Role of Nicotine Dependence on the Relationship between Variants in the Nicotinic Receptor Genes and Risk of Lung Adenocarcinoma

Tung-Sung Tseng¹, Jong Y. Park², Jovanny Zabaleta³, Sarah Moody-Thomas¹, Melinda S. Sothern¹, Ted Chen⁴, David E. Evans⁵, Hui-Yi Lin⁶

¹ Behavioral and Community Health Sciences, School of Public Health and Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA, United States of America, ² Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, United States of America, ³ Department of Pediatrics and Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, LA, United States of America, ⁴ Department of Global Community Health and Behavioral Sciences, Tulane University, New Orleans, LA, United States of America, ⁵ Department of Health Outcomes and Behavior, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, United States of America, ⁶ Department of Biostatistics and Bioinformatics, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, United States of America

Abstract

Several variations in the nicotinic receptor genes have been identified to be associated with both lung cancer risk and smoking in the genome-wide association (GWA) studies. However, the relationships among these three factors (genetic variants, nicotine dependence, and lung cancer) remain unclear. In an attempt to elucidate these relationships, we applied mediation analysis to quantify the impact of nicotine dependence on the association between the nicotinic receptor genetic variants and lung adenocarcinoma risk. We evaluated 23 single nucleotide polymorphisms (SNPs) in the five nicotinic receptor related genes (CHRNA3, CHRNA6, and CHRNA5/A3/B4) previously reported to be associated with lung cancer risk and smoking behavior and 14 SNPs in the four ‘control’ genes (TERT, CLPTM1L, CYP1A1, and TP53), which were not reported in the smoking GWA studies. A total of 661 lung adenocarcinoma cases and 1,347 controls with a smoking history, obtained from the Environment and Genetics in Lung Cancer Etiology case-control study, were included in the study. Results show that nicotine dependence is a mediator of the association between lung adenocarcinoma and gene variations in the regions of CHRNA3/A3/B4 and accounts for approximately 15% of this relationship. The top two CHRNA3 SNPs associated with the risk for lung adenocarcinoma were rs1051730 and rs12914385 (p-value = 1.9 × 10⁻¹⁰ and 1.1 × 10⁻¹⁰, respectively). Also, these two SNPs had significant indirect effects on lung adenocarcinoma risk through nicotine dependence (p = 0.003 and 0.007). Gene variations rs2736100 and rs2853676 in TERT and rs401681 and rs31489 in CLPTM1L had significant direct associations on lung adenocarcinoma without indirect effects through nicotine dependence. Our findings suggest that nicotine dependence plays an important role between genetic variants in the CHRNA3/A3/B4 region, especially CHRNA3, and lung adenocarcinoma. This may provide valuable information for understanding the pathogenesis of lung adenocarcinoma and for conducting personalized smoking cessation interventions.

Introduction

Lung cancer was the second leading cause of cancer incidence (14%) and the first leading cause of cancer deaths (27%) for Americans in 2014 [1]. There are two major histological categories for lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is classified into three subtypes: adenocarcinoma (ADC), squamous cell carcinoma (SQC), and large cell carcinoma (LC). Worldwide, ADC is the most common type of lung cancer, with an increasing trend of incidence over time for both males and females [2]. Most lung cancer patients are diagnosed at advanced stages, and the 5-year survival rate for these patients is less than 10% [3,4]. Despite its negative impact on public health, effective early detection tools for lung cancer are still under development. Chest X-ray screening with or without sputum cytologic analysis have shown no reduction in lung-cancer mortality based on several randomized trials [5]. In another large scale clinical trial with more than 53,000 participants, low-dose computed tomography (CT) scans can reduce mortality from lung cancer by 20% relative to chest X-ray screening among the high-risk group with a history of heavy smoking. However, CT scan screening showed high false-positive results (96%) [6]. Thus, there is an urgent need for identifying additional biomarkers in order to improve prediction accuracy of lung cancer. While exploring biomarkers of lung cancer, smoking needs to be taken into consideration.

Smoking, which is a well-known modifiable behavior, is the leading risk factor of lung cancer. The impact of smoking on lung cancer is enormous. Both quantity and duration of smoking increase lung cancer risk [7]. The historical cigarette smoking
prevalence can be used to predict the trend of lung cancer incidence in both men and women based on a study using 1920–1990 data [8]. A similar trend impact of smoking is also shown in lung ADC [9]. Smoking is generally involved in the development of ADC [10], although the risk is less for lung ADC than for SQC and SCLC [11].

Although smoking is the most important behavioral factor of lung cancer, the impact of smoking on the associations between variants in nicotine related genes and lung cancer is still understudied. In attempts to elucidate these relationships, we have used mediation analysis in this study to quantify the mediation effect of nicotine dependence. CHRNA5/A3/B4, CHRNB3, and CHRNA6 genes are nicotinic receptor genes, encoding nicotine acetylcholine receptor subunits. The nicotinic cholinergic receptor subunits expressed in the human brain form various types of functional receptors by different subunit composition and play a vital role in modulation of dopaminergic function and sensitivity to nicotine [12]. These five genes have been reported to be associated with both smoking behavior and lung cancer risk [13,14]. CHRNB3 and CHRNA6 are in the chromosome 8p11 region. Single nucleotide polymorphisms (SNPs) in CHRNB3 were associated with nicotine dependence [15] and cigarettes smoked per day [16]. Variations in CHRNA6 are associated with nicotine dependence [15] and tobacco phenotypes [17]. Based on the National Human Genome Research Institute (NHGRI) GWAS Catalog [18], SNPs in CHRNA5/A3/B4 are associated with overall lung cancer, lung ADC, and smoking; SNPs in CHRNA3 are associated with cigarettes smoked per day.

Another four genes (TERT, CLPTM1L, CYP1A1, and TP53) are associated with lung cancer risk. In several GWA studies, SNPs in TERT and CLPTM1L are associated with overall lung cancer and lung ADC [18]. Genetic variants of the CYP1A1 exon7 are reported to be associated with lung cancer in the overall population, especially in Asians, Caucasians, females, and smokers [19]. The CYP1A1 polymorphisms [Ile462Val (rs1048943) and T6255C (rs4649005)] are associated with lung cancer risk, especially for lung SQC in Asian populations [20]. Another meta-analysis indicates that TP53 codon 72 and intron 6 (rs1625895) polymorphisms are associated with lung cancer risk [21].

There was a limited number of related mediation studies to evaluate the impact of smoking (number of cigarettes smoked per day or smoking pack-year) on the relationship between variants (especially rs1051730) in CHRNA5/A3/B4 and overall lung cancer or chronic obstructive pulmonary disease (COPD) [22–24]. However, nicotine dependence and its mediation impact on lung ADC remain unexplored. Lung cancer is a heterogeneous disease so it is important to evaluate specific histological categories of lung cancer separately in genetic studies. In addition, nicotine dependence, which largely contributes to persistent smoking, has a large impact on lung cancer. Previous studies show that 60–70% of the variance in smoking persistence and nicotine dependence result from genetic impact [25–27]. In our study, nicotine dependence was measured using the Fagerstrom Test [20], which correlates well with various nicotine withdrawal symptoms, level of smoking urges, and self-rated addiction [29–31]. Therefore, the objective was to characterize the mediation effects of nicotine dependence on the relationship between genetic variants in the five nicotinic receptor genes (CHRNA5/A3/B4, CHRNB3, and CHRNA6) and lung ADC risk among ever smokers. In order to evaluate robustness of the mediation analysis, we also included four ‘control’ genes (TERT, CLPTM1L, CYP1A1, and TP53), which are not the nicotinic receptor genes and were not reported in the smoking GWA studies.

Methods

Study population and measurements

A total of 661 lung adenocarcinoma (ADC) cases and 1,347 controls with a smoking history obtained from the Environment and Genetics in Lung Cancer Etiology (EAGLE) case-control study were included in this study. Due to the heterogeneity of lung cancer, we focused only on the most common histology type: Lung ADC. This dataset is a part of the GENEVA/GEI lung cancer and smoking GWS dataset (dbGaP accession number: phs000093.v2.p2). DNA samples were genotyped on the Illumina HumanHap550v3_B BeadChips (Illumina, San Diego, CA, USA). SNPs with minor allele frequencies (≤5%), completion rates (≥95%), or a p-value of Hardy-Weinberg equilibrium in controls< 10−7 were excluded [32]. Included were 37 SNPs in nine candidate genes (CHRNB3, CHRNA6, CHRNA5/A3/B4, TERT, CLPTM1L, CYP1A1, and TP53).

All participants, enrolled in Italy between 2002 and 2005, were self-identified as White. We included only ever smokers, which was defined as those who smoked at least 100 cigarettes in their entire lifetime. Ever smokers were chosen because (1) the mechanism of lung cancer development may be different for ever smokers and never smokers [13], and (2) ever smokers are commonly used as
the targeted population for early detection of lung cancer. Nicotine dependence was measured using the 6-item Fagerstrom Test [28], with a score range of 0 to 10. Higher scores indicated greater nicotine dependence. For former smokers who quit smoking 6 months previously, nicotine dependence at the time in which they smoked the most was reported. Pack-year, estimated by number of cigarette packs (20 cigarettes = 1 pack) times years of smoking, was also included. The details of this study are presented at http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000093.v2.p2.

Statistical analysis

Participants’ demographic and smoking characteristics by disease status were compared using t-tests for continuous variables and chi-square tests for categorical variables. SNPs were treated as an additive model with a minor allele count. The binary nicotine dependence status (high/low) was used in the analyses. High nicotine dependence was defined as a Fagerstrom score $\geq 6$, which is commonly used in other studies [33–36]. Mediation analyses based on the Baron and Kenny method [37] were performed. In these analyses, the SNP was the independent variable; lung cancer was the outcome variable; and nicotine dependence was the proposed mediator. In our mediation analysis, logistic regressions were applied for modeling the nicotine dependence and lung cancer risk. The mediated effect is robust in terms of the logistic assumption [38]. All models were adjusted for age and gender. The odds ratios (ORs) per minor allele were calculated.

For each SNP, the mediation analyses were based on the following three logistic models (Equations 1–3). For simplicity, the covariates of age and gender are not shown in the equations.

$$ND = a_{SNP} + \epsilon_1$$

$$L = c_{SNP} + \epsilon_2$$

$$L = b_{ND} + \epsilon_{SNP} + \epsilon_3$$

Here, ND represents nicotine dependence, L represents lung cancer, and $\epsilon_1$–$\epsilon_3$ represent the error terms for each equation. In these equations and Figure 1, $a$ was the coefficient of nicotine dependence on an SNP (Equation 1). The total effect, denoted as $c$, was the coefficient of lung cancer on an SNP without considering nicotine dependence (Equation 2). Using a logistic model of lung cancer with nicotine dependence and a given SNP (Equation 3), $b$ was the coefficient on nicotine dependence, and $c'$ was the coefficient on the SNP. The indirect effect of an SNP on lung cancer risk is defined as $a \times b$, the direct effect as $c'$, and the total effect as $c$. When the logistic regressions were applied for the binary mediator and/or binary outcome, the coefficients in the mediation analysis have different scales. Thus, standardized coefficients ($a_{SNP}$, $b_{SNP}$, $c'_{SNP}$) are needed in order to make these coefficients comparable across models. The standardized coefficient was calculated by multiplying the original coefficient by the standard deviation of the same predictor variable in the model and then dividing by the standard deviation of the outcome variable.

| Characteristics | Control (n = 1,347) | Adenocarcinoma (n = 661) | p-value$^1$ |
|-----------------|--------------------|--------------------------|-------------|
| **Age**         |                    |                          |             |
| $\leq 59$       | 328 (24.4)         | 189 (28.6)               | 0.113       |
| 60–64           | 253 (18.8)         | 127 (19.2)               |             |
| 65–69           | 307 (22.8)         | 144 (21.8)               |             |
| 70–74           | 283 (21.0)         | 111 (16.8)               |             |
| **Gender**      |                    |                          |             |
| Male            | 1143 (84.9)        | 527 (79.7)               | 0.004       |
| Female          | 204 (15.1)         | 134 (20.3)               |             |
| **Smoking status** |                |                          |             |
| Former          | 858 (63.7)         | 312 (47.2)               | <.0001      |
| Current         | 489 (36.3)         | 349 (52.8)               |             |
| **Cigarette pack-year** |           |                          |             |
| 0.1–30          | 832 (61.8)         | 201 (30.4)               | <.0001      |
| 31–50           | 343 (25.5)         | 253 (38.3)               |             |
| 50+             | 172 (12.8)         | 207 (31.3)               |             |
| **Nicotine dependence** |       |                          |             |
| Mean ± SD       | 2.8±2.5            | 4.5±2.5                  |             |
| Low (<6)        | 1118 (83.0)        | 414 (62.6)               | <.0001      |
| High (≥6)       | 229 (17.0)         | 247 (37.4)               | <.0001      |

$^1$t-test for continuous variables and chi-square test for categorical variables.
$^2$measured using the Fagerstrom Test, with a score range of 0 to 10. Higher scores indicated greater nicotine dependence. SD: standard deviation.
Table 2. Gene variations associated with lung adenocarcinoma in ever smokers.

| Ch1 | Gene | SNP1 | A/a2 | MAF2 | Total effect3 | p-value | Direct effect3 | p-value | IND p-value4 | Med %4 |
|-----|------|------|------|------|--------------|---------|---------------|---------|--------------|--------|
| 5   | TERT | rs2736122 | C/T 0.22 | 1.02 (0.87–1.19) | 0.849 | 1.01 (0.86–1.19) | 0.868 | 0.889 | -           |
|     |      | rs4975605 | C/A 0.48 | 0.94 (0.83–1.07) | 0.380 | 0.97 (0.85–1.11) | 0.686 | 0.037 | -           |
|     |      | rs2736100 | G/T 0.44 | 0.76 (0.67–0.87) | 8.2 x 10^-5 | 0.76 (0.66–0.88) | 1.2 x 10^-5 | 0.405 | -           |
|     |      | rs2853676 | G/A 0.31 | 1.17 (1.02–1.34) | 0.029* | 1.17 (1.01–1.35) | 0.031* | 0.722 | -           |
| 5   | CLPTM1 | rs402710 | C/T 0.31 | 0.99 (0.85–1.14) | 0.351 | 0.98 (0.84–1.14) | 0.774 | 0.690 | -           |
|     |      | rs10073340 | C/T 0.16 | 1 (0.83–1.2) | 0.975 | 1 (0.83–1.2) | 0.973 | 0.987 | -           |
|     |      | rs401681 | C/T 0.44 | 0.83 (0.72–0.95) | 0.007* | 0.84 (0.73–0.97) | 0.015* | 0.143 | -           |
|     |      | rs31489 | C/A 0.40 | 0.85 (0.74–0.97) | 0.018* | 0.85 (0.74–0.98) | 0.024* | 0.419 | -           |
| 8   | CHRNA3 | rs6474414 | C/A 0.22 | 0.87 (0.74–1.02) | 0.092 | 0.9 (0.76–1.06) | 0.222 | 0.033 | -           |
|     |      | rs7012713 | C/T 0.03 | 0.84 (0.58–1.22) | 0.351 | 0.85 (0.58–1.25) | 0.416 | 0.512 | -           |
|     |      | rs2470893 | G/A 0.20 | 1.06 (0.9–1.25) | 0.492 | 1.02 (0.86–1.21) | 0.797 | 0.061 | -           |
|     |      | rs6495306 | A/G 0.33 | 0.86 (0.75–0.98) | 0.030* | 0.86 (0.75–1) | 0.043* | 0.439 | -           |
|     |      | rs680244 | G/A 0.35 | 0.85 (0.74–0.98) | 0.027* | 0.86 (0.75–0.99) | 0.038* | 0.450 | -           |
|     |      | rs621849 | A/G 0.33 | 0.86 (0.75–0.99) | 0.030* | 0.86 (0.75–0.99) | 0.042* | 0.479 | -           |
| 15  | CYP1A1 | rs4646421 | C/T 0.11 | 1.22 (0.99–1.51) | 0.056 | 1.16 (0.94–1.44) | 0.161 | 0.020 | -           |
|     |      | rs2470893 | G/A 0.20 | 1.06 (0.9–1.25) | 0.492 | 1.02 (0.86–1.21) | 0.797 | 0.061 | -           |
|     |      | rs6993006 | A/G 0.33 | 0.86 (0.75–0.98) | 0.030* | 0.86 (0.75–1) | 0.043* | 0.439 | -           |
|     |      | rs12910984 | A/G 0.24 | 0.68 (0.57–0.8) | 3.4 x 10^-6 | 0.7 (0.59–0.83) | 4.1 x 10^-5 | 0.005* | 16.8       |
|     |      | rs105730 | C/T 0.41 | 1.56 (1.36–1.78) | 1.9 x 10^-10 | 1.51 (1.31–1.74) | 6.9 x 10^-9 | 0.003* | 12.7       |
|     |      | rs3743077 | G/A 0.35 | 0.84 (0.73–0.96) | 0.014* | 0.84 (0.73–0.97) | 2.0 x 10^-2 | 0.422 | -           |
|     |      | rs936485 | T/C 0.23 | 0.67 (0.57–0.79) | 2.6 x 10^-6 | 0.7 (0.59–0.83) | 2.8 x 10^-5 | 0.007* | 15.7       |
|     |      | rs12914385 | C/T 0.44 | 1.56 (1.36–1.78) | 1.1 x 10^-10 | 1.52 (1.33–1.75) | 2.4 x 10^-9 | 0.007* | 11.1       |
|     |      | rs8042374 | A/G 0.24 | 0.67 (0.57–0.79) | 2.1 x 10^-6 | 0.7 (0.59–0.82) | 2.6 x 10^-5 | 0.005* | 16.3       |
|     |      | rs3743075 | G/A 0.31 | 0.85 (0.73–0.98) | 0.027* | 0.85 (0.73–0.99) | 0.034* | 0.565 | -           |
|     |      | rs8192475 | G/A 0.04 | 0.86 (0.61–1.22) | 0.394 | 0.87 (0.61–1.24) | 0.432 | 0.769 | -           |
|     |      | rs6495309 | C/T 0.23 | 0.66 (0.56–0.78) | 1.5 x 10^-6 | 0.69 (0.58–0.82) | 2.0 x 10^-5 | 0.004* | 16.3       |
| 15  | CHRNA4 | rs1948 | C/T 0.30 | 0.81 (0.7–0.93) | 0.004* | 0.81 (0.69–0.94) | 0.006* | 0.502 | -           |
|     |      | rs950776 | T/C 0.30 | 0.81 (0.7–0.94) | 0.004* | 0.8 (0.69–0.93) | 0.005* | 0.745 | -           |
|     |      | rs11636753 | G/T 0.34 | 0.78 (0.68–0.9) | 0.001* | 0.78 (0.67–0.91) | 0.001* | 0.456 | -           |
|     |      | rs12441998 | A/G 0.22 | 0.68 (0.57–0.8) | 7.7 x 10^-6 | 0.7 (0.59–0.83) | 4.7 x 10^-5 | 0.025 | -           |
|     |      | rs1316971 | G/A 0.22 | 0.71 (0.6–0.84) | 5.4 x 10^-5 | 0.72 (0.61–0.86) | 2.3 x 10^-4 | 0.049 | -           |
| 17  | TPS3 | rs12951053 | A/C 0.06 | 1.06 (0.82–1.38) | 0.666 | 1.14 (0.87–1.48) | 0.355 | 0.049 | -           |
Results

Samples from the 661 lung ADC patients and 1,347 controls in the GENEVA/GEI lung cancer and smoking study were analyzed. The distributions of age at diagnosis for cases or at review for controls were similar (Table 1). There were more female (20.3% vs. 15.1%, p = 0.004) and more current smokers (52.8% vs. 36.3%, p < 0.001) for the ADC patients than the controls. As expected, the ADC patients had significantly higher pack-year and higher nicotine dependence (mean score 4.5 vs. 2.8) than the controls. Over 30% of the ADC patients (vs. 13% controls) had more than 50 pack-years. Cigarette pack-year and nicotine dependence were highly correlated (Spearman correlation = 0.70).

Most of the variations in CHRNA5/A3/B4 had significant total effects on lung ADC (Table 2 and Figure 2), and rs12441998 in CHRN4B4 was significantly associated with nicotine dependence (a coefficient in Table 3). In this region, most of the SNPs in CHRNA3 had significant indirect effects on lung ADC through nicotine dependence, and their relationship is shown in Figure 1c. Three SNPs in CHRNA5 (rs6495306, rs680244 and rs621849), in complete LD ($r^2 = 0.99$), had a significant total effect (p = 0.03, FDR q = 0.023) and direct effects (p = 0.04, FDR q = 0.034), but the indirect effect through nicotine dependence was not significant. Nine SNPs in CHRNA3 had significant total and direct effects; seven of these had significant indirect effects on lung ADC through nicotine dependence. The top two SNPs associated with lung ADC (total effect) were with a strong LD ($r^2 = 0.93$); these were rs1051730 (OR = 1.56 per T-allele, p = 1.9e-10, FDR q = 0.023) and rs12914385 (OR = 1.56 per T-allele, p = 1.1e-10, FDR q = 1.5e-10). Their effects mediated through nicotine dependence were significant (p = 0.003 and 0.007, and FDR q-value = 0.023 and 0.024, respectively). The relative indirect effects for these top two SNPs were 13% and 11%, respectively. The mediation proportions of SNPs in CHRNA3 were 11–17% (mean = 13.1%). The indirect effects and their 95% confidence intervals were shown in Table S1. All five SNPs in CHRNA3 had significant total and direct effects, and only two SNPs (rs12441998 and rs1316971) had a raw p-value < 0.05 for the indirect effect. However, they became insignificant after considering multiple comparisons (FDR q-value = 0.056 and 0.072, respectively).

Some genetic variations in TERT and CLPTM1L, in the region of chromosome 5p15.33, had a direct association with lung ADC risk, but the mediation effect of nicotine dependence was not significant (relationship shown in Figure 1a). Two SNPs in TERT (rs2736100 and rs2835676) directly influenced lung cancer risk (OR = 0.76 per T allele, p = 1.2x10^-4, FDR q = 2.5x10^-4, and OR = 1.17 per A allele, p = 0.031, FDR q = 0.031, respectively). The indirect effects of these two SNPs in TERT on lung cancer risk though nicotine dependence were not significant. In
| Chr | gene  | SNP  | SNP associated with ND<sup>1</sup> | ND associated with lung ADC<sup>2</sup> | SNP associated with lung ADC<sup>3</sup> |
|-----|-------|------|-------------------------------|---------------------------------|---------------------------------|
| 5   | TERT  | rs2736122 | 0.012                         | 0.889                           | 1.107                           |
|     |       | rs4975605 | -0.159                        | 0.033                           | 1.090                           |
|     |       | rs2736100 | -0.063                        | 0.403                           | 1.107                           |
|     |       | rs2853676 | 0.028                         | 0.722                           | 1.086                           |
| 5   | CLPTM1L | rs402710 | 0.033                         | 0.690                           | 1.103                           |
|     |       | rs10073340 | 0.002                         | 0.987                           | 1.108                           |
|     |       | rs401681 | -0.113                        | 0.139                           | 1.105                           |
|     |       | rs31489 | -0.062                        | 0.417                           | 1.106                           |
| 8   | CHRNA3 | rs6474414 | -0.294                        | 0.029                           | 1.102                           |
|     |       | rs7012713 | -0.138                        | 0.511                           | 1.107                           |
| 8   | CHRNA6 | rs892413 | -0.015                        | 0.879                           | 1.107                           |
|     |       | rs16891604 | -0.016                        | 0.908                           | 1.108                           |
|     |       | rs16891620 | 0.055                         | 0.641                           | 1.108                           |
|     |       | rs4646421 | 0.273                         | 0.017*                          | 1.100                           |
| 15  | CYP1A1 | rs2470893 | 0.172                         | 0.057                           | 1.107                           |
|     |       | rs6495306 | -0.061                        | 0.437                           | 1.104                           |
|     |       | rs800244 | -0.059                        | 0.449                           | 1.102                           |
|     |       | rs621849 | -0.056                        | 0.478                           | 1.112                           |
| 15  | CHRNA3 | rs578776 | -0.243                        | 0.006*                          | 1.088                           |
|     |       | rs12910984 | -0.273                        | 0.003*                          | 1.093                           |
|     |       | rs1051730 | 0.241                         | 0.002*                          | 1.083                           |
|     |       | rs3743077 | -0.063                        | 0.420                           | 1.106                           |
|     |       | rs938682 | -0.261                        | 0.005*                          | 1.086                           |
|     |       | rs12914385 | 0.211                         | 0.005*                          | 1.084                           |
|     |       | rs8042374 | -0.275                        | 0.003*                          | 1.078                           |
|     |       | rs3743075 | -0.047                        | 0.565                           | 1.105                           |
|     |       | rs8192475 | -0.058                        | 0.769                           | 1.107                           |
| 15  | CHRNA4 | rs6495309 | -0.282                        | 0.003*                          | 1.085                           |
|     |       | rs1948 | -0.056                        | 0.501                           | 1.107                           |
|     |       | rs950776 | -0.027                        | 0.745                           | 1.110                           |
|     |       | rs11636753 | -0.060                        | 0.455                           | 1.108                           |
|     |       | rs12441998 | -0.217                        | 0.021*                          | 1.092                           |
|     |       | rs1316971 | -0.187                        | 0.045                           | 1.095                           |

<sup>1</sup> Significant association with nicotine dependence (ND) at p-value < 0.05. <sup>2</sup> Significant association with lung adenocarcinoma (ADC) at p-value < 0.05. <sup>3</sup> Significant association with lung ADC at p-value < 0.05.
CLPTM1L, two SNPs (rs401681 and rs31489) were directly associated with lung ADC risk (OR = 0.84 per T allele, p = 0.015, FDR q = 0.018, and OR = 0.85 per A-allele, p = 0.024, FDR q = 0.026, respectively), but none had a significant mediation effect through nicotine dependence. Thus, most of these SNPs were associated with lung ADC risk through channels other than nicotine dependence. One SNP (rs4646421) in CYP1A1 was significantly associated with nicotine dependence (p = 0.017, FDR q = 0.043) but its indirect effect on lung ADC became insignificant after multiple comparison justification. The impact of SNPs in another three genes (CHRN3, CHRNA6, and TP53) on nicotine dependence and lung ADC was not statistically significant.

**Discussion**

To our knowledge, this is the first study to report the significant impact of nicotine dependence on the relationship between genetic variants in the region of CHRNA5/A3/B4 and lung ADC risk. Most of the SNPs in CHRNA3 had a significant indirect effect on lung ADC through nicotine dependence. Nicotine dependence is the mediator and contributor of approximately 15% of the association between lung ADC and gene variations of CHRNA3. Only variants in the nicotinic receptor genes (CHRNA5/A3/B4) had a significant indirect effect on lung ADC through nicotine dependence and variants in the control genes did not. This demonstrated robustness of the mediation analyses in identifying the casual relationship. The significant mediation impact of smoking on the association between rs1051730 in CHRNA3 and lung related diseases [overall lung cancer and chronic obstructive pulmonary disease (COPD)] were reported previously [22–24]. However, the results between these studies and ours are not directly comparable because other studies had different outcomes [lung cancer overall (instead of lung ADC) or COPD] and different mediators (number of cigarettes smoked per day or smoking pack-year). SNP rs1051730 had both a direct effect on overall lung cancer risk and indirect effects through smoking pack-year, and the mediation proportion was 7.6% in ever smokers. This SNP is also significantly associated with COPD, and the mediation proportion through cigarette pack-years is 24% [22]. Two linked variants (rs1051730 and rs8034191) in the AGPHD1/CHRNA3 cluster are strongly associated with COPD, and the mediation effect is 11–12% through number of cigarettes smoked per day and 26–42% through cigarette pack-years [24]. However, another study based on meta-analyses of four projects had inconsistent results. The impact of two SNPs, rs8034191 and rs1051730, on 15q25.1 on lung cancer through cigarettes smoked per day is not significant [23].

Our findings are consistent with other studies that show that variations in CHRNA5/A3/B4 are significantly associated with lung ADC risk and nicotine dependence. In our study, the majority of SNPs in the CHRNA5/A3/B4 region are significantly associated with both nicotine dependence and lung ADC risk. As shown in Table 3, the top two CHRNA3 SNPs (rs1051730 and rs12914385, $r^2 = 0.93$) associated with lung ADC risk also had significant indirect effects through nicotine dependence (a coefficient, p = 0.002 and 0.005, respectively). Many previous studies show that genetic variations of CHRNA5/A3/B4 relate to overall lung cancer [16,44–46], lung ADC [32,45], smoking quantities [16,44], and nicotine dependence [44]. In a meta-analysis study combining ten studies [32], two CHRNA3 SNPs (rs1051730 and rs12914385) were shown to be significantly associated with lung ADC (p = 7.1 × 10^-19 and 3.3 × 10^-16, respectively). The impact of these two CHRNA3 SNPs on lung

### Table 3. Cont.

| Chr | gene | SNP | a coef | b coef | c coef | p-value | r2 | p-value |
|-----|------|-----|--------|--------|--------|---------|----|---------|
| 17  | TP53 | rs1296103 | -0.24  | -0.06  | 1.113   | 0.045   | 0.93 | 0.001   |
| 17  | TP53 | rs2909410 | -0.06  | 0.326  | 1.108   | 0.001   | 0.93 | 0.003   |
| 17  | TP53 | rs16379544 | -0.380 | -0.485 | 1.108   | 0.001   | 0.93 | 0.003   |
| 17  | TP53 | rs2078486 | -0.396 | -0.059 | 1.108   | 0.001   | 0.93 | 0.003   |

1. Chr: chromosome; SNP: single nucleotide polymorphism.
2. Logistic regression coefficients (coef) of (1) ND = a SNP + e1, and (2) L = b ND + c SNP + e2.
3. Statistical significance: False Discover Rate (FDR) q-value < 0.05.

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ADC risk was only significant in ever smokers \cite{32}. Two SNPs (rs1051730 and rs8034191, both with P < 1 \times 10^{-15}) with strong LD in the regions of 15q25.1 containing CHRNA3, CHRNA5, and PSMA4 are strongly associated with lung cancer risk among ever smokers \cite{46}. SNP rs1051730 in the CHRNA3 gene was strongly associated with lung cancer risk (p = 1.5 \times 10^{-16}) and cigarettes per day (P = 5 \times 10^{-16}) \cite{44}. Based on two GWA meta-analyses, rs1051730 is also strongly associated with the number of cigarettes smoked per day for Liu JZ’s study \cite{47} (p = 1.7 \times 10^{-46}) and the Tobacco and Genetics Consortium study \cite{48} (P = 2.8 \times 10^{-73}). The haplotype A_C of rs16969698 and rs680244 in the region of CHRNA5/A3/B4 is associated with larger quantities of cigarettes smoked per day and later age at smoking cessation \cite{49}. The three genes of CHRNA5/A3/B4, encoding nicotine acetylcholine receptor subunits (\alpha_5, \alpha_3 and \beta_4) are in the region of chromosome 15q24-25.1. The nicotinic acetylcholine receptor belongs to the superfamily of ligand-gated ion channels, and it is activated by acetylcholine, choline, and nicotine. These proteins are involved in the regulation of nicotine and tobacco nitrosamines, which are the main carcinogens responsible for smoking related lung cancer \cite{50}. In addition, the nicotinic acetylcholine receptors are involved in several pathways and influence cancer progression, such as cell proliferation, apoptosis, invasion, and angiogenesis \cite{51,52}. Schuller et al (2009) suggested that carcinogenesis may be triggered by regulating a complex network of neurotransmitters by altered signaling of the nicotinic acetylcholine receptors \cite{53}.

Among our four ‘control’ genes, gene variations in TERT (rs2736100 and rs2853676) and CLPTM1L (rs401681 and rs31489) have a significant direct association with lung ADC risk but not an indirect effect through nicotine dependence. This means that the association between the SNPs in these two genes is not explained by nicotine dependence but could be explained by other, unmeasured factors. The identified associations between genetic variations in TERT and CLPTM1L and lung ADC are consistent with previous studies. In a large-scale GWA study \cite{54}, rs2736100 in TERT associated with lung cancer reached GWA significance (p = 4 \times 10^{-6}) and was replicated in an independent set. The significant association between rs2736100 and lung ADC is also reported in a study combining several GWA studies and meta-analyses \cite{32}. In CLPTM1L, rs402710 (p = 2 \times 10^{-7}), rs31489 (p = 8 \times 10^{-7}), and rs401681 (p = 2 \times 10^{-6}), were associated with lung cancer risk \cite{54}. Two of these SNPs (rs401681 and rs31489) in CLPTM1L are associated with lung cancer risk in ever smokers [32]. Two SNPs (rs1051730 and rs8034191, both with P < 1 \times 10^{-15}) with strong LD in the regions of 15q25.1 containing CHRNA3, CHRNA5, and PSMA4 are strongly associated with lung cancer risk among ever smokers [46]. SNP rs1051730 in the CHRNA3 gene was strongly associated with lung cancer risk (p = 1.5 \times 10^{-16}) and cigarettes per day (P = 5 \times 10^{-16}) [44]. Based on two GWA meta-analyses, rs1051730 is also strongly associated with the number of cigarettes smoked per day for Liu JZ’s study [47] (p = 1.7 \times 10^{-46}) and the Tobacco and Genetics Consortium study [48] (P = 2.8 \times 10^{-73}). The haplotype A_C of rs16969698 and rs680244 in the region of CHRNA5/A3/B4 is associated with larger quantities of cigarettes smoked per day and later age at smoking cessation [49]. The three genes of CHRNA5/A3/B4, encoding nicotine acetylcholine receptor subunits (\alpha_5, \alpha_3 and \beta_4) are in the region of chromosome 15q24-25.1. The nicotinic acetylcholine receptor belongs to the superfamily of ligand-gated ion channels, and it is activated by acetylcholine, choline, and nicotine. These proteins are involved in the regulation of nicotine and tobacco nitrosamines, which are the main carcinogens responsible for smoking related lung cancer [50]. In addition, the nicotinic acetylcholine receptors are involved in several pathways and influence cancer progression, such as cell proliferation, apoptosis, invasion, and angiogenesis [51,52]. Schuller et al (2009) suggested that carcinogenesis may be triggered by regulating a complex network of neurotransmitters by altered signaling of the nicotinic acetylcholine receptors [53].

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smokers ($p = 1 \times 10^{-3}$; and $p = 2.1 \times 10^{-5}$)[55]. Genetic variations in TERT and CLPTM1L at chromosome 3p13.33 are associated with lung ADC risk in smokers [92] and never smokers [55]. However, studies evaluating the association between variations in TERT and CLPTM1L and smoking behavior are sparse. The TERT is the reverse transcriptase component of telomerase. The role of telomerases is to preserve the integrity of the genome during cellular replication [56]. Telomere shortening often leads to chromosomal instability, mutagenesis [57], tumorigenesis [58–63], and progression of cancer [66–70]. Telomere length may be considered as a biological regulator and a predictive indicator of disease risk, progression, and premature mortality [71]. We recently reported significant associations between changes of telomere lengths and cancer risk [72,73]. Like the TERT gene, CLPTM1L is also located at 3p15.33, which is a susceptible region for various cancers, including lung cancer [74]. The CLPTM1L was originally identified among the genes involved in resistance to the anticancer agent cisplatin in cancer cell lines [75]. Overexpression of CLPTM1L mRNA has been observed in all cisplatin-resistant cell lines examined. However, CLPTM1L over-expression doesn’t seem to have any effect on cisplatin-resistant cells and cause apoptosis in cisplatin-sensitive cell lines. Although the exact function of the CLPTM1L is not known, it appears that there is an association with resistance to cisplatin and activation of the mitochondrial apoptotic pathway. CLPTM1L was expressed in lung tumor tissue, most intensely in ADC tissue, especially in the mitochondria [74]. CLPTM1L expression was strongly associated with the tumor grades of differentiation but not smoking status [74].

Translating these findings of genetic variants in the CHRNA5/A3/B4 region into public health practice may be used to identify high risk groups and could lead to tailored smoking cessation interventions. For individuals with high-risk genetic variants, which have both direct and indirect effects through nicotine dependence on lung ADC, a customized intervention can be applied to assist them to quit smoking and then reduce risk of developing lung ADC. A recent smoking cessation trial in heavy smokers (smoked $\geq 10$ cigarettes per day) shows that those with the high-risk haplotype (A_C allele of rs16969968 and rs680244) in the CHRNA5/A3/B4 region are biologically predisposed to difficulty in quitting but tended to respond better to pharmacotherapy treatment of smoking cessation than those with low-risk haplotype [49].

The study limitations include self-reported nicotine dependence measurements and a limited sample size. Although self-reported nicotine dependence score based on the Fagerstrom Test [29], which is easy to obtain in practice, is strongly associated with nicotine withdrawal [31], it may not completely capture smoking intensity and concomitant carcinogens. Other objective smoking phenotypes [such as NNK (a nicotine metabolite) and puff volume] have been recommended [76,77]. Larger and follow-up studies for Whites and other race groups, and an objective smoking phenotype, are warranted to further test the potential applications. In addition, it has been shown that there may be a potential bias in estimating the association between the exposure and the mediator (indirect effects) because the binary mediator is not selected using the principals of a case-control study design [78–80]. For adjusting for this bias, the sampling weighting approach has been suggested [23,91]. Our study did not apply this sampling weighting approach because it is a challenge for obtaining the prevalence of lung ADC in ever smokers in Italy. In summary, our results show that genetic variants in the CHRNA5/A3/B4 region may impact lung ADC through nicotine dependence. These identified SNPs information may help to detect lung ADC at earlier stages and provided promising support for genetic-guided smoking cessation and lung cancer prevention.

Supporting Information

Table S1 Indirect effects and their bootstrap confidence intervals of SNPs on lung adenocarcinoma through nicotine dependence. (DOCX)

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Author Contributions

Conceived and designed the experiments: TST HYL. Analyzed the data: TST HYL. Contributed reagents/materials/analysis tools: HYL. Wrote the paper: TST HYL. Assisted with the interpretation of data and edited the drafts: JYP JZ SM MSS TC DEE.
31. DiFranza JR, Wellman RJ, Savageau JA, Beccia A, Ursprung WWSA, et al.
30. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
28. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The
26. Sullivan PF, Kendler KS (1999) The genetic epidemiology of smoking. Nicotine
23. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
22. Wang J, Spitz MR, Amos CI, Wilkinson AV, Wu X, et al. (2010) Mediating
20. Lee KM, Kang D, Clapper ML, Ingelman-Sundberg M, Ono-Kihara M, et al.
46. Amos CI, Wu X, Broderick P, Gorlov IP, Gu J, et al. (2008) Genome-wide
45. Hung RJ, McKay JD, Gaborieau V, Boffetta P, Chabrier A, et al. (2008) Lung
164.
717–731.
70. Widmann T, Kneer H, Konig J, Herrmann M, Pfreundschuh M (2008)
68. Montgomery E, Argani P, Hicks JL, DeMarzo AM, Meeker AK (2004)
67. Stack MS, Shaw AS, Zhi Z, Zwaal RF, Chiu LS, et al. (2005) Telomere length
66. Park JY, Park G, Lee SG, Park H, Lee SH, et al. (2007) Role of telomere
65. Wang Y, Broderick P, Gao JQ, Qiao Q, Fielder A, et al. (2009) Telomere
64. Shay JW, Wright WE (2001) Telomeres and telomerase: implications for cancer
63. Londono-Vallejo JA (2008) Telomere instability and cancer. Biochimie 90: 73–
62. Artandi SE, DePinho RA (2000) Mice without telomerase: what can they teach
59. Wang Y, Zhi Z, Ruggiero S, Shafey M, Chen Y, et al. (2008) Increased telomere
58. Artandi SE, Chellappan SP (2006) Nicotine-mediated cell proliferation and
57. Coffee L, Zimm TL, Le Marchand L, Davis AM, Walker D, et al. (2007) A
56. Wang J, Shi X, Dennis JW, Shin DM, Lefkowith JB, et al. (2006) Telomere
55. Wang Y, Broderick P, Matakidou A, Eisen T, Houlston RS (2010) Role of
54. Hecht SS (2008) Progress and challenges in selected areas of tobacco
carcinogenesis. Chem Res Toxicol 21: 160–171.
53. Bleehen NM, Smith AM, Peto J, Korsbakken J, Williams J, et al. (2004) Small
52. Dasgupta P, Chellappan SP (2006) Nicotine-mediated cell proliferation and
48. Tobacco and Genetics Consortium. (2010) Genome-wide meta-analyses identify
47. Chen LS, Baker TB, Piper ME, Breslau N, Cannon DS, et al. (2012) Interplay of
gene risk factors (CHRNA5+CHRNAS+CHRNB4) and cessation treatments in
smokers and GGTTSs. Am J Psychiatry 169: 735–742.
46. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
45. Hung RJ, McKay JD, Gaborieau V, Boffetta P, Chabrier A, et al. (2008) Lung
44. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
43. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
42. Characteristic of telomere suppression in smokers. Tumour Biol. 29: 193–200.
41. Shahab L, West R (2010) Strength of urges to smoke as a measure of
40. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
39. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
38. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
37. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
36. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
35. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
34. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
33. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
32. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
31. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
30. Fidler JA, Shahab L, West R (2011) Strength of urges to smoke as a measure of
29. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
28. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO (1991) The
27. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
26. Sullivan PF, Kendler KS (1999) The genetic epidemiology of smoking. Nicotine
25. Hecht SS (2008) Progress and challenges in selected areas of tobacco
carcinogenesis. Chem Res Toxicol 21: 160–171.
24. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
23. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
22. Wang J, Spitz MR, Amos CI, Wilkinson AV, Wu X, et al. (2010) Mediating
effects of smoking and chronic obstructive pulmonary disease on the relation
between the CHRNA5-A5 genetic locus and lung cancer risk. Cancer Epidemiol
Biomarkers Prev 17: 1120–1126.
21. Ye-XH, Bu ZB, Feng J, Peng L, Liao XB, et al. (2013) Association between the
Taq1B polymorphism and lung cancer risk: a meta-analysis. Med Biol Rep.
20. Lee KM, Kang D, Clapper ML, Ingelman-Sundberg M, Ono-Kihara M, et al.
19. Xu CH, Wang Q, Qian Q, Zhan P, Yu LK. (2013) CVPA1 exon7 polymorphism is associated with lung cancer risk among the female population and among smokers: a meta-analysis. Tumour Biol.
18. Wang J, Zhang Q, Zhang C, Zhai H, He Y, et al. (2010) The genetic basis of
17. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
16. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
15. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
14. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
13. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
12. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
11. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
10. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
9. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
8. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
7. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
6. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
5. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
4. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
3. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
2. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
1. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
0. VanderWeele TJ, Asomaning K, Tchetgen Tchetgen EJ, Han Y, Spitz MR, et
the secondary phenotypes associated with primary diseases. Genet Epidemiol 35: 739–743.

79. Wang J, Shete S (2011) Estimation of odds ratios of genetic variants for the secondary phenotypes associated with primary diseases. Genet Epidemiol 35: 190–200.

80. Wang J, Spitz MR, Amos CI, Wu X, Wetter DW, et al. (2012) Method for evaluating multiple mediators: mediating effects of smoking and COPD on the association between the CHRNA5-A3 variant and lung cancer risk. PLoS One 7: e47705.

81. Vanderweele TJ, Vansteelandt S (2010) Odds ratios for mediation analysis for a dichotomous outcome. Am J Epidemiol 172: 1339–1348.