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Genome-wide association study confirms lung cancer susceptibility loci on chromosome 5p15 and 15q25 in an African-American population

Krista A. Zanetti[1,*], **Zhaoming Wang**[2,*], **Melinda Aldrich**[3,4], **Christopher I. Amos**[5], **William J. Blot**[3], **Elise D. Bowman**[6], **Laurie Burdette**[2], **Quiyin Cai**[3], **Neil Caporaso**[2], **Charles C. Chung**[2], **Elizabeth M. Gillanders**[1], **Christopher A. Haiman**[7], **Helen M. Hansen**[8], **Brian E. Henderson**[7], **Laurence N. Kolonel**[9], **Loic Le Marchand**[9], **Shengchao Li**[2], **Lorna Haughton McNeill**[10], **Bríd M. Ryan**[6], **Ann G. Schwartz**[11], **Jennette D. Sison**[8], **Margaret R. Spitz**[12], **Margaret Tucker**[2], **Angela S. Wenzlaff**[11], **John K. Wiencke**[8], **Lynne Wilkens**[9], **Margaret R. Wrensch**[8], **Xifeng Wu**[13], **Wei Zheng**[3], **Weiyin Zhou**[2], **David Christiani**[14], **Julie R. Palmer**[15], **Trevor M. Penning**[16,17], **Alyssa G. Rieber**[18], **Lynn Rosenberg**[15], **Edward A. Ruiz-Narvaez**[15], **Li Su**[14], **Anil Vachani**[16,19], **Yongyue Wei**[14], **Alexander S. Whitehead**[16,17], **Stephen J. Chanock**[2,**], and **Curtis C. Harris**[5,**]

Krista A. Zanetti: zanettik@mail.nih.gov; Zhaoming Wang: wangzha@mail.nih.gov; Melinda Aldrich: melinda.aldrich@Vanderbilt.Edu; Christopher I. Amos: Christopher.I.Amos@Dartmouth.edu; William J. Blot: blotw@iei.us; Elise D. Bowman: bowmane@intra.nci.nih.gov; Laurie Burdette: burdettl@mail.nih.gov; Quiyin Cai: qiuyin.cai@Vanderbilt.Edu; Neil Caporaso: caporasn@mail.nih.gov; Charles C. Chung: chungcc@mail.nih.gov; Elizabeth M. Gillanders: lgilland@mail.nih.gov; Christopher A. Haiman: haiman@usc.edu; Helen M. Hansen: helen.hansen@ucsf.edu; Laurence N. Kolonel: lkolonel@crch.hawaii.edu; Loic Le Marchand: Loic@cc.hawaii.edu; Shengchao Li: shengchao.li@nih.gov; Lorna Haughton McNeill: lmcneill@mdanderson.org; Bríd M. Ryan: ryanb@mail.nih.gov; Ann G. Schwartz: schwarta@karmanos.org; Jennette D. Sison: jennette.sison@ucsf.edu; Margaret R. Spitz: spitz@bcm.edu; Margaret Tucker: tuckerp@mail.nih.gov; Angela S. Wenzlaff: wenzlaff@karmanos.org; John K. Wiencke: john.wiencke@ucsf.edu; Lynne Wilkens: lynne@cc.hawaii.edu; Margaret R. Wrensch: Margaret.Wrensch@ucsf.edu; Xifeng Wu: xwu@mdanderson.org; Wei Zheng: wei.zheng@vanderbilt.edu; Weiyin Zhou: zhouw@mail.nih.gov; David Christiani: dchris@hsph.harvard.edu; Julie R. Palmer: jpalmer@bu.edu; Trevor M. Penning: PENNING@UPENN.EDU; Alyssa G. Rieber: arieber@mdanderson.org; Lynn Rosenberg: lrosenbe@bu.edu; Edward A. Ruiz-Narvaez: eruiznar@bu.edu; Li Su: lisu@hsph.harvard.edu; Anil Vachani: avachani@mail.med.upenn.edu; Yongyue Wei: ywei@hsph.harvard.edu; Alexander S. Whitehead: aswhiteh@mail.med.upenn.edu; Stephen J. Chanock: chanocks@mail.nih.gov; Curtis C. Harris: harrisc@mail.nih.gov

[1]Division of Cancer Control and Population Sciences, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20892, USA

[2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20892, USA

Corresponding Authors: Curtis C. Harris, M.D., National Cancer Institute, Laboratory of Human Carcinogenesis, Building 37, Room 3068, 37 Convent Drive, MSC 4258, Bethesda, MD 20892-4258, Tel: 301-496-2048, Curtis_Harris@nih.gov. Stephen Chanock, M.D., National Cancer Institute, Division of Cancer Epidemiology and Genetics, 9609 Medical Center Drive, Room 7E412, Rockville, MD 20892, Tel: 240-276-7150, chanocks@mail.nih.gov.

*These authors contributed equally to this work

** These authors contributed equally to this work

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[3]Division of Epidemiology, Vanderbilt University Medical Center, 1161 21st Avenue South D-3100 Medical Center North, Nashville, TN 37232, USA

[4]Department of Thoracic Surgery, Vanderbilt University Medical Center, 609 Oxford House, 1313 21st Ave South, Nashville, TN 37232-4682, USA

[5]Department of Biomedical Data Science, Geisel School of Medicine, 1 Rope Ferry Road, Dartmouth, Lebanon, NH 03755-1404, USA

[6]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Building 37, Room 3068A, Bethesda, MD 20892, USA

[7]Department of Preventive Medicine, Keck School of Medicine, University of Southern California and Norris Comprehensive Cancer Center, 1975 Zonal Avenue, Los Angeles, CA 90033, USA

[8]Department of Neurological Surgery, University of California, 505 Parnassus Ave., Room 779 M, San Francisco, San Francisco, CA 94143-00112, USA

[9]Epidemiology Program, Cancer Research Center, University of Hawaii, 701 Ilalo Street, Honolulu, HI 96813, USA

[10]Department of Health Disparities Research, Division of OVP, Cancer Prevention and Population Sciences, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 91, Houston, TX 77030, USA

[11]Department of Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, 4100 John R, Detroit, MI 48201, USA

[12]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Mail Stop BCM225, Houston, TX 77030 USA

[13]Department of Epidemiology, Division of OVP, Cancer Prevention and Population Sciences, The University of Texas MD Anderson Cancer Center, Unit 1340, PO Box 301439, Houston, TX 77230-1439, USA

[14]Harvard School of Public Health, Massachusetts General Hospital/Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA

[15]Slone Epidemiology Cancer Center at Boston University, 1010 Commonwealth Avenue, 4th Floor, Boston, MA 02215, USA

[16]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, 3535 Market Street, Mezzanine, Philadelphia PA 19104, USA

[17]Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, 3535 Market Street, Mezzanine, Philadelphia PA 19104, USA

[18]Department of General Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

[19]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, 3535 Market Street, Mezzanine, Philadelphia, PA 19104, USA

Abstract

Objectives—Genome-wide association studies (GWAS) of lung cancer have identified regions of common genetic variation with lung cancer risk in Europeans who smoke and never-smoking Asian women. This study aimed to conduct a GWAS in African Americans, who have higher rates of lung cancer despite smoking fewer cigarettes per day when compared with Caucasians. This population provides a different genetic architecture based on underlying African ancestry allowing the identification of new regions and exploration of known regions for finer mapping.

Materials and Methods—We genotyped 1,024,001 SNPs in 1737 cases and 3602 controls in stage 1, followed by a replication phase of 20 SNPs ($p<1.51\times10^{-5}$) in an independent set of 866 cases and 796 controls in stage 2.

Results and Conclusion—In the combined analysis, we confirmed two loci to be associated with lung cancer that achieved the threshold of genome-wide significance: 15q25.1 marked by rs2036527 ($p = 1.3 \times 10^{-9}$; OR = 1.32; 95% CI = 1.20–1.44) near *CHRNA5*, and 5p15.33 marked by rs2853677 ($p = 2.8 \times 10^{-9}$; OR = 1.28; 95% CI = 1.18–1.39) near *TERT*. The association with rs2853677 is driven by the adenocarcinoma subtype of lung cancer ($p = 1.3 \times 10^{-8}$; OR = 1.37; 95% CI = 1.23–1.54). No SNPs reached genome-wide significance for either of the main effect models examining smoking - cigarettes per day and current or former smoker. Our study was powered to identify strong risk loci for lung cancer in African Americans; we confirmed results previously reported in African Americans and other populations for two loci near plausible candidate genes, *CHRNA5* and *TERT*, on 15q25.1 and 5p15.33 respectively, are associated with lung cancer. Additional work is required to map and understand the biological underpinnings of the strong association of these loci with lung cancer risk in African Americans.

Keywords

Genome-wide Association Study; Lung Neoplasms; Smoking; African Americans; Telomerase; Receptors; Cholinergic

1. Introduction

Lung cancer is the second most common cancer in US men and women and has the highest death rate [1]. There is a substantial disparity in African-American men, who have higher age-adjusted incidence rates (99.9 per 100,000) when compared with Caucasian and Asian men (76.4 and 52.2 per 100,000, respectively), as well as higher age-adjusted death rates (African American 82.6 per 100,000; Caucasian 65.3 per 100,000; Asian 35.9 per 100,000) [2]. Smoking represents the major risk factor for lung cancer [3–5]. Known differences in smoking-related exposures for African Americans could contribute to the observed differences in incidence, including age of smoking initiation, intensity of smoking, and quitting and relapse behaviors [6]. It has been estimated that approximately eighty-five percent of lung cancer risk is attributed to cigarette smoking; however, the higher incidence seen in African Americans occurs even though they smoke fewer cigarettes per day than their Caucasian counterparts [7]. Family and twin studies, as well as lung cancer genome-wide association studies (GWAS) have confirmed an underlying genetic contribution to lung cancer risk as well. To date, GWAS performed in populations of European and Asian descent have identified over 15 lung cancer risk loci [8–21] (Supplementary Table 1). Interestingly, other than the hTERT region on chromosome 5p15, the regions identified in

non-smoking Asian women do not overlap with the regions identified in Europeans who smoke, suggesting distinct genetic contributions to primary lung carcinogenesis and smoking-related carcinogenesis. Although African Americans have higher lung cancer incidence and poorer lung cancer survival when compared with other racial and ethnic populations in the United States, no GWAS has been conducted in this population. In addition, African admixture in African Americans could provide an opportunity to identify new lung cancer risk alleles [22]. Smoking behavior also has a significant genetic component, as reported in family, twin and GWAS studies [12, 23–26]. Given differences in smoking exposures and genetic architecture in African Americans as compared to Europeans and Asians, studies of genetic variation contributing to risk of lung cancer in African Americans might help explain the racial difference observed in lung cancer incidence and survival, and identify novel markers of lung cancer susceptibility.

Studies have been conducted to further interrogate chromosomal regions 2q31.1, 5p15.33, 6p22.1-p21.31, and 15q25.1 in African Americans, including fine mapping studies [27–31]. The 15q25.1 region harbors three nicotinic acetylcholine receptor subunit genes (*CHRNA3*, *CHRNA4*, and *CHRNA5*), which are associated with lung cancer [9, 11, 12], smoking behavior [32], nicotine addiction [33], and increased exposure to specific nicotine metabolites [34]. These studies have identified SNPs potentially associated with lung cancer risk in African Americans, either weakly associated with or independent of smoking behavior [28, 29]. Additionally, variants on 5p15.33 and 6p21.33 were observed to have histology-specific associations with lung cancer risk in African Americans [31, 35]. Specifically, variants on 5p15.33 appear to have a stronger association with adenocarcinoma, whereas variants on 6p21.33 had a specific association with squamous cell carcinoma.

In light of the current evidence suggesting that a genetic contribution to lung cancer in African Americans, we conducted a two-stage GWAS comprised of 1737 cases and 3602 controls (stage 1) and followed by replication of the most significantly associated genotypes in an independent sample set of 866 cases and 796 controls (stage 2).

2. Materials and Methods

2.1 Study populations

A two-stage case-control study was designed to evaluate the association between common genetic variants and the risk of lung cancer. Detailed study descriptions of the eight studies included in the discovery phase (stage 1) GWAS and seven studies in the replication phase (stage 2) can found in Supplementary Information. The stage 1 studies included 1737 cases and 3602 controls from the following studies: MD Anderson Lung Cancer Epidemiology Study, The Multiethnic Cohort Study (MEC), NCI-MD Lung Cancer-Case Control Study, Northern California Lung Cancer Study, Project CHURCH (Creating a Higher Understanding of Cancer Research & Community Health), Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), Southern Community Cohort Study (SCCS), and the Karmanos Cancer Institute at Wayne State University (KCI/WSU). The Project CHURCH dataset contained controls only; therefore, these data were combined with the MD Anderson Lung Cancer Epidemiology Study to generate one analytical unit for the analyses (designated as MDA). The stage 2 studies included an independent set of 866 cases and 796

controls from the following studies: The Black Women's Health Study (BWHS), The Harvard-MGH Lung Cancer Susceptibility Study (HLCS), MD Anderson Lung Cancer Epidemiology Study, MD Anderson/LBJ Hospital Biorepository, NCI-MD Lung Cancer Case-Control Study, Northern California Lung Cancer Study, Philadelphia Lung Cancer Study on Gene Environment Interactions (Plus-Gene), Southern Community Cohort Study (SCCS), and KCI/WSU. The MD Anderson Lung Cancer Epidemiology Study and MD Anderson/LBJ Hospital Biorepository were provided as one dataset from the investigators, so were analyzed as one analytical unit (designated as MDA).

Each participating study obtained informed consent from study participants and approval of the study from its IRB; studies also obtained IRB certification permitting data sharing in accordance with the US National Institutes of Health (NIH) Policy for Sharing of Data Obtained in NIH-Supported or -Conducted Genome-Wide Association Studies (GWAS).

2.2 Genome-wide SNP genotyping

Genome-wide SNP genotyping of lung cancer cases from MD Anderson Lung Cancer Epidemiology Study, NCI-MD Lung Cancer Case-Control Study, Northern California Lung Cancer Study, Project CHURCH, PLCO, SCCS, and KCI/WSU (Supplementary Table 2a) was conducted using the Illumina HumanHap 1M Duo chip at the NCI Cancer Genomics Research Laboratory (CGR) in the Division of Cancer Epidemiology and Genetics (DCEG) at the National Cancer Institute. SNPs from the Multi-ethnic Cohort (MEC) were genotyped using the same chip at University of Southern California and the primary iDat files were shared for the combined genotype calling.

2.3 Quality control assessment

SNPs with less than a 90% completion rate were excluded from further analysis. Samples were excluded on the basis of (i) completion rates lower than 94% (n = 556 samples); (ii) abnormal heterozygosity values of less than 24% or greater than 32% (n = 54); (iii) discordant expected duplicates (n=10); (iv) abnormal X-chromosome heterozygosity including gender discordant samples (n = 52); and (v) phenotype exclusion (due to ineligibility or incomplete information) (n = 114).

Using the high quality data based on the sample level filters above, we performed an assay concordance analysis and identified all 164 expected duplicates with average concordance rate > 99.9%. Genotypes for all subject pairs were also examined for close relationships (presence of first- and second-degree relatives) using the Genotyping Library and Utilities (GLU) version 1.0 *qc.ibds* module with an IBD0 threshold of 0.70, and 122 first-degree relatives were identified and excluded from analysis.

Using a set of population informative SNPs [36] and data from HapMap 27, we excluded 27 subjects that displayed either Asian admixture coefficients >20% or European admixture coefficients >90% [37], as determined on the basis of the GLU *strct.admix* module with the HapMap I+II CEU, YRI, ASA (JPT +CHB) samples used as reference populations [38] (Supplementary Figure 1). The final association analysis included 1737 cases and 3602 controls of African American ancestry. After quality control filtering, data from 1,024,001 SNPs were available.

2.4 Replication and TaqMan genotyping

A total of 20 SNPs with $p < 1.51 \times 10^{-5}$ based on the adjusted trend model was advanced for replication in stage 2. TaqMan genotyping assays (ABI) were optimized in the CGR pipeline stage 2 genotyping, which included BWHS, HLCS, MD Anderson Lung Cancer Epidemiology Study, MD Anderson/LBJ Hospital Biorepository, NCI-MD Lung Cancer Case-Control Study, Plus-Gene, KCI/WSU, and the Northern California Lung Cancer Study (Supplementary Table 2b). SCCS genotyped their samples at Vanderbilt using the Sequenom platform for 21 assays where rs17568263 was substituted with rs2128611 [a perfect linkage disequilibrium (LD) surrogate] and rs55781567 was genotyped as a redundant assay because it is in LD with rs2036527. rs2036527 was not genotyped in Northern California Lung Cancer Study. We analyzed a total of 20 assays in 866 cases and 796 controls drawn from 7 studies, including additional samples from 5 of the stage 1 studies and 355 controls pooled from an African American renal cancer GWAS [39].

2.5 Statistical analysis

Associations between SNPs and risk of lung cancer (overall, adenocarcinoma only, and squamous cell carcinoma only) were estimated using multivariate logistic regression assuming a trend genetic effect (i.e. linear increase in risk per affected allele) and adjusting for study, age, gender, smoking status (never/former/current), and admixture coefficient for YRI (stage 1); and study, age, gender, smoking status (never/former/current) (stage 2). When included in the null model (baseline model and covariates only), none of the top 10 eigenvectors is significantly ($P < 0.05$) associated with the case/control outcome, and therefore were they were not included in the final main effect model as adjustment for population substructure. For smoking status, we ran a logistic regression model for current versus former smokers assuming linear trend for each SNP and adjusting for study, age, gender, admixture coefficient for YRI, one significant eigenvector, and lung cancer case/control status. We also ran a linear regression model for log transformation of cigarette smoked per day (CPD) and assuming a trend effect for SNP and adjusting for study, age, gender, admixture coefficient for YRI and lung cancer case/control status. To examine the association between smoking behavior and lung cancer, we performed linear regression analysis for CPD and pack years as the outcomes, adjusting for study, age, gender, coefficient for YRI, current smokers, and eigenvectors.

2.6 Data analysis

Data analysis and management were performed with GLU (https://code.google.com/p/glu-genetics/).

3. Results

The stage 1 analysis included 1737 cases and 3602 controls among African Americans drawn from eight studies of lung cancer (Supplementary Table 2a). We observed no evidence of systematic genomic inflation of the test statistic ($\lambda = 1.003$) for the 1,024,001 genotyped SNPs analyzed in stage 1 and had no excess of small p values beyond what was expected (Supplementary Figure 2).

Three SNPs were observed to be associated with lung cancer below the threshold of genome-wide significance ($p > 5 \times 10^{-8}$) in stage 1 (rs55781567, rs3019885, and rs6580649) (Supplementary Figure 3). The most significant association was observed in rs55781567 ($p = 7.1 \times 10^{-9}$; OR=1.34; 95% CI=1.21–1.48; MAF = 0.25 in cases and 0.308 in controls), located in the 5′-untranslated region (UTR) of the gene *CHRNA5* (cholinergic receptor, nicotinic, alpha 5) on chromosome 15q25. Twenty promising SNPs ($p < 1.51 \times 10^{-5}$) from stage 1 were genotyped in stage 2 in 866 cases and 796 controls from 7 studies, including additional samples from 5 of the stage 1 studies and 355 controls pooled from an African American renal cancer GWAS [39] (Supplementary Table 2b).

We selected the 20 SNPs for replication in stage 2 (Supplementary Table 3). SNP rs55781567 failed the assay design; however, rs2036527, also located in the 5′-untranslated region (UTR) of the gene *CHRNA5* on chromosome 15q25, is a surrogate (r^2=1 and 0.48 in 1000 Genomes Project CEU and YRI data, respectively) for rs55781567 and was genotyped in the replication phase. The surrogate selection was based on rs2036527 being the next most significant SNP observed in our scan in the same region. Among the 20 SNPs evaluated, the stage 2 ORs were similar to or somewhat attenuated from the stage 1 ORs for 11, but in different directions for 9.

Combining results from stages 1 and 2, two SNPs were significant at the genome-wide significance level. rs2036527 ($p = 1.3 \times 10^{-9}$; OR = 1.32; 95% CI = 1.20–1.44) and rs2853677 ($p = 2.8 \times 10^{-9}$; OR=1.28; 95% CI = 1.18–1.39), which are located 511 base pairs from the initiation sequence of *CHRNA5* and in the first intron of telomerase gene (*TERT*), respectively (Figure 1, Table 1, Supplementary Table 3). SNPs rs3019885 and rs6580649 did not replicate. When stratified by histology, we observed an association with adenocarcinoma below the threshold for genome-wide significance ($p = 1.3 \times 10^{-8}$; OR=1.37; 95% CI = 1.23–1.53), but not squamous cell carcinoma ($p = 0.42$; OR=1.06; 95% CI = 0.92–1.23). Notably, four loci that failed to replicate displayed substantial differences in allele frequencies between stage1 (GWAS scan) and stage 2 (TaqMan or Sequenom together). Moreover, the allele frequencies of stage 1 were quite different from those estimates from HapMap samples (CEU or YRI); whereas the allele frequencies based on stage 2 genotypes for all four loci were very similar (Supplementary Table 3). In this regard, we consider that the Illumina genotype assays may be problematic for these four SNPs and thus not reliable [40].

We examined whether SNPs were associated with smoking, including cigarettes smoked per day and smoking status (former vs. current smokers). No SNPs reached genome-wide significance for either of the main effect models examining smoking behavior. When comparing current with former smokers, the most significant association was observed with rs1293936 ($p = 5.41 \times 10^{-7}$; OR = 1.28, 95% CI = 1.16 – 1.41), which is located in *ESR1* (estrogen receptor 1) on chromosome 6q25.1 (data not shown). When examining cigarettes smoked per day (log transformed) in a linear regression model, the most significant association was observed with rs1372626 ($p = 2.5 \times 10^{-6}$), which is located in the intron of Deleted in Colorectal Cancer (DCC) encoding netrin 1 receptor (data not shown).

Additionally, we evaluated the association between smoking behavior (CPD and pack years) and lung cancer case-control status. We observed that lung cancer is strongly associated with both CPD and pack years in this population (Supplementary Tables 4a and 4b).

4. Discussion

Herein, we report the first GWAS of common genetic variation for lung cancer and smoking phenotypes in 1737 African American cases and 3602 controls, followed by a replication phase including an independent set of 866 cases and 796 controls. Two loci, previously reported in GWAS in other populations, were significant at the genome-wide significance level when combining the discovery and replication phases (Table 1); 15q25.1 marked by rs2036527 near *CHRNA5*, and 5p15.33 marked by rs2853677 near *TERT*. The first SNP marker, rs2036527 on 15q25.1 is located 511 base pairs from the initiation sequence for *CHRNA5*, in the gene cluster *CHRNA5-CHRNA3-CHRNA4* that codes for the α5, α3, β5 subunits of the nicotinic acetylcholine receptor (nAChR). Nicotinic acetylcholine receptors initiate the primary brain and peripheral responses to smoking. A number of studies have implicated the nAChR gene cluster in smoking-related phenotypes, including cigarettes smoked per day [12, 32, 41–49], smoking 100 cigarettes or more in a lifetime [42], persistent smoking [50], heavy smoking [32], quitting attempts [42], age at smoking initiation [33, 51, 52], pleasurable early smoking experience [53], reported physical effects after smoking first cigarette [54] and serum cotinine levels [44]. These observations have been reported primarily in populations of European ancestry. Some of the above-mentioned studies include African Americans [45, 52, 53, 55]; yet, small sample size was an issue for some [52, 53]. However, rs2036527 was observed to be associated with smoking quantity in African Americans through a GWAS meta-analysis of 32,389 subjects from the Study of Tobacco in Minority Populations Genetics Consortium [56]. This SNP has also been associated with nicotine dependence in the Finnish Twin Cohort Study [57] and cigarettes smoked per day [43]. However, we did not see a similar association in our data when examining current versus former smokers (p=0.29), and only nominally significant (p=0.038) if modeling cigarettes smoked per day, perhaps due to the small sample size and that African Americans on average smoke cigarettes differently than Caucasians. It has been shown that CPD is less predictive of smoke intake in African-American smokers when compared with Caucasian smokers [58]. When examining carbon monoxide (CO), a biomarker of cigarette smoke, Bloom et al. observed that the correlation between CO and CPD is significantly lower in African Americans when compared with European Americans [59]. Additionally, we recognize that comparing current versus former smokers or examining CPD is not an ideal surrogate for nicotine dependency; however, in the assembled dataset, these are the best measures available. Furthermore, the relationship between rs2036527 and smoking behavior is not likely to be mediated solely through CPD.

rs16969968, a nonsynonomous SNP in *CHRNA5* that is in LD with rs2036527 (r^2 = 0.88, 1000 Genomes Project CEU data [60]) in populations of European descent [61], replaces an aspartic acid with an asparagine at codon 398 of the protein. This SNP has been associated with smoking behavior in European populations and been shown to have functional consequences, including partial loss of the function of the protein [50, 62]. rs16969968 has been reported to be associated with smoking behavior and nicotine dependence in several

small studies with African Americans subjects [32, 61, 63]. In 2012, a large meta-analysis in African American smokers showed a significant association between rs2036527 and self-reported CPD, but not between rs16969968 CPD [56]. However, a more recent meta-analysis showed rs16969968 is associated with increased risk for nicotine dependence in European and African Americans and provided evidence that the A allele increases risk for heavy smoking [64]. In another study, rs16969968 was not found to be consistently associated with smoking abstinence in African Americans; whereas rs2036527 was associated with active pharmacotherapy [65]. Additionally, rs588765 in high LD with rs2036527, and was observed to be associated with CHRNA5 mRNA expression levels, using a diploytpe analysis [66]. Therefore, it is possible that rs16969968 and/or rs588765 are the functional variants in *CHRNA5* underlying the associations we have observed with rs2036527. Although these SNPs may be functional variants, they only partially capture variation in nicotine dependency and are one driver of heavy smoking. For example, recent report observed that rs2273500-C, a splice site acceptor variant in *CHRNA4* on chromosome 20 accounts for some nicotine dependency [67].

The SNP marker, rs2036527 has been previously shown to be associated with lung cancer in African Americans in a fine mapping study of the nAChR region [28]. It has been shown that rs2036527 is associated with tumor DNA methylation levels in the promoter of *CHRNB4,* suggesting that genotype-specific methylation may be associated with susceptibility to lung cancer [68]. Because this SNP has been associated with smoking habits, it is not clear whether smoking may also play a role in the epigenetic deregulation. Further investigations into the functional implications of this relationship are warranted.

The second genome-wide significant SNP observed in our study, rs2853677, is located in the first intron of *TERT* on chromosome 5p15.33. In a case-control study of Caucasian and African-American women, rs2853677 was associated with non-small cell lung cancer in both populations when the data were stratified by race [69]. Furthermore, rs2853677 has been shown to be associated with lung cancer susceptibility in a Japanese GWAS [20]. In a recent report, the A allele of rs2853677 displayed pleiotropy between different cancer types. The same allele is positively associated with testicular germ cell tumor and pancreatic cancer, but negatively associated with glioma and lung cancers, which includes some of the African-American individuals in this study [70]. Additionally, concordant with earlier work, we observed that rs2853677 had a histology-specific association with risk of adenocarcinoma. Variants on 5p15.33 detected upstream of, and within, intron 1 of *TERT* (rs2735940 and rs2736100) have been observed to be associated with risk of adenocarcinoma [31, 35]. rs2853677 is in moderate linkage disequilibrium (LD) with rs2735940 and rs2736100 in 1000 Genome Project CEU data (r^2 = 0.42 and 0.56, respectively) as compared with moderate to low LD (r^2 = 0.34 and 0.04, respectively) in 1000 Genomes Project YRI data [60]. This suggests that using an African-American population can refine the association signals in this complex region. We have further confirmed its association with lung cancer in African Americans at a genome-wide significance level by replicating the finding in additional samples of African Americans. Further functional interrogation is warranted.

5. Conclusions

Our study was powered to identify strong risk loci for lung cancer in African Americans; we confirmed data previously reported in African Americans and other populations for two loci near plausible candidate genes, *CHRNA5* and *TERT*, rs2036527 and rs2853677 on 15q25.1 and 5p15.33 respectively, are associated with lung cancer. Additional work is required to map and understand the biological underpinnings of the strong association of these loci with lung cancer risk in African Americans. Further studies of African-American smokers and never-smokers are warranted to investigate the underlying genetic contribution of common variants to lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Confirmed previous reports that two loci on 15q25.1 and 5p15.33 are associated with lung cancer.
- Two loci associated with lung cancer are near plausible candidate genes, *CHRNA5* and *TERT*.
- No SNPs reached genome-wide significance for the main effect model examining cigarettes per day.

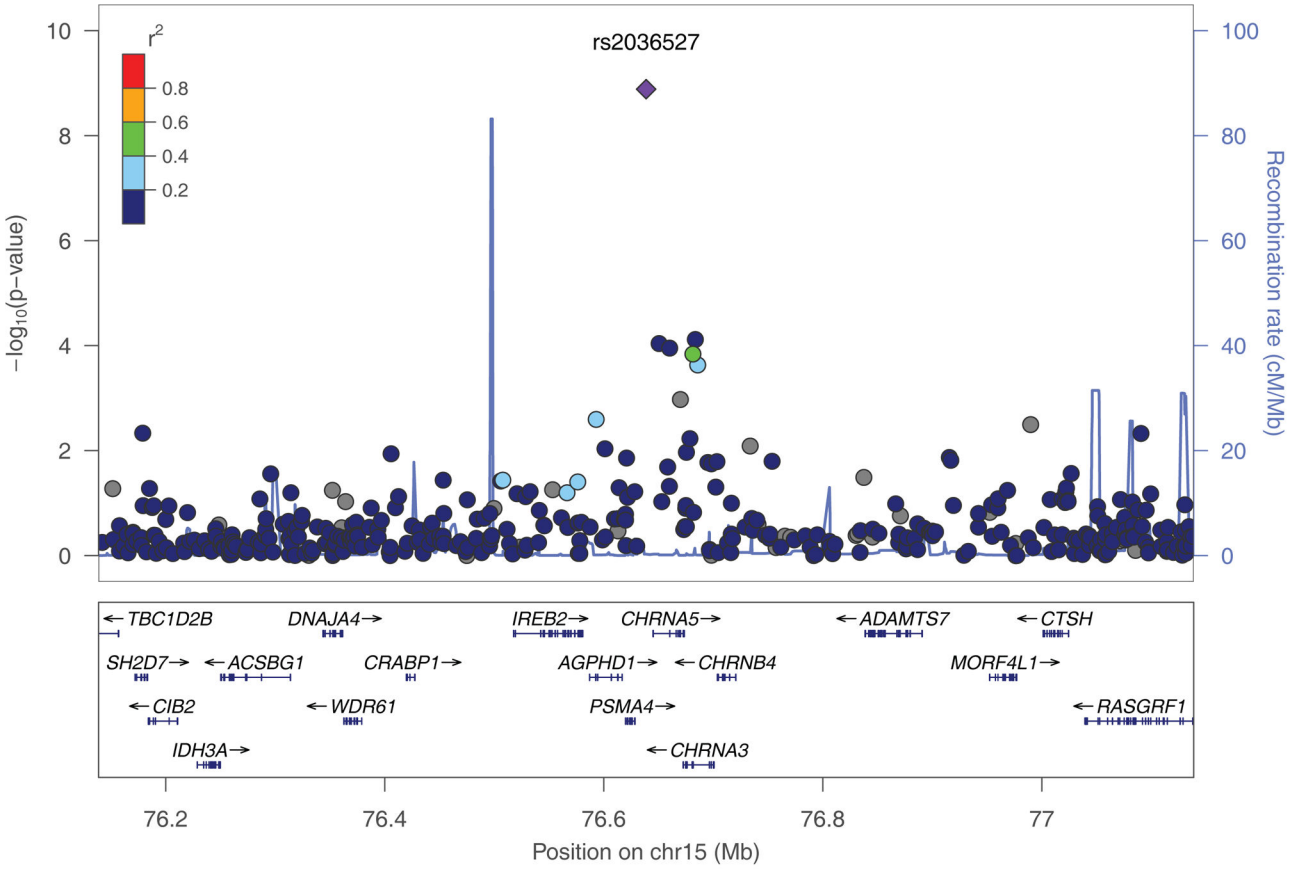

Figure 1a

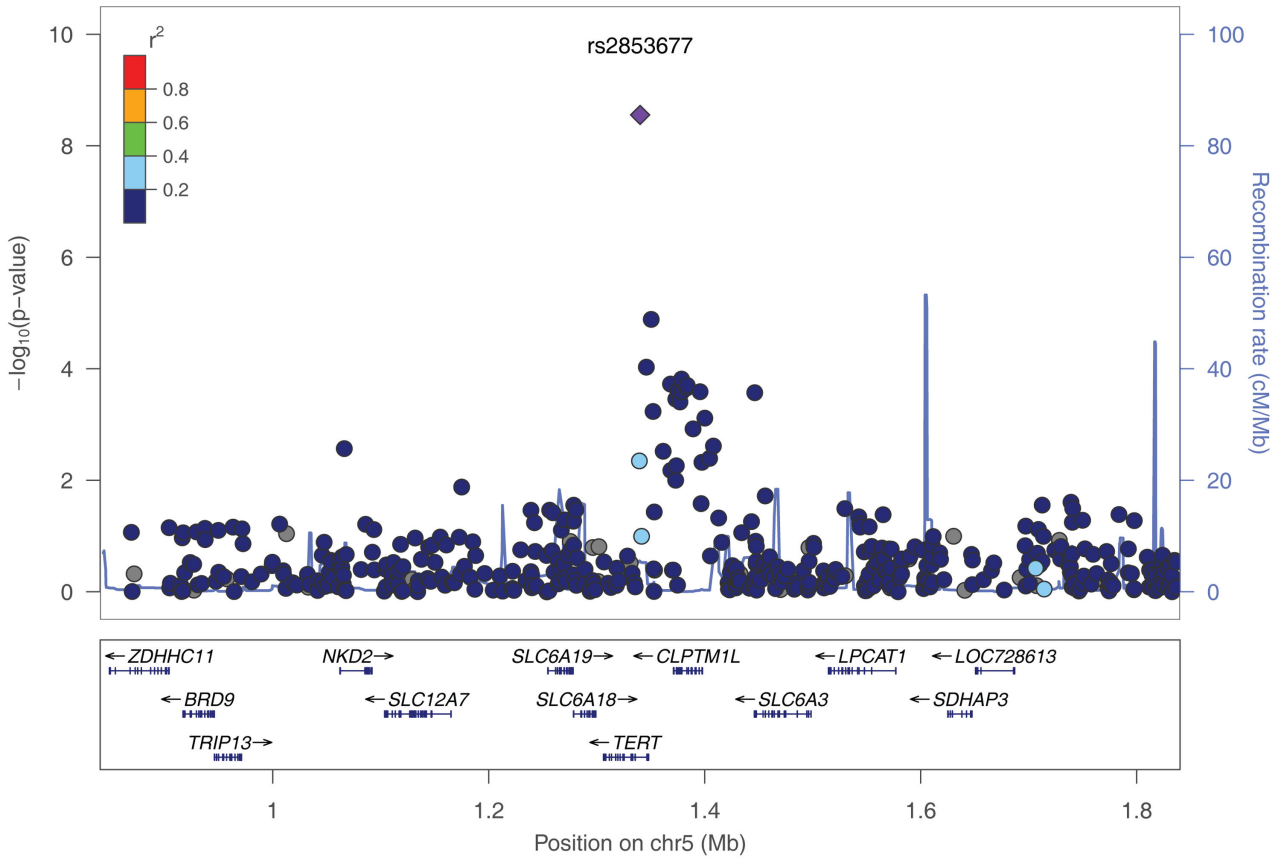

Figure 1b

Fig. 1.

Regional plots of association results, recombination rates, and linkage disequilibrium for the (a) 15q25.1 marked by rs2036527; (b) 5p15.33 marked by rs2853677. Association results from a log-additive genetic model in −log10 *P*-values of the SNPs were shown according to their chromosomal positions (hg18) within 1Mb region centering around the index SNP. *P* values were based on the stage 1 only except for both rs2036527 and rs2853677 where the combined *P* values were shown. Linkage Disequilibrium (LD) r^2 values were estimated on 1000 Genomes Project data June 2010 YRI population (hg18). The colors of the dots reflect the LD with the index SNP as shown in the legend box. The figures were generated using locusZoom (http://csg.sph.umich.edu/locuszoom/).

Table 1

Two genome-wide significant SNPs for lung cancer risk after combining stage 1 and 2

Locus	Genes	SNP (reference, effect)	Model	Stage	Controls	Cases	EAF in control[c]	EAF in cases[c]	OR	95%CI	P	Phet
15q25.1	CHRNA5	rs2036527 (C,T)[a]	Overall	Stage1	3601	1736	0.212	0.255	1.33	(1.20–1.48)	1.03E-07	
				Stage2	1062	762	0.222	0.282	1.28	(1.09–1.51)	2.92E-03	
				Combined[b]	4663	2498	0.214	0.263	1.32	(1.20–1.44)	1.30E-09	0.70432
			Adeno only	Stage1	3601	739	0.212	0.254	1.28	(1.12–1.47)	4.06E-04	
				Stage2	1062	284	0.222	0.282	1.31	(1.05–1.64)	1.83E-02	
				Combined[b]	4663	1023	0.214	0.262	1.29	(1.15–1.45)	2.28E-05	0.87615
			Squamous only	Stage1	3601	399	0.212	0.237	1.25	(1.04–1.51)	1.91E-02	
				Stage2	1062	176	0.222	0.259	1.24	(0.93–1.66)	1.36E-01	
				Combined[b]	4663	575	0.214	0.244	1.25	(1.07–1.46)	5.59E-03	0.96628
5p15.33	TERT	rs2853677 (T,C)	Overall	Stage1	3600	1736	0.277	0.320	1.26	(1.14–1.38)	4.28E-06	
				Stage2	1137	851	0.261	0.321	1.35	(1.16–1.56)	1.15E-04	
				Combined[b]	4737	2587	0.273	0.320	1.28	(1.18–1.39)	2.80E-09	0.45653
			Adeno only	Stage1	3600	739	0.277	0.341	1.34	(1.18–1.52)	4.44E-06	
				Stage2	1137	311	0.261	0.339	1.45	(1.17–1.78)	5.55E-04	
				Combined[b]	4737	1050	0.273	0.340	1.37	(1.23–1.53)	1.27E-08	0.55297
			Squamous only	Stage1	3600	400	0.277	0.278	1.04	(0.87–1.25)	6.34E-01	
				Stage2	1137	192	0.261	0.276	1.11	(0.85–1.46)	4.44E-01	
				Combined[b]	4737	592	0.273	0.277	1.06	(0.92–1.23)	4.16E-01	0.69993

[a] Study design failed; rs2036527 was used as surrogate for the original top SNP rs55781567 in stage 1.

[b] For the combined stage, the odds ratio and p values were generated using a fixed-effects meta-analysis approach. Heterogeneity was assessed using *p*-values for Cochran's Q statistic.

[c] Effective allele frequency in controls or cases