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New associations of the genetic polymorphisms in nicotinic receptor genes with the risk of lung cancer

Anna Chikova^{a,b}, Hans-Ulrich Bernard^c, Igor B. Shchepotin^d, and Sergei A. Grando^{a,e}

^aDepartment of Dermatology, University of California, Irvine, CA, USA

^bThe D.I. Ivanovsky Institute of Virology of The Ministry of Health of The Russian Federation, Moscow, Russia

^cDepartment of Molecular Biology and Biochemistry, University of California, Irvine, CA, USA

^dNational Cancer Institute, Kyiv, Ukraine

^eCancer Center and Research Institute, University of California, Irvine, CA, USA

Abstract

Aims—Previous studies revealed association of lung cancer risk with single nucleotide polymorphisms (SNPs) in chromosome 15q25 region containing CHRNA5-CHRNA3-CHRNA4 nicotinic acetylcholine receptor (nAChR) subunit gene cluster. The genetic variations in other lung nAChRs remained unknown. In this study, we perform case-control analysis of CHRNA9 and CHRNA3 genes using 340 non-small cell lung cancer cases and 435 controls.

Main methods—All exons, 3'UTR, intron 1 and parts of other introns surrounding exons 2–5 of CHRNA9 gene as well as exons 2, 3 of CHRNA3 gene and parts of surrounding intronic regions were sequenced. The study was controlled for gender, age and ethnicity related differences. Each SNP in analyzed groups was assessed by allele frequency, genotype distribution and haplotype analysis.

Key findings—The case-control analysis revealed that an increased risk is associated with two SNPs in CHRNA9, rs56159866 and rs6819385, and one in CHRNA3, rs8040868. The risk was reduced for three SNPs in CHRNA9, rs55998310, rs56291234, and newly discovered ss410759555, and also in carriers of the haplotype NP_060051.2 containing ancestral N442 variant of $\alpha 9$.

Significance—The nonsynonymous substitutions can produce receptors exhibiting unique ligand-binding and downstream signaling characteristics, synonymous as well all intronic SNPs may affect protein production at the transcriptional and/or translational levels, or just manifest association with cancer by genetic linkage to other alleles. Elucidation of the mechanisms by which individual genetic variations in $\alpha 9$ affect predisposition to lung cancer may lead to development of personalized approaches to cancer prevention and treatment as well as protection against tobacco consumption.

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Correspondence to: Sergei A. Grando, MD, PhD, DSc, University of California Irvine, 134 Sprague Hall, Irvine, CA 92697, USA. Telephone: (949) 824-2713; Fax: (949) 824-2993, sgrando@uci.edu.

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Keywords

lung cancer; CHRNA3; CHRNA9; $\alpha 3$ and $\alpha 9$ nicotinic acetylcholine receptors; single nucleotide polymorphisms

Introduction

The genome wide studies of American, European and Asian populations revealed association of lung cancer risk with single nucleotide polymorphisms (SNPs) in the chromosome 15q25 region containing the CHRNA5-CHRNA3-CHRNA4 nicotinic acetylcholine receptor (nAChR) subunit gene cluster, which is also involved in tobacco addiction (reviewed in (Linnoila, 2010)). The association with lung cancer might, therefore, simply reflect smoking behavior. Most recently, however, the association of these nAChR SNPs with lung cancer was confirmed both in smokers and non-smokers, and with all histological types of lung cancer (Shiraishi et al., 2009). A limitation of previous studies was the very high linkage disequilibrium in the analyzed region of chromosome 15q25, and it remains unclear whether all, some or none of the genes coding for $\alpha 3$, $\alpha 5$, and $\beta 4$ nAChR subunits are directly involved in the development and progression of lung cancer. The detected SNPs may be linked to other functional genetic polymorphisms in the 15q25 cluster. The genetic variations in other nAChR subunits expressed in lung epithelia remain largely unknown. The knowledge about low frequency SNPs in the nAChR genes is limited. Many SNPs are presented by single entry in NCBI without validation or frequency analysis. Furthermore, most previous studies were performed using SNP arrays, and even when direct sequencing was employed, only selected SNPs were targeted.

Activation of nAChRs stimulates the growth of lung cancer cells and suppresses apoptosis (Cattaneo et al., 1997, Maneckjee and Minna, 1990, 1994, Novak et al., 2000, Schuller et al., 1990, Schuller et al., 2003, Song et al., 2003, West et al., 2003), indicating that nicotine and its carcinogenic derivatives can contribute to the development of lung cancer by acting as tumor promoters that facilitate the outgrowth of cells with genetic damage. Thus, smokers who carry certain nAChR variants are expected to be at increased risk for lung cancer, compared with smokers who do not carry these alleles even if they smoke the same number of cigarettes (Le Marchand et al., 2008). In non-smokers, the mechanism should be the same, because lung nAChRs are constantly stimulated by the physiologic doses of auto/paracrine acetylcholine produced by bronchial cells (Klapproth et al., 1997). Environmental exposure to tobacco smoke may aggravate the aberrant signaling, thus facilitating tumorigenic transformation.

We focused this study on the human CHRNA9 gene encoding $\alpha 9$ nAChR that was originally cloned by us from human epidermal keratinocytes (Nguyen et al., 2000). Recent publications have demonstrated overexpression and activation of the $\alpha 9$ nAChR in human breast epithelial cells during tumorigenesis (Lee et al., 2010), and antitumor effects of inhibition of $\alpha 9$ signaling in human breast cancer cells (Wu et al., 2011). We also evaluated association of several SNPs in the CHRNA3 gene encoding $\alpha 3$ nAChR subunit with lung cancer measuring frequencies of minor alleles and genotype distribution. The physiological roles of $\alpha 3$ and $\alpha 9$ nAChRs stems from their differential, yet synergistic regulation of regulation of vital cell functions, including viability, proliferation, cell-cell and cell-substrate attachment, differentiation and programmed cell death (apoptosis) of normal and malignant respiratory cells (reviewed in (Tournier and Birembaut, 2011, Wessler and Kirkpatrick, 2008)).

Materials and Methods

Design and limitations of the case-control study

A total of 340 non-small cell lung cancer cases and 435 matching controls were included in this study. Lung cancer samples were obtained from Ontario Tumor Bank (<http://www.ontariotumourbank.ca/>) and control DNA samples were from Coriell Institute for Medical Research (<http://www.coriell.org/>). Ontario region Census 2006 was used to determine ethnicity of the local population and select matching controls. Over 90% of the study subjects were Caucasians of various ethnic origin. As a sample of the Northern American population, we used the following control groups: the "Caucasian 200" and AGPLONG4 panel of 100, Southern European panel (Greeks 8, Iberians 10, Italians 10, Basques 10, Hungarians 10), Northern Europeans panel (Icelandic panel of 12, Northern European panel of 10), Eastern European panel (Czechs/Slovaks 10, Russians of Zversky District Northeast of Moscow 9, Russians of Krasnodar Region 8), Africans and African American panel (South of Sahara group of 9, African American group of 10), Chinese (version 2) panel of 10, and Middle Eastern (version 1) panel of 10. The median age was 70 and 40 years old, in the case and control groups, respectively, and the male/female ratios—59/41% and 49/51%, respectively Supplemental Table S1. Case group included 86 current and 168 former tobacco smokers, 16 non-smokers and 70 with unknown smoking status. Since the smoking status was not available for control group, we could not analyze associations of nAChR SNPs with the smoking status.

The control group might include about 7% of potential lung cancer victims, because the lifetime risk of the lung cancer has been estimated as 9.6% for men and 4.3% for women (Villeneuve and Mao, 1994). Possible presence of such subjects in control group was ignored as negligible, because it might only slightly reduce differences between case and control groups but could not cause artificial differences or increase odds ratios.

DNA purification and sequencing

DNA was purified using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Valencia, CA). The oligonucleotides used for DNA amplification and sequencing are shown in Supplemental Table S2. From 20 to 30 ng of total genomic DNA were used for each PCR reaction with 8 pM of each primer and Platinum PCR SuperMix High Fidelity (Invitrogen, Carlsbad, CA). At least 2 negative controls for each PCR reaction were included in each 96-well plate. The amplified products were prepared for sequencing using the ExoSAP-IT mix (USB, Santa Clara, CA). Each fragment was sequenced in both directions. Results were analyzed by the "Geneious" software (Biomatters development team, Auckland, New Zealand). Heterozygous SNPs were confirmed by visual analysis of both forward and reverse sequences. For approximately 1% of the reactions, PCR and DNA sequencing was repeated for validation. Detected SNPs and their heterozygosity were reproducible. All exons, 3'UTR, intron 1 and parts of other introns surrounding exons 2–5 of CHRNA9 gene as well as exons 2, 3 and 4 of CHRNA3 gene and parts of surrounding intronic regions were sequenced. Each SNP in the analyzed groups was assessed by allele frequency, genotype distribution and haplotype analysis.

Statistical analysis

All statistical analyses were performed by "Prism" software (GraphPad Software Ink., La Jolla, CA). Two-tailed Fisher's exact test was used in allele frequency analysis. Chi-square test and two-tailed Fisher's exact test were used for genotype distribution analysis. Linkage was assessed with use of the Pearson correlation coefficient. Differences were deemed significant if the *p* value calculated with 95% confidence interval was less than 0.05.

Results

SNPs in CHRNA9 associated with an increased risk of lung cancer

Frequency of the synonymous substitution at amino acid position Val55 (rs56159866) located in exon 2 was found to be 0.26 in the lung cancer group and 0.198 in controls. A significant association with an increased risk of lung cancer (odds ratio = 1.34) was confirmed by Fisher's exact test (Table 1).

The SNP rs6819385 located in intron 3 displayed significant increase in allele frequency in the lung cancer cases with odds ratio 1.27 (Table 1), the number of homozygotes of this allele in the lung cancer cases was also significantly higher than in controls (Table 2 and Supplemental Table S3). The genotype AA in this allele was detected in 126 of 340 cancer patients, while in the control group only 102 of 435 individuals had this genotype. Thus, homozygous rs6819385 is associated with an increased risk of lung cancer with the odds ratio of 1.65.

SNPs in CHRNA9 associated with a decreased risk of lung cancer

The synonymous SNP rs55998310 in exon 3 at position Thr78 was found to be significantly associated with reduced risk of lung cancer with the odds ratio of 0.14. Frequency of the minor allele in the controls was 0.01, whereas a 6.7-fold lower frequency was detected in the lung cancer cases (Tables 1 and 2, and Supplemental Table S3).

The frequency of another SNP, rs56291234, located in 3'UTR was also significantly reduced in lung cancer cases (allele frequency 0.04), compared to controls (allele frequency 0.075) with odds ratio of 0.52 (Tables 1 and 2, and Supplemental Table S3).

In control group, we discovered the low frequency single nucleotide deletion ss410759555 in intron 2, which was not seen in any of 340 cancer samples. Statistical analysis demonstrated significant association of this new SNP with reduced risk of lung cancer with odds ratio of 0.08 (Table 1).

Haplotype analysis of CHRNA9 gene

Four haplotypes associated with three amino-acid substitutions were detected in the exon 5 of CHRNA9 gene. The linkage among polymorphisms affecting protein sequence is particularly important, since each amino acid substitution may influence properties of the protein. The reference sequence NP_060051.2 containing N442 without any amino-acid substitutions was found with frequency of 0.078 and 0.05 in control and lung cancer groups, respectively (Table 3). This observation indicated that the N442 haplotype is associated with a reduced in risk for lung cancer (odds ratio 0.62).

The most frequent haplotype S442 (rs10009228) detected with an average frequency of 0.8 did not show significant differences between the case and control groups (Table 3). The frequencies of the haplotype producing a protein with amino acids N442/A315V (rs55633891) in lung cancer cases and controls were 0.14 and 0.13, respectively, and that of haplotype coding for the protein N442/A312T (rs56210055) was 0.004 and 0.007, respectively (Table 3). Although rs55633891, rs56210055 and rs10009228 SNPs are located in the same exon within less than 400 nucleotide fragment, no linkage among these SNPs was detected.

SNP rs8040868 in CHRNA3 is associated with increased risk of lung cancer

Synonymous SNP rs8040868 (Val53Val) in exon 2 was associated with significantly increased risk of lung cancer. Association with G allele had the odds ratio of 1.38 (Table 1),

and the homozygous genotype GG that of 2.0 (Table 2). This finding is consistent with results of the haplotype based association analysis of 194 familial lung cases and 219 cancer free controls from the Genetic Epidemiology of Lung Cancer Consortium collection that showed that a haplotype including rs8040868 was significantly associated with lung cancer (Liu et al., 2009). Our study, however, demonstrated that rs8040868 is by itself significantly associated with lung cancer, regardless of the haplotype.

Age- and gender – association analysis of polymorphisms in CHRNA9 and CHRNA3 genes

To address potential problems due to age and gender mismatches, we performed age and gender based analysis for each SNP. No significant variations in population frequency of analyzed SNPs was found among different age and gender groups (Supplemental Tables S4 and S5).

Ethnic-specific polymorphisms in CHRNA9 and CHRNA3 genes

To test for possible influence of ethnic distribution on the frequency of nAChR polymorphisms, all detected SNPs in CHRNA9 and the sequences of exons 2, 3 and 4 with adjacent parts of introns of CHRNA3 were analyzed based on the ethnic origin using 336 DNA samples from distinct anthropologically defined human populations. Statistically significant differences in the minor allele frequencies among various ethnical groups were observed for 15 of 26 evaluated SNPs (Table 4). Significant ethnic specific differences were also observed in genetic linkage.

Analysis of the CHRNA9 gene showed significant differences among Caucasians, Africans, Middle Easterners and Chinese (Table 4). The non-synonymous amino acid substitution Ala312Thr (rs56210055) was detected only in individuals of African ($p < 0.0001$) and Middle Eastern ($p = 0.0022$) origin with minor allele frequency 0.1. Interestingly, rs10938433 at 3'UTR showed significantly different frequencies among Africans ($p = 0.03$) and Chinese ($p = 0.0023$). The 4.7-fold difference in frequency between these two groups was also significant ($p = 0.0002$).

In CHRNA3, the SNPs rs71581736 ($p = 0.0073$), rs113048580 ($p = 0.0006$) and rs71581740 ($p = 0.0073$) were detected only in Africans and African Americans. rs71581741 ($p = 0.0018$) and rs3743076 0.048 ($p = 0.048$) were detected in Chinese and African Americans ($p < 0.0001$ and $p = 0.0073$, respectively), but not in other groups (Table 4). A significant difference in CHRNA3 at rs77574318 (Arg110His) was found between the "Caucasian 200" group and Eastern Europeans ($p = 0.014$) but not Southern and Northern European groups. This SNP was seen only in Russians with heterozygosity 0.06. Its total frequency in Eastern European group was 0.037. It is therefore likely that the "Caucasian-200" panel includes only a small number, if any, of Northern Americans of Russian descent.

Ethnic-specific differences were also detected in linkage among SNPs in CHRNA3. The rs8192475 and rs71148543 SNPs in CHRNA3 gene were significantly ($p < 0.0001$) linked in all ethnic groups except for Africans and African Americans. In these groups, rs71581741 and rs71148543 showed a significant linkage ($p = 0.0002$). The SNPs rs3743076 and rs71581736 were linked only in Africans and African Americans ($p < 0.0001$). The rs71581741/rs71148543 haplotype was detected in African descendants with heterozygosity 0.13, while rs71148543 had total frequency of 0.16.

Discussion

The results of this case control study revealed new associations of genetic polymorphisms of nAChRs with the risk for lung cancer risk. An increased risk was found to be associated two SNPs in CHRNA9, rs56159866 and rs6819385, and one CHRNA3 SNP, rs8040868. The

risk was reduced for three carriers of three SNPs in *CHRNA9*, rs55998310, rs56291234, and the newly discovered ss410759555, and those with the N442 (NP_060051.2) $\alpha 9$ nAChR haplotype. The latter observation is consistent with our experimental data showing that overexpression of the N442 $\alpha 9$ variant protects human bronchial cells from neoplastic transformation induced by a tobacco nitrosamine (Chikova and Grando, 2011). DNA for the cancer samples was purified from the tumor tissues to evaluate for possible somatic mutations in *CHRNA9* and *CHRNA3* genes, but no tumor-associated somatic mutations were detected in the sequenced regions.

In contrast to *CHRNA3*, the polymorphisms in *CHRNA9* gene have never been studied in relation to the risk of lung cancer in the past, but it was reported that SNPs in *CHRNA9* are associated with nicotine dependence (Greenbaum et al., 2006). While some authors felt that the SNPs in the *CHRNA5-CHRNA3-CHRNA4* gene cluster influence the individual's predisposition to lung cancer regardless of the tobacco smoking status (Shiraishi, Kohno, 2009), the others opined that this genetic cluster influences only the nicotine addiction (Yang et al., 2010). Previous research of the influence of rs8040868 in *CHRNA3* on nicotine addiction brought interesting results. While several large studies did not show association of this SNP with nicotine addiction (Sherva et al., 2010, Wessel et al., 2010), a family-based study of 402 African Americans and 671 European Americans, and the case-control study including nearly 9,000 Koreans demonstrated its significant association with nicotine dependence in African Americans and Koreans but not in European Americans (Li et al., 2010a, Li et al., 2010b). The study of 1,075 individuals of Caucasian, Hispanic and African origin showed significant association of rs8040868 with the early age of tobacco initiation (Schlaepfer et al., 2008). However, in all these studies association did not remain significant after correction for multiple testing. Therefore, all previous reports agreed that there is no significant association between rs8040868 genotype and nicotine addiction in Caucasians. Since the population analyzed in our study was represented by approximately 90% Caucasians, with only approximately 5% of African descent, it can be concluded that in Caucasians the rs8040868 SNP is associated with the risk of lung cancer not through nicotine addiction but due to a putative direct influence on cancer predisposition. Unfortunately, insufficient information regarding the smoking status of studied subjects did not allow us to specifically address this important issue in the present study.

The differences between lung cancer cases and controls detected in this study reflect the risk of lung cancer rather than the ethnic differences between studied groups. The results of case control study did not correlate with the differences revealed by the ethnicity-based analysis, except for rs56159866 whose frequency was increased among lung cancer patients and reduced among Africans. Likewise, frequency of rs10022491 was also significantly lower among Africans and African Americans but this SNP did not show association with the risk of lung cancer ($p=0.96$). Thus, the results of our case-control study were not biased by ethnicity age or gender. On the other hand, we demonstrated that sequences of nAChR genes may vary among distinct ethnic groups. Despite relatively small number of individuals from each ethnic group, statistical significance of observed differences suggests that these SNPs are fairly frequent in selected populations. Noteworthy, according to the CDC data from 2007 (<http://www.cdc.gov>), 203,536 people were diagnosed with lung cancer and 158,683 people died of lung cancer that year, with the highest incidence rate and mortality in African American men. Moreover, an increased susceptibility of African Americans and Native Hawaiians to lung cancer among cigarette smokers (Haiman et al., 2006) suggests that sensitivity to the oncogenic effect of tobacco products vary among different ethnic groups. Therefore, the minor alleles found to be significantly different from Caucasians in this study, may be important for epidemiologic study of association with lung cancer in African and African American populations.

As the consequences of the SNPs found in $\alpha 3$ and $\alpha 9$ subunits of nAChRs, several effects can be expected to occur in their physiological roles that could lead to cancer risk increase or reduction. For instance, we have recently demonstrated that naturally occurring isoforms of CHRNA9 mRNA differentially influence cellular proliferation and transformation (Chikova and Grando, 2011). Synonymous and intronic SNPs that do not affect amino acid sequence may affect transcription and/or translation of receptor protein. For example, Valor *et al.*, (Valor *et al.*, 2003) demonstrated the regulatory sequences in 5' region of CHRNA9 responsive to Sox9 and Sox10 proteins that affect levels of $\alpha 9$ expression. The protein production can be also affected by changes in binding microRNAs in the area of polymorphism. Since different tissues produce different spectra of tRNAs, synonymous SNPs in exons may affect protein production in a tissue specific manner. Intronic and synonymous SNPs may also affect RNA splicing. Thus, abnormal nAChR signaling may represent one of the several well-known genetic conditions associated with lung cancer. The principal molecular changes in lung cancer are seen in tumor suppressor genes, proto-oncogenes, growth factors, telomerase activity, and methylation status of promoters (reviewed in (Duarte and Paschoal, 2006)).

Conclusion

The obtained results suggest that $\alpha 9$ and $\alpha 3$ nAChR play important role in the genetically determined susceptibility to lung cancer. The nonsynonymous substitutions can produce receptors exhibiting unique ligand-binding and downstream signaling characteristics, the synonymous as well all intronic SNPs may affect protein production at the transcriptional and/or translational levels, influence RNA splicing or just manifest association with cancer by genetic linkage to other alleles. Further studies of the genetic polymorphisms in nAChRs should provide important information for cancer research. Elucidation of the mechanisms by which individual genetic variations in nAChRs affect predisposition to lung cancer may lead to development of personalized approaches to cancer prevention and treatment as well as protection against tobacco consumption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Minor allele frequencies in CHRNA9 and CHRNA3 genes*

Gene	SNP	Allele change	Type	Cancer	Control	P-value	Odds Ratio (95% confidence interval)
CHRNA9	rs56241474	C>T	Ile13Ile	0.3	0.29	0.5	
	rs56159866	C>T	Val55Val	0.26	0.198	0.019	1.343 (1.06–1.7)
	rs55974552	G>A	Thr63Thr	0.014	0.0186	0.7	
	rs10022491	C>T	Ser43Ser	0.43	0.42	0.96	
	rs10015231	C>T	intron	0.23	0.22	0.7	
	rs6447332	G>C	intron	0.043	0.056	0.24	
	rs55998310	T>C	Thr78Thr	0.0015	0.01	0.027	0.14 (0.02–1.0)
	rs56299220	G>A	intron	0.0015	0.001	1	
	rs56171884	G>A	Gln66Gln	0.003	0.001	0.59	
	rs6819385	G>A	intron	0.58	0.52	0.026	1.27 (1.03–1.6)
	ss410759555	A T	intron	<0.001	0.008	0.02	0.08 (0.005–1.5)
	rs55853173	V T	intron	0.04	0.048	0.5	
	rs60714708	C>T	intron	0.007	0.017	0.1	
	rs10938433	C>T	3'-UTR	0.5	0.5	0.9	
	rs56291234	C>T	3'-UTR	0.04	0.075	0.0058	0.52 (0.33–0.83)
	rs4861327	T>A	3'-UTR	0.05	0.06	0.66	
CHRNA3	rs6819816	G>A	3'-UTR	0.26	0.22	0.07	
	rs10009228	A>G	Asn442Ser	0.81	0.79	0.44	
	rs55633891	C>T	Ala315Val	0.14	0.13	0.76	
	rs56210055	G>A	Ala312Thr	0.004	0.007	0.45	
	rs113048580	A>G	Intron 1	0.003	0.003	1	
	rs72648883	A>G	Intron 1	0.003	0.002	1	
	rs71581736	G>A	Intron 1	0.003	0.007	0.46	
	rs8192475	G>A	Arg37His	0.046	0.037	0.44	
	rs8040868	A>G	Val53Val	0.48	0.39	0.0003	1.38 (1.13–1.7)

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Gene	SNP	Allele change	Type	Cancer	Control	P-value	Odds Ratio (95% confidence interval)
	rs3743073	A>C	Intron 2	0.37	0.37	0.96	
	rs3743074	T>C	Intron 2	0.37	0.37	0.96	
	rs3743075	G>A	Lys97Lys	0.37	0.37	0.96	
	rs77574318	G>A	Arg110His	0.006	0.005	0.96	
	rs8192479	G>A	K115K	0.04	0.027	0.24	
	rs71581741	G>C	Intron 3	0.043	0.056	0.24	
	rs3743076	A>T	Intron 3	0.012	0.009	0.26	

* P values were calculated by two-tailed Fisher's exact test with 95% confidence intervals. Significant (p<0.05) differences are highlighted with bold font.

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Table 2

Odd ratios for selected genotypes in CHRNA9 and CHRNA3 genes*

Gene	SNP	Allele change	Cancer	Control	P-value	Odds Ratio (95% confidence interval)
CHRNA9	rs56159866	TT	35	22	0.024	1.9 (1.1–3.4)
		CC+CT	305	403		
	rs6819385	AA	126	102	0.002	1.65 (1.2–2.3)
		AG+GG	209	279		
CHRNA3	rs8040868	GG	81	56	0.0003	2 (1.38–2.9)
		AG+AA	255	380		

* P values were calculated by two-tailed Fisher’s exact test with 95% confidence intervals.

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Table 3

Haplotype frequencies of the CHRNA9 gene*

NCBI ID	Protein sequence	Cancer	Control	P-value	Odds Ratio (95% confidence interval)
Reference sequence	NP_060051.2	0.05	0.078	0.03	0.62 (0.4–0.9)
rs10009228	S442	0.81	0.79	0.44	
rs55633891	A315V/N442	0.14	0.13	0.76	
rs56210055	A312T/N442	0.004	0.007	0.45	

* Table shows frequencies of SNPs detected in 340 lung cancer and 430 control samples. P-values were calculated by two-tailed Fisher's exact test with 95% confidence intervals. Differences were considered significant if P<0.05. Significant differences are shown in bold font.

Table 4

Minor allele frequencies in various ethnical groups.*

Gene	SNP	Allele change	Type	Caucasians	South Europeans	Northern Europeans	Eastern Europeans	Chinese	Sub Sahara Africans and Americans	Middle Easters
CHRNA9	rs56159866	C>T	Val55Val	0.22	0.31	0.19	0.29	0.05	0.05	0.2
	rs10022491	C>T	Ser43Ser	0.41	0.36	0.45	0.44	0.75	0.14	0.3
	rs10015231	C>T	intron	0.25	0.23	0.26	0.24	0.1	0.16	0.25
	rs56241474	C>T	Ile131Ile	0.29	0.36	0.31	0.32	0.05	0.26	0.35
	rs6447332	G>C	intron	0.06	0.068	0.06	0.028	0	0.09	0.15
	rs6819385	A>G	intron	0.55	0.48	0.48	0.43	0.7	0.25	0.33
	rs10938433	C>T	3'-UTR	0.5	0.57	0.45	0.53	0.15	0.71	0.7
	rs56291234	C>T	3'-UTR	0.08	0.075	0.063	0.03	0	0.16	0.15
	rs6819816	G>A	3'-UTR	0.22	0.32	0.31	0.29	0.05	0.05	0.25
	rs10009228	G>A	Asn442Ser	0.79	0.76	0.80	0.77	0.95	0.56	0.7
	rs55633891	C>T	Ala315Val	0.14	0.14	0.14	0.12	0.05	0.16	0.15
	rs56210055	G>A	Ala312Thr	0	0	0	0	0	0.1	0.1
	rs559745522	G>A	Thr63Thr	0.025	0.045	0.06	0	0	0	0
	rs4861327	T>A	3'-UTR	0.07	0.045	0.05	0.09	0	0.079	0.05
CHRNA3	NP_060051.2	Reference sequence	Asn442	0.08	0.07	0.07	0.12	0	0.16	0.05
	rs3743073	A>C	intron	0.35	0.37	0.34	0.39	0.15	0.47	0.35
	rs71581741	C>G	intron	0	0	0	0	0.1	0.13	0
	rs77574318	G>A	Arg110His	0	0	0	0.037	0	0	0
	rs3743076	A>T	intron	0	0	0	0	0.05	0.06	0
	rs8040868	T>C	Val53Val	0.4	0.48	0.39	0.41	0.2	0.34	0.5
	rs8192475	G>A	Arg37His	0.04	0.04	0.07	0.074	0	0.026	0
	rs71148543	insertion T	intron	0.04	0.04	0.07	0.074	0	0.16	0.05
	rs71581740	deletion TG	intron	0	0	0	0	0	0.053	0

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Gene	SNP	Allele change	Type	Caucasians	South Europeans	Northern Europeans	Eastern Europeans	Chinese	Sub Sahara Africans and African Americans	Middle Easters
	rs113048580	T>C	intron	0	0	0	0	0	0.079	0
	rs72648883	G>A	intron	0	0	0	0	0	0.026	0
	rs71581736	C>T	intron	0	0	0	0	0	0.053	0
	rs1051730	C>T	Tyr215Tyr	0.34	0.38	0.3	0.37	0.05	0.1	0.45
Chromosomal sample number				400	96	44	54	20	38	20

Heterozygosity of each SNP is shown. Frequency of each SNP for every group was compared to the largest tested group "Caucasian 200" in Fisher's exact test. Significant (p<0.05) differences are highlighted with bold font.