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ASSOCIATION OF THE CYTOCHROME P450 AND ARYLAMINE N-ACETYLTRANSFERASE GENE POLYMORPHISMS WITH THE INCIDENCE OF HEAD AND NECK CANCER IN POLISH POPULATION

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Abstract
Objectives: Head and neck cancer (HNC) is one of the most common cancers. Most exogenous HNC is head and neck squamous cell carcinomas. Scientists are striving to develop diagnostic tests that will allow the prognosis of HNC. The aim of the study was to determine the risk of HNC. The research concerned changes caused by polymorphisms in genes encoding proteins responsible for the metabolism of xenobiotics. **Material and Methods:** In group of 280 patients with HNC, the occurrence of polymorphic variants in *NAT1*(rs72554606), *NAT2*(rs1799930), *CYP1A*(rs1799814), *CYP2D*(rs3892097) were studied with TaqMan technique. The control group consisted of 260 cancer free people. The TNM scale was analyzed. Gene interactions of genotyped polymorphisms were investigated. The effects of smoking and alcohol consumption on HNC were assessed. **Results:** The results indicated an increased risk of HNC in *NAT1* polymorphisms in the *GC* genotype (OR = 1.772, 95% CI: 1.184–2.651, p = 0.005) and *NAT2* polymorphism in the *GA* genotype (OR = 1.506, 95% CI: 1.023–2.216, p = 0.037). The protective phenomenon in the *CYP1A* polymorphism the *GT* genotype (OR = 0.587, 95% CI: 0.381–0.903, p = 0.015) and the *TT* genotype (OR = 0.268, 95% CI: 0.159–0.452, p = 0.001). The coexistence of *GA-GC* polymorphisms (OR = 2.687, 95% CI: 1.387–5.205, p = 0.003) in *NAT2-NAT1* genes increases the risk of HNC. Risk-reducing effect in the polymorphism *GG-GT* (OR = 0.340, 95% CI: 0.149–0.800, p = 0.011), *GG-TT* (OR = 0.077, 95% CI: 0.028–0.215, p < 0.0001), *GA-TT* (OR = 0.250, 95% CI: 0.100–0.622, p = 0.002), *AA-GT* (OR = 0.276, 95% CI: 0.112–0.676, p = 0.002) in *NAT2-CYP1A* genes. In the *CYP2D-CYP1A* genes in the polymorphisms *CT-CC* (OR = 0.338, 95% CI: 0.132–0.870, p = 0.020), *TT-GG* (OR = 0.100, 95% CI: 0.027–0.359, p = 0.001, *TT-GC* (OR = 0.190, 95% CI: 0.072–0.502, p = 0.0004), *TT-CC* (OR = 0.305, 95% CI: 0.107–0.868, p = 0.024). Correlation was noted between cigarette smoking and HNC (OR = 7.297, 95% CI: 4.989–10.674, p < 0.0001) and consuming alcohol (OR = 1.572, 95% CI: 1.003–2.464, p = 0.047). **Conclusions:** The *CYP1A* polymorphism shows a protective association with HNC. On the other hand, *NAT2, NAT1* polymorphism influence the susceptibility to developing HNC. The coexistence of the *NAT2-NAT1* genotypes increases the risk of HNC. In contrast, *NAT1-CYP1A* and *CYP1A-CYP2*D reduce this risk. Smoking and alcohol consumption increase the incidence of HNC. Int J Occup Med Environ Health. 2023;36(6):812–24

Key words:
xenobiotics, head and neck cancer, *CYP1A*, *NAT2*, *CYP2D*, *NAT1*

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INTRODUCTION

Head and neck cancer (HNC) are classified into oral, laryngeal and pharyngeal cancer. Most exogenous HNC are head and neck squamous cell carcinomas (HNSCC). In 2012 according to Global Cancer Observatory (GLOBOCAN) there were 500 000 new HNC cases, HNC was the sixth most common cancer after breast, prostate, lung, cancer, colon cancer [1]. According to statistics, men are more often affected because of their lifestyle. About 80% of HNC squamous cell carcinomas occur with smoking and alcohol abuse. There is also evidence that the human papilloma virus (HPV) is responsible for HNSCC. Studies confirm the presence of HPV in about half of oropharyngeal cancers [2]. Despite the increasing awareness of people and the promotion of a healthy lifestyle, the incidence of HNC remains high. [3].

The incidence factors of head and neck cancer can be divided into a group of external and internal factors. External factors include smoking and excessive alcohol consumption.

The listed factors are considered to be the main cause of HNC [4]. Human papilloma virus and ultraviolet radiation are factors that increase the incidence of HNC [5]. The presence of this type of neoplasm in patients whose history did not indicate smoking or drinking alcohol indicated the possibility of hereditary features of HNSCC. These include: genetic syndromes, Falconi anemia and congenital dyskeratosis, nuclear DNA mutations, Li-Fraumeni syndrome are internal factors [6].

The diagnosis of cancer itself is based on computed tomography or resonance imaging. Endoscopic examinations are performed. There are no effective screening tests for this type of cancer yet. Scientists are trying to find the right test for the detection or progression of the disease.

The research is directed to analysis of changes caused by polymorphisms in genes encoding proteins responsible for the metabolism of xenobiotics [7]. Xenobiotics are divided into 2 groups:

- phase I enzymes (cytochrome P450 family: CYP2E1, CYP2C19, CYP2D6 and mEH), which metabolically activate potentially carcinogenic forms,
- phase II enzymes (N-acetyl- and glutathione-S-transferases families: NAT1, NAT2 , GSTT1, GSTM1 and GSTP1) [8].

Cytochromes (CYP) are proteins that belong to the superfamilies that contain heme as a cofactor. Their other name is hemoproteins and they are used as substrates in enzymatic reactions. Cytochromes play a significant role in the first phase of the enzymatic reaction, which consists of detoxification, metabolic activation through oxidation, reduction, and hydroxylation. These enzymes are involved in the metabolism of arachidonic acid and its derivatives, including prostaglandins, prostacyclins, leukotrienes, thromboxanes, cholecalciferol, etc. Many CYP are involved in epoxidation, reduction, and oxidation of more than 100 eicosanoid moieties involved in maintaining homeostasis. The CYP enzymes are involved in the metabolism of xenobiotic substances [9]. The CYP catalyzes many oxidation and reduction reactions involving broad substrate specificity. Thus, a single compound may be metabolized by various CYP isoenzymes in a complex biotransformation possible through multiple pathways resulting in multiple metabolites. Conversely, a unique compound can be metabolized by a single CYP from different metabolites [10]. The activation of CYP enzymes is influenced by many factors, including the environment, medical condition, alcohol abuse and taking medications. Arylamine N-acetyltransferases (NAT) catalyze an acetylation reaction in which the acetyl group from the acetyl coenzyme A (acetyl-CoA) cofactor is transferred to substrates including drugs and carcinogens [11,12]. The *NAT1* is found in many tissues, the greatest amount is found in the colon, *NAT2* is in the liver and intestine and is mainly responsible for the metabolism of drugs. The *NAT2* participates in the initial biotransformation metabolism of aromatic amines and hydrazines and catalyzes the trans-

fer of the acetyl group from acetyl-CoA to the substrate of the nitrogen. Selected *NAT2* polymorphisms lead to amino acid substitutions, which may result in impairment of enzyme activity [13]. The *NAT2* polymorphism determines the risk of certain diseases and the rate of inactivation of various xenobiotics and drugs [14]. The reports to date indicate a possible contribution of *CYP* and *NAT* gene polymorphisms in the increased risk of breast, lung and colorectal cancers [15]. Also for HNC, the possible influence of these polymorphisms on the increased risk of cancer has been noticed [16].

In the present study, 2 groups of polymorphisms were investigated: *CYP* rs1799814 and rs3892097 polymorphisms and *NAT* rs72554606 and rs1799930 polymorphism selected single nucleotide polymorphisms (SNPs) for *CYP* genes may affect the metabolic capacity of endogenous substances, leading to physiological or pathological changes [17]. Further, the authors have also evaluated the gene–gene interactions that might provide further information on the HNC cancer risk associated with the combinations of genes that are involved in the biotransformation and transport of xenobiotics. The gene–gene interaction or epistasis is a unique component of the genetic architecture of common diseases, such as cancer [18]. The effect of SNP might be less, compared to the genetic effect of combinations of functionally relevant SNPs that may additively or synergistically contribute to the increased cancer risk.

MATERIAL AND METHODS

Study of group

The source of DNA was lymphocytes from peripheral blood. In this study 280 patients of the oncological laryngology department of the Copernicus Hospital in Lodz, Łódź, Poland, were included. Before sample collection HNC was confirmed histopathologically in case of every patient and any other neoplastic disease was the exclusion criterion. One hundred ninety-seven men and 83 women (with the age of M±SD 40±8 years) were enrolled in the study. The group consisted of 203 people smoking nicotine products and 40 people consuming alcohol at least once a week. Data collected during the medical interview. The study group included 90 people with stage I cancer, in group II – 98 people, in group III – 62 and in group IV – 30 people. Each case was also assessed by the TNM scale. Cases were randomly selected from patients diagnosed in the period of 2015–2020. Cancer free patients (N = 260) admitted to the hospital for other reasons served as control group (age corresponding to the age of the studied group, $p < 0.05$). History of any neoplastic disease was the exclusion criterion for the control group. The Table 1 shows the clinical data. Research was approved by the bioethics committee of the Medical University of Lodz, Łódź, Poland.

DNA isolation and genotyping

Blood Mini (A&A Biotechnology, Gdańsk, Poland) was used to isolate DNA in accordance with the manufacturer's instructions; 200 µl of blood was used for each isolation. Polymorphisms rs1799930 of *NAT2* gene, rs72554606 of *NAT1* gene, rs1799814 of *CYP1A* gene and rs3892097 of *CYP2D* gene were studied with TaqMan technique. The authors used 20 µl of reaction mixture: 1 µl of isolated DNA, 1 µl TaqMan probes, 10 µl of premix with polymerase and 8 µl of water. Thermocycler CFX Connect (Bio-Rad, Berkeley, California) was used to perform the reaction. Reference SNP cluster IDs and thermal conditions are shown in Table 2.

Reaction conditions: 10 min of initial denaturation at 95°C, 42 cycles of 15 s at 92°C and 42 cycles of 1 min at 60°C. The dyes in this reaction were FAM and VIC. The reference dye was FAM.

Statistics

Hardy-Weinberg equilibrium was examined using a χ^2 test with 1 degree of freedom. Risk modulation of HNC by selected SNPs was calculated using logistic regression

Table 1. Demographic characteristics and risk factors and head and neck cancer (HNC) tumor stages in patients participating in the study, Copernicus Hospital in Lodz, Łódź, Poland, 2020

Variable	Participants (N = 540)			p***
	studied group (N = 280)		control group (N = 260) [n (%)]	
	n	%		
Gender				
male	197	70	178 (64)	0.633
female	83	30	82 (36)	
Age distribution				
<40 years	75	27	134 (52)	0.0001
>40 years	205	73	126 (48)	
Smoking*				
no	77	28	191 (73)	0.0001
yes	203	72	69 (27)	
Alcohol consumption**				
no	240	86	206 (79)	0.471
yes	40	14	54 (21)	
Tumor stage TNM scale				
I	90	32		
II	98	35		
III	62	24		
IV	30	9		
Assessment the stage of cancer				
T1				
T1N0M0	44			
T1N1-3M0	30			
T1N1-3M1	16			
T2				
T2N0M0	48			
T2N1-3M0	42			
T2N1-3M1	8			
T3				
T3N0M0	7			
T3N1-3M0	39			
T3N1-3M1	16			
Assessment the stage of cancer – cont.				
T4				
T4N0M0	4			
T4N1-3M0	8			
T4N1-3M1	18			

* Smoking – comprises daily or occasional smoking, including use of electric cigarettes.
** People who drank alcoholic beverages at least once a week were defined as drinkers, and non-drinkers as those who more rarely consumed alcohol.
*** Pearson's χ^2 test for gender, age, smoking, and alcohol drinking.

analysis and presented as odds ratio (OR) with 95% confidence interval (CI) and the p-value. Additionally, multiple logistic regression was conducted to estimate the relative risk of HNC associated with each SNP adjusted for age, gender, smoking, alcohol drinking as possible confounding variables. Similar analysis was conducted for selected pairs of genes and polymorphisms to characterize joint effect (interaction) of pairs of the polymorphisms in 2 genes.

RESULTS

The results of genotyping indicate that the *GA* genotype of the rs1799930 polymorphism of the *NAT 2* gene (Table 3) increases the risk of HNC (OR = 1.506, 95% CI: 1.023–2.216, p = 0.037). The GC genotype of the rs72554606 polymorphism of the *NAT1* gene (Table 3) increases the risk of HNC (OR = 1.772, 95% CI: 1.184–2.651, p = 0.005). There was no significant effect of the rs3892097 polymorphism of the *CYP2D* gene (Table 3) on the modulation of the risk of HNC.

The effect of reducing the probability of HNC can be observed in the *GT* genotype of the rs1799814 polymorphism of the *CYP1A* gene (Table 3) (OR = 0.587, 95% CI: 0.381–0.903, p = 0.015). A significant effect can be observed

Table 2. The Single Nucleotide Polymorphism Database ID (dbSNP ID) used in the polymerase chain reaction (PCR) reaction in 280 patients with head and neck cancer (HNC), Copernicus Hospital in Lodz, Łódź, Poland, 2020

Variable	Gene			
	NAT2	*NAT1*	*CYP2D*	*CYP1A*
Polimorphism	*GA*	*GC*	*CT*	*GT*
dbSNP ID	rs1799930	rs72554606	rs3892097	rs1799814
Position	chr:8:18400593	chr:8:18222397	chr:22:42128945	chr:15:74720646
Alleles	G>A,C	GG>CC	C>T	G>A,T

in the *TT* genotype of the same polymorphism (Table 6) (OR = 0.268, 95% CI: 0.159–0.452, p = 0.001). The same effect can be observed in the Allele *T* of this polymorphism (OR = 0.551, 95% CI: 0.433–0.701, p = 0.001) The study of intergenic interactions showed that the coexistence of *CT-GC* genotypes in *NAT2-NAT1* (Table 4) gene polymorphisms increases the risk of HNC (OR = 2.687, 95% CI: 1.387–5.205, p = 0.002). No significant role of gene interaction was found in *NAT2-CYP2D* genes implicated in HNC (Table 4). On the other hand, the interaction of the genotypes *CC-GT* (OR = 0.344, 95% CI: 0.148–0.800, p = 0.011), *CC-TT* (OR = 0.077, 95% CI: 0.028–0.215, p < 0.001), *CT-TT* (OR = 0.249, 95% CI: 0.100–0.621, p = 0.002), *TT-GT* (OR = 0.275, 95% CI: 0.112–0.676, p = 0.004) in polymorphisms of *NAT2-CYP1A* (Table 4) genes lowered the risk of HNC tumors.

No significant role of gene interaction was found in *NAT1-CYP2D* genes implicated in HNC (Table 4). A similar situation was noticed in the interaction of genotypes of polymorphisms of genes *NAT1-CYP1A* (Table 4) where *GG-TT* (OR = 0.137, 95% CI: 0.043–0.428, p = 0.003), *CC-TT* (OR = 0.155, 95% CI: 0.049–0.491, p = 0.001) and in the interaction of *CYP1A-CYP2D* (Table 4) where *GT-CC* (OR = 0.338, 95% CI: 0.131–0.869, p = 0.021), *TT-GG* (OR = 0.100, 95% CI: 0.027–0.359, p = 0.001), *TT-GC* (OR = 0.190, 95% CI: 0.072–0.501, p = 0.001), *TT-CC* (OR = 0.304, 95% CI: 0.107–0.867, p = 0.023). As above, the interaction of these genotypes reduce the occurrence of HNC tumors.

Effects of additional lifestyle variables, such as smoking or drinking alcohol, were also analyzed. A significant correlation was noted between cigarette smoking and HNC neoplasms (OR = 7.297, 95% CI: 4.989–10.674, p = 0.001). A similar relationship occurred in people consuming alcohol (OR = 1.572, 95% CI: 1.003–2.464, p = 0.047) (Table 5). There was no significant correlation between gender and HNC risk (OR = 1.093 95% CI: 0.758–1.577, p = 0.631). A different conclusion was drawn in a study of the correlation between age and HNC risk, where an association was found (OR = 2.906, 95% CI: 2.029–4.163, p < 0.0001). However, these variables had only little influence on the estimated associations between the genotype and HNC, because crude and adjusted odds ratios for HNC in all studied genotypes were similar, as shown in Tables 3–6.

The distribution of genotypes for different *NAT1, NAT2, CYP1A* and *CYP2D* at different stages of the disease is presented in Table 6. Among the clinical parameters of HNC, it did not show any relationship with the genotypes *NAT1, NAT2, CYP1A, CYP2D* (Table 6).

DISCUSSION

Head and neck cancer offers a unique opportunity to study the impact of genes responsible for the metabolism of carcinogens on the risk of disease. In this study, the authors investigated the potential relationship between the occurrence of polymorphisms of the *NAT1, NAT2, CYP1A, CYP2D* genes and HNC risk assessment in the Polish population. In the literature, there are many

Table 3. Distribution of genotypes and frequency of alleles and analysis of the odds ratio for the polymorphism of the genes in patients with head and neck cancer (HNC) and in the control group, Copernicus Hospital in Lodz, Łódź, Poland, 2020

Genotype/allele	Participants (N = 540)				OR (95% CI)	p	Adjusted OR (95% CI)[a]	p**
	studied group (N = 280)		control group (N = 260)					
	n	frequency	n	frequency				
rs1799930 polymorphism of the *NAT2* gene*								
G/G	78	0.28	90	0.35	1 (ref.)		1 (ref.)	
G/Ab	154	0.55	118	0.45	1.506 (1.023–2.216)	0.037	1.219 (1.005–1.478)	0.04
A/A	48	0.17	52	0.2	1.065 (0.649–1.749)	0.806	1.033 (0.796–1.341)	0.887
G	310	1.11	298	1.15	1 (ref.)		1 (ref.)	
A	250	0.89	222	0.85	1.082 (0.851–1.377)	0.517	1.038 (0.925–1.165)	0.559
rs72554606 polymorphism of the *NAT 1* gene**								
G/G	64	0.23	81	0.31	1 (ref.)		1 (ref.)	
G/Cb	168	0.6	120	0.46	1.772 (1.184–2.651)	0.005	1.771 (1.184–2.651)	0.007
C/C	48	0.17	63	0.24	0.964 (0.586–1.587)	0.888	0.979 (0.739–1.297)	1
G	296	1.06	282	1.08	1 (ref.)		1 (ref.)	
C	264	0.94	246	0.95	1.022 (0.806–1.297)	0.862	1.010 (0.900–1.134)	0.920
rs3892097 polymorphism of the *CYP2D* gene***								
C/C	63	0.23	60	0.23	1 (ref.)		1 (ref.)	
C/T	152	0.54	130	0.5	1.114 (0.729–1.702)	0.617	1.052 (0.858–1.289)	0.698
T/T	65	0.23	70	0.27	1.2592 (0.8349–1.899)	0.271	0.94 (0.735–1.201)	0.708
C	278	0.99	250	0.96	1 (ref.)		1 (ref.)	
T	280	1	270	1.04	0.933 (0.734–1.184)	0.566	0.966 (0.861–1.085)	0.610
rs1799814 polymorphism of the *CYP1A* gene****								
G/G	85	0.30	44	0.17	1 (ref.)		1 (ref.)	
G/Tb	153	0.55	135	0.52	0.587 (0.381–0.903)	0.015	0.806 (0.683–0.950)	0.019
T/Tb	42	0.15	81	0.31	0.268 (0.159–0.452)	0.001	0.542 (0.415–0.706)	0.001
rs1799814 polymorphism of the *CYP1A* gene****								
G	323	1.15	223	0.86	1 (ref.)		1 (ref.)	
Tb	237	0.85	297	1.14	0.551 (0.433–0.701)	0.001	0.750 (0.667–0.844)	0.001

Chi-square test p-value: * 0.242; ** 0.159; *** 0,981; **** 0.334.

[a] Odds ratio adjusted for age, gender, smoking, and alcohol drinking by multiple logistic regression.

[b] Variables that statistically significant modulate the risk of HNC.

references to the association of xenobiotics with bladder cancer, colorectal cancer or lung cancer. The authors can find studies on HNC, but so far there have been no studies on the comprehensive analysis of polymorphisms of the *NAT1*, *NAT2*, *CYP1A* and *CYP2D* genes and the interactions between these genes.

Table 4. Genotype distribution and odds ratio analysis for intra-gene interactions: in patients with head and neck cancer (HNC) tumors and in the control group, Copernicus Hospital in Lodz, Łódź, Poland, 2020

Genotype	Participants (N = 540)		OR (95% CI)	p	Adjusted OR (95% CI)[a]	p*
	studied group (N = 280)	control group (N = 260)				
NAT2-NAT1						
G/G-G/G	19	30	1 (ref.)		1 (ref.)	
G/G-G/C	42	38	1.745 (0.847–3.596)	0.129	1.353 (0.899–2.038)	0.182
G/G-C/C	17	22	1.220 (0.519–2.869)	0.647	1.124 (0.681–1.855)	0.806
G/A-G/G	31	38	1.288 (0.611–2.714)	0.507	1.158 (0.747–1.795)	0.631
G/A-G/C[b]	97	57	2.687 (1.387–5.205)	0.003	1.624 (1.119–2.356)	0.004
G/A-C/C	26	23	1.785 (0.799–3.985)	0.156	1.368 (0.881–2.123)	0.223
A/A-G/G	14	12	1.842 (0.704–4.819)	0.210	1.388 (0.841–2.290)	0.314
A/A-G/C	29	25	1.832 (0.8353–4.016)	0.129	1.385 (0.900–2.129)	0.187
A/A-C/C	5	15	0.526 (0.164–1.685)	0.275	0.644 (0.279–1.488)	0.416
NAT2-CYP2D						
G/G-G/G	18	20	1 (ref.)		1 (ref.)	
G/G-G/C	40	41	0.922 (0.426–1.995)	0.841	1.042 (0.698–1.557)	1
G/G-C/C	20	29	0.707 (0.345–1.448)	0.343	0.617 (0.345–1.105)	0.423
G/A-G/G	36	29	1.272 (0.661–2.449)	0.471	1.169 (0.783–1.744)	0.559
G/A-G/C	87	59	1.511 (0.875–2.611)	0.138	1.258 (0.877–1.804)	0.241
G/A-C/C	31	30	1.059 (0.545–2.059)	0.862	1.072 (0.707–1.626)	0.887
A/A-G/G	9	11	0.839 (0.314–2.241)	0.729	0.95 (0.527–1.712)	0.920
A/A-G/C	25	30	0.854 (0.430–1.697)	0.655	0.959 (0.616–1.494)	1
A/A-C/C	14	11	1.304 (0.529–3.215)	0.5657	1.182 (0.729–1.915)	0.680
NAT2-CYP1A						
G/G-G/G	29	10	1 (ref.)		1 (ref.)	
G/G-G/T[b]	40	40	0.340 (0.149–0.800)	0.011	0.672 (0.505–0.895)	0.019
G/G-T/T[b]	9	40	0.077 (0.028–0.215)	<0.0001	0.247 (0.133–0.458)	<0.0001
G/A-G/G	44	24	0.632 (0.264–1.515)	0.300945	1.020 (0.806–1.29)	0.920
G/A-G/T	89	65	0.472 (0.215–1.037)	0.058	0.777 (0.618–0.976)	0.086
G/A-T/T[b]	21	29	0.250 (0.100–0.622)	0.002	0.564 (0.388–0.821)	0.004
A/A-G/G	12	10	0.414 (0.137–1.249)	0.113	0.733 (0.480–1.120)	0.193
A/A-G/T[b]	24	30	0.276 (0.112–0.676)	0.004	0.275 (0.421–0.848)	0.007
A/A-T/T	12	12	0.345 (0.118–1.011)	0.049	0.672 (0.432–1.044)	0.089

Table 4. Genotype distribution and odds ratio analysis for intra-gene interactions: in patients with head and neck cancer (HNC) tumors and in the control group, Copernicus Hospital in Lodz, Łódź, Poland, 2020 – cont.

Genotype	Participants (N = 540)		OR (95% CI)	p	Adjusted OR (95% CI)[a]	p*
	studied group (N = 280)	control group (N = 260)				
NAT1-CYP2D						
G/G-G/G	12	17	1 (ref.)		1 (ref.)	
G/G-G/C	33	40	1.169 (0.489–2.792)	0.729	1.092 (0.661–1.803)	0.887
G/G-C/C	19	23	1.170 (0.450–3.046)	0.752	1.093 (0.633–1.887)	0.920
G/C-G/G	41	32	1.815 (0.759–4.3399)	0.177	1.357 (0.841–2.189)	0.259
G/C-G/C	92	60	2.172 (0.969–4.870)	0.056	1.462 (0.931–2.298)	0.088
G/C-C/C	35	28	1.771 (0.727–4.315)	0.206	1.342 (0.825– 2.183)	0.298
C/C-G/G	10	11	1.288 (0.415–3.991)	0.663	1.150 (0.616–2.147)	0.887
C/C-G/C	27	30	1.275 (0.516–3.147)	0.597	1.144 (0.685–1.910)	0.764
C/C-C/C	11	19	0.820 (0.288–2.338)	0.708	0.886 (0.467–1.679)	0.920
CYP1A-CYP2D						
G/G-G/G	21	8	1 (ref.)		1 (ref.)	
G/G-G/C	47	22	0.814 (0.312–2.123)	0.671	0.940 (0.713–1.240)	0.862
G/G-C/C	17	14	0.463 (0.157–1.360)	0.158	0.757 (0.512–1.119)	0.252
G/T-G/G	37	33	0.427 (0.167–1.093)	0.072	0.729 (0.532– 1.000)	0.115
G/T-G/C	84	66	0.485 (0.202–1.164)	0.100	0.773 (0.592–1.008)	0.150
G/T-C/C[b]	32	36	0.339 (0.132–0.870)	0.022	0.649 (0.463–0.910)	0.038
T/T-G/G[b]	5	19	0.100 (0.028–0.360)	0.00018	0.287 (0.127–0.647)	0.0005
T/T-G/C[b]	21	42	0.190 (0.072–0.502)	0.0004	0.460 (0.303–0.697)	0.001
T/T-C/C[b]	16	20	0.305 (0.107–0.868)	0.024	0.613 (0.399–0.942)	0.044
NAT1-CYP1A						
G/G-G/G	21	12	1 (ref.)		1 (ref.)	
G/G-G/T	37	43	0.492 (0.213–1.133)	0.092	0.726 (0.512–1.031)	0.140
G/G-T/T[b]	6	25	0.137 (0.044–0.4283)	0.00034	0.304 (0.141–0.652)	0.0008
G/C-G/G	49	22	1.273 (0.533–3.036)	0.584	0.304 (0.141–0.652)	0.0008
G/C-G/T	89	64	0.794 (0.365–1.731)	0.560	0.914 (0.683–1.222)	0.698
G/C-T/T	30	34	0.504 (0.213–1.195)	0.118	0.736 (0.510–1.063)	0.176
C/C-G/G	15	10	0.857 (0.294–2.497)	0.777	0.942 (0.625–1.4222)	1
C/C-G/T	27	28	0.551 (0.227–1.335)	0.185	0.771 (0.531–1.119)	0.269
C/C-T/T[b]	6	22	0.156 (0.049–0.491)	0.00094	0.336 (0.158–0.716)	0.002

[a] Odds ratio adjusted for age, gender, smoking, and alcohol drinking by multiple logistic regression.
[b] Variables that statistically significant modulate the risk of HNC.

Table 5. The risk of head and neck cancer (HNC) depending on smoking, alcohol consumption, demographic character, age distribution, Copernicus Hospital in Lodz, Łódź, Poland, 2020

Variable	Participants (N = 540) [n (%)]		OR (95% CI)	p
	studied group (N = 280)	control group (N = 260)		
Smoking				
no	77 (28)	191 (73)	1 (ref.)	
yes[a]	203 (72)	69 (27)	7.297 (4.989–10.674)	<0.0001
Alcohol consumption				
no	240 (86)	206 (79)	1 (ref.)	
yes[a]	40 (14)	54 (21)	1.572 (1.003–2.464)	0.047
Gender				
female	83 (30)	82 (36)	1 (ref.)	
male	197 (70)	178 (64)	1.093 (0.758–1.577)	0.631
Age				
≤40 years	75 (27)	134 (52)	1 (ref.)	
>40 years[a]	205 (73)	126 (48)	2.906 (2.029–4.163)	<0.0001

[a] Variables that statistically significant modulate the risk of HNC.

The results of this study indicate that there is an increased risk of HNC in the *NAT2* polymorphism in *GA*. This is largely in line with most of the available literature. Interestingly, Gutpa et al. came to a similar conclusion, pointing to an increased risk of HNC in the *NAT2* gene polymorphism, while their research indicates an increased risk of this risk in the *AA* genotype (OR = 2.69, CI: 1.38–5.23, p = 0.004) and *allele A* (OR = 1.45 95% CI: 1.11–1.90, p = 0.006) [19]. The difference between these results and other respondents may be due to the culture of drug use in India. In India, chewing tobacco is a popular method, according to the respondents, it is used by 48% of respondents. In Poland, it is not practiced, but smoking is common – as many as 72% of respondents confirmed smoking. Comparing to India, smoking was reported by 46% of respondents. Research results may also differ due to the number of people in a particular TNM group. The authors' research was based on the predominance of people in the first and second stages of the disease (32% and 35%, respectively), while the Indian researchers had an advantage in the third and fourth stages of the disease (25% and 49%, respectively).

In the research, in the polymorphism of the *NAT1* gene in the *GC* genotype, the authors observe an increase in the risk of developing HNCs. The available studies often indicate that the *NAT1* gene polymorphism is not related to the risk of HNC. Such conclusions were reached by Majumder et al. [20] and Khlifi et al. [21]. This may be due to the difference in the number of study groups. Such a large difference in the results may be related to the large difference between the study group and the control group of the authors' respondents. Bidyut presented the division of groups into 310 people with cancer and 389 people from the control group. In Khlifi et al. 169 people were tested and 261 people from the control group. Results in this study are presented in a different way. Unlike other

Table 6. Distribution of different genotypes of *NAT1, NAT2, CYP1A, CYP2D* polymorphisms among different tumor stages

Genotype	Tumor stage distribution									
	I [n]	II [n]	III [n]	IV [n]	II vs. I (OR (95% CI))	p	IV vs. III (OR (95% CI))	p	I+II+III vs. IV (OR (95% CI))	p
NAT 1										
GG	21	22	13	8	1 (ref.)		1 (ref.)		1 (ref.)	
GC	55	59	39	11	0.976 (0.484–1.970)	1	2.181 (0.721–6.594)	0.162	1.987 (0.760–5.193)	0.155
CC	14	17	10	7	0.862 (0.341–2.178)	0.75	0.879 (0.237–3.248)	0.841	0.836 (0.280–2.492)	0.751
G	97	103	65	27	1 (ref.)				1 (ref.)	
C	83	93	59	25	0.579 (0.230–1.459)	0.241	1.020 (0.533–0.950)	1	0.957 (0.540–1.696)	0.887
NAT 2										
GG	26	28	18	6	1 (ref.)				1 (ref.)	
GA	50	53	34	17	1.016 (0.525–1.963)	1	0.666 (0.223–1.987)	0.466	0.671 (0.253–1.777)	0.420
AA	14	17	10	7	0.886 (0.365–2.151)	0.791	0.476 (0.125–1.812)	0.273	0.488 (0.153–1.550)	
G	102	109	70	26	1 (ref.)				1 (ref.)	
A	78	87	54	31	0.958 (0.637–1.440)	0.841	0.647 (0.344–1.215)	0.174	0.653 (0.377–1.133)	0.127
CYP2D										
CC	22	21	12	8	1 (ref.)		1 (ref.)		1 (ref.)	
CT	49	54	37	12	0.866 (0.424–1.765)	0.689	2.055 (0.679–6.215)	0.197	1.697 (0.658–4.376)	0.269
TT	19	23	13	10	0.788 (0.336–1.849)	0.583	0.866 (0.256–2.925)	0.823	0.8 (0.293–2.179)	0.662
C	93	96	61	28	1 (ref.)		1 (ref.)		1 (ref.)	
T	87	100	63	32	0.898 (0.599–1.346)	0.603	0.903 (0.487–1.675)	0.751	0.875 (0.511–1.496)	0.624
CYP1A										
GG	28	31	18	8	1 (ref.)		1 (ref.)		1 (ref.)	
GT	51	55	34	13	1.026 (0.542–1.941)	0.92	1.162 (0.406–3.321)	0.777	1.118 (0.444–2.817)	0.806
TT	11	12	10	9	1.014 (0.386–2.662)	1	0.493 (0.144–1.683)	0.256	0.381 (0.135–1.073)	0.061
G	107	117	70	29	1 (ref.)		1 (ref.)		1 (ref.)	
C	73	79	54	31	1.023 (0.560–1.867)	0.92	0.721 (0.388–1.339)	0.3	0.655 (0.383–1.121)	0.121

researchers, the authors have more people in the study group (280 people) compared to the control group (260 people). In addition, the difference between the study and control groups is small in contrast to the others. In the polymorphism of the *CYP1A* gene in the *GT* and *TT* genotypes, the research showed a protective effect on the occurrence of HNCs. Interestingly, the available literature reports that *CYP1A* polymorphism may or may not increase the risk of HNC. An example where the results of studies in the literature suggest an increased risk of HNC are the studies by Sabitha et al. [22]. This may be due to differences in the studied groups due to ethnic differences between Poland and India. The way of eating, the use of stimulants or alcohol. The main reason may be the smoking of Bidi (raw tobacco wrapped in tobacco leaves) by the inhabitants of India, which present a much higher level of benzopyrenes compared to cigarettes smoked in Poland [22].

Studies in which the *CYP1A* gene polymorphism does not affect the risk of HNC were indicated by Marques

et al. [23]. This study results may differ due to ethnic differences between Poland and Brazil. The research conducted in Brazil included the examination of patients according to skin color, age or gender. The difference in the studies may be due to the diversity of breeds present in Brazil. The study of Brazil covered the races: whites (56%), mulattoes (26%) and blacks (18%). An interesting relationship was found in the polymorphisms of NAT2-CYP1A genes in the *CC-GT, CC-TT, CT-TT* and *TT-GT* genotypes. Although the *CT* in the *NAT2* polymorphism increases the risk of HNC tumors, it exceeds the silencing effect observed in the *TT* genotype of the *CYP1A* polymorphism. This effect when combined increases a reduction in HNC predisposition. Despite the absence of other relevant genotypes in *NAT2*, the coexistence with the *CYP1A* genotypes in the above-mentioned combinations works to reduce the incidence of HNC tumors. A similar effect was observed in the polymorphisms of the *NAT1-CYP1A* and *CYP1A-CYP2D* genes. In the *NAT1-CYP2D* polymorphisms, despite the genotypes in *NAT1* increasing the incidence of the tumor in question, no influence on its risk was found. This corresponds to the theory that data differences represent the degree of complexity and duplication of factors responsible for neoplastic transformation which, as in the above case, lead to different genotypic outcomes for the same gene.

A literature review reports similar results for assessing intergenomic interactions to the authors' ones. An example may be the research by Demokan et al., where the interaction of *NAT2-NAT1* increases the risk of HNC [24]. Similar conclusions to the authors' were also made by the above-mentioned Khlifi et al., who noticed the *NAT2-NAT1* interaction increasing the risk of HNC as well as *CYP2D-CYP1A*, where coexistence creates a protective effect [21]. However, they found no significant relationship between *NAT1-CYP1A* and *NAT2-CYP1A*. This may result, as it was mentioned, from the number of the research group, environmental factors, the number of people smoking and consuming alcohol, or the difference of ethnic groups. This research has shown a strong link between smoking and HNCs. The link was also investigated by McCarter et al. [25] where they showed a strong correlation between smoking and a predisposition to HNCs. The group was larger than the number of men, as in this study. The same conclusions were reached by Mayne et al. [26], who showed a clear link between smoking and the occurrence of HNCs.

Interestingly, the aforementioned researchers also conducted research on alcohol consumption, which was associated with a predisposition to the cancer in question. This study results also confirmed the theses of these independent research centers. Due to the limited amount of literature on the polymorphisms of individual genes discussed above, it is advisable to extend research into the risk of HNC. Further research in this direction, intensified by more patients, could help to develop a diagnosis of HNC risk. This results are promising, with significant associations between the effects of *NAT2, NAT1*, and *CYP1A* polymorphisms on HNC risk.

CONCLUSIONS

The *GA* genotypes of the *NAT2* gene and the *GC* genotypes of the *NAT1* gene may increase the risk of HNC, while the *GT* and *TT* polymorphism of the *CYP1A* gene reduce the risk of HNC. At the same time, the *GC* and *CC* genotype of the *CYP2D* gene does not influence the risk of a given tumor. The authors believe the results are promising, but more research is needed to establish a compelling relationship between a given polymorphism and its phenotypic effect. Additionally, the intergenic interaction in *NAT2-NAT1* in genotypes *GA-GC* increases the risk of HNC. Another situation is in the *NAT2-CYP1A* interaction, where despite the situation where the authors are dealing with a genotype increasing the likelihood of cancer, the second genotype, which is protective, outweighs the risk increase effect, and in connection with

the effect of reducing the risk of HNCs. Similar in *NAT1-CYP1A*. *CYP1A-CYP2D*, where the risk-lowering effect is shown in genotypes in coexistence, leads to an enhancement of the tumor risk-lowering effect. Alcohol as well as smoking are factors that aggravate HNCs.

Author contributions

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