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 444 of the ERCC4 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 polymorphism 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 x 10⁶ cells/ml. Genomic DNA was extracted from PBLs by phenol/chloroform extrac-
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 χ²-test. Genotype frequencies in cases and controls were compared by χ² or Fisher’s exact tests. The genotype-specific risks were estimated as odds ratios (ORs) by unconditional logistic regression. As reference, we used the Arg/Arg genotype. Because of small number of Gln/Gln variant genotypes, ORs 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 SigmaStat, 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**


**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.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>number of patients/controls</th>
<th>ORa/p</th>
</tr>
</thead>
<tbody>
<tr>
<td>never</td>
<td>12/50</td>
<td>12/56</td>
</tr>
<tr>
<td>ever</td>
<td>33/30</td>
<td>46/33</td>
</tr>
<tr>
<td>moderate</td>
<td>17/12</td>
<td>17/15</td>
</tr>
<tr>
<td>heavy</td>
<td>31/13</td>
<td>36/9</td>
</tr>
<tr>
<td>Drinkers</td>
<td>never</td>
<td>24/45</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>54/51</td>
</tr>
<tr>
<td></td>
<td>heavy</td>
<td>15/9</td>
</tr>
</tbody>
</table>

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).