Original article

Genetic Polymorphism of DNA repair gene ERCC2/XPD (Arg 156 Arg) (A22541C) and Lung Cancer Risk in Northern Romania

Polimorfismul Arg 156 Arg (A22541C) al genei de reparare a ADN-ului ERCC2/XPD şi riscul de a dezvolta cancer pulmonar în nordul României

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Abstract

Latest studies suggest that polymorphic DNA repair encoding genes could interfere with the etiopathogeny and therefore individual susceptibility to develop lung cancer. Because ERCC2/XPD gene is generally involved in carcinogenesis, certain mutant polymorphism might be important risk factors in exposure to carcinogens. The purpose of this case-control study is to evaluate for the first time in the Romanian population, ERCC2/XPD (A22541C) allele frequency among lung cancer patients. 65 patients with lung cancer (histological diagnosis) were randomly included in the study group, while 107 unrelated individuals served as controls. ERCC2/XPD (A22541C) allele genotyping was performed using the RFLP molecular technique (restriction fragment length polymorphism), preceded by PCR amplification and enzyme restriction digestion. The results of the study suggest that ERCC2/XPD Arg 156Arg (A22541C) null allele frequency in lung cancer patients, compared to the control group has no significant difference (p=0,127), although XPD mutant genotypes vary according to tumor histological type. XPD Arg 156Arg, AA null allele seems to be associated with lung adenocarcinoma. Our major finding suggests that AA mutant genotype could to be associated with lung adenocarcinoma in smokers.

Key words: *DNA repair genes, genetic polymorphism, lung adenocarcinoma*

Rezumat

Polimorfismele genelor care codifica proteine implicate in repararea ADN-ului ar putea contribui la variabilitatea individuală si susceptibilitatea de a dezvolta cancer pulmonar. Rolul genei ERCC2/XPD în carcinogeneza pulmonara ar putea fi importanta în expunerea la agenți cancerigeni precum fumul de tigara.. Studiul prospectic, randomizat, caz-control care evaluaza frecvența alelei mutante Arg 156 Arg (A22541C) a genei ER-

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CC2/XPD in randul pacienților cu cancer pulmonar. Studiul a inclus 65 de pacienți diagnosticați cu cancer pulmonar (diagnostic confirmat prin examen histopatologic) și 107 de controale (subiecți fara neoplasm pulmonar sau alte patologii cronice pulmonare). Genotiparea ERCC2/XPD a necesitat amplificarea PCR a fragmentelor de interes, fiind urmata de digestia enzimatica specifica. Detectarea alelei Arg 156 Arg (A22541C) a genei ERCC1/XPD a fost determinată prin analiza polimorfismului lungimii fragmentelor de restrictie (RFLP), urmată de electroforeză în gel. Analiza moleculară nu a evidențiat o frecvență crescută a alelei mutante Arg 156Arg (A22541C) ERCC2/XPD în grupul de studiu, comparativ cu grupul de control (p = 0,127). Se pare că polimorfimul genei XPD poate varia în funcție de subtipul histologic tumoral.Varianta alelica nula AA, Arg 156Arg, XPD a fost asociata cu un risc crescut de de a dezvolta adenocarcinom pulmonar. Principala constatare a studiului nostru este aceea că genotipul mutant AA, Arg 156Arg (A22541C) al genei ERCC2/XPD pare să fie asociat cu un risc crescut pentru a dezvolta adenocarcinom pulmonar.

Cuvinte cheie: *gene de reparare a ADN, polimorfism genetic, adenocarcinom pulmonar*

Introduction

Lung cancer is the most common cancer in the world with 1.61 million new cases diagnosed every year (1). Lung cancer is considered to be a multifactorial disease that results from complex interactions between many genetic and environmental factors (2).

Tobacco smoke is the established major cause of lung cancer; cigarette smoke contains many chemicals that are known to chemically modify DNA (3) and lead to different mutations (4).

DNA repair systems are responsible for maintaining the integrity of the genome and play a critical role in protecting against mutations involved in cancer pathogenesis (5-7).

Several medical studies have investigated whether reduced DNA repair capacity is associated with an increased risk of cancer (8).

There are at least four different pathways of DNA repair that operate on specific types of damaged DNA. The nucleotide excision repair (NER) is a versatile, sophisticated DNA damage removal pathway; it is one of the basic mechanisms that mammalian cells use as a major defense system against the carcinogenic effects of UV radiation and chemical exposure (cigarette smoke).

One of the major proteins of NER pathway is XPD, a well conserved helicase, essential for transcription initiation, nucleotide excision repair, cell cycle control and apoptosis. Mutations in XPD gene reduces helicase activity and leads to defects in nucleotide excision repair. Single nucleotide polymorphisms (SNPs) of ERCC2/XPD gene have been described in the last decade. The silent polymorphism, C→A leading to Arg156Arg in exon 6, the G→A polymorphism Asp312Asn in exon 10 and the A→C polymorphism Lys 751Gln in exon 23, are three polymorphisms of ERCC2/XPD gene that have been intensely studied (9).

Recently, several medical studies have shown that inter individual differences in NER pathway like ERCC2/XPD single nucleotide polymorphisms may be associated with lung cancer and basal cell carcinoma (10-14). The results of a well documented study reported a significant association of XPD Arg156Arg and lung cancer risk, suggesting a gene-environmental interaction (15).

In the current study, we aimed to test the hypothesis of an association between genetic variations of Arg156 (A22541C) polymorphism of ERCC/XPD gene and lung cancer susceptibility in Northern Romanian smoking population.

Material and methods

Study population

This is a cross-sectional, randomized case control study evaluating 65 cases with lung cancer and 107 people without lung cancer (control group). Patients diagnosed with lung cancer were recruited between 2009 and 2011, at *Leon Danielo* Pneumology Hospital from Cluj Napoca, Romania. Randomly selected controls were counted in from Medical Clinic I,

Emergency Hospital Cluj. All of the subjects have signed a written informed consent and each participant was personally interviewed by specialist doctors to obtain detailed information on lifetime and family history, associated pathology and of course tobacco use.

Primary lung cancer was confirmed in all cases by pathological examination of a lung tissue sample. We must underline that all patients included in the study are active smokers (active smokers for more than 10 years). A sample of 2 ml of venous blood was then collected from all patients.

Genotyping *(16, 17)*

DNA was extracted from 300 µl peripheral blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, USA).

The ERCC2/XPD Arg156Arg (A22541C) polymorphism was detected using a PCR-RFLP method (polymerase chain reaction followed by enzymatic restriction fragment length polymorphism). The PCR primers were synthesized by Eurogentec (Belgium). Lyophilized primers were dissolved in a 10X volume of water and another 1:10 stock dilution for the final primer in use:

- *Forward primer* 5`- TGG AGT GCT ATC GCA CGA TCT CT - 3'
- *Reverse primer* 5'- CCA TGG GCA TCA AAT TCC TGG GA - 3'

A total amount of 100 ng of genomic DNA was amplified in a total volume of 25µl reaction mixture containing 1.5 nM MgCl2, 20 pM of each primer, 200 µM of each dNTPs and 0.5 units of conventional Taq DNA polymerase (Fermentas).

PCR was carried out using a thermal cycler (Eppendorf Mastercycler Thermal Cycler). The amplification steps consisted of an initial 1 minute denaturation at 96 °C, followed by 30 cycles of denaturation at 94 °C for 30 seconds each, primer annealing at 60 °C for 30 seconds, primer extension at 72 °C for 1 minute and finally, a 2 minutes extension at 72 °C.

The PCR amplification products were digested with 4 units of *Tfi* enzyme (Fermentas) for 6 hours; resulted fragments were then separated on a 2% agarose gel and transilluminated with UV light. ERRC2/XPD Arg156Arg polymorphism A mutant allele presents a *Tfi* I cutting site within the 644–base pair (bp) amplicon product. The *Tfi*I enzyme has an additional restriction site that is used as an internal control for digestion.

Gel electrophoresis defines 3 distinct banding patterns each correspondent for 3 possible genotypes: CC wild type homozygous genotype (corresponding for a 587 bp and 57 bp fragments), AC, heterozygous genotype (corresponding for a 587 bp, 474 bp, 113 bp and 57 bp fragments) and AA mutant homozygous genotype (corresponding for a 474 bp, 113 bp and 57 bp fragments).

Statistical analysis

For statistical analysis we used SPSS 18.0 for Windows (SPSS, Inc., Chicago, IL). Hardy-Weinberg equilibrium test and ORs were determined for a proper evaluation of the relationship between Arg156Arg polymorphism of ERCC2/XPD gene and lung cancer risk. For genotype / tumoral type association we used Adjusted Residual Analysis, Crosstabulation between tumoral type and genotype.

Results

Our study included 65 cases and 107 controls. Patient and control group statistical analysis revealed that there were no significant differences in mean gender and age distribution among controls and lung cancer diagnosed patients.

Molecular analysis of ERCC2/XPD Arg 156Arg (A22541C) did not reveal a statistically increased frequency of mutant allele in the study group compared to the control group (p=0.127). Statistically, mutant allele A frequency was the same among cases and control group (p=0.68).

We evaluated the risk for each of three major lung cancer subtypes: squamous cell carcinoma (39 cases, 60%), small cell carcinoma (12 cases, 18.46 %) and adenocarcinoma (14 cases, 21.53 %). The variant mutant allele A of XPD Arg 156Arg polymorphism was associated with an increase risk of lung adenocarcinoma. The adjusted residual analysis with

Table 1. Association between tumoral type and genotype - Adjusted Residual Analysis

Gender	Tumoral type	Genotype			Total
		AA	AC	CC	
F $\chi^2 = 0.405$ p = 0.247	*None*	-0.2	0.0	0.2	(36)
	ADK	-0.8	-1.2	2.0	(2)
	SC	-0.1	0.9	-0.9	(9)
	S	1.9	-0.9	-0.7	(1)
	Total, n	(11)	(20)	(17)	(48)
M $\chi^2 = 0.287$ p = 0.116	*None*	-2.4*	0.8	1.1	(71)
	ADK	0.8	0.7	-1.3	(12)
	SC	2.2*	-2.0*	0.4	(30)
	S	0.1	1.0	-1.2	(11)
	Total, n	(21)	(61)	(42)	(124)

*p < 0.05
ADK = adenocarcinoma, SC = scuamous cell carcinoma, S = small cell carcinoma

crosstabulation between tumoral type and genotype, holding constant sex, revealed an increased AA genotype frequency among patient with adenocarcinoma comparing to other histological types of cancer (*Table 1*).

Another finding is that AA genotype is associated with an increased risk for scuamous cell carcinoma comparing to other histological subtypes, while CC genotype seems to be protective against this histological subtype.

Discussions

ERCC2/XPD is involved in transcription-coupled nucleotide excision repair; the protein product has a dual function in basal transcription and NER pathway (17). The XPD156-22541C→A single nucleotide polymorphism has been investigated in only a few studies. Recent medical findings suggest that there is no causal relationship between ERCC2/XPD polymorphisms, unrepaired DNA damage, and increased cancer risk (18).

The Arg156Arg (A22541C) polymorphism of ERCC2/XPD gene has no structural abnormalities of aminoacids as consequences, therefore it seems that enzymatic function of the gene product should not be affected by the mutation that may rather influence the rate of translation by altering codon usage or reduce XPD protein levels through an effect on mRNA stability (11, 16).

The complicated pathogenesis of lung cancer involves the association of multiple complex molecular abnormalities that interact over a long period of time (20, 21). Among other genetic mutations involved in lung cancer etiopathogenesis ERCC2/XPD gene encodes several other genes for DNA repair such as ERCC1, XRCC3 and LIGI (22, 23).

Two studies have suggested an association between *XPD*156-22541C (a Caucasian Danish study for basal cell carcinoma (19) and a Chinese study for bladder cancer (24),while other studies found no statistical association between *XPD*156-22541C and cancer (Chinese study for lung cancer) (15).

The results of our study suggest that XPD Arg156Arg (A22541C) polymorphism is not significantly associated with lung cancer risk. Our main finding is that mutant A allele seems to be associated with an increased risk of lung adenocarcinoma

comparing to other histological subtypes. One of the possible explanations would be that adenocarcinoma is regarded as being less related to smoking the major environmental risk factor than small cell or squamous cell carcinoma subtypes.

Our study is the first to investigate the relationship between XPD Arg156Arg (A22541C) and lung cancer risk in northern Romanian population. In conclusion, the results of our study prompt that XPD Arg156Arg (A22541C) polymorphic variant may be considered as a potential risk factor for lung adenocarcinoma in this Romanian population.

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