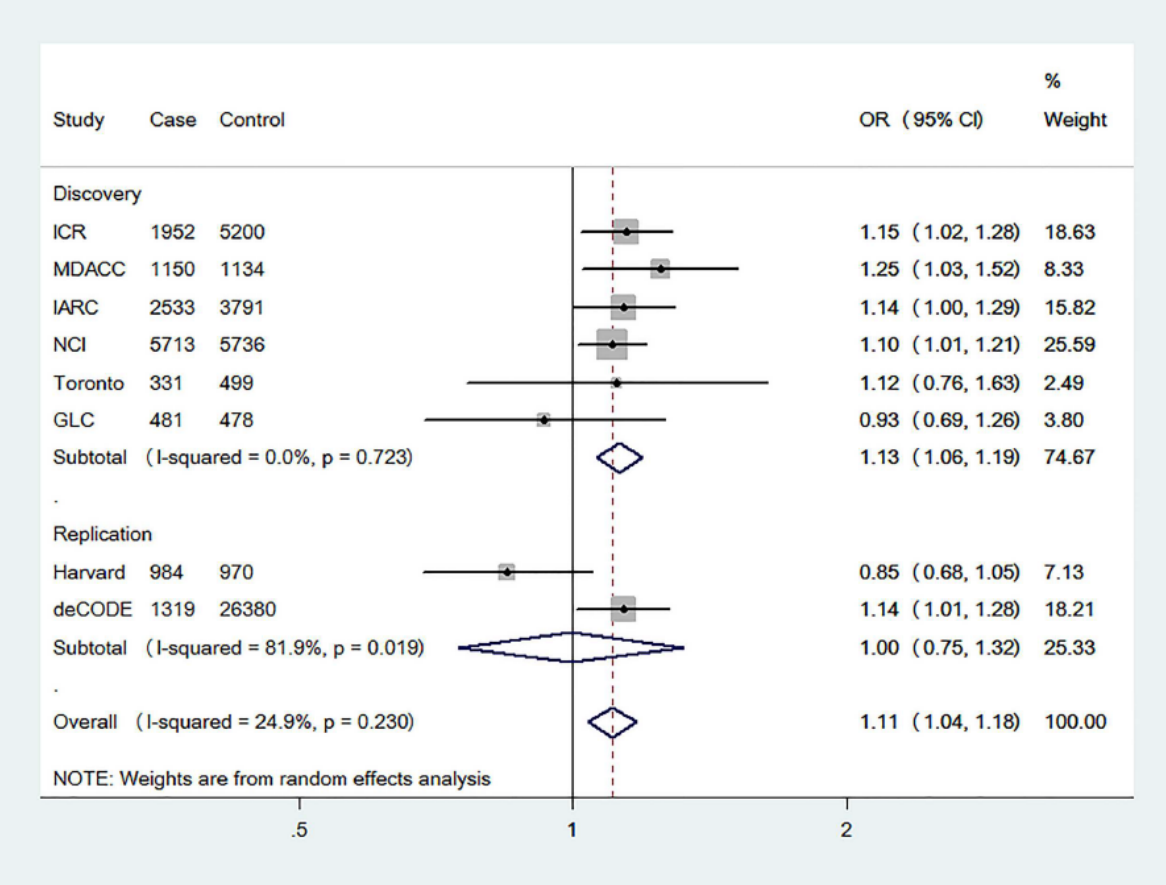
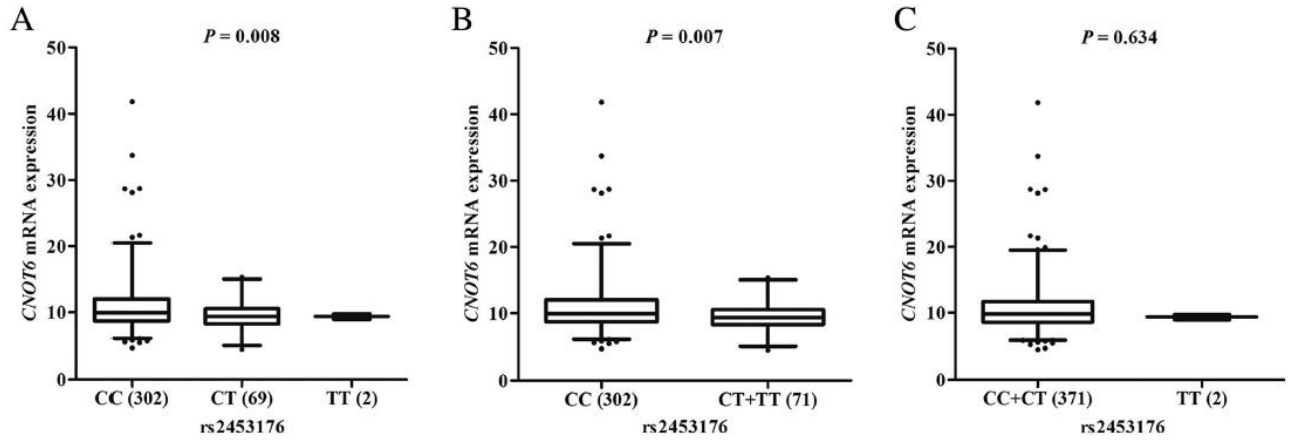
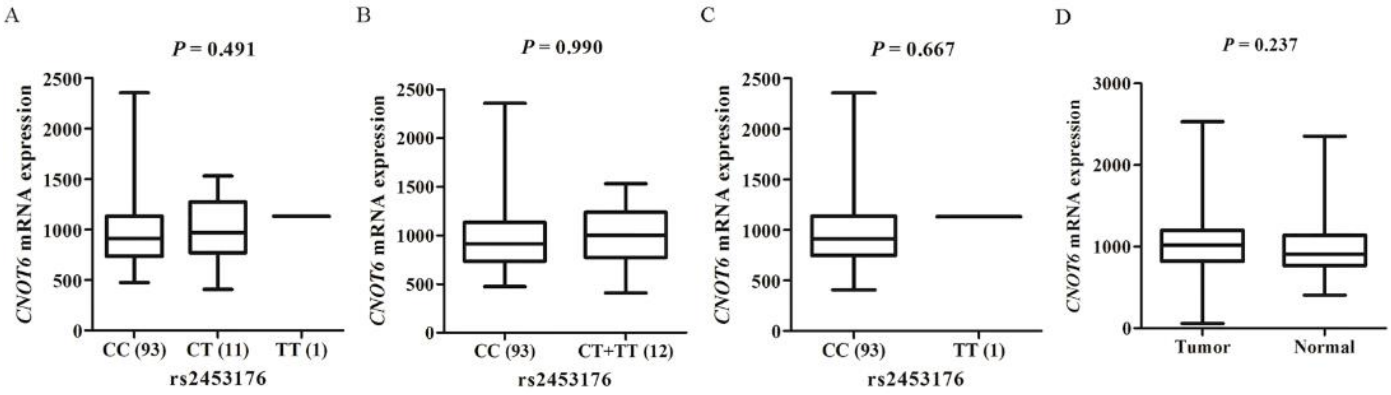


**Figure 4. The eQTL analysis of *CNOT6* mRNA expression for rs2453176 with lymphoblastoid cell data of 373 Europeans from 1000 Genomes Project.** A. additive model, $P = 0.008$; B. dominant model, $P = 0.007$; C. recessive model, $P = 0.634$.



**Supplemental Figure S1 A-C. The eQTL analysis of *CNOT6* mRNA expressions for rs2453176 in the 105 adjacent normal lung cancer tissue samples from the TCGA database.** A. additive model, $P = 0.491$, B. dominant model, $P = 0.990$, C. recessive model, $P = 0.667$; **D, The mRNA expression of *CNOT6* in the 107 paired lung cancer and normal adjacent tissue samples from the TCGA database** ($P = 0.237$).



**Supplementary Figure S2. The ENCODE project data of rs2453176 from UCSC browser (NCBI137/hg19).**

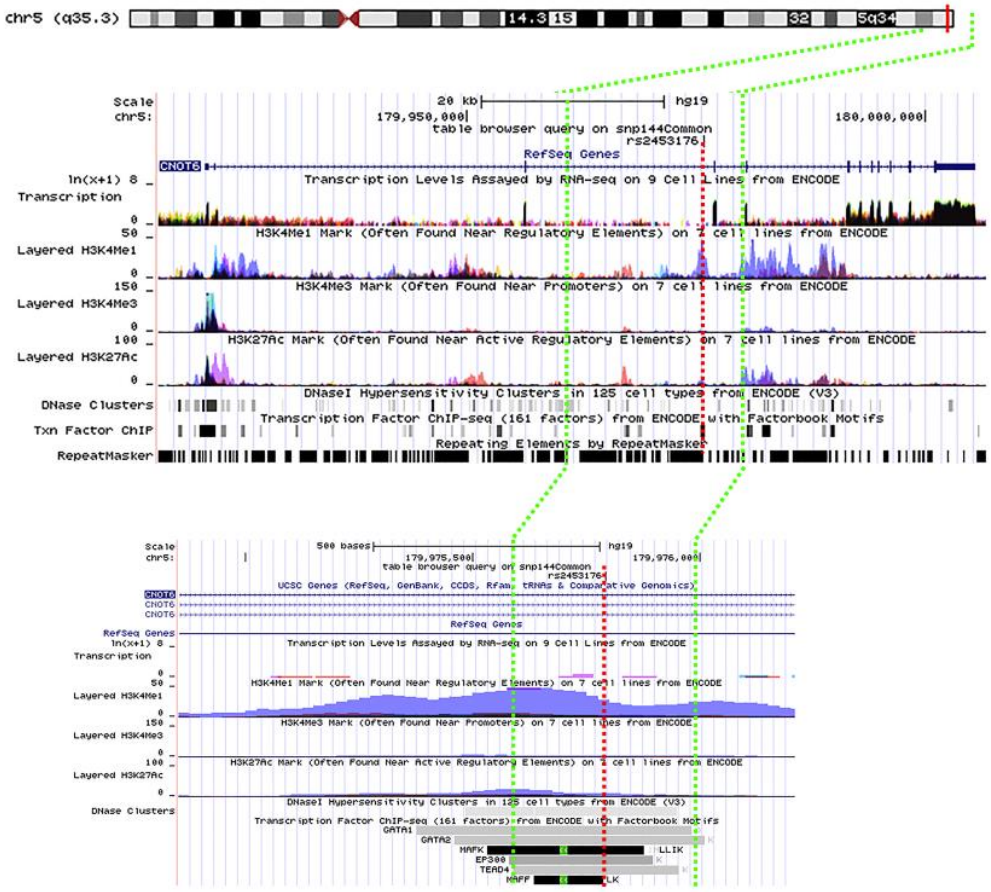


**Table 1** The mRNA degradation pathway gene sets

| Dataset | Name of pathway | Gene number | Gene name |
|---|---|---|---|
| KEGG* | KEGG_RNA_DEGRADATIONE | 59 | *C1D*, *C1DP2***, *C1DP3***, *CNOT1*, *CNOT10*, *CNOT2*, *CNOT3*, *CNOT4*, *CNOT6*, *CNOT6L*, *CNOT7*, *CNOT8*, *DCP1A*, *DCP1B*, *DCP2*, *DCPS*, *DDX6*, *DIS3*, *EDC3*, *EDC4*, *ENO1*, *ENO2*, *ENO3*, *EXOSC1*, *EXOSC10*, *EXOSC2*, *EXOSC3*, *EXOSC4*, *EXOSC5*, *EXOSC6*, *EXOSC7*, *EXOSC8*, *EXOSC9*, *HSPA9*, *HSPD1*, *LSM1*, *LSM2*, *LSM3*, *LSM4*, *LSM5*, *LSM6*, *LSM7*, *MPHOSPH6*, *NAA38*, *PAPD7*, *PAPOLA*, *PAPOLB*, *PAPOLG*, *PARN*, *PATL1*, *PNPT1*, *RQCD1*, *SKIV2L*, *SKIV2L2*, *TTC37*, *WDR61*, *XRN1*, *XRN2*, *ZCCHC7*. |
| Reactome | REACTOME_DEADENYLATION_DEPENDENT_MRNA_DECAY | 48 | *C2orf29***, *CNOT10*, *CNOT2*, *CNOT3*, *CNOT4*, *CNOT6*, *CNOT7*, *CNOT8*, *DCP1A*, *DCP1B*, *DCP2*, *DCPS*, *DDX6*, *DIS3*, *EDC3*, *EDC4*, *EIF4A1*, *EIF4A2*, *EIF4A3*, *EIF4B*, *EIF4E*, *EIF4G1*, *EXOSC1*, *EXOSC2*, *EXOSC3*, *EXOSC4*, *EXOSC5*, *EXOSC6*, *EXOSC7*, *EXOSC8*, *EXOSC9*, *LOC645139***, *LOC645947***, *LOC651789***, *LOC652607***, *LSM1*, *LSM2*, *LSM3*, *LSM4*, *LSM5*, *LSM6*, *PABPC1*, *PAIP1*, *PARN*, *PATL1*, *RQCD1*, *TNKS1BP1*, *XRN1*. |
| Reactome | REACTOME_DEADENYLATION_OF_MRNA | 22 | *C2orf29***, *CNOT10*, *CNOT2*, *CNOT3*, *CNOT4*, *CNOT6*, *CNOT7*, *CNOT8*, *EIF4A1*, *EIF4A2*, *EIF4A3*, *EIF4B*, *EIF4E*, *EIF4G1*, *LOC645139***, *LOC651789***, *LOC652607***, *PABPC1*, *PAIP1*, *PARN*, *RQCD1*, *TNKS1BP1*. |
| Reactome | REACTOME_MRNA_DECAY_BY_3_TO_5_EXORIBONUCLEASE | 11 | *DCPS*, *DIS3*, *EXOSC1*, *EXOSC2*, *EXOSC3*, *EXOSC4*, *EXOSC5*, *EXOSC6*, *EXOSC7*, *EXOSC8*, *EXOSC9*, |
| Reactome | REACTOME_MRNA_DECAY_BY_5_TO_3_EXORIBONUCLEASE | 15 | *DCP1A*, *DCP1B*, *DCP2*, *DDX6*, *EDC3*, *EDC4*, *LOC645947*, *LSM1*, *LSM2*, *LSM3*, *LSM4*, *LSM5*, *LSM6*, *PATL1*, *XRN1*. |
| PID* | NO DATA | 0 | |
| GO* | NO DATA | 0 | |
| BioCarta | NO DATA | 0 | |
| Literature | | 2 | *PAN2*, *PAN3* |
| Total | | 68*** | |

*KEGG, Kyoto encyclopedia of genes and genomes; GO, gene ontology; PID, pathway interaction database;
**Pseudo gene: *C1DP2*, *C1DP3*, *LOC645139*; same gene with different name: *C2orf29*; withdrawn by updated NCBI: *LOC645947*, *LOC651789*, *LOC652607*.
***After removing the duplicate genes and those genes mentioned in **;
Search keyword: mRNA degradation; Search Filters: Collection, canonical pathways + GO gene sets; Organism, Homo sapiens; Contributor, all contributors.

**Table 2** Associations between SNPs in the general mRNA degradation pathway and lung cancer risk with FDR < 0.050 in TRICL GWASs

| SNP | Gene | Chr. | Position (hg19) | Allele[a] | EAF | Q[b] | $I^2$ |
|---|---|---|---|---|---|---|---|
| rs115834633 | *LSM2* | 6 | 31765984 | G/A | 0.11 | 0.200 | 30.79 |
| rs114312980 | *LSM2* | 6 | 31768799 | A/C | 0.11 | 0.230 | 26.77 |
| rs115801685 | *LSM2* | 6 | 31772093 | C/A | 0.11 | 0.220 | 27.36 |
| rs115489726 | *LSM2* | 6 | 31766660 | C/T | 0.11 | 0.240 | 25.69 |
| rs114637560 | *LSM2* | 6 | 31765864 | T/A | 0.15 | 0.260 | 22.42 |
| rs114984862 | *SKIV2L* | 6 | 31936668 | C/T | 0.27 | 0.290 | 18.64 |
| rs9800264 | *CNOT6* | 5 | 179940091 | G/A | 0.10 | 0.750 | 0.00 |
| rs2387281 | *CNOT6* | 5 | 179988283 | T/C | 0.10 | 0.743 | 0.00 |
| rs6877400 | *CNOT6* | 5 | 179996111 | T/C | 0.10 | 0.747 | 0.00 |
| rs116188106 | *SKIV2L* | 6 | 31927342 | G/A | 0.27 | 0.298 | 17.82 |
| rs114011334 | *SKIV2L* | 6 | 31928799 | C/T | 0.27 | 0.297 | 17.92 |
| rs115002281 | *SKIV2L* | 6 | 31929014 | C/A | 0.27 | 0.297 | 17.95 |
| rs10052782 | *CNOT6* | 5 | 179975104 | C/T | 0.10 | 0.723 | 0.00 |
| rs6422334 | *CNOT6* | 5 | 179982151 | C/T | 0.10 | 0.734 | 0.00 |
| rs2453176 | *CNOT6* | 5 | 179975792 | C/T | 0.10 | 0.723 | 0.00 |
| rs2387285 | *CNOT6* | 5 | 179982278 | A/G | 0.10 | 0.700 | 0.00 |
| rs2447734 | *CNOT6* | 5 | 179968674 | G/C | 0.10 | 0.720 | 0.00 |
| rs76820754 | *CNOT6* | 5 | 179936737 | G/A | 0.10 | 0.735 | 0.00 |
| rs780126 | *CNOT6* | 5 | 179963034 | C/T | 0.13 | 0.758 | 0.00 |
| rs812956 | *CNOT6* | 5 | 179953048 | G/C | 0.10 | 0.653 | 0.00 |

SNP: single nucleotide polymorphism; FDR: false discovery rate; TRICL: Transdisciplinary Research in Cancer of the Lung; GWAS: genome-wide association study; Chr.: chromoso
[a]Reference allele/effect allele;
[b]Fixed effect models were used when no heterogeneity was found between studies (Q-test $P > 0.100$ and $I^2 < 50.0\%$); otherwise, random effect models were used;
[c] "+" means a positive association, and "-" means a negative association.

**Table 3** Linkage disequilibrium between the 11 SNPs of *CNOT6* in European populations included in the 1000 Genomes Project and imputation quality scores

| SNP | Position (hg19) | D' | $r^2$ | Function prediction | | Imputation quality | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SNPinfo[a] | Regulome DB[b] | Info ICR | Rsq MDACC | Rsq IARC | Info NCI | Info Toronto | Rsq GLC |
| rs2453176 | 179975792 | | | -- | 1f | 1.000 | 0.999 | 1.000 | 1.000 | 1.000 | 1.000 |
| rs780126 | 179963034 | 1.00 | 0.71 | -- | -- | 0.874 | 0.751 | 0.703 | 0.859 | 0.857 | 0.784 |
| rs2387281 | 179988283 | 1.00 | 0.97 | -- | -- | 0.998 | 0.972 | 0.969 | 0.996 | 0.990 | 0.967 |
| rs6877400 | 179996111 | 1.00 | 0.97 | Splicing site | 5 | 0.998 | 0.964 | 0.965 | 0.996 | 0.990 | 0.953 |
| rs2387285 | 179982278 | 1.00 | 0.97 | -- | 4 | 0.990 | 0.966 | 0.923 | 0.988 | 0.981 | 0.964 |
| rs812956 | 179953048 | 1.00 | 0.97 | -- | 6 | 0.991 | 0.961 | 0.962 | 0.988 | 0.978 | 0.976 |
| rs9800264 | 179940091 | 1.00 | 0.99 | -- | -- | 0.999 | 0.970 | 0.977 | 0.998 | 0.993 | 1.000 |
| rs6422334 | 179982151 | 1.00 | 0.99 | -- | 5 | 0.999 | 0.982 | 0.976 | 0.997 | 0.994 | 0.979 |
| rs10052782 | 179975104 | 1.00 | 1.00 | -- | 6 | 1.000 | 0.998 | 1.000 | 1.000 | 1.000 | 1.000 |
| rs76820754 | 179936737 | 1.00 | 1.00 | -- | 6 | 1.000 | 0.970 | 0.971 | 0.999 | 0.998 | 0.999 |
| rs2447734 | 179968674 | 1.00 | 1.00 | -- | -- | 1.000 | 0.994 | 0.999 | 0.999 | 0.997 | 0.999 |

SNP: single nucleotide polymorphism;
Imputation quality: Rsq: MaCH r-squared; Info: IMPUTE2 information score;
ICR: the Institute of Cancer Research Genome-wide Association Study, UK;
MDACC: the MD Anderson Cancer Center Genome-wide Association Study, US;
IARC: the International Agency for Research on Cancer Genome-wide Association Study, France;
NCI: the National Cancer Institute Genome-wide Association Study, US;
Toronto: the Samuel Lunenfeld Research Institute Genome-wide Association Study, Toronto, Canada;
GLC: German Lung Cancer Study, Germany;
[a]https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm;
[b]http://regulomedb.org/.

**Table 4** Associations between of *CNOT6* rs2453176 (C >T) and lung cancer risk stratified by histologic types in all eight lung cancer GWASs from ILCCO-TRICL

| Study | Overall | | | | AD | | | | SC | | | | $P^*$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Case | Control | OR (95% CI) | *P* | Case | Control | OR (95% CI) | *P* | Case | Control | OR (95% CI) | *P* | |
| ICR | 1952 | 5200 | 1.15 (1.02-1.28) | 0.020 | 465 | 5200 | 1.38 (1.12-1.70) | 0.002 | 611 | 5200 | 1.14 (0.95-1.38) | 0.158 | 0.181 |
| MDACC | 1150 | 1134 | 1.25 (1.03-1.52) | 0.027 | 619 | 1134 | 1.06 (0.92-1.47) | 0.206 | 306 | 1134 | 1.45 (1.08-1.94) | 0.013 | 0.102 |
| IARC | 2533 | 3791 | 1.14 (1.00-1.29) | 0.053 | 517 | 2824 | 1.13 (0.90-1.42) | 0.301 | 911 | 2968 | 1.18 (0.98-1.41) | 0.081 | 0.771 |
| NCI | 5713 | 5736 | 1.10 (1.01-1.21) | 0.025 | 1841 | 5736 | 1.17 (1.03-1.33) | 0.016 | 1447 | 5736 | 1.04 (0.91-1.20) | 0.543 | 0.220 |
| Toronto | 331 | 499 | 1.12 (0.76-1.63) | 0.057 | 90 | 499 | 1.48 (0.83-2.64) | 0.186 | 50 | 499 | 1.00 (0.44-2.25) | 0.998 | 0.442 |
| GLC | 481 | 478 | 0.93 (0.69-1.26) | 0.064 | 186 | 478 | 1.25 (0.86-1.83) | 0.240 | 97 | 478 | 0.90 (0.52-1.54) | 0.695 | 0.330 |
| **Discovery combined** | 12160 | 16838 | 1.13 (1.06-1.19) | 4.33E-05 | 3818 | 15871 | 1.21 (1.11-1.32) | 2.04E-05 | 3424 | 16015 | 1.13 (1.03-1.23) | 0.009 | 0.268 |
| Harvard | 984 | 970 | 0.85 (0.68-1.05) | 0.133 | 597 | 970 | 0.79 (0.62-1.01) | 0.130 | 216 | 970 | 1.08 (0.75-1.56) | 0.678 | 0.164 |
| deCODE | 1319 | 26380 | 1.14 (1.01-1.28) | 0.032 | 547 | 26380 | 1.02 (0.85-1.21) | 0.858 | 259 | 26380 | 1.11 (0.86-1.43) | 0.436 | 0.592 |
| **Replication combined** | 2303 | 27350 | 1.00 (0.75-1.32) | 0.098 | 1144 | 27350 | 0.91 (0.71-1.16) | 0.449 | 475 | 27350 | 1.10 (0.89-1.36) | 0.381 | 0.252 |
| **Overall** | 14463 | 44188 | 1.11 (1.04-1.18) | 0.001 | 4862 | 43221 | 1.13 (1.00-1.27) | 0.050 | 3897 | 43365 | 1.12 (1.03-1.22) | 0.006 | 0.905 |

GWAS: genome-wide association study; ILCCO: International Lung Cancer Consortium; TRICL: Transdisciplinary Research in Cancer of the Lung; AD, adenocarcinoma; SC, squamous cell carcinoma; OR: odds ratio; CI: confidence interval.

*Homogeneity tests suggest that there is no heterogeneity between the subgroups of AD and SC in each GWAS and overall result ($P > 0.05$).

ICR: the Institute of Cancer Research Genome-wide Association Study, UK;

MDACC: the MD Anderson Cancer Center Genome-wide Association Study, US;

IARC: the International Agency for Research on Cancer Genome-wide Association Study, France;

NCI: the National Cancer Institute Genome-wide Association Study, US;

Toronto: the Samuel Lunenfeld Research Institute Genome-wide Association Study, Toronto, Canada;

GLC: German Lung Cancer Study, Germany;

Harvard: Harvard Lung Cancer Study;

DeCODE: Icelandic Lung Cancer Study.

**Table 5** Associations between of *CNOT6* rs2453176 (C >T) and lung cancer risk stratified by smoking status in six lung cancer GWASs from ILCCO-TRICL Consortia

| Study | Smoker | | | | Non-smoker | | | | *P** |
|---|---|---|---|---|---|---|---|---|---|
| | Case | Control | OR (95% CI) | *P* | Case | Control | OR (95% CI) | *P* | |
| MDACC | 1150 | 1134 | 1.25 (1.03-1.52) | 0.027 | | | | | |
| IARC | 2367 | 2508 | 1.12 (0.97-1.29) | 0.131 | 159 | 1253 | 1.40 (0.94-2.09) | 0.096 | 0.303 |
| NCI | 5342 | 4336 | 1.10 (1.00-1.22) | 0.058 | 350 | 1379 | 0.99 (0.72-1.37) | 0.972 | 0.540 |
| Toronto | 236 | 272 | 1.14 (0.70-1.86) | 0.606 | 95 | 217 | 1.13 (0.61-2.11) | 0.702 | 0.983 |
| GLC | 433 | 258 | 1.00 (0.67-1.49) | 0.995 | 35 | 220 | 1.64 (0.71-3.82) | 0.250 | 0.298 |
| Harvard | 892 | 809 | 0.87 (0.70-1.09) | 0.221 | 92 | 161 | 0.71 (0.38-1.33) | 0.288 | 0.549 |
| **Overall** | 10420 | 9317 | 1.09 (1.02-1.17) | 0.011 | 731 | 3230 | 1.10 (0.89-1.36) | 0.363 | 0.936 |

GWAS: genome-wide association study; ILCCO: International Lung Cancer Consortium; TRICL: Transdisciplinary Research in Cancer of the Lung; OR: odds ratio; CI: confidence interval.
MDACC: the MD Anderson Cancer Center Genome-wide Association Study, US;
IARC: the International Agency for Research on Cancer Genome-wide Association Study, France;
NCI: the National Cancer Institute Genome-wide Association Study, US;
Toronto: the Samuel Lunenfeld Research Institute Genome-wide Association Study, Toronto, Canada;
GLC: German Lung Cancer Study, Germany;
Harvard: Harvard Lung Cancer Study;
*Homogeneity tests suggest there is no heterogeneity between the subgroups of smoker and non-smoker in each GWAS and overall result ($P > 0.05$).

**Supplemental Table S1** Summary of characteristics in the eight lung cancer genome-wide association studies of the ILCCO-TRICL Consortia

| Variable | ICR[1] | MDACC[2] | IARC[3] | NCI[4] | Toronto[5] | GLC[6] | Harvard[7] | deCODE[8] |
|---|---|---|---|---|---|---|---|---|
| **Case** | 1952 | 1150 | 2533 | 5713 | 331 | 481 | 984 | 1319 |
| AD | 465 | 619 | 517 | 1841 | 90 | 186 | 597 | 547 |
| SC | 611 | 306 | 911 | 1447 | 50 | 97 | 216 | 259 |
| Smoker | | 1150 | 2367 | 5342 | 236 | 433 | 892 | |
| Non-smoker | | | 159 | 350 | 95 | 35 | 92 | |
| **Control** | 5200 | 1134 | 3791 | 5736 | 499 | 478 | 970 | 26380 |
| Smoker | | 1134 | 2508 | 4336 | 272 | 258 | 809 | |
| Non-smoker | | | 1253 | 1379 | 217 | 220 | 161 | |

ILCCO: International Lung Cancer Consortium;TRICL: Transdisciplinary Research in Cancer of the Lung; AD: adenocarcinoma, SC: squamous cell carcinoma;

[1] ICR: the Institute of Cancer Research Genome-wide Association Study, UK;

[2] MDACC: the MD Anderson Cancer Center Genome-wide Association Study, US;

[3] IARC: the International Agency for Research on Cancer Genome-wide Association Study, France;

[4] NCI: the National Cancer Institute Genome-wide Association Study, US;

[5] Toronto: the Samuel Lunenfeld Research Institute Genome-wide Association Study, Toronto, Canada;

[6] GLC: German Lung Cancer Study, Germany;

[7] Harvard: Harvard Lung Cancer Study, US;

[8] deCODE: Icelandic Lung Cancer Study, Iceland.