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SHORT COMMUNICATIONS

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POLYMORPHISMS OF THE DNA BASE EXCISION REPAIR GENE *MUTYH* IN HEAD AND NECK CANCER

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Background: **Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. The major risk factors of HNSCC are smoking and alcohol consumption. Genetic polymorphisms of DNA repair enzymes may lead to genetic instability and carcinogenesis. *MUTYH* gene encodes a DNA glycosylase that can initiate the base excision repair (BER) pathway and prevent G:C > T:A transversion by excising adenine mispaired with 8-hydroxyguanine produced by reactive oxygen species (ROS). *Aim:* to perform a case-control study to test the association between polymorphism in the *MUTYH* gene: Tyr165Cys and head and neck cancer risk progression. *Methods:* Genotypes were determined in DNA from peripheral blood lymphocytes of 193 patients (among them 97 subjects with precancerous hyperplastic laryngeal lesions and 96 subjects with head and neck cancer) and 140 age, sex and ethnic-matched cancer-free controls by tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR). *Results:* We found an association between head and neck cancer risk and the Tyr165Tyr variant of the *MUTYH* gene (OR 2.18; 95% CI 1.19–3.97). For Tyr165Tyr genotype we also observed positive correlation with cancer progression assessed by tumor size (OR 4.56; 95% CI 1.60–12.95). We did not observe any correlation between Tyr165Cys polymorphism of *MUTYH* gene and precancerous hyperplastic laryngeal lesions risk. *Conclusion:* The Tyr165Tyr polymorphic variant of the *MUTYH* gene may be associated with head and neck cancer in Polish population.**

Key Words: ***MUTYH*****, gene polymorphism, head and neck cancer.**

Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. Overall survival is low, especially in developing countries, and the major risk factors of HNSCC are smoking and/or alcohol consumption [1]. Tobacco and alcohol consumption are the main etiological factors in head and neck carcinogenesis [2]. Head and neck carcinogenesis is associated with abnormalities in DNA repair, apoptosis, carcinogen metabolism and cell-cycle control [3–5].

Polymorphisms of DNA repair genes may influence variation in individual DNA repair capacity, which is crucial for preventing genomic instability and in turn may be associated with risk of cancer [6]. Among the various DNA repair pathways, base excision repair (BER) is considered to play a key role by removing DNA damage from oxidation, deamination, and ring fragmentation [7]. Exposure to tobacco smoking can increase production of reactive oxygen species (ROS), which have the potential to induce oxidative damage, like the stable guanine adduct 8-oxoguanine [8]. Moreover, the variation in ability of glycosylases MUTYH or OGG1 involving in BER may be associated with cancer risk occurrence [9]. MUTYH excises the misincorporated adenine opposite 8-oxoguanine, and thus prevents 8-oxoguanine-induced mutagenesis. An increased susceptibility to spontaneous carcinogenesis of the liver, lung or intestine was observed in MUTYH-null mice [10]. Moreover Germ-line MUTYH mutations predispose persons to a recessive phenotype, multiple adenomas, or polyposis coli [11].

In this paper we described a case-control study performed to test the association between single nucleotide polymorphisms (SNPs) of *MUTYH* gene and the occcurence as wel as progression of head and neck cancer. We investigated *MUTYH* polymorphism of a G → A transition producing a Tyr → Cys substitution at codon 165 located in chromosome 1 (the Tyr165Cys polymorphism; rs34612342) in patients with precancerous hyperplastic laryngeal lesions (PHLL), patients with head and neck cancer and cancer-free controls.

Patients. Blood samples were obtained from 193 patients, among them 97 subjects had precancerous hyperplastic laryngeal lesions (80 men and 17 women; median age 52, quartiles: 37, 74 years) and 96 subjects have been diagnosed with head and neck cancer (83 men and 13 women; median age 54, quartiles: 37, 76 years). Control samples consisted of age- (± 5 years) and sex-matched 140 cancer-free blood donors. The diagnosis of cancer was made after histopathological examination of patients biopsies. Patients with precancerous hyperplastic laryngeal lesions were selected during direct laryngoscopy with the minimal clinical follow-up 10 years. Patients and controls subjects enrolled to the examination were also matched in smoking attitude or alcohol consumption. Prior to examination, the patients

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Abbreviations used: BER – base excision repair; HNSCC – head and neck squamous cellcarcinoma; OGG1 – 8-oxoguanin DNA glycosylase; PHLL – precancerous hyperplastic laryngeal lesions; ROS – reactive oxygen species; SNPs – single nucleotide polymorphisms; T-ARMS-PCR – tetra primer amplification refractory mutation system PCR.

and control subjects, did not receive medicaments like antibiotics or steroids. All patients and controls subjects were recruited from three medical units of Head and Neck Neoplasm Surgery Departments, Medical University of Lodz, Poland. All subjects included into the study were unrelated Caucasians and inhibited Lodz district, Poland. The study was approved by the Local Ethic Committee and written consent was obtained from each patient or healthy blood donor before enrolling into the study.

Chemicals and reagents. QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA was obtained from Qiagen (Chatsworth, CA, USA). All reagents for PCR reaction were from Qiagen. Elektrophoresis was conducted in TAE buffer.

Genotyping. Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit for isolation of high-molecular-weight DNA. Multiplex Tetra-Primer Amplification Refractory Mutation System PCR was used to detect the genotypes of the Tyr165Cys polymorphism of the *MUTYH* gene associated with head and neck cancer. T-ARMS-PCR amplified both wild-type and mutant alleles, together with a control fragment, in a single tube PCR reaction. The region flanking the mutation was amplified by 2 common (outer) primers, producing a non–allele-specific control amplicon 200 bp in length:

Fo 5'GGGACTGACGGGTGATCTCTTTGACCTCTG 3'
Ro 5'CCTCTACCACCTGATTGGAGTGCAAGACTC 3'

Two allele-specific (inner) primers:

Fi(G) 5'GGTGAATCAACTCTGGGCTGGCCTGGGATG 3'
Ri(A) 5'CTGCAGCCGCCGGCCACGAGAATCGT 3'

were designed in opposite orientation and, in combination with the common primers, simultaneously amplified both the wild-type and the mutant amplicons 100 bp and 155 bp in length respectively. PCR primers and conditions for amplification was described previously by Piccili et al. [12]. The 2 allele-specific amplicons have different lengths were separated by 8% polyacrylamide gel electrophoresis. More than 10% of the samples were repeated, and the results were 100% concordant.

Data analysis. Distribution of genotypes and alleles between groups were tested using chi-square tests. Potential linkage between genotype and cancer was assessed by the logistic regression. Analyses were performed using STATISTICA 6.0 package (Statsoft, Tulsa, OK, USA).

The genotypes of patients and controls were scored according to Tyr165Cys polymorphism of *MUTYH* gene (Figure). All distributions did not differ significantly ($P > 0.005$) from those predicted by the Hardy-Weinberg equilibrium. An statistically significant association between head and neck cancer occurrence and the Tyr165Tyr genotype was found (OR 2.18; 95% CI 1.19–3.97) (Table 1). Additionally, there were statistically significant differences in the frequency of Tyr^{165} allele between HNSCC patients and controls (OR 1.81; 95% CI 1.05–3.13), while there were not observed any differences in the frequency of the Cys^{165} allele (see Table 1). Finally, we did not observe any correlation between Tyr165Cys polymorphism of *MUTYH* gene and precancerous hyperplastic laryngeal lesions risk.

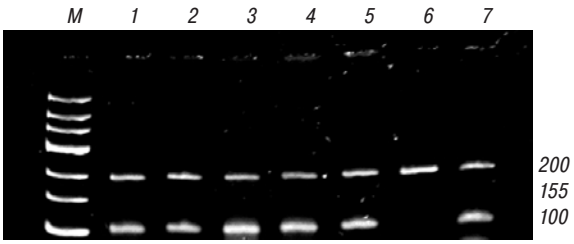

Figure. Representative T-ARMS-PCR for the Tyr165Cys polymorphism of *MUTYH* gene. Lane M, DNA marker 100 bp; lanes: 1, 2, 5, 7 wild-type control 100 bp band; lanes: 3, 4 Tyr165Cys heterozygote; 100 and 155 bp bands and lane: 6 Cys165Cys homozygote 155 bp band. In each line 200 bp product indicate a positive control of PCR. T-ARMS-PCR products were electrophoresed on a 8% polyacrylamide gel containing ethidium bromide. *MUTYH* T-ARMS-PCR band sizes are indicated on the right of panel

Table 2 shows the distribution of genotypes and frequency of alleles in groups of patients suffer head and neck cancer with different tumour size (T) and node status (N). We found statistically significant differences in distribution of the Tyr165Tyr genotype (OR 4.56; 95% CI 1.60–12.95) and frequency of Tyr^{156} allele (OR 17.39; 95% CI 7.10–42.59) in group of patients with different tumour size, when patients with T3 and T4 grades were compared with patients with T1 and T2 grade. Additionally, there was no differences in the distribution of genotypes and frequency of alleles in patients with positive (N1 + N2 + N3 + N4) and negative (N0) lymph node.

Table 1. The genotype and allele frequency and odds ratio (OR) of the Tyr165Cys polymorphism of the *MUTYH* gene in head and neck cancer (HNSCC) and precancerous hyperplastic laryngeal lesions (PHLL)

	HNSCC (n = 96)	Controls (n = 140)		PHLL (n = 97)	
Genotype or Allele	Frequency	Frequency	OR (95% CI)	Frequency	OR (95% CI)
Tyr165Tyr	0.80	0.64	**2.18 (1.19–3.97)**	0.53	0.66 (0.39–1.12)
Tyr165Cys	0.19	0.36	0.43 (0.23–0.79)	0.43	1.28 (0.75–2.17)
Cys165Cys	0.01	–	–	0.04	–
165Tyr	0.90	0.82	**1.81 (1.05–3.13)**	0.74	0.66 (0.42–1.03)
165Cys	0.10	0.18	0.55 (0.32–0.95)	0.26	1.51 (0.97–2,36)

Notes: "CI" – confidence interval; "–" – not estimated.

Table 2. The genotype and allele frequency and odds ratios (OR) of the Tyr165Cys polymorphism of *MUTYH* gene in patients with head and neck cancer with different tumor size and lymph node status

	Tumour size			Node status		
Genotype or Allele	T3 + T4 Frequency	T1+ T2 Frequency	OR (95% CI)	N1 + N2 + N3 + N4 Frequency	N0 Frequency	OR (95% CI)
Tyr/Tyr	0.89	0.63	**4.56 (1.60–12.95)**	0.81	0.78	1.14 (0.41–3.21)
Tyr/Cys	0.11	0.34	0.25 (0.09–0.71)	0.19	0.20	0.96 (0.34–2.73)
Cys/Cys	–	0.03	–	–	0.02	–
Tyr	0.94	0.80	**17.39 (7.10–42.59)**	0.90	0.88	1.23 (0.47–3.20)
Cys	0.06	0.20	0.06 (0.02–0.14)	0.10	0.12	0.82 (0.31–2.13)

Notes: "CI" – confidence interval; "–" – not estimated.

An association between the risk of occurrence and progression of the head and neck cancer and the Tyr165Cys polymorphism of the *MUTYH* gene of BER-repair DNA pathway has not been investigated previously. Our results suggest that this polymorphism may increase the risk of head and neck cancer in Polish population (OR 2.18 for homozygous Tyr165Tyr). We also found an association between Tyr165Tyr genotype of *MUTYH* gene and head and neck cancer progression assessed by tumour size (OR 4.56). However, we did not observe any correlation between Tyr165Cys polymorphism of *MUTYH* gene and precancerous hyperplastic laryngeal lesions risk. Thus, this polymorphism may not reflect the progression of PHLL as it is allowed to be determined by classic histological grading.

There is only a few data reported an investigation of *MUTYH* gene polymorphisms in association with cancer occurrence. Previously, it was found that recessive mutations of the *MUTYH* gene predispose to multiple colorectal adenomas and carcinoma [13–18]. Other reports have suggested that the Gln324His variant of *MUTYH* gene is strongly associated with colorectal cancer in Japanese population [19, 20]. In contrast, Gorgens et al. have not found any correlation between polymorphisms of *MUTYH* gene and the etiology of a head and neck cancer [21], but they investigated Val22Met, Gln324His and Val315Met variants and they did not investigated Tyr165Cys. The other reason that no correlation was found by Gorgens et al. might be extremely small number of subjects enrolled to their study (29 patients and 30 controls). In our study we investigated an association of Tyr165Cys polymorphism of *MUTYH* gene in group of 193 patients (among them 97 subjects with precancerous hyperplastic laryngeal lesions and 96 subjects with head and neck cancer) and 140 cancer-free controls. Our study is the first that reported an assaociation of Tyr165Tyr polymorphic variant of *MUTYH* gene with head and neck cancer.

Evidences consistently linking SNPs in the DNA repair genes with pathogenesis of head and neck cancer are still limited. In the post-genomic era we think about orchestration of the expression of the genome rather, than about acts of expression of individual genes. However, to construct a reliable microarray dedicated to any cancer a large set of SNPs data should be obtained. We suggest that the Tyr165Cys polymorphism of the *MUTYH* gene may be considered as an early diagnostic marker in occurrence and progression of the head and neck cancer.

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