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NNK Reduction Pathway Gene Polymorphisms and Risk of Lung Cancer

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Abstract

The tobacco-specific nitrosamine NNK is a potent carcinogen found in tobacco smoke and implicated in the development of lung cancer. The major route of NNK metabolism is carbonyl reduction by AKR1C1, AKR1C2, CBR1, and 11p-HSD1 to form NNAL. This study investigated the potential role of variants in this pathway on lung cancer risk by examining 53 tag-SNPs representing the common variations in *AKR1C1*, *AKR1C2*, *CBR1*, and *HSD11B1* in 456 lung cancer cases and 807 controls. One SNP in *CBR1* (rs2835267) was significantly associated with overall risk of lung cancer, but did not pass multiple testing adjustment (OR: 0.76 95% CI: 0.58–0.99, $P = 0.048$, FDR $P = 0.20$). After stratification and multiple testing correction, three SNPs showed significance. One SNP (rs2835267) in *CBR1* showed a significant decreased risk for smokers with a high pack-years (OR: 0.3595% CI: 0.17–0.69, $P = 0.018$) and in SCC (OR: 0.4895% CI: 0.29–0.76, $P = 0.018$). Another SNP located in *CBR1* (rs3787728) also showed a significant decreased risk in SCC (OR: 0.4695% CI: 0.26–0.80, $P = 0.024$) and small cell carcinoma (only in current smokers) (OR: 0.06895% CI: 0.01–0.42, $P = 0.028$). The *HSD11B1* SNP (rs4844880) showed a significant increased risk for adenocarcinoma within former smokers (OR: 3.9495% CI: 1.68–9.22, $P = 0.011$). Haplotype analysis found significance with six haplotypes and lung cancer risk. These findings indicate that select variants in genes in the carbonyl reduction pathway of NNK may alter the risk of lung cancer.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Keywords

NNK; NNAL; genetic polymorphism; AKR; CBR1; HSD11B1; lung cancer

INTRODUCTION

It is estimated that lung cancer will cause 159,260 deaths, about 27% of all cancer deaths, in 2014 [1]. Cigarette smoking is the leading cause of lung cancer, accounting for over 85% of all lung cancer related deaths. The relative risk for lung cancer is 20-times higher for smokers than for non-smokers, with a 30% increased risk for those exposed to second-hand smoke [2]. However, only 15% of lifetime smokers develop lung cancer [3]. The discrepancy between cause and incidence of lung cancer among smokers suggests that individual susceptibility to tobacco carcinogens may be modified by genetic factors. Candidate gene studies have identified multiple gene polymorphisms that may be associated with lung cancer risk, and genome-wide association studies (GWAS) have uncovered low penetrant variants, most of which have no known functional significance. Other approaches to identifying important genetic cancer risk modifiers that have recently gained favor include pathway analysis that replaces studying variations in single genes with a network of genes that regulate a specific biological function [4].

Tobacco smoke contains over 7000 chemical substances, of which over 70 are known carcinogens [5]. The tobacco-specific nitrosamines (TSNA), including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and the polycyclic aromatic hydrocarbons (PAH), including benzo[a]pyrene are among the most potent and abundant carcinogens found in tobacco smoke implicated in the development of lung cancer [6]. PAHs are a group of chemicals that are formed during the incomplete burning of tobacco and other organic substances [7,8]. NNK is derived from nicotine and forms during the tobacco curing process. Independent of the route of administration, NNK selectively induces adenocarcinoma tumors of the lungs in all rodent models tested and frequently at low doses [9].

The major route of NNK metabolism is carbonyl reduction to form 4-(methylnitrosamino)-1-(3-pyr-idyl)-1-butanol (NNAL) (Figure 1) [10], which undergoes glucuronidation by UDP-glucuronyl transferases (UGTs) that allows for its excretion in urine or bile [11–13]. Both NNK and NNAL undergo α -hydroxylation by cytochrome P450s (CYPs), leading to DNA adduct formation [8,14] and cohort studies have shown that total NNAL levels (NNAL plus NNAL-glucuronide) are associated with increased lung cancer risk in Chinese male smokers [15,16]. The carbonyl reduction of NNK to NNAL is believed to be primarily catalyzed by the short chain dehydrogenase/reductase (SDR) family of enzymes. 11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD1), a microsomal protein, was the first enzyme identified to catalyze the reduction of NNK to NNAL [17,18]. However, the formation of NNAL was observed in red blood cells and cytosolic fractions as well as in microsomes, suggesting that other non-membrane-bound enzymes also contribute to the carbonyl reduction of NNK [19,20]. Further investigation has shown that the cytosolic enzymes carbonyl reductase (CBR1) and three aldo-keto reductases, AKR1C1, AKR1C2,

and AKR1C4, can also metabolize NNK to NNAL [21–24]. Inter-individual variation in NNK carbonyl reduction has been observed [25,26], but the relative contribution of the different enzymes is not well understood. 11 β -HSD1 is predicted to be the major enzyme for NNK reduction in lung as microsomes are responsible for three times the NNAL production per mg of protein compared to cytosol in lung tissue, while the cytosolic and microsomal carbonyl reduction is nearly identical in liver [19]. In the cytosol of both tissues, CBR1 may be the dominant enzyme for NNK carbonyl reduction based on enzyme kinetics and relative abundance, with approximate contributions of NNAL formation from CBR1 of 60%, AKR1C1 and AKR1C2 at 20% each, and AKR1C4 at 1% [21].

There are different isomers of NNAL produced in smokers [23] and it is not known whether any important gene variants might differentially affect the production of these specific NNAL isomers. Since NNK is a prochiral compound, its metabolism to NNAL results in the formation of two enantiomers, (S)-NNAL and (R)-NNAL. Although structurally similar, the carcinogenicity, metabolism, and pharmacokinetics of these two enantiomers have been shown to be vastly different. Based on the data available, it has been hypothesized that the persistence of (S)-NNAL in lung tissue coupled with its apparent ability to re-oxidize to NNK, may be important to the lung-specific carcinogenicity of NNK [23]. The general mechanism is also believed to involve NNK being inhaled via smoking and preferentially metabolized to (S)-NNAL, which is retained in the lung tissue [27]. (S)-NNAL can then be re-oxidized back to NNK and bioactivated to form pyridyloxobutyl POB-DNA adducts. Thus, the formation of (S)-NNAL may prolong the exposure of the lung to NNK, which may be critical to the lung carcinogenicity of NNK. In addition, while two enantiomers, (S)-NNAL and (R)-NNAL are formed; (R)-NNAL is more readily eliminated from the body via the glucuronidation pathway, suggesting that (S)-NNAL may be the more relevant carcinogen [27]. Microsomes from both liver and lung produce mostly (R)-NNAL (70–90%), while the cytosolic fraction from both tissues produce mostly (S)-NNAL [23]. As AKR1C1, AKR1C2, and CBR1 are found in the cytosol, this suggests that they are likely to play an integral role in NNK activation and carcinogenicity.

In the current study, the potential role of genetic variants in the NNK reduction pathway on lung cancer risk was investigated by examining 53 tag-single nucleotide polymorphisms (tag-SNPs) that represent the common variations in the *AKR1C1*, *AKR1C2*, *CBR1*, and *HSD11B1* genes. The apparent differences among individuals in their ability to metabolize NNK make these gene polymorphisms of great interest.

MATERIALS AND METHODS

Subjects

The case-control study was conducted at the H. Lee Moffitt Cancer Center (Tampa, FL) from 2000 to 2003 as previously described [28]. All subjects were white. Lung cancer cases ($n = 456$) were from newly diagnosed subjects with histologically confirmed lung cancer and no past history of other tobacco-related cancers. Controls ($n = 807$) were selected from community residents attending the Lifetime Cancer Screening facility of the Moffitt Cancer Center. Control subjects were randomly selected from thousands of community residents who underwent prostate-specific antigen testing, skin examinations, endoscopy, or

mammography. Spiral computed tomography for lung cancer was not done at the clinic. The Lifetime Cancer Screening facility conducts community outreach and educational programs throughout the Tampa Bay area, including lecture series, screening events, health fairs, literacy programs, and community-based partnerships. A list of control IDs were matched against the hospital patient database to identify any subjects, if any, who might have developed cancer. All control subjects with a new cancer diagnosis were excluded from this study. Ninety-nine percent of the hospital patients and 97% of clinic patients who were asked to participate in the study consented. All study subjects signed a consent form approved by the institutional review board. A trained interviewer administered a structured questionnaire that obtained lifestyle and smoking history information including levels of education, occupation, year of smoking onset, current smoking status, number of cigarettes smoked per day, and years since quitting (former smokers). The medical charts of the case subjects were reviewed to obtain diagnostic and pathology records.

Genotyping

Oral buccal cell swabs were collected for genomic DNA (gDNA) isolation and gDNA was isolated from oral buccal cell swabs using standard phenol: chloroform isolation. Pico Green® analysis was used to quantify the amount of double stranded DNA (dsDNA) for each genomic sample (Life Technologies, Grand Island, NY).

Tag-SNPs were selected for the four major NNK-metabolizing genes (*AKR1C1*, *AKR1C2*, *CBR1*, and *HSD11B1*) by analyzing the CEPH individuals (representing individuals with European ancestry) from Hapmap.org [29] using the Tagger program implemented in Haploview software [30] according to the following criteria: (a) SNPs were located in one of the four genes or within the 5-kb 5' or 3' flanking region, (b) had a minor allele frequency 0.05, and (c) the other unselected SNPs could be captured by one of the tagging SNPs with a linkage disequilibrium (LD) $r^2 \geq 0.80$ (mean $r^2 = 0.98$).

Fifty-three tag-SNPs were genotyped: 9 from *AKR1C1*, 26 from *AKR1C2*, 7 from *CBR1* (1 SNP located in the 3'-UTR), and 11 from *HSD11B1*. A custom 48-plex Illumina Veracode GoldenGate (Illumina, San Diego, CA) genotyping panel was designed and run on the Illumina Bead Express instrument. Five additional SNPs (rs3923936, rs2904802, rs2961611, rs6650153, rs7076886) were genotyped using pre-designed Taqman real-time PCR genotyping assays (Life Technologies) and run on the Applied Biosystems 7900HT Fast Real-Time PCR system (Life Technologies) and SDS 2.4 software (Life Technologies) was used for automated calling of genotypes. Illumina GenomeStudio (Illumina). In genome studio, the project threshold for each golden gate assay was set to 0.25. All samples with call rates lower than 90% were removed along with any SNP data where there with no clear clusters or any SNP that failed Hardy-Weinberg equilibrium (HWE).

Statistical Analysis

HWE analysis and the identification of allele frequencies and haplotype blocks (Figure 2) was conducted using the control sample set from the present study in the Haploview software, defining blocks by the solid spine of linkage disequilibrium (LD). SNPs were excluded if the call rate was <90% and/or a Hardy-Weinberg equilibrium $P < 1 \times 10^{-3}$. SAS

PROC HAPLOTYPE (SAS Institute, Inc., Cary, NC) procedure was used to conduct the haplotype analysis using the haplotype block definitions from the Haploview software. The procedure utilizes the Expectation Maximization (EM) algorithm to generate maximum likelihood estimates of haplotype frequencies given a multi-locus sample of genetic marker genotypes under the assumption of HWE. The initializing method was INIT = RANDOM, which initializes haplotype frequencies with random values from a uniform (0,1) distribution. The haplotype frequency threshold was set to 0.05, and haplotypes with a lower frequency were excluded from subsequent logistic regression analysis. The standard errors and the confidence intervals for each haplotype were estimated by default, under a binomial assumption. The total probability of an individual having a particular haplotype compared to all other haplotype possibilities was created. These values were used in the following statistical analysis assuming an additive statistical model (comparing the probability of one haplotype to all other haplotype possibilities).

Unconditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between individual SNPs and haplotypes and lung cancer risk. Three statistical models were tested for individual SNP logistic regression analysis: additive (BB) > (BA) > (AA), dominant [(BB) + (BA)] vs. (AA), and recessive (BB) vs. [(BA) + (BB)], with B = minor allele. Demographic characteristics between cases and controls were compared using the χ^2 -test for categorical variables and non-parametric Wilcoxon rank sum test for continuous variables. If demographic variables appeared non-normally distributed then appropriate transformation was performed (for example, log-transformation) to normalize their distributions. T-tests were used on log-transformed data and then confirmed by the Wilcoxon rank sum test. Likelihood ratio tests were used to evaluate the fit of each model. Reported P-values are 2-sided after correcting for the effects of multiple testing within each genomic region using SAS PROC MULTITEST. The False Discovery Rate (FDR) method described by Benjamini and Hochberg [31] was used for single SNP analysis only and controls the rate at level $< (m_0/m)\alpha < \alpha$ when you have independent P-values that are uniformly distributed under their respective null hypotheses. A P-value <0.05 was considered significant for all tests.

Multivariate models were adjusted for potential confounding variables that were selected a priori: age (continuous), sex (male, female), pack-years of smoking, and education (no college degree, college degree or above). Stratified analysis was performed by sex, pack-years of smoking (above and below the median), smoking status (former smoker, current smoker). Subset analysis was performed by histology, by comparing histologic-specific cases to the entire control group.

All statistical analyses were performed with SAS version 9.2/9.3 (SAS Institute, Inc.), R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) and JMP Pro 10 (SAS Institute, Inc.).

RESULTS

The basic demographic characteristics of the lung cancer case and control subjects are shown in Table 1. Twelve hundred and sixty-three samples were geno-typed (456 lung

cancer cases and 807 controls). All subjects were of white race and measures of LD and genotype frequency are consistent among various populations of white individuals. Men comprised 54% of cases and 53% of controls, and the mean age of cases and controls were 64 and 58, respectively. A higher percentage of cases than controls were current smokers (40% vs. 20%) or former smokers (51% vs. 42). The mean pack-years of smoking were 57 for cases and 24 for controls. The most frequent lung cancer histology was adenocarcinoma (38%), followed by squamous cell carcinoma (SCC) (24%).

The number of representative SNPs for each gene is shown in Table 2. Nine SNPs were excluded from the analysis for either not meeting the call rate criteria of >90% or HWE of $<1 \times 10^{-3}$, rs4143631, rs760951, rs846910, rs846906, rs10795222, rs12565406, rs12769178, rs17295755, and rs6601882. Five SNPs were excluded from the analysis because they were not found to be SNPs in this study population, rs1937888, rs2801885, rs2835264, rs12261648, and rs28488494. One SNP in *CBR1* (rs2835267), was significantly associated with overall risk of lung cancer, but did not pass multiple testing adjustment ($p_{\text{add}} = 0.048$, FDR $p_{\text{add}} = 0.20$; $p_{\text{rec}} = 0.048$, FDR $p_{\text{rec}} = 0.26$). None of the other individual SNPs were associated with risk for lung cancer in the overall study population after multiple testing correction (Table S1). After stratification and multiple testing correction, three SNPs showed significant associations: two in *CBR1* and one in *HSD11B1* (Table 3). One SNP (rs2835267) located on the *CBR1* gene showed a significant decreased risk for smokers with a high number of pack-years ($p_{\text{add}} = 0.003$, FDR $p_{\text{add}} = 0.018$) and in SCC ($p_{\text{rec}} = 0.003$, FDR $p_{\text{rec}} = 0.018$). Another SNP located in *CBR1* (rs3787728) also showed a significant decreased risk in SCC ($p_{\text{add}} = 0.008$, FDR $p_{\text{add}} = 0.024$) and small cell carcinoma (only in current smokers) ($p_{\text{dom}} = 0.004$, FDR $p_{\text{dom}} = 0.028$). The *HSD11B1* SNP (rs4844880) showed a significant increased risk for adenocarcinoma within former smokers ($p_{\text{dom}} = 0.002$, FDR $p_{\text{dom}} = 0.011$). SNPs with a *P*-value <0.05 before multiple testing correction are shown in Table S1.

Haplotype analysis found six haplotypes approaching significance with lung cancer risk: two in *CBR1*, one in *AKR1C1*, one in *AKR1C2*, and two in *HSD11B1* (Table 4). The two significant haplotypes for *CBR1* are associated with SCC, but differ by three alleles for rs3787728, rs2835266, and rs2835267 with the A-T-G-G-G-T haplotype exhibiting decreased risk while the A-C-A-G-G-C haplotype exhibits an increased risk. For *HSD11B1*, both haplotypes are in the same block (block 2), differing by only one allele in rs3753519. The A-A haplotype exhibits a protective effect for all lung cancer for subjects with low cigarette pack-years, while the A-G haplotype is associated with increased risk for SCC. For the AKR genes, the *AKR1C2* haplotype C-C-A-A-C-C-T-A-A-C-A in block 2 shows a significant increased risk for SCC while the *AKR1C1* haplotype C-T-T-G-A-G-C in block 1 shows a significant decreased risk for small cell carcinoma.

DISCUSSION

In the current case-control study of SNPs in the *AKR*, *HSD11B1*, and *CBR1* genes, one SNP in *CBR1* (rs2835267) was found to be significantly associated with overall lung cancer risk, but did not pass a multiple testing adjustment. However, after stratification by smoking status/pack-years and lung cancer histology, along with multiple testing correction, three

single SNPs (rs2835267 and rs3787728 in *CBR1*, and rs4844880 in *HSD11B1*) were found to be significant. Although the SNPs genotyped in this study may not have any known functional roles, the significant association indicates that the haplotypes are tagging a variant that does have functional relevance. Haplotype analysis found six significant haplotypes among the four genes tested. Although a multiple testing correction on each analysis to account for potential false positives was performed in this study, the stratification analysis is limited in power and will need further validation.

While the current study examined polymorphisms and haplotypes within the genes responsible for NNK reduction on lung cancer risk, the results showed both single SNPs and haplotypes that were associated with SCC. Previous data show that NNK primarily causes lung adenocarcinoma in rodent models, while SCC is associated with the induction of PAHs [32]. The association of the *AKR* and *CBR1* SNPs with SCC may be due to their additional roles in the metabolism of PAHs [33,34]. There are three major pathways for the activation of mutagenic benzo[a]pyrene (B[a]P), which result in the formation of radical cations, diol epoxides, and electrophilic and redox-active α -quinones (Figure 3) [35]. In the α -quinone pathway of PAH activation, the trans-dihydrodiols are oxidized by AKRs to yield ketols. These ketols then spontaneously rearrange to form catechols, like B[a]P 7,8-catechol, which is not stable and undergoes autoxidation to yield B[a]P-7,8 dione. AKRs also catalyze the two electron reduction of PAH α -quinones back to the corresponding cognate PAH catechols, creating a redox cycle which results in the formation of reactive oxygen species and subsequent oxidative DNA damage in human lung cells [35]. *CBR1* has been shown to catalyze the two electron reduction of PAH α -quinones as well, but at a reduced activity [36]. A PAH-mediated role for AKRs in lungis further supported by the fact that *AKRs 1C1* and *1C2* are found to be highly expressed in human lung tissue [37] and can be induced by PAH [38,39].

Polymorphisms in the four major genes responsible for carbonyl reduction of NNK have not been extensively studied previously. In a recent study of NNK-metabolizing gene polymorphisms, three tag-SNPs in *HSD11B1*, (rs2235543, rs3753519, and rs10863782), and one in *AKR1C4* (rs7083869) were found to be associated with decreased urinary NNAL levels in a population of 87 smokers, although two of these SNPs were not statistically significant when using multiple testing adjustments. The *HSD11B1* SNPs tag a haplotype with 5% frequency in the studied population [40]. However, none of the SNPs in that study were examined in the current study. Those results were limited by the number of smokers in the study population (87) and the number of SNPs selected from the four major metabolizing genes (two in *AKR1C1*, three in *AKR1C2*, none for *CBR1*, and five in *HSD11B1*).

In summary, our findings indicate that select variants in genes in the carbonyl reduction pathway of NNK, of which the AKRs and *CBR1* also play a role in PAH metabolism, appear to have an important role in lung cancer risk. Validation of these results with an independent dataset will be needed to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and impact:

This article identifies significant associations of polymorphisms in several genes involved in the metabolism of a tobacco-specific nitrosamine NNK, a potent carcinogen, with lung cancer risk. This article provides evidence that genetic variability in these NNK carbonyl reductases may contribute to inter-individual variability in lung cancer risk.

Figure 1.
Metabolism of NNK to NNAL.

Figure 2.

Linkage disequilibrium (LD) structure of *AKRIC1* and *AKRIC2* (a), *CBR1* (b), and *HSD11B1* (c). CEPH (US residents with Northern and Western Europe ancestry) genotypes from HapMap were downloaded and LD was determined using Haploview. D' values are displayed in the squares (empty squares have a pairwise $D' = 1.00$). Red squares show high pairwise LD, gradually coloring down to white squares of low pairwise LD. Blue squares indicate high LD, but low significance. The black triangles indicate the haplotype blocks.

Figure 3.
The three major pathways of PAH metabolism.

Table 1.**Study Population Demographics in Cases and Controls**

	Cases <i>n</i> = 456	Controls <i>n</i> = 807	<i>P</i>-value
Mean age ^a	64±11	58±10	<0.001
Men (%)	248 (54)	427 (53)	0.639
Women (%)	208 (46)	380 (47)	0.639
Pack-years ^a	57±39	24±31	<0.001
Smoking status (%)			<0.001
Never smokers	39 (9)	311 (38)	
Former smokers	232 (51)	337 (42)	
Current smokers	185 (40)	159 (20)	
Education level (%)			
<High school degree	70 (15)	35 (4)	0.016
High school degree	171 (38)	183 (23)	<0.001
Some college	120 (26)	273 (34)	<0.001
College degree	63 (14)	190 (24)	0.006
Post graduate degree	32 (7)	126 (16)	<0.001
Histology (%)			
Adenocarcinoma	173 (38)		
Squamous cell carcinoma (SCC)	111 (24)		
Other non-small cell carcinoma (NSCCA)	84 (18)		
Small cell carcinoma	42 (9)		
Large cell carcinoma	29 (6)		
Other	16 (4)		

^aMean ± SD.

Table 2.

List of Genes and Tagging SNPs

Gene symbol	Gene formal name	Chromosome	SNPs
<i>AKR1C1</i>	Aldo-keto reductase family 1, member C1	10	9
<i>AKR1C2</i>	Aldo-keto reductase family 1, member C2	10	26
<i>CBR1</i>	Carbonyl reductase 1	21	7
<i>HSD11B1</i>	Hydroxysteroid (11-beta) dehydrogenase 1	1	11

Table 3.
Association Between Significant SNPs and Lung Cancer by Genetic Model and Lung Cancer Histology

Gene	SNP	Reference allele/minor allele	MAF (controls/cases)	Stratification	Genetic MOI ^d	OR	95% CI	P-value	FDR P-value
<i>CBR1</i>	rs2835267	T/C	0.394/0.349	SCC	Add	0.48	0.29–0.76	0.003	0.018
				High Cig pack-years	Rec	0.35	0.17–0.69	0.003	0.018
<i>CBR1</i>	rs3787728	T/C	0.27/0.231	SCC	Add	0.46	0.26–0.80	0.008	0.024
				Small cell (current smokers) ^b	Dom	0.068	0.01–0.42	0.004	0.028
<i>HSD11B1</i>	rs4844880	A/T	0.2/0.165	Adenocarcinoma (former smokers) ^b	Dom	3.94	1.68–9.22	0.002	0.011

^a Genetic mode of inheritance (MOI): add, additive model; rec, recessive model; dom, dominant model. All models were adjusted by age (continuous), sex (male, female), pack-years of smoking, and education (no college degree, college degree or above).

^b Stratified within current versus former smokers.

Table 4.

Association Between Haplotypes and Lung Cancer Risk

Gene	Haplotype	Block	SNPs	Stratification	Frequency in controls	OR	95% CI	P-value
<i>CBR1</i>	A-C-A-G-G-C	1	rs3761354	SCC	0.051	5.46	1.10–27.2	0.038
			rs3787728					
			rs2835266					
			rs9024					
			rs998383					
	A-T-G-G-G-T	1	rs2835267	SCC	0.255	0.32	0.12–0.91	0.033
			rs3761354					
			rs3787728					
			rs2835266					
			rs9024					
	C-C-A-A-C-T-A-A-C-A	2	rs998383	SCC	0.140	3.16	1.1–9.06	0.033
			rs2835267					
			rs7915338					
			rs11252866					
			rs11252867					
	C-T-T-G-A-G-C	1	rs4344395	Small cell	0.352	0.26	0.08–0.81	0.020
			rs2854466					
			rs11816204					
			rs2854482					
			rs1937868					
			rs1937865					
			rs3902925					
			rs28488494					
			rs2904803					
			rs2904802					
<i>AKR1C1</i>	C-T-T-G-A-G-C	1	rs7076886	Small cell	0.352	0.26	0.08–0.81	0.020
			rs2904804					
			rs2961611					

Gene	Haplotype	Block	SNPs	Stratification	Frequency in controls	OR	95% CI	P-value
<i>HSD11B1</i>	A-A	2	rs3923936	Low pack-years	0.113	0.16	0.03–0.80	0.025
			rs6650153					
			rs4844880					
			rs3753519					
			rs4844880					
	A-G	2	rs4844880	SCC	0.074	0.07	0.01–0.96	0.047
			rs3753519					

Haplotype analysis was adjusted by age (continuous), sex (male, female), pack-years of smoking, and education (no college degree, college degree or above).