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The Role of Chosen Polymorphisms in Genes Coding Xenobiotic Metabolizing Enzymes and DNA Repair Proteins in Laryngeal Cancers

Znaczenie wybranych polimorfizmów w genach kodujących enzymy metabolizujące ksenobiotyki i białka naprawy DNA w raku krtani

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Abstract
Background. Laryngeal cancer (LSCC – larynx squamous cell carcinoma) is the most common head and neck cancer (HNSCC – head and neck squamous cell carcinoma). The etiology of laryngeal cancer is complex, influenced by inter alia genetic alterations (e.g. SNPs, single nucleotide polymorphisms).
Objectives. The authors investigated the association between laryngeal cancer and 22 polymorphisms of 8 chosen genes involved in: *i)* Phase I of xenobiotic metabolism, such as: 1) *CYP1A1,* 2) *CYP2A6* and 3) *CYP2D6* and *ii)* mechanisms of DNA repair, such as 1) *XRCC1*, *OGG1* (BER, base excision repair), 2) *XPC*, *XPD* (NER, nucleotide excision repair) and 3) *XRCC3* (HR, homologous recombination repair). The next step of our analysis was to look for possible associations between the clinical course of the disease and the polymorphisms mentioned above.
Material and Methods. The authors studied a group of 60 patients with primary LSCC and 100 cancer-free individuals formed the control group. The clinical course of 30 randomly-selected patients was observed. The laryngeal cancer patients and cancer-free controls were genotyped by PCR or PCR-RFLP, as appropriate. Fisher's exact test for association and the Cochran-Armitage test were used to investigate the association between the chosen polymorphisms and cancer. The Benjamini-Hochberg procedure for multiple testing was adopted. The Mann-Whitney test and Kendall test for correlation were also used (using the R and SPSS statistical packages).
Results. The authors found nine associations between the polymorphisms of the studied genes (*CYP1A1, CYP2A6* and *XPC* PAT, *XRCC3, OGG1)* and laryngeal cancer. The association of *XPC* PAT and several *CYP1A1* polymorphisms with cancer was particularly clear, remaining significant after the use of the Benjamini-Hochberg procedure for multiple testing. In addition, the authors found a few statistically significant associations between the polymorphisms analyzed and the available clinical data. However, none of the associations was significant after the application of the Benjamini-Hochberg procedure.
Conclusions. The results indicate an important role of polymorphisms of *CYP1A1* and *XPC, XRCC3, OGG1* genes in determining the risk of LSCC. However to clarify possible associations between polymorphisms and clinical data, further large studies are needed (**Adv Clin Exp Med 2011, 20, 5, 583–590**).

Key words: DNA repair, xenobiotic metabolizing enzymes (XME), genetic polymorphisms, LSCC – laryngeal squamous cell carcinoma, SNP – single nucleotide polymorphism.

Streszczenie

Wprowadzenie. Rak krtani (LSCC – płaskonabłonkowy rak krtani) należy do najczęstszych nowotworów głowy i szyi (HNSCC – płaskonabłonkowy rak głowy i szyi). Etiologia raka krtani jest złożona, obejmuje m.in. wpływ zmian genetycznych (np. SNP, polimorfizm pojedynczych nukleotydów).
Cel pracy. Badano związek między rakiem krtani a 22 polimorfizmami wybranych 8 genów zaangażowanych w *i)* metabolizm ksenobiotyków pierwszej fazy, jak: 1) *CYP1A1,* 2) *CYP2A6,* 3) *CYP2D6*, i *ii)* mechanizmy naprawy

DNA, jak 1) *XRCC1*, *OGG1* (BER – naprawa przez wycięcie zasady); 2) *XPC*, *XPD* (NER – naprawa przez wycięcie nukleotydu); 3) *XRCC3* (HR – naprawa przez homologiczną rekombinację). Dodatkowo przeprowadzono analizę korelacji między przebiegiem klinicznym a wyżej wymienionymi polimorfizmami.

Materiał i metody. Grupą badaną było 60 pacjentów z pierwotnym LSCC, grupą kontrolną 100 zdrowych osób. Przebieg kliniczny analizowano u 30 wyselekcjonowanych pacjentów z LSCC. Genotypowanie przeprowadzono w grupie badanej i kontrolnej metodą PCR lub PCR-RFLP. Analizę statystyczną przeprowadzono w programach R i SPSS z użyciem testów Fischera, Manna-Whitneya, Kendalla, Cochran-Armitage oraz procedury Benjamini-Hochberg.

Wyniki. Znaleziono 9 pozytywnych korelacji między określonymi polimorfizmami badanych genów (*CYP1A1, CYP2A6* i *XPC* PAT, *XRCC3, OGG1)* i rakiem krtani. Stwierdzono dodatkowo kilka statystycznie istotnych korelacji między analizowanymi polimorfizmami i dostępnymi danymi klinicznymi. Niemniej żadna z nich nie była statystycznie znacząca po zastosowaniu procedury Benjaminiego-Hochberga do testowania wielokrotnego.

Wnioski. Wyniki badań wskazują na znaczącą rolę zarówno polimorfizmów genu *CYP1A1*, jak i *XPC, XRCC3, OGG1* w modyfikacji ryzyka LSCC. Aby wyjaśnić możliwe asocjacje między polimorfizmami a danymi klinicznymi, wskazane jest rozszerzenie badań **(Adv Clin Exp Med 2011, 20, 5, 583–590)**.

Słowa kluczowe: naprawa DNA, enzymy metabolizujące ksenobiotyki (XME), polimorfizm genetyczny, LSCC – płaskonabłonkowy rak krtani, SNP – polimorfizm pojedynczych nukleotydów.

Laryngeal cancer (LSCC – larynx squamous cell carcinoma) is the most common head and neck cancer (HNSCC – head and neck squamous cell carcinoma). Males are more susceptible to LSCC than females (a male/female ratio of 10/1) [1]. The etiology of laryngeal cancer is complex, influenced by both genetic alterations (e.g. SNPs, single nucleotide polymorphisms) and exposure to carcinogens. Tobacco smoking and alcohol consumption are known environmental risk factors. Their metabolites may contribute to DNA damage, for example directly by oxidative stress and strand breaks. Moreover, genetic variants (SNPs) in DNA repair genes may affect an individual's susceptibility to LSCC. Therefore, we selected several polymorphisms of genes from the cytochrome P-450 (CYP) subfamily and from three DNA repair pathways, base excision repair (BER), nucleotide excision repair (NER) and homologous recombination repair (HR) [2].

Cytochrome P-450 1A1 (CYPA1A), 2A6 (CYP2A6) and 2D6 (CYP2D6) – oxidative enzymes – are involved in Phase I of xenobiotic metabolism. Their products are usually more carcinogenic than xenobiotics alone. It has been proven that SNPs in genes encoding xenobiotic metabolizing enzymes (XMEs) can modulate cancer risk [2].

BER, NER and HR are different DNA repair pathways involved in the repair of various and nonspecific DNA damage e.g. simple base modifications, nonbulky adducts and double-strand breaks [3]. Numerous studies have suggested a relationship between DNA repair genes and the risk of HNSCC [4–6].

The study was aimed at: 1) elucidating the possible associations between laryngeal cancer and polymorphisms of selected genes: *i)* involved in Phase I of xenobiotic metabolism, such as: *CYP1A1, CYP2A6* and *CYP2D6,* and *ii)* various mechanisms of DNA repair, such as 1) BER: *XRCC1, OGG1,* 2) NER: *XPC*, *XPD* and 3) HR: *XRCC3*; 2) searching for associations between the clinical course of the disease and the polymorphisms mentioned above.

Material and Methods

In the first step of the study, the authors analyzed a group of 60 Caucasian patients diagnosed with primary LSCC (55 male and 5 female, with a mean age of 57.70 years and a standard deviation of 6.93 years) in regard to the following polymorphisms of DNA repair genes – 1) BER: *XRCC1* $Arg^{399}Gln$, *OGG1* $Ser^{326}Cys$; 2) NER: *XPC* Lys^{939} Gln, *XPC* C/A (i11), *XPC* PAT, *XPD* $Asp^{312}Asn$, *XPD* $Lys^{751}Gln$; 3) HR: *XRCC3* $Thr^{241}Met$.

Blood samples from the study group were collected before chemotherapy and/or radiotherapy at the Department of Otolaryngology, Wroclaw Medical University, Poland.

In the second step the authors selected a group of 30 patients (30 men) with available clinical data, including histopathological as well clinical data (the localization of the tumor, TNM classification), and the clinical course of the disease (period of treatment, progression or remission of the disease, recurrence of laryngeal tumor, second primary tumors).

Summary of clinical data:

- location of cancer: supraglottis – 19 cancers, glottis – 11 cancers;
- histopathological examination showed 20 cases of keratinizing laryngeal squamous cell carcinoma and 10 cases of nonkeratinizing laryngeal squamous cell carcinoma;
- second TNM classification: 9 cases of T1–T2, 14 of T3 and 7 cases of T4; in 27 cases there were no lymph metastases (N0 – 27 cancers, N > 0 – 3 cancers); M0 – all cases
- the median follow-up time was 34.9 months (range, 5 to 68 months);
- the recurrence of LSCC was observed in 8 patients (more than 26% of patients); progres-

sion of the disease in 7 patients (more than 23%) and incomplete remission in 1 person; in 22 people complete remission was observed;
– lung cancer (5 patients), malignant melanoma (1 patient) and oral cancer (1 patients) were diagnosed as second primary tumors.

For these patients, analysis was performed on chosen polymorphisms encoding xenobiotic metabolizing enzymes, such as: 1) *CYP1A1*: M1/ M3 T/C, M2 A/G, M4 C/A, M5 G/A, M6 C/T, M7 G/A; 2) *CYP2A6*: 1013 A/G; 3) *CYP2D6* *2 G/C; *3 delA, *4 G/A, *5 deletion of the whole gene, *10 C/T, *14G/A, *17 C/T.

The control group consisted of 100 individuals (70 female and 30 male) from the Department of Internal Diseases, Clinical Hospital, Świebodzice, Poland. No one had cancer or a family history of cancer. None had been exposed to any known mutagens in the 3 months prior to blood donation. The average age of the individuals in the control group was 74.89 years with a standard deviation of 7.63 years. Because the most common age of larynx cancer patients in the population is 55–69 years of age, the authors decided that the control group should be older.

The design of the study was accepted by Wroclaw Medical University's Ethics Committee. All the individuals involved were informed about the study plan, which was documented in written form.

Analysis of Polymorphisms

Genomic DNA was extracted from peripheral blood lymphocytes using a standard proteinase K/ /phenol method [7]. The polymorphisms in the

Table 1. Frequency of chosen polymorphisms in DNA repair genes in the study and control groups

Tabela 1. Częstość występowania polimorfizmów genów naprawy DNA w grupie badanej i kontrolnej

Gene/allele (Gen/allele)	Genotype (Genotyp)	Control group % (Grupa kontrolna)	Study group % (Grupa badana)
OGG1 Ser326Cys	Cys/ Cys	0 (0.0)	4 (6.6)
	Ser/ Cys	33 (33.3)	25 (41.7)
	Ser/ Ser	67 (67.7)	31 (51.7)
XRCC1 Arg399Gln	G/G	37 (37.0)	15 (25.4)
	A/G	51 (51.0)	31 (52.5)
	A/A	12 (12.0)	13 (22.0)
XPC Lys939Gln	A/A	43 (43.0)	17 (28.4)
	A/C	46 (46.0)	32 (53.3)
	C/C	11 (11.0)	11 (18.3)
XPC C/A (i11)	A/A	35 (35.0)	20 (33.3)
	A/C	50 (50.0)	31 (51.7)
	C/C	15 (15.0)	9 (15.0)
XPC PAT	–/–	37 (37.0)	10 (16.7)
	+/–	47 (47.0)	23 (38.3)
	+/+	16 (16.0)	27 (45.0)
XPD Asp312Asn	Asp/ Asp	32 (32.7)	19 (31.7)
	Asp/ Asn	55 (56.1)	30 (50.0)
	Asn/ Asn	11 (11.2)	11 (18.3)
XPD Lys751Gln	A/A	32 (32.0)	20 (33.4)
	A/C	46 (46.0)	26 (43.3)
	C/C	22 (22.0)	14 (23.3)
XRCC3 Thr241Met	C/C	51 (51.0)	17 (28.3)
	C/T	36 (36.0)	33 (55.0)
	T/T	13 (13.0)	10 (16.7)

selected DNA repair genes were determined by a PCR-RFLP method as previously described [8]. In order to genotype *OGG1* Ser^{326}Cys, the authors used the method described by Le Marchand et al. [9].

Statistical Analysis

Fisher's exact test for association and the Cochran-Armitage test were used to investigate the association between the chosen polymorphisms and cancer. The Benjamini-Hochberg procedure for multiple testing was adopted. The Mann-Whitney test and Kendall test for correlation were also used (using the R and SPSS statistical packages). The Peto method was used to estimate odds ratios (calculated relative to wild type homozygotes).

Results

In the first step of the analysis, the authors analyzed the frequencies of the chosen polymorphisms of DNA repair genes in the study group (n = 60) compared to the control group (n = 100) (Table 1).

The authors found a significant association between the following polymorphisms and susceptibility to cancer (in each case Fisher's exact test of association was used): a) *XPC* PAT (p = 0.000173). It was noted that individuals with at least one + allele are more susceptible than those with the -/- genotype, OR = 2.65 [1.31,5.34] and those with a +/+ genotype are particularly susceptible to cancer, OR = 5.45, 95% CI = [2.36, 12.57]) b) those with a T allele (^{241}Met allele) at the *XRCC3* Thr^{241}Met (C722T substitution) locus are more susceptible to cancer (p = 0.00530, OR = 2.51, 95% CI = [1.32, 4.79]), c) *OGG1* Ser^{326}Cys (p = 0.0110, individuals with at least one Cys allele seem to be more susceptible than those with the Ser/Ser genotype, OR = 1.90, 95% CI = [0.99,3.66]). The association between *XPC* PAT and cancer remains significant after the application of the Benjamini-Hochberg procedure for multiple testing.

In the next step, the frequency of chosen polymorphisms of XME-s genes in the group of clinically well-characterized patients (n = 30) was compared to the controls (n = 100) (Table 2).

Using Fisher's exact test of association, the following polymorphisms were found to be strongly associated with cancer after the application of the Benjamini-Hochberg correction for multiple testing. The associations are:

- *CYP1A1* M2 A/G – Individuals with at least one G allele are more susceptible to LSCC, p = 1.85×10^{-15} (i.e. the first non-zero digit appears 15 places after the decimal point, OR = 38.97, 95% CI = (16.42, 96.80));
- *CYP1A1* M4 C/A – Individuals with at least one A allele are more susceptible to LSCC, p = 1.08×10^{-9} , OR = 64.13, 95% CI = (19.26, 213.55);
- *CYP1A1* M6 C/T – Individuals with at least one T allele are more susceptible to LSCC, p = 9.2×10^{-9}, OR = 11.33, 95% CI = (2.21, 42.33);
- *CYP1A1* M1/M3 T/C – Individuals with at least one C allele are more susceptible to LSCC, p = 7.77×10^{-6} , OR = 11.33, 95% CI = (4.21, 30.44);
- *CYP1A1* M5 G/A – Individuals with at least one A allele are more susceptible to LSCC, p = 0.003, OR = 3.62, 95% CI = (1.58, 8.33);
- *CYP2A6* 1013 A/G – Individuals with at least one G allele are more susceptible to LSCC p = 9.156×10^{-7} , OR = 8.50, 95% CI = (3.67, 19.67).

The following significant associations were found between the clinical data and the studied polymorphisms. However, none of these associations are significant after using the Benjamini-Hochberg procedure for multiple testing. Note that only the two most common histopathologies, keratinizing and nonkeratinizing, were considered in the analysis:

- size of tumor (T) and *CYP1A1* M5 G/A (the number of G alleles is positively associated with the value of T, p = 0.014, Kendall test of correlation);
- histopathology and *OGG1* Ser^{326}Cys (the Cys allele is associated with keratinizing LSCC and the Ser allele with nonkeratinizing, p = 0.014, Cochran-Armitage test);
- histopathology and *XPC* Lys^{939}Gln (heterozygotes are associated with nonkeratinizing LSCC, p = 0.0098, Fisher's exact test);
- histopathology and *XPC* (i11) (heterozygotes are associated with nonkeratinizing LSCC, p = 0.019 Fisher's exact test). In relation to the previous result, it should be noted that the *XPC* Lys^{939}Gln and *XPC* (i11) polymorphisms are in clear linkage disequilibrium in both groups;
- recurrence and *CYP1A1* M5 G/A (heterozygotes are less likely to have a recurrence (p = = 0.0077, Fisher's exact test);
- remission and *CYP2A6* 1013 AG (the A allele is associated with progression, p = 0.016, Cochran-Armitage test).

The authors observed an interesting correlation between drinking habits and *XPC* (i11) polymorphism in the patient group (the C allele is associated with higher levels of drinking). It might be that those who have the CC genotype but do not drink are less susceptible to cancer than those who have the AA genotype, while those who have

Table 2. Frequency of chosen polymorphisms in XME genes in the study and control groups

Tabela 2. Częstość wybranych polimorfizmów genów XME w grupie badanej i kontrolnej

Gene/allele (Gen/allele)	Genotype (Genotyp)	Control group % (Grupa kontrolna)	Study group % (Grupa badana)
CYP1A1 M1/M3 T/C	T/T	88 (88.0)	14 (46.7)
	T/C	12 (12.0)	14 (46.7)
	C/C	0 (0.0)	2 (6.7)
CYP1A1 M2 A/G	A/A	88 (88.0)	3 (10.0)
	A/G	11(11.0)	15 (50.0)
	G/G	1 (1.0)	12 (40.0)
CYP1A1 M4 C/A	C/C	97 (98.0)	15 (50.0)
	C/A	2 (2.0)	14 (46.7)
	A/A	0 (0.0)	1 (3.3)
CYP1A1 M5 G/A	G/G	67 (67.7)	11 (36.7)
	G/A	29 (29.3)	16 (53.3)
	A/A	3 (3.0)	3 (10.0)
CYP1A1 M6 C/T	C/C	88 (88.0)	14 (46.7)
	C/T	12 (12.0)	8 (26.7)
	T/T	0 (0.0)	8 (26.7)
CYP1A1 M7 G/A	G/G	93 (93.0)	26 (86.7)
	G/A	7 (7.0)	3 (10.0)
	A/A	0 (0.0)	1 (3.3)
CYP2A6 1013 A/G	A/A	74 (74.0)	7 (23.3)
	A/G	26 (26.0)	10 (33.3)
	G/G	0 (0.0)	13 (43.3)
CYP2D6 *2 G/C	G/G	41 (41.0)	16 (53.3)
	G/C	47 (47.0)	11 (36.7)
	C/C	12 (12.0)	3 (10.0)
CYP2D6 *3 del A	G/G	95 (95.0)	25 (83.3)
	G/A	5 (5.0)	4 (13.4)
	A/A	0 (0.0)	1 (3.3)
CYP2D6 *4 G/A	G/G	89 (89.0)	24 (80.0)
	G/A	10 (10.0)	7 (20.0)
	A/A	1 (1.0)	0 (0.0)
CYP2D6 *10 C/T	C/C	29 (96.7)	25 (83.3)
	C/T	1 (3.3)	5 (16.7)
	T/T	0 (0.0)	0 (0.0)
CYP2D6 *14 G/A	G/G	80 (80.0)	19 (63.3)
	G/A	19 (19.0)	11 (36.7)
	A/A	1 (1.0)	0 (0.0)

CYP2D6 *5 deletion of the whole gene and *CYP2D6* *17 C/T were not analyzed due to their lack of variability.

the CC genotype and drink are more susceptible to cancer. However, since the authors have no data on the drinking habits of the control group, this question requires further study.

Discussion

Laryngeal cancer has become one of the most common cancers in the developed world [10]. The carcinogenesis process of LSCC depends largely on exposure to environmental carcinogens, mainly alcohol consumption and tobacco smoking. Therefore, the activity of enzymes involved in xenobiotic metabolism and DNA-repair underlies an individual's susceptibility to cancer [11]. Single nucleotide polymorphisms may alter carcinogen metabolism, as well as DNA repair capacity, thus modulating the risk of cancer.

This study analyzed 22 variants of XMEs and DNA-repair genes. The authors found associations between nine of these polymorphisms (*CYP1A1, CYP2A6, CYP2D6* and *XPC* PAT, *XRCC3, OGG1)* and laryngeal cancer. The activity of DNA-repair enzymes is crucial in the maintenance of genome integrity. The reported that +/+ genotypes of *XPC* PAT [an intronic and biallelic poly(AT) insertion/deletion polymorphism] modify the risk of LSCC. The authors have confirmed the results obtained by Shen et al. that *XPC* PAT+ polymorphism may contribute to a higher risk of HNSCC [12]. However Yang et al. found an association between this polymorphic variant and an increased risk for HNSCC in non-Hispanic whites, but revealed no effect of the *XPC* PAT polymorphism on HNSCC risk in the Korean population [13]. The other authors, Uchida et al. [14] and Qiu et al. [15], suggested that some haplotypes *XPC* may lead to decreased DNA-repair activity and thus affect an individual's risk of cancer. For example, haplotype *XPC* (i11)/ /*XPC* PAT/ *XPC* Lys^{939}Gln (exon 15) is consistent with data from Martin et al., who have shown that the A allele of *XPC* (i11) is associated with the PAT+ and the C allele of exon 15, and the C allele *XPC* (i11) with PAT- and the A allele of exon 15 [16]. The *XPC* (i11) polymorphism (A homozygote) leads to an increased frequency of exon 12 skipping and as a result decreases DNA-repair activity (NER pathway). It has been suggested that decreased DNA-repair capacity may modulate cancer risk [15].

The authors have found an association between the T allele (^{241}Met allele) of the of *XRCC3* gene and a higher risk of LSCC. The *XRCC3* Thr^{241}Met polymorphism (^{241}Met allele) is associated with increased DNA adduct levels in healthy donors and nonsmokers and a predisposition to cancer, inter alia to HNSCC [4, 17]. The results of the last study of Sliwinski et al. indicated that the CT and TT genotype did increase both the risk of hyperplastic laryngeal lesions and head and neck squamous cell carcinomas. The authors suggest the use of these polymorphisms as a predictive factor of precancerous lesions for HNSCC in the Polish population [5]. No effect on HNSCC cancer risk with the *XRCC3* Thr^{241}Met polymorphism was found by Huang et al. [18]. This result is consistent with data in oral/pharynx and larynx cancer cases from Benhamou et al. [19] and Shen et al. [12].

In the present study the authors also observed an association between the *OGG1* ^{326}Cys polymorphism and the risk of laryngeal cancer. The OGG1 enzyme appears to play an important role in defining the risk of HNSCC, especially in orolaryngeal cancer. Elahi et al., who demonstrate that OGG1 is expressed in the larynx, found an association between the *OGG1* genotype and the risk of smoking- and alcohol-related orolaryngeal cancer [20]. Two authors found a significant association between the *OGG1* ^{326}Cys homozygote and HNSCC [20, 21]. This result is consistent with present data, where ^{326}Cys may play an important role in laryngeal cancer. Paz-Elizur et al. suggest that low-activity OGG (which may be associated with *OGG1* Ser^{326}Cys) may be useful in the early detection of HNSCC in smokers [22]. However, no significant associations between the *OGG1* Ser^{326}Cys polymorphism, tumor characteristics and radiotherapy results were observed by Monteiro et al. [23]. Those authors conclude that this polymorphism is not an important factor in treatment decisions for patients with laryngeal cancer. No association between *OGG1* Ser^{326}Cys polymorphism and the risk of HNSCC was found by Zhang et al. in a large hospital-based case-control study [24]. The discrepancies between the results of Zhang et al. and Elahi et al. may be due to differences in the study and control populations (sample size and the frequencies of variant alleles) or the type of HNSCC cancer [20, 24].

In addition to the previously described correlations, the authors found 6 associations between polymorphisms of XMEs (*CYP1A1, CYP2A6*) and LSCC. CYP1A1 is a crucial enzyme involved in Phase I of the xenobiotic metabolism of, among other things, tobacco procarcinogens (i.e. polycyclic aromatic hydrocarbons -PAHs and aromatic amines). This study revealed that among the six studied SNPs in *CYP1A1*, five are associated with laryngeal cancer. Similar results to those presented were recently published by Tai et al. [25]. Their data highlighted the strong association between both *CYP1A1* A^{4889}G (M2) and ^{6235}C (M1) variants and laryngeal and hypopharyngeal cancer in the Han Chinese population. It has been proven that

the $T^{6235}C$ (M1/M3) transition and $A^{4889}G$ (M2) transition of the *CYP1A1* gene are associated with higher enzymatic activity and have been described as a factor of genetic susceptibility to lung cancer [26]. Moreover, the $A^{4889}G$ (M2) SNP influences the frequency of chromosomal aberrations (CA) in non-smokers [27].

The authors have confirmed the results obtained by Gajecka et al. concerning the M2 polymorphism of the *CYP1A1* gene [28]. They investigated six XME genes, *CYP1A1, CYP2D6, CYP2E1, NAT2, GSTM1* and *GSTT1,* in 289 laryngeal cancer patients and 316 cancer-free controls (all Polish). However, the meta-analysis carried out by Zhuo et al. on 35 studies regarding *CYP1A1* and *GSTM1* polymorphisms among laryngeal cancer patients revealed that among Caucasians, only the *CYP1A1* MspI (M2) polymorphism increases the risk of developing laryngeal cancer. They did not find any relationship between the *CYP1A1* M2 (exon 7) and *GSTM1* null polymorphisms and laryngeal cancer in Asians and Caucasians [29].

Gajecka et al. found a correlation only between the *CYP1A1* M4 polymorphism and laryngeal cancer [28]. Indeed, the authors have confirmed the opinion that the A allele of the *CYP1A1* gene may play an important role in determining the risk of developing LSCC. To the best of authors knowledge, this is the first publication that mentions the significance of the T allele of the *CYP1A1* M6 polymorphism in the etiology of LSCC.

The authors have found that the G allele of the *CYP2A6* $A^{1013}G$ polymorphism increases the risk of laryngeal cancer. There is no data revealing the relationship between this polymorphism and LSCC. However, as the CYP2A6 enzyme is responsible for the initiation of nicotine metabolism, there is a positive correlation between the polymorphism of *CYP2A6* and a variety of cancers, i.e. lung, oral colorectal and gastric [30].

In this analysis of 22 polymorphic sites in both XMEs and DNA-repair genes, the authors also took into consideration available clinical data. They did not find any clearly statistically significant correlations. Because of the low number of patients, to draw any strong conclusions, it would be necessary to increase the sample size.

Regarding the association between drinking habits and the *XPC* int11 polymorphism in the study group, the authors do not have data about drinking habits in the control group, so they cannot draw any conclusions. None of the other associations between the clinical data and particular polymorphisms were significant after the application of the Benjamini-Hochberg procedure (and also, the data sets are small).

In conclusion, it seems reasonable to continue the study on a larger group of patients with LSCC.

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