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XRCC3 THR241MET POLYMORPHISM IS NOT ASSOCIATED WITH LUNG CANCER RISK IN A ROMANIAN POPULATION

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Abstract

Background and aims. *Deoxyribonucleic Acid (DNA) repair mechanisms play a critical role in protecting the cellular genome against carcinogens. X-ray cross-complementing gene 3 (XRCC3) is involved in DNA repair and therefore certain genetic polymorphisms that occur in DNA repair genes may affect the ability to repair DNA defects and may represent a risk factor in carcinogenesis. The purpose of our study was to investigate the association between XRCC3 gene substitution of Threonine with Methionine in codon 241 of XRCC3 gene (Thr241Met) polymorphism and the risk of lung cancer, in a Romanian population.*

Methods. *We recruited 93 healthy controls and 85 patients with lung cancer, all smokers. Thr241Met, XRCC3 gene genotyping was determined by multiplex Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR–RFLP).*

Results. *Statistical analysis (OR, recessive model), did not revealed an increased risk for lung cancer, for the variant 241Met allele and Thr241Met genotypes (p=0.138, OR=0.634, CI=0.348-1.157; p=0.023, OR=0.257, CI=0.085-6.824). Also, there were no positive statistical associations between Thr241Met polymorphism of XRCC3 gene, gender, tobacco and various histopathological tumor type of lung cancer.*

Conclusion. *In conclusion, the results of the study suggest that the XRCC3 gene Thr241Met polymorphism is not associated with an increased risk for the development of lung cancer in this Romanian group.*

Keywords*:* lung cancer, XRCC3gene, Thr241Met polymorphism

Introduction

Lung cancer is currently the major cause of neoplastic related deaths in the world [1]. In the past century, lung cancer has been transformed from a rare disease [2] into a global problem [3]. Lung neoplasia causes more deaths than breast, colorectal and prostate cancers all together. An estimated 158,040 Americans are expected to die from lung cancer in 2015, accounting for approximately 27 percent of all neoplastic related deaths [4]. Over the last two decades, the incidence of lung cancer has increased steadily in Romania, ranking second as cause of death following cardiovascular diseases [5].

Although lung cancer is not considered a genetic disorder, there are some predisposing genetic risk factor, involved in carcinogen induced genotoxicity like DNA

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stability mechanisms that were associated with a higher risk for lung cancer development in environmentally exposed individuals [6,7]. It has become clear that the susceptibility to lung cancer varies from one individual to another, and is highly related to smoking that result in carcinogenic adducts that block the transcription of critical genes or result in "hot spots" mutations. Nevertheless, only 10% of cigarette smokers and individuals of second-hand smoke develop lung cancer in their lifetime, which may be partially attributed to polymorphisms in certain genes involved in (GSTs, Kras, XPA, and ERCC2) [8,9].

The X-ray repair cross-complementing group 3 (XRCC3) belongs to a family of genes responsible for repairing DNA double strand breaks caused by normal metabolic processes and/or exposure to ionizing radiation. It encodes an important protein that functions in the homologous recombination repair of a DNA double-strand break [10]. In recent years, there have been several studies and meta-analyses for XRCC3 genetic polymorphisms and lung cancer, but results are conflicting and thus inconclusive [11,12,13]. Also, there is little information regarding the connection of *XRCC3*, Thr241Met polymorphism and lung cancer in the Eastern European Caucasian population which is why we considered it appropriate to conduct a study in a group of Romanian population. The purpose of the study was to establish the frequency of Thr241Met gene variant of *XCRR3* gene in a Romanian population group and to investigate whether this polymorphism is involved in the susceptibility to lung cancer in this population group.

Materials and methods

Patients and controls

All subject included in the current study gave the written informed consent approved by the Ethics Committee of the conducting institution (Iuliu Hatieganu University of Medicine and PharmacyCluj-Napoca, Romania). The study was conducted according to the Helsinki Declaration.

A group of 178 individuals were included in the study. The study group included 85 cases diagnosed with lung cancer, all smokers (active smokers with more than 15 cigarettes/day). Lung malignancy was confirmed by imaging (CT scan) and histopathological examination and subtype tumour classification was made according to World Health Organization (WHO) criteria [14]. The controls included 93 healthy volunteers, also smokers, with no history of any malignancy or chronic lung pathology.

Genotypic analysis of XRCC3 gene

Genomic DNA was extracted from 400 µl venous blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, USA) and ZymoBead Genomic DNA Kit (ZymoResearch, USA). Thr241Met polymorphisms of *XRCC3* gene was genotyped using a multiplex PCR–RFLP technique. 100 ng of genomic DNA was amplified in a total volume of 25 µl reaction mixture containing reaction buffer of 1.5 nM MgCl2, 10 pmol of each primer (F5'-GGTCGAGTGACAGTCCAAAC3' and R5'-TGCAACGGCTGAGGGTCTT3') (Eurogentec Belgium) 200 µm of each dNTPs and 0.5 units of Taq polymerase. Termocycling conditions were carried out as follows: 94° C for 5 minutes followed by 35 cycles each of 94° C for 40 seconds, 63° C for 1 minute, 72° C for 50 seconds and a final polymerization step at 72° C for 10 minutes (Gradient Mastercycler, Eppendorf, Germany). The amplified DNA products were submitted for a 10 minute digestion with 4 U of *NlaIII* restriction enzyme (Fast digest NlaIII, Fermentas, ThermoScientific Biosciences GmbH, Germany). Resulting fragments were separated on a 2.5 % agarose gel (MetaPhor®, FMC BioProducts, Rockland, ME, USA), allowing detection by ethidium bromide staining. There are 3 possible genotypes each defined by 3 distinct banding patterns: wild type Thr/Thr (316,140 bp), heterozygous Thr/Met (316,211, 140,105 bp) and variant homozygous Met/Met (211,140,195 bp).

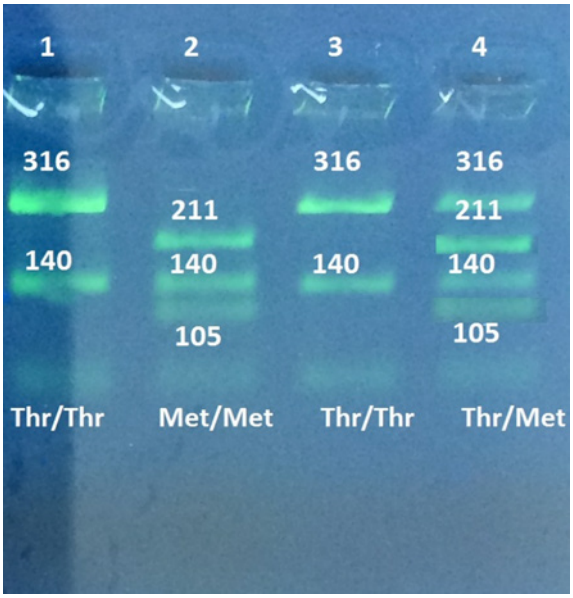

Figure 1. Gel electrophoresis for the analysis of Thr241Met polymorphisms of XRCC3 gene.
(lane 1 – homozygous Thr/Thr genotype, lane 2- homozygous Met/Met genotype,
lane 3 - homozygous Thr/Thr genotype, lane 4 – heterozygous Thr/Met genotype).

Statistical analysis

The distribution of genotype and allele frequency of Thr241Met *XRCC3* polymorphisms between different groups was compared by the Fisher's exact test, followed by comparative analysis according dominant and recessive models. For estimation of the relative risk and strength of association we calculate odds ratio (OR) at 95% confidence interval (CI). We considered statistically significant a p-value less than 0.05. Statistical analysis was carried out using SPSS 18.0 for Windows software (SPSS, Inc., Chicago, Il., USA).

Results

The common tumor type identified for lung cancer group was squamous cell carcinoma (70.6%), with a frequency of 13.6% in women and 86.4% men, followed by adenocarcinoma (20,1%), of which 27.2% women and 72.8% men. Small cell squamous carcinoma was the rarest diagnosed histological type (9.3%). The average age of diagnosis was 64.3 years with the lowest average age of onset of clinical symptoms recorded in patients with adenocarcinoma (ADK) 57 years (CI = 52.8 to 63.6 years).

Genotype distribution and allele frequency of Thr241Met *XRCC3* polymorphism in patients with lung cancer and controls are presented in Table I.

Comparative analysis (Fisher Test) following dominant and recessive models for variant carriers lung cancer risk is presented in Table II.

Comparative analysis to assess the risk for lung cancer in the study group compared with the control group for variant *XRCC3* gene 241Met allele carriers (Fisher test analysis Odd ratios dominant model), does not identifies a statistical significance risk for the study group (p=0.138, OR=0.634, CI=0.348-1.157). Also, statistical comparative analysis (Odd ratios Fisher test for recessive model), did not reveal any significant differences among patients and controls, for homozygous Met241Met genotype (p=0.023, OR=0.257, CI=0.085-6.824). Comparative analysis of Trh241Met polymorphisms of *XRCC3* gene in patients with lung cancer according to gender and histopathological type did not reveal any statistical significant association between variant 241Met allele and different histological types of lung cancer. I would like to specify that both patients and controls are active smokers consuming 15 to 45 cigarettes a day. In terms of the amount of tobacco consumed in relation to Trh241Met polymorphisms of *XRCC3* gene and tumor subtypes there were no statistical differences identified in this study group.

Table I. Genotype distribution and allele frequency of Trh241Met polymorphisms of *XRCC3* gene in patients with lung cancer and controls..

Polymorphisms	***Variant***	***Lung cancer n (%)***	***Controls n (%)***	***OR (95%CI)***	***p value***
XRCC3 Trh241Met	*Trh/Trh*	*52 (61.2)*	*45 (50)*	*0.3 (0.160-0.589)*	*0.048*
	Trh/Met	*20 (23.5)*	*41 (45.6)*	*0.3 (0.090-0.969)*	*0.044*
	Met/Met	*13 (15.3)*	*4 (4.4)*	*3.88 (1.212-12.427)*	*0.020*
	Trh allele frequency	*122 (72.2)*	*131 (72.7)*	*0.81 (0.499-1.320)*	*0.402*
	Met allele frequency	*47 (27.8)*	*49(27.3)*	*2.27(1.451-3.539)*	*0.943*

**Statistical significant for p<0.050*

Table II. Comparative analysis of Thr241Met polymorphisms of *XRCC3* gene in patients with lung cancer (Fisher Test).

Genotype	**Model**	**Z**	**p-value**	**OR**	**CI**
XRCC3 Thr241Met	*Dominant*	*1.483*	*0.138*	*0.634*	*0.348-1.157*
	Recessive	*2.285*	*0.023*	*0.257*	*0.085-6.824*

*Statistical significance for p<0.050

Table III. Comparative analysis of Thr241Met polymorphisms of *XRCC3* gene in patients with lung cancer according to gender and histopathological type. (Fisher's Exact Test).

Histopathological type	***Allele***	***Males***	***Females***	***p***
Squamous cell carcinoma	*241 Trh*	43	7	0.629
	241 Met	8	2	
Adenocarcinoma	*241 Trh*	8	1	*0.102*
	241 Met	4	4	
Small cell carcinoma	241 Trh	4	3	0.634
	241 Met	1	0	

Discussion

Lung cancer remains the most lethal cancer worldwide, despite recent improvements in diagnostic and therapeutic techniques [1]. As cigarette smoke contains several thousand chemicals that are known to chemically modify DNA and therefore lead to mutations in targeted cells, smoking remains one of the major risk factors in lung cancer etiology [15]. NER (Nucleotide Excision Repair) is a versatile DNA repair system that removes a wide range of DNA lesions and play a critical role in the cellular response that counteracts the carcinogenic effects of DNA damage, maintaining genome integrity, therefore genetic polymorphisms in DNA repair genes may contribute to susceptibility to lung cancer [16]. One of these polymorphisms, located in exon 7, causing the replacement of Thr241Met in 241 site, which consequently has an effect on the enzyme activity as well as its restoration function of damaged DNA, has been linked to neoplastic disease. Many studies have examined the association between the *XRCC3* gene Thr241Met polymorphism and lung cancer risk in various populations, but their results have been inconsistent, especially that most of the available data regards Asian populations [9,10,11].

To our knowledge, this is the first study that has performed a comparative analysis of Thr241Met polymorphisms of *XRCC3* gene in patients with lung cancer in Romania. Overall, our study provides evidence that Thr241Met XRCC3 gene variant is not associated with lung cancer susceptibility in this Caucasian group of Romanian subjects.

A recent meta-analysis taking into account a total of 21 studies, including 6880 lung cancer cases and 8329 controls, showed that there is no clear evidence showing a significant correlation between XRCC3 Thr241Met polymorphism and lung cancer risk in total population and stratified analysis by ethnicity [11]. Other meta-analysis including 17 independent case-control studies involving 12,610 subjects with different ethnicities did not support any appreciable association between the XRCC3 Thr241Met polymorphism and lung cancer risk in studied populations [17,18,13].

As the results of the present study highlight that *XRCC3* Thr241Met polymorphism is not associated with a higher risk for lung cancer, our results come in agreement with reported data in literature, which do not associate this genetic variant with lung cancer, although these information are mostly linked to Asian population which might present certain genetic particularities compared to Caucasian population. Currently, there is no specialized data about the distribution of the Trh241Met polymorphism alleles and genotypes in Romanian population, but the results of the current study highlights that there are no significant differences between the studied groups compared with the existing data in the literature.

Given that all subjects in the study are smokers and tobacco smoke induces the formation of oxidative DNA damage, we investigated whether reduced repair of oxidative DNA damage is associated with lung cancer, but there was no evidence of any connection between the amount of consumed tobacco and *XRCC3* Thr241Met polymorphism.

We also assessed a possible connection between Trh241Met polymorphisms of *XRCC3* gene in patients with lung cancer according histopathological type but there were no statistical significant association between the variant allele and different histological types. Literature data regarding the variant allele 241Met of *XRCC3* gene in different histological types of lung cancer are also ambiguous. A study including 291 Asian of origin lung cancer diagnosed patients, suggested that 241Thr allele of *XRCC3* gene has a weak protective effect on adenocarcinoma [19]. Another study assumes that Thr241Met genotype of *XRCC3* gene may act as a favorable prognostic indicator for lung squamous cell carcinoma patients [20]. Unfortunately, one of the limitations of our study is that it does not follow the evolution of patients, to evaluate a possible connection between certain genotypes or variant alleles and different histopathological types of lung cancer in our patients. Still, we tried to see if there is any link between gender, variant Trh241Met alleles of *XRCC3* gene and lung cancer susceptibility, but we did not find any statistical relevant results. An older study revealed that Thr241Met polymorphism of *XRCC3* gene is associated with a protective effect on lung cancer in non-smoking women, and possibly an increased lung cancer risk among smoking men, but still further studies did not reach statistical significance [20]. Other researchers, admit that there might be a positive correlation between this Thr241Met variant of the *XRCC3* gene and lung adenocarcinoma in non-smoker Asian women [21]. Because our study has a small number of women as subjects and all are smokers, we cannot draw any valid conclusions regarding a possible association between the investigated genetic polymorphism and gender associated lung cancer risk.

In conclusion, our results showed no evidence of a relationship between the Trh241Met polymorphism of *XRCC3* gene and lung cancer risk in smoker individuals. Taking into account the small number of investigated subject and lack of data regarding the possible genetic particularities of the East European Caucasian population, the precise relationship between the *XRCC3* gene Thr241Met variant and lung cancer risk needs further confirmation in future studies with larger available data.

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