RESEARCH ARTICLE

Novel Mutations of the CHRNA3 Gene in Non-Small Cell Lung Cancer in an Iranian Population

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Abstract

Background: Lung cancer, the leading cause of cancer-related worldwide deaths, largely results from the combined effects of smoking exposure and genetic susceptibility. CHRNA3, a nicotinic acetylcholine receptor gene, is associated with lung cancer risk. This study sought to identify variations in exon 3 of CHRNA3 in an Iranian population with non-small cell lung cancer (NSCLC). **Materials and methods:** A case-control study including 147 individuals with lung cancer and 145 healthy individuals was conducted. As mobility shift caused by nucleotide substitutions might be due to a conformational change of single-stranded DNAs, we designated these as single-strand conformation polymorphisms (SSCPs). PCR amplified products with SSCP were subjected to DNA sequencing. **Results:** The sequencing results showed 3 polymorphisms in exon 3 of CHRNA3, rs8040868, rs763384023 and rs2869547 , the latter two of which have not been reported in NSCLC, previously. **Conclusion:** It appears that the rs8040868 may be considered as a pathogenic mutation associated with the clinical phenotype. Polymorphisms are important factors for development of cancers and may provide additional insights into mechanisms underlying NSCLC.

Keywords: Non-small cell lung cancer- CHRNA3- SSCPs- polymorphism- rs8040868

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Introduction

Non-small-cell lung cancer is the leading cause of cancer death in Iran and worldwide (Zappa et al., 2016). Lung cancer remains by far the single most common cause of cancer-related mortality with nearly 1.6 million deaths worldwide in 2012 or nearly 20% of cancer mortality as a whole (Chan et al., 2015). It is widely accepted that lung cancer is a complex multifactorial disease, attributed to the interaction of genetic factors with environmental factors. Chronic smoking, occupational exposure, air pollution and other factors are risk factors for lung cancer. In addition, genetic factors also contribute to the risk of lung cancer (Gu et al., 2012).

In recent years, several studies have set out to determine whether there is an association between genetic polymorphisms and lung cancer susceptibility. According to the recent genome-wide association (GWA) studies, the human cholinergic receptor nicotinicα3 (CHRNA3) gene, encoding the neuronal nicotinic acetylcholine receptor, located on the chromosome 15q25.1 region is a hotspot for lung cancer susceptibility (Vink et al., 2009; Kaur et al., 2014). Several polymorphisms in the gene have been identified, such as the rs6495309, rs8034191 and rs1051730. However, the effect of CHRNA3 genetic variants on lung cancer risk was not consistent even conflicting among different studies (Xiao et al., 2014). As other developing countries, studies show that different malignancies such as breast cancer in females and lung cancer in males tends to accumulate among Iranian population (Shiryazdi et al., 2014; Almasi et al., 2016). According to the last research, the incidence of lung cancer in the North West and West provinces was higher than in other regions. This is, to our knowledge, the first case-control study that investigated the mutations in the CHRNA3 gene exon 3 in an Iranian population with lung cancer.

Materials and methods

Study population

Institutional review board of Yazd University, Yazd, Iran approved this case control study. In addition, informed consent for participation in this study was obtained from all patients. A total of 147 NSCLC patients and 145 unrelated healthy controls were recruited from Shahid Sadoughi hospital of, Yazd, Iran, between November 2013 and January 2015. All cases and controls were of Iranian Persian origin and lived in the same geographic region in Yazd city, a city in the center of Iran. Exclusion criteria included a history of previous primary cancer other than lung cancer. Clinical data of patients involved were obtained from the inpatient and outpatient medical records. The patients were selected each from a different family. These families were found to be not related to each other as per the records.

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DNA Samples

Genomic DNA was extracted from each sample. Exons 2 to 3 of the CHNRNA3 were amplified using PCR or single-strand conformation polymorphism- polymerase chain reaction (SSCP-PCR) methods. PCR for genomic DNA was done using the following primer pair designed by Gene Runner software. They were (5'TGGACACCTCGAAATGGATGAT 3') [forward primer]/(3'TAGATGGGTGATGTTTCTGCTG5') [reverse primer]. The composition of the 10X buffer contained 100 mM Tris-HCl pH 8.8, 15 mM MgCl 2, 750 mM KCl. PCR was performed in 25 µl solutions containing 100 ng of genomic DNA, 1X PCR buffer, 100 µM dNTPs, 10 pmol of each primer and 0.5 U of Taq DNA polymerase. The reaction program was set in a thermal cycler (Eppendorf™ Mastercycler™) to an initial denaturation of 2 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, and then a final extension of 5 min at 72 °C.

SSCP analysis

Eight percent neutral polyacrylamide gel electrophoresis was performed as previously described (Zhu et al., 2006). Briefly, 3 mL 40% acrylamide solution, 3 mL 5 × TBE solution, 3 mL 50% glycerin, 6 mL ddH2O, 75 µL 10% ammonium persulfate, 7 µL TEMED, were mixed and poured into the gel and concreted for 1 h at room temperature. Four µL PCR product and 6 µL formamide sample were mixed. The mixture was centrifuged for 15 s, denatured at 95°C for 10 min, bathed in ice for 10 min, put on an 8% neutral polyacrylamide gel, and electrophoresed with 1 × TBE buffer for 8 h at 14°C and 300 V. The fixation solution was infused into a flat utensil, into which gel was immerged, vibrated for 10 min, and washed 3 times (2 min each time) with ddH2O. The gel was immerged into a staining solution, vibrated for 10 min, washed 3 times (20 s each time) with ddH2O. The gel was then immerged into a display solution, vibrated until the sample signal became brown and the background became transparent yellow, and rinsed with tap water to stop display. The staining results were observed and photographs were taken. According to the PCR-SSCP results of genome DNA, the difference in the single strand strip number and electrophoresis transference location, also known as the mobility shift, was considered PCR-SSCP positive.

Direct sequencing of PCR products

Genome DNA from positive PCR-SSCP samples was amplified again in 80 µL reaction system. The amplicons were purified using QIAquick PCR purification kit (Qiagen) and subjected to automated DNA sequencing (ABI 377; Applied Biosystems) using the manufacturer's suggested protocols. Sense and antisense strand sequencing were done to confirm all mutations.

Results

Finally, DNAs from patients and controls with a conformation shift on the SSCP were sequenced. The sequencing results showed three SNPs rs8040868, rs763384023 and rs2869547 in CHRNA3 exon 3 loci, which the last two SNPs were not reported in NSCLC, previously (Table 1).

Discussion

In the current study, for first time we investigated exon 3 of CHRNA3 SNPs in 147 NSCLC patients and 145 age-matched healthy controls for association with NSCLC risk in an Iranian population. In this study three SNPs on CHRNA3 exon 3 of the patients including rs8040868, rs763384023 and rs2869547 were identified which the last two SNPs were not reported in NSCLC, previously.

A first examination of the single SNP analysis results points to the rs8040868 SNP of the CHRNA3 gene as a variation that might influence both alcohol and tobacco phenotypes (Schlaepfer et al., 2008; Lou et al., 2014). More interestingly, results obtained with the "tobacco age first use" phenol type and the CHRNA5/A3/B4 gene cluster in the CADD pooled sample indicate that rs8040868 (CHRNA3), and some other SNPs are significantly associated with the age of tobacco initiation. Therefore, these results indicate that rs8040868 with rs8023462, and rs1948 SNPs of the cluster overlap in their putative association with the age of tobacco and alcohol initiation phenotypes (Schlaepfer et al., 2008).

The data showed that there is a conflicting association between rs8040868 SNPs and NSCLC risk in different population (Lou et al., 2014). Previously, some studies reported that rs8040868 was significantly associated with lung cancer. In a haplotype based association study of 194 familial lung cases and 219 cancer free controls from the Genetic Epidemiology of Lung Cancer Consortium collection, Liu et al., 2009 showed that the rs8040868 SNP in a haplotype significantly was associated with lung cancer. However, Lou et al., found that CHRNA3 rs8040868 SNP was not associated with NSCLC among Chinese males (Lou et al., 2014). It is important to note that, in their previous study, they have found that CHR¬NA3 polymorphism was not associated with NSCLC among non-smoking Chinese (Li et al., 2013). In both mentioned studies, it was suggested that CHRNA3 SNPs were no associated with NSCLC risk in Southern Chinese. In addition, Lou et al., demonstrated that rs8040868 is by itself significantly associated with lung cancer, regardless of the haplotype (Lou et al., 2014).

In the current study, the minor allele frequency (MAF) of CHRNA3 rs8040868 was 0.350 which was inconsistent with the HapMap database. There is a

Table 1. Characteristic of the Identified Polymorphism in the CHRNA3 Exon 3

SNPs	Description	Position	Allele	Function	Protein residue
rs763384023	exon 2	64	T/C	missense	Ser
rs8040868	exon 2	53	A/G	missense	Val
rs2869547	intron	-	T/C	unknown	-

great variability in MAF of CHRNA3 rs8040868 in the HapMap database among different populations. For example, the MAF of rs8040868 was 0.305 among CHB (Han Chinese in Beijing, China), 0.353 among CHD (Chinese in Metro-politan Denver, Colorado), and 0.367 among ASW (Afri¬can ancestry in Southwest USA); the MAF of rs9390123 was 0.500 among CHB, 0.446 among CHD, and 0.337 among ASW (African ancestry in Southwest USA) (www.ncbi.nlm.nih.gov). The reasons for this variability are unknown; however, it is becoming increasingly apparent that ethnic background can play an important role in determining how different alleles are associated with risk of cancer (Jing et al., 2014). In other words, the essentiality of racial diversities may account for the candidate genes for association with NSCLC. Since MAF of SNPs varies significantly between populations, association based on these SNPs will be particularly sensitive to ethnic variability (Zhang et al., 2012).

In summary, it seems that the CHRNA3 rs8040868 may be considered as a pathogenic mutation and associated with clinical phenotype. Polymorphisms are important precursors to development of cancers. Therefore, further clarification of the role of ethnic background in affecting the association of variants with cancer risk is needed.

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