

POLYMORPHISMS OF THE DNA REPAIR GENES *XRCC1* AND *ERCC4* ARE NOT ASSOCIATED WITH SMOKING- AND DRINKING-DEPENDENT LARYNX CANCER IN A POLISH POPULATION

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Background: Tobacco smoking and alcohol drinking generate oxidative DNA damage and may contribute to larynx carcinogenesis. The X-ray repair cross complementing 1 (*XRCC1*) and excision repair cross-complementing rodent repair deficiency, complementation group 4 (*ERCC4*/*XPF*) genes are important components of DNA excision repair systems, which repair DNA damage induced by various factors, including tobacco smoking and alcohol. **Aim:** To investigate the association between the genotypes of the *XRCC1*-Arg399Gln (rs25487) and *ERCC4*-Arg415Gln (rs1800067) polymorphisms and smoking- and drinking-related larynx cancer in a Polish population. **Methods:** The polymorphisms were determined by PCR-RFLP method in 253 patients with squamous cell carcinoma of the larynx and 253 sex- and age-matched controls. **Results:** We did not find any association between the investigated polymorphisms and larynx carcinoma, dependent on either smoking or drinking status. No association was found between these polymorphisms and larynx cancer grade, stage or age at diagnosis. **Conclusions:** The results indicated that Arg399Gln polymorphism of *XRCC1* gene and Arg415Gln polymorphism of *ERCC4* gene may not be associated with smoking- and drinking-related larynx cancer in Polish population. **Key Words:** smoking, alcohol consumption, *XRCC1*, *ERCC4*, larynx cancer, gene polymorphism.

Tobacco smoking and alcohol exposure are well known risk factors in the development of squamous cell carcinoma of the larynx. It was suggested that single nucleotide polymorphisms (SNPs) in the DNA repair genes may alter the ability of cells to repair damaged DNA, and initiate malignant transformation. X-ray repair cross-complementing 1 (*XRCC1*) gene coding for the XRCC1, one of a key protein of base excision repair (BER) pathway. Excision repair cross-complementing rodent repair deficiency, complementation group 4 (*ERCC4*/*XPF*) gene coding for the ERCC4/*XPF* protein one of essential parts of nucleotide excision repair (NER) pathway. A G → A substitution at 28152 of the *XRCC1* gene, exon 10, producing an Arg to Gln change in codon 399 (the Arg399Gln polymorphism) has been linked to slightly (marginally statistically significant) increased risk of laryngeal carcinoma among Caucasians [1]. The ERCC4/*XPF* G1244A polymorphism is a G-to-A change in the exon 8 (Arg415Gln, rs1800067) that results in a change from arginine to glutamine at 415 codon. This polymor-

phism has been reported to be associated with an increased risk for breast cancer [2].

In this paper, we have used a hospital-based case-control study in Poland to assess the potential association of the *XRCC1*-Arg399Gln and *ERCC4*-Arg415Gln SNPs with the development and clinical pathological parameters of larynx cancer.

Patients. Blood samples were obtained from 253 patients: 194 men and 59 women with larynx cancer (squamous cell carcinoma) treated in the Department of Head and Neck Cancer, Medical University of Lodz, Lodz, Poland and Department of Otolaryngology, Medical University of Poznan, Poznan, Poland in 2005–2009 and 253 cancer-free age and sex matched controls. The patients ranged in age from 48 to 86 years (median age 63 years). Grade of differentiation (G) was evaluated in all cases (according to World Health Organization criteria). There were 138 cases of 1 grade, 104 cases of 2 grade and 11 of 3 grade in total. According to TNM staging there were 60 cases of stage I, 33 cases of stage II, 113 cases of stage III, 41 cases of stage IVA and 6 of stage IVB. The study was approved by Ethic Committee of Medical University of Lodz and Medical University of Poznan, and each patient gave a written consent.

DNA isolation. Peripheral blood lymphocytes (PBLs) were isolated by centrifugation in a density gradient of Histopaque-1077 (15 min, 280g). The pellet containing PBLs was resuspended in Tris-EDTA buffer, pH 8, to give about 1–3 × 10⁵ cells/ml. Genomic DNA was extracted from PBLs by phenol/chloroform extrac-

Received: January 11, 2011.

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Abbreviations used: BER – base excision repair; *ERCC4* (*XPF*) – excision repair cross-complementing rodent repair deficiency, complementation group 4 gene; NER – nucleotide excision repair; OR – odds ratio; PBLs – peripheral blood lymphocytes; PCR – RFLP – polymerase chain reaction - restriction fragment length polymorphism; SNPs – single nucleotide polymorphisms; *XRCC1* – the X-ray repair cross complementing 1 gene.

tion and proteinase K digestion. The final samples were kept in Tris-EDTA buffer, pH 8, at -20°C until use.

Genotype determination. The PCR — restriction fragment length polymorphism method (PCR – RFLP) was used to detect the genotypes of the Arg399Gln polymorphism of *XRCC1* gene and the Arg415Gln polymorphism of *ERCC4* gene as described previously [3, 4].

Data analysis. For each genotype, deviation of the frequencies in the controls from those expected under Hardy — Weinberg equilibrium was assessed using the standard χ^2 -test. Genotype frequencies in cases and controls were compared by χ^2 or Fisher's exact tests. The genotype-specific risks were estimated as odds ratios (ORs) by unconditional logistic regression. Wild type Arg/Arg genotype was used as the reference. Because of small number of Gln/Gln variant genotypes of both polymorphisms odds ratios were calculated for Gln allele carriers (Arg/Gln + Gln/Gln genotypes) p -values < 0.05 were considered to be significant.

All statistical analyses were performed with Sigma-Stat, ver. 8.0 (Systat Software Inc, San Jose, CA, USA).

We showed previously that heavy alcohol drinking and tobacco smoking increased significantly the risk of laryngeal carcinoma independently of any genetic variation (OR = 28; $p < 0.0001$ for smoking and drinking heavily) [5]. Tobacco smoke contains many kinds of carcinogens that can cause DNA lesions, and variations in repair of tobacco carcinogen-induced DNA lesions may contribute to the variation in susceptibility to cancer [6].

It was suggested that SNP at codon 399 of the *XRCC1* gene may alter the ability of *XRCC1* to repair damaged DNA [7]. It was also shown that the 399Gln homozygote genotype was associated with increased levels of bulky-DNA adducts in leucocytes of non-smokers [8].

From the PCR analysis, 253 larynx cancer patients and 253 controls were divided into three genotypes: Arg/Arg, Arg/Gln and Gln/Gln for both gene polymorphisms. Distributions of genotypes did not differ significantly from those predicted by the Hardy-Weinberg equilibrium. There were no significant differences in the frequencies of both polymorphisms genotypes between patients and controls ($p > 0.05$). Additionally, odds ratio analysis did not show any relation between this polymorphisms and larynx cancer (data not shown).

All 253 larynx cancer cases were stratified by grade of differentiation and stage of disease. Grades 2 and 3 were grouped together for the purposes of statistical analysis. There were no significant differences between

distributions of genotypes in subgroups assigned to histological grades and TNM stages (data not shown).

To check whether DNA repair gene polymorphisms were associated with tobacco smoking, participants were categorized into groups according to smoking habits (Table). There were no significant differences inside these groups in the Arg399Gln and Arg415Gln polymorphisms genotypes distribution ($p > 0.05$). Additionally, we did not find any association of investigated polymorphisms and drinking status (Table). We did not find any association of drinking and smoking status and laryngeal cancer for carriers of both variant genotypes of *XRCC1* and *ERCC4* genes (data not shown).

Our results indicated that Arg399Gln polymorphism of *XRCC1* gene and Arg415Gln polymorphism of *ERCC4* gene may not be associated with smoking- and drinking-related larynx cancer in Polish population.

ACKNOWLEDGEMENTS

This work was supported by the grants N403 2955 33 from the Ministry of Science and Higher Education and 505/376 from the University of Lodz, Poland.

REFERENCES

1. Yang Y, Tian H, Zhang ZJ. Association of the *XRCC1* and *hOGG1* polymorphisms with the risk of laryngeal carcinoma. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2008; **25**: 211–3 (In Chinese).
2. Smith TR, Levine EA, Perrier ND, et al. DNA-repair genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1200–4.
3. Romanowicz-Makowska H, Smolarz B, Kulig A. Polymorphisms in *XRCC1* and *ERCC4/XPF* DNA repair genes and associations with breast cancer risk in women. *Pol Merkur Lekarski* 2007; **22**: 200–3 (In Polish).
4. Krupa R, Blasiak J. An association of polymorphism of DNA repair genes *XRCC1* and *XRCC3* with colorectal cancer. *J Exp Clin Cancer Res* 2004; **23**: 285–94.
5. Pawlowska E, Janik-Papis K, Rydzanicz M, et al. The Cys326 allele of the 8-oxoguanine DNA N-glycosylase 1 gene as a risk factor in smoking- and drinking-associated larynx cancer. *Tohoku J Exp Med* 2009; **219**: 269–75.
6. Friedberg EC. DNA damage and repair. *Nature* 2003; **421**: 436–40.
7. Duell EJ, Wiencke JK, Cheng TJ, et al. Polymorphisms in the DNA repair genes *XRCC1* and *ERCC2* and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis* 2000; **21**: 965–71.
8. Matullo G, Palli D, Peluso M, et al. *XRCC1*, *XRCC3*, *XPB* gene polymorphisms, smoking and 32P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 2001; **22**: 1437–45.

Table. Genotype distribution and odds ratio (OR) for Arg399Gln polymorphism of *XRCC1* gene and Arg415Gln polymorphism of *ERCC4* gene for laryngeal cancer patients and controls

	Arg399Gln - <i>XRCC1</i> , number of patients/controls				Arg415Gln - <i>ERCC4</i> number of patients/controls			
	Arg/Arg	Arg/Gln	Gln/Gln	OR ^a / p	Arg/Arg	Arg/Gln	Gln/Gln	OR ^a / p
<i>smokers</i>								
never	12/50	12/56	8/17	1.23/0.30	29/106	2/17	1/0	1.13/0.69
ever	33/30	46/33	22/13	0.83/0.30	88/70	11/6	2/0	1.07/0.80
moderate	17/12	17/15	7/4	0.81/0.30	37/28	4/3	0/0	1.06/0.85
heavy	31/13	36/9	12/1	0.76/0.19	67/20	9/3	3/0	0.98/0.95
<i>drinkers</i>								
never	24/45	26/50	10/17	1.32/0.17	55/99	4/13	1/0	1.05/0.88
moderate	54/51	62/55	34/15	0.83/0.30	130/108	16/13	4/0	1.03/0.77
heavy	15/9	23/8	5/3	0.81/0.30	36/17	6/3	1/0	1.09/0.79

Note: ^a – odds ratio adjusted with age and sex; odds ratio calculated for Arg/Gln + Gln/Gln genotype carriers, Arg/Arg genotype served as the reference (OR = 1.00).