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PRACA ORYGINALNA
ORIGINAL PAPER

Rs401681 and rs402710 polymorphisms of *CLPTM1L* gene in cancerous and healthy lung tissues in patients with lung adenocarcinoma

Polimorfizmy rs401681 i rs402710 genu *CLPTM1L* w tkance zmienionej nowotworowo i w tkance zdrowej płuc u chorych z gruczolakorakiem płuca

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ABSTRACT

INTRODUCTION: The search for factors influencing the survival of patients with lung cancer is still ongoing. It may potentially be a polymorphism of the cleft lip and palate transmembrane 1-like (*CLPTM1L*) gene, which is involved in the process of carcinogenesis. The aim of the study was to assess the distribution of genotypes and alleles of selected polymorphisms of the *CLPTM1L* gene – rs401681 and rs402710 – in cancerous and healthy lung tissue in patients with lung adenocarcinoma and their relationship with patient survival.

MATERIAL AND METHODS: The study included 133 patients with an average age of lung cancer diagnosis of 65 years, who had undergone lung adenocarcinoma surgery in the past. Genetic material – deoxyribonucleic acid (DNA) – was isolated from paraffin-protected specimens of cancerous and healthy lung tissue, and genotyping of *CLPTM1L* polymorphisms was performed. The obtained results were analyzed along with demographic data, history of smoking, family history of cancer, stage of the disease in the tumor, node, metastasis (TNM) classification, clinical stage of the cancer and the survival time of the patients.

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RESULTS: The mean follow-up period was 44.5 months. The patients who died lived an average of 22.6 months from the time of cancer diagnosis. There were no significant differences between the distribution of genotypes or alleles in the cancerous and healthy tissues and their relationship with the survival of the patients. The age at the time of diagnosis of cancer, category N in the TNM classification and high clinical advancement of the cancer were the only factors influencing the survival of the patients.
CONCLUSIONS: No relationships between the polymorphic variability of rs401681 and rs402710 in cancerous and healthy lung tissue and the survival of patients were found.

KEYWORDS

lung adenocarcinoma, *CLPTM1L* gene, rs401681, rs402710, survival analysis

STRESZCZENIE

WSTĘP: Poszukiwanie czynników wpływających na przeżycie chorych z nowotworami płuc jest stale aktualne. Takim potencjalnym czynnikiem jest polimorfizm genu *CLPTM1L* (*cleft lip and palate transmembrane 1-like*), włączony w proces karcynogenezy. Celem pracy była ocena rozkładu genotypów i alleli wybranych polimorfizmów genu *CLPTM1L* – rs401681 i rs402710 – w tkance zmienionej nowotworowo i w tkance zdrowej płuc u chorych z gruczolakorakiem płuca oraz ich związku z przeżyciem chorych.
MATERIAŁ I METODY: Badaniami objęto 133 chorych w wieku ujawnienia nowotworu płuca wynoszącym średnio 65 lat, u których w przeszłości dokonano usunięcia gruczolakoraka płuc. Z zabezpieczonych w parafinie próbek tkanki zdrowej płuc i z tkanki zmienionej chorobowo wyizolowano materiał genetyczny – kwas deoksyrybonukleinowy (DNA) – oraz przeprowadzono genotypowanie polimorfizmów *CLPTM1L*. Uzyskane wyniki poddano analizie wraz z danymi demograficznymi, wywiadem dotyczącym palenia tytoniu, wywiadem rodzinnym obciążonym nowotworowo, stadium choroby w klasyfikacji TNM (*tumor, node, metastasis*), zaawansowaniem klinicznym nowotworu i czasem przeżycia chorych.
WYNIKI: Średni czas obserwacji wynosił 44,5 miesiąca. Chorzy, którzy zmarli, żyli średnio 22,6 miesiąca od czasu postawienia diagnozy nowotworu. Nie stwierdzono znamiennych różnic pomiędzy rozkładem genotypów ani alleli w tkankach chorej i zdrowej płuc oraz ich związku z przeżyciem chorych. Czynnikami wpływającymi na przeżycie chorych były wiek w czasie postawienia diagnozy nowotworu, kategoria N w klasyfikacji TNM oraz wysokie zaawansowanie kliniczne nowotworu.
WNIOSKI: Nie stwierdzono związku pomiędzy zmiennością polimorficzną rs401681 i rs402710 w tkance chorej i zdrowej płuc a przeżyciem chorych.

SŁOWA KLUCZOWE

gruczolakorak płuca, gen *CLPTM1L*, rs401681, rs402710, analiza przeżycia

INTRODUCTION

According to World Health Organization (WHO), lung cancer is the most frequent neoplasm all over the world, being the cause of over 13% all new incidents of cancers, mainly (about 68%) among males. Even though women develop it still less frequently, a rising trend of the incidence of lung malignancy is observed in Europe. Lung carcinoma causes about 1.2 million deaths in the world every year, which equals 17% of all deaths from cancers [1]. The low patient survival rate is secondary to the long-time asymptomatic course of the disease and to a delayed start of diagnostic procedures, usually at an advanced stage of neoplasm. Among 67 countries participating in the CONCORD-2 study, the survival rate of 5 years after lung carcinoma diagnosis (2005–2009yrs) varies from 2.2% in Libya, to 16.5% in Switzerland, 18.7% in USA and 30.1% in Japan. From the 1990s, the survival rate increased from 11.4% to 13.4% in Poland [2]. It is estimated that about 85–90% of deaths caused by lung cancer is closely connected with tobacco smoking [3]. The exposure to other carcinogens (for example professional risk factors or air pollution) in the cases of lung carcinoma in the population of non-smokers was demonstrated. Here the relevance of changes in the genetic profile are particularly emphasised [4,5,6].

The genetic and epigenetic changes accumulating with the time in the cells by the changing of its structures and function are the cornerstone of long-term ongoing carcinogenesis, leading finally to cancer development. These unfavourable changes occur in normal healthy cells as a reaction to different mutagenic factors, both exogenous and endogenous. They could be of chemical (in tobacco smoke), physical (UV radiation) or biological origin (viruses), but also metabolic waste products or replication mistakes.

Genetic instability is one of characteristic features of cancer cells. It can appear as allele instability, so-called loss of heterozygosity (LOH). In this case malignant cells, originally heterozygous, lost one from two alleles

of gene polymorphism [7]. It occurs either by the simple deletion of one allele (copy-loss LOH), or by the deletion of one allele accompanied by duplication of the remaining allele (copy-neutral LOH). As a result, this reduction of heterozygosity creates genetic changes between neoplasm and healthy tissues, which leads to further functional changes specific to carcinogenesis. Malignant tissue could be composed of cells remaining at various stages of malignancy. Therefore, usually only partial loss of allele heterozygosity in cancerous tumors is observed (20–80%). Studies indicate that genetic changes concern not only tumor tissue but also its "healthy environment", which does not present any histopathological deviations. LOH on chromosome 5, both in the *p* and *q* arms, has been reported in non-small cell lung carcinoma [8,9].

Several genome-wide association studies reported different genetic variants, among them single nucleotide polymorphisms, that are associated with the risk of malignancy [5,6,10,11]. In the light of recent data, *CLPTM1L* gene polymorphisms seem to be an interesting focus for studies concerning risk factors for the development of different neoplasms [10,12]. *CLPTM1L* was determined to be overexpressed in cisplatin-resistant ovarian cancer cells and promotes this apoptosis [13]. The results of a large-scale genome--wide gene-gene interaction study of lung cancer susceptibility in Europeans published in 2022 (including over 445 thousands participants) demonstrated the relation of the *CLPTM1L* gene to lung malignancy [14].

The *CLPTM1L* gene encodes a protein linked to cisplatin resistance and is associated with the susceptibility to cleft lip palate. It is located on chromosome 5 near the telomerase reverse transcriptase (*TERT*) gene, which is closely related to lung cancerogenesis. The *CLPTM1L* genome assembly GRCh38.p13, location: 5:1317752-1345099 and the cytogenetic region: 5p15.33 are as follows (https://www.ebi.ac.uk/gwas/search?query=CLPTM1L). In the cell it is mainly localized in the perinuclear region of the cytoplasm.

Two single nucleotide polymorphisms (SNPs) of *CLPTM1L*, rs401681 and rs402710, are located in cytogenic region 5p15.33, respectively Chr.5 1321972 and 1320607 on GRCh38. Rs401681 is the intron variant, while rs402710 is the non-coding transcript exon variant. For both of these SNPs allele T is MAF, i.e. a minor allele frequency (https://www.ebi.ac.uk/gwas/search?query=CLPTM1L). The allele distribution of the studied polymorphisms are different in various populations (https://www.ncbi.nlm.nih.gov/snp/). In Europe, for instance, according to the ALFA Project, the distribution of the rs401681 *CLPTM1L* gene allele is as follows: T 0.44 and C 0.56, while rs402710 respectively T 0.34 and C 0.66. The comparable data of T and C allele distribution in Asians for rs401681 *CLPTM1L* are: 0.33 and 0.67, whereas for rs402710 – 0.31 and 0.69.

The estimation of the rs401681 and rs402710 *CLPTM1L* gene genotypes, as well as allele distribution in both neoplastic and healthy lung tissue samples in patients with lung adenocarcinoma was the purpose of the study. Its relationship with patient survival, taking into consideration the patient's age and sex, tobacco smoking, family history of malignancy and staging of neoplasm were also investigated.

MATERIAL AND METHODS

The study protocol was approved by the Ethics Committee of the Medical University of Silesia in Katowice. Patient consent was waived due to the fact that it was a retrospective study based on tissue speciments stored in the Department of Pathomorphology archives.

Two paraffin-embedded lung tissue specimens (one obtained from a cancerous tumor and the second from healthy tissue) collected during operative procedures from all of the 150 patients treated in the thoracic surgery unit were subjected to further analysis. The lung adenocarcinoma was diagnosed based on histological examination in all the patients. Patients were excluded from the final analysis in the case when their survival was impossible to establish. In the end, the study group consisted of 133 patients (88.7%), whose data were further analysed.

SNP selection

Two SNPs in the *CLPTM1L* gene, suspected of being associated with a predisposition for cancer, i.e. loci rs401681 and rs402710, were selected for analysis based on PubMed references and the bioinformatics database (https://www.ncbi.nlm.nih.gov/snp/; accessed on 15 September 2023).

Genotyping

DNA was isolated for every patient from his own two tissue samples, protected with paraffin, stored in the Department of Pathomorphology archives: one 10 μm piece from a histologically confirmed neoplastic tumor and the second piece from healthy lung tissue – both obtained during surgery. The Maxwell 16 System and the Maxwell 16 FFPE plus LEV DNA Purification Kit were used for DNA extraction. The concentration and quality of the extracted DNA was verified with DeNovix equipment. The DNA purification was done with gDNA Clean-up (Syngen). Genotyping was carried out on a Roche Light 96 with TaqMan and Master Mix FastStart Essentials DNA Probes, Master and ThermoFisher Scientific kits. The primers Context

Sequence [VIC/FAM] of the rs401681 *CLPTM1L* gene was CTGCTATCCAGACAACTTCAGAGTC**[C/T]**ATCATG GTGTGAAGCAGCTTTCTGG. The primers Context Sequence [VIC/FAM] of the rs402710 *CLPTM1L* gene was GGAGCAACGGCCGAGCATACGCAGC**[C/T]**GCACT CACCACCGCTGGTACAGGTA.
In all the samples the genotypes of rs401681 and rs402710 of the *CLPTM1L* gene were determined.

Additional data

Based on archival medical documentation, the information on the age at the time of neoplasm diagnosis, tobacco smoking (expressed in packs of cigarettes during all the years of the habit), alcohol drinking, family history of malignancy, as well as TNM classification and neoplasm staging (based on 8th ed. of the Union for International Cancer Control – UICC – criteria from 2018), were obtained for each patient [15].

Statistical analysis

All the data and genetic tests results underwent statistical analysis with Statistica 12 Software and Statistical Analysis System (SAS). After the initial checking of data distribution, the obtained results were compared with the χ^2 test / Fischer test. The survival analysis was performed with Kaplan-Meier plots. Univariate and multivariate Cox regression analyses regarding patient survival were also done. P-values less than 0.05 ware taken as statistically significant.

RESULTS

Characteristics of patients

The group of 133 patients with complete survival data consisted of 71 men and 62 women in the mean age of 64.96 ± 8.44 years at lung carcinoma diagnosis. They were divided into two subgroups "deceased" (75 persons, i.e. 56.4%) or "living" during the survey (58 individuals, i.e. 43.6%) for the purpose of analysis. These subgroups did not differ significantly one from another as far as the age of neoplasm diagnosis (64.71 ± 9.80 vs 65.29 ± 7.60 years, p = 0.6934) was concerned, and also as regards tobacco smoking expressed as the number of cigarette packages during all the years of the habit (30.00 {15–40} vs 30 {15–40}, p = 0.4289). 113 patients (85%) were smokers and 33 patients (24.8%) had a positive family history for malignancy. The distribution of the patients according to neoplasm staging at the time of making the histopathological diagnosis was 73 (54.9%), 44 (33.1%) and 16 (12%), respectively for I stage, II stage and III stage or above. Because of the small number of cases (1%) a history of drinking alcohol was excluded from the analysis.

Genotype and allele distribution – comparison between neoplastic and normal tissues

We did not find any differences between the neoplastic and healthy lung tissue as far as the frequency of either the genotype or the allele of rs401681 and also rs402710 were concerned (Table I).
No significant differences in the tissue genotype or the allele distribution of the studied SNPs between the persons diagnosed with adenocarcinoma who had died and those who survived were demonstrated (Table II).
The analysis taking into account the sex of the patients, as well as the smoking addiction did not show any statistically significant variations between the genotype and the allele distribution of either the studied SNPs in the neoplastic or healthy lung tissues (Tables III and IV). Nevertheless, the borderline tendency for differences in the rs401681 genotype distribution in adenocarcinoma tissue was visible between men and women (Table III).

Table I. Comparison of genotype (CC, CT, TT) and allele (C, T) distribution of rs401681 and rs402710 *CLPTM1L* gene in neoplastic and healthy tissues in whole study group; χ^2 test
Tabela I. Porównanie rozkładu genotypów (CC, CT, TT) oraz alleli (C, T) polimorfizmów rs401681 i rs402710 genu *CLPTM1L* w tkance zmienionej nowotworowo i w tkance zdrowej w całej grupie badanej; test χ^2

Genotype/Allele	Polymorphism					
	rs401681			rs402710		
	adenocarcinoma tissue	healthy tissue	χ^2 test p-value	adenocarcinoma tissue	healthy tissue	χ^2 test p-value
CC	45 (34.09%)	47 (36.15%)	0.9161	54 (40.60%)	62 (46.62%)	0.5302
CT	69 (52.27%)	67 (51.54%)		69 (51.88%)	64 (48.12%)	
TT	18 (13.64%)	16 (12.31%)		10 (7.52%)	7 (5.26%)	
C	159 (60.23%)	161 (61.92%)	0.6906	177 (66.54%)	188 (70.68%)	0.3041
T	105 (39.77%)	99 (38.08%)		89 (33.46%)	78 (29.32%)	

Table II. Genotype (CC, CT, TT) and allele (C, T) distribution of rs401681 and rs402710 *CLPTM1L* gene in neoplastic and healthy tissues according to patients' survival; χ^2 test
Tabela II. Rozkład genotypów (CC, CT, TT) oraz alleli (C, T) polimorfizmów rs401681 i rs402710 genu *CLPTM1L* w tkance zmienionej nowotworowo i w tkance zdrowej w zależności od przeżycia chorych; test χ^2

Polymorphism/Tissue	Genotype/Allele	All n	Death NO n (%)	Death YES n (%)	χ2 test p-value	Fisher's test p-value
rs401681 adenocarcinoma tissue	CC	45	20 (34.5%)	25 (33.8%)	0.8289	0.8069
	CT	69	29 (50%)	40 (54.1%)		
	TT	18	9 (15.5%)	9 (12.2%)		
	C		69/116 (59.48%)	90/148 (60.81%)	0.8286	0.8993
	T		47/116 (40.52%)	58/148 (39.19%)		
rs401681 healthy tissue	CC	47	20 (35.7%)	27 (36.5%)	0.5095	0.5075
	CT	67	27 (48.2%)	40 (54.1%)		
	TT	16	9 (16.1%)	7 (9.5%)		
	C		67/112 (59.82%)	94/148 (63.51%)	0.5438	0.6063
	T		45/112 (40.18%)	54/148 (36.49%)		
rs402710 adenocarcinoma tissue	CC	54	22 (37.9%)	32 (42.7%)	0.1875	0.1875
	CT	69	34 (58.6%)	35 (46.7%)		
	TT	10	2 (3.4%)	8 (10.7%)		
	C		78/116 (67.24%)	99/150 (66.0%)	0.8315	0.8960
	T		38/116 (32.76%)	51/150 (34.0%)		
rs402710 healthy tissue	CC	62	24 (41.4%)	38 (50.7%)	0.4956	0.4525
	CT	64	30 (51.7%)	34 (45.3%)		
	TT	7	4 (6.9%)	3 (4%)		
	C		78/116 (67.24%)	110/150 (73.33%)	0.2791	0.2817
	T		38/116 (32.76%)	40/150 (26.67%)		

Table III. Genotype (CC, CT, TT) and allele (C, T) distribution of rs401681 and rs402710 *CLPTM1L* gene in neoplastic and healthy tissues according to gender; χ^2 test
Tabela III. Rozkład genotypów (CC, CT, TT) oraz alleli (C, T) polimorfizmów rs401681 i rs402710 genu *CLPTM1L* w tkance zmienionej nowotworowo i w tkance zdrowej w zależności od płci; test χ^2

Polymorphism/Tissue	Genotype/Allele	All n	Women n (%)	Men n (%)	χ2 test p-value	Fisher's test p-value
rs401681 adenocarcinoma tissue	CC	45	23 (37.1%)	22 (31.4%)	0.0771	0.0824
	CT	69	35 (56.5%)	34 (48.6%)		
	TT	18	4 (6.5%)	14 (20%)		
	C		81/124 (65.32%)	78/140 (55.71%)	0.1114	0.1308
	T		43/124 (34.68%)	62/140 (44.29%)		
rs401681 healthy tissue	CC	47	23 (38.3%)	24 (34.3%)	0.1934	0.2006
	CT	67	33 (55%)	34 (48.6%)		
	TT	16	4 (6.7%)	12 (17.1%)		
	C		79/120 (65.83%)	82/140 (58.57%)	0.2293	0.2504
	T		41/120 (34.17%)	58/140 (41.43%)		
rs402710 adenocarcinoma tissue	CC	54	27 (43.5%)	27 (38%)	0.2085	0.2229
	CT	69	33 (53.2%)	36 (50.7%)		
	TT	10	2 (3.2%)	8 (11.3%)		
	C		87/124 (70.16%)	90/142 (63.38%)	0.2423	0.2974
	T		37/124 (29.84%)	52/142 (36.62%)		
rs402710 healthy tissue	CC	62	30 (48.4%)	32 (45.1%)	0.7404	0.743
	CT	64	28 (45.2%)	36 (50.7%)		
	TT	7	4 (6.5%)	3 (4.2%)		
	C		88/124 (70.97%)	100/142 (70.42%)	0.9224	1.000
	T		36/124 29.03%)	42/142 (29.58%)		

Table IV. Genotype (CC, CT, TT) and allele (C, T) distribution of rs401681 and rs402710 *CLPTM1L* gene in neoplastic and healthy tissues according to tobacco smoking; χ^2 test
Tabela IV. Rozkład genotypów (CC, CT, TT) oraz alleli (C, T) polimorfizmów rs401681 i rs402710 genu *CLPTM1L* w tkance zmienionej nowotworowo i w tkance zdrowej w zależności od wywiadu dotyczącego palenia tytoniu; test χ^2

Polymorphism/Tissue	Genotype/Allele	All n	Tobacco smoking NO n (%)	Tobacco smoking YES n (%)	χ2 test p-value	Fisher's test p-value
rs401681 adenocarcinoma tissue	CC	45	8 (40%)	37 (33%)	0.7741	0.7441
	CT	69	9 (45%)	60 (53.6%)		
	TT	18	3 (15%)	15 (13.4%)		
	C		25/40 (62.5%)	134/224 (59.82%)	0.7499	0.8612
	T		15/40 (37.5%)	90/224 (40.18%)		
rs401681 healthy tissue	CC	47	8 (44.4%)	39 (34.8%)	0.7314	0.7754
	CT	67	8 (44.4%)	59 (52.7%)		
	TT	16	2 (11.1%)	14 (12.5%)		
	C		24/36 (66.67%)	137/224 (61.16%)	0.5277	0.5830
	T		12/36 (33.33%)	87/224 (38.84%)		
rs402710 adenocarcinoma tissue	CC	54	9 (45%)	45 (39.8%)	0.8492	0.9307
	CT	69	10 (50%)	59 (52.2%)		
	TT	10	1 (5%)	9 (8%)		
	C		28/40 (70.0%)	149/226 (65.93%)	0.6150	0.7174
	T		12/40 (30.0%)	77/226 (34.07%)		
rs402710 healthy tissue	CC	62	10 (50%)	52 (46%)	0.5184	0.7816
	CT	64	10 (50%)	54 (47.8%)		
	TT	7	0 (0%)	7 (6.2%)		
	C		30/40 (75.0%)	158/226 (69.91%)	0.5147	0.5734
	T		10/40 (30.0%)	68/226 (30.09%)		

The investigation of the genotypes and alleles of both the SNPs conducted between the subgroups divided by sex (women vs men), tobacco smoking (Yes/No) and survival (Yes/No) did not establish statistical variations either in the neoplastic or healthy tissue specimens (not shown).

Patient survival analysis

The median observation time was 44.55 (21.29−61.17) months. The patients who died (56.4%) survived for 22.6 (12.06−34.43) months from the time of a lung cancer diagnosis. The survival analysis based on Kaplan-Meier curves did not demonstrate differences in the subgroups divided by either gender (Figure 1), tobacco smoking or family history (not shown).

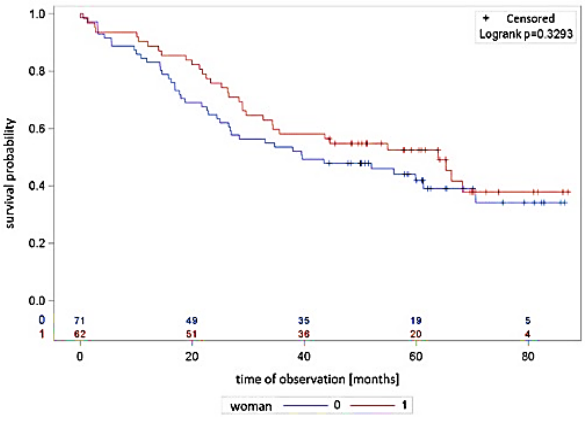

Fig. 1. Patient survival depending on sex; Kaplan-Meier curves.
Ryc. 1. Przeżycie chorych w zależności od płci; krzywe Kaplana i Meiera.

The patient's age at the time of diagnosis, the N (nodulus) category of TNM classification as well as high clinical staging appeared to be the only factors significant different between the subgroups (Figures 2 and 3).

There were no differences of survival depending on the genotype or allele distribution of rs401681 (Figures 4 and 5) or rs402710 (Figures 6 and 7) of the *CLPTM1L* gene.

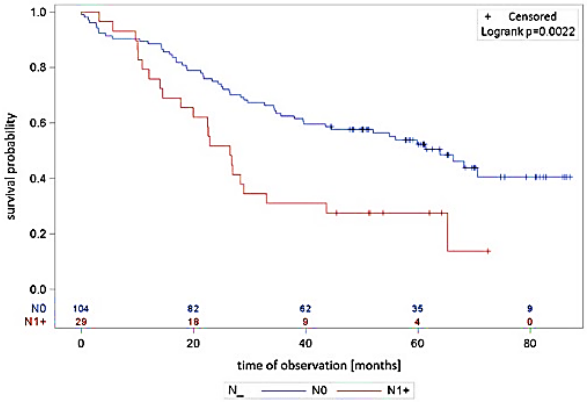

Fig. 2. Patient survival depending on N category of tumor, node, metastasis (TNM) neoplasm staging; Kaplan-Meier curves.
Ryc. 2. Przeżycie chorych w zależności od kategorii N według klasyfikacji TNM (*tumor, node, metastasis*); krzywe Kaplana i Meiera.

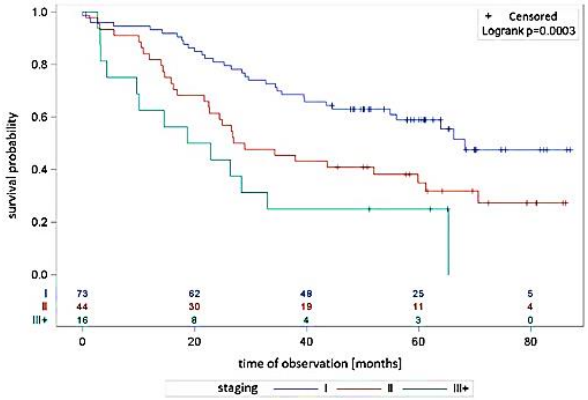

Fig. 3. Patient survival depending on neoplasm staging; Kaplan-Meier curves.
Ryc. 3. Przeżycie chorych w zależności od zaawansowania nowotworu; krzywe Kaplana i Meiera.

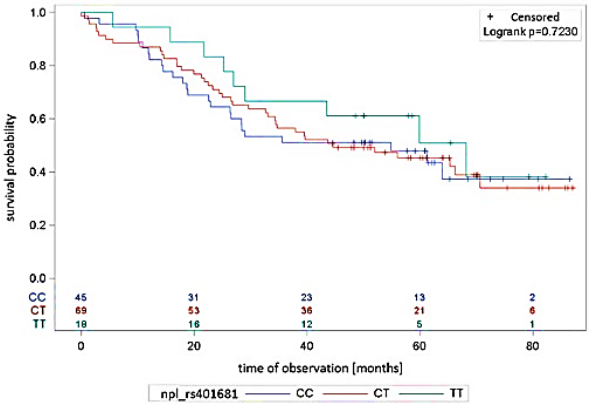

Fig. 4. Patient survival depending on rs401681 genotype in neoplastic lung tissue; Kaplan-Meier curves.
Ryc. 4. Przeżycie chorych zależnie od genotypu polimorfizmu rs401681 w tkance płuc zmienionej nowotworowo; krzywe Kaplana i Meiera.

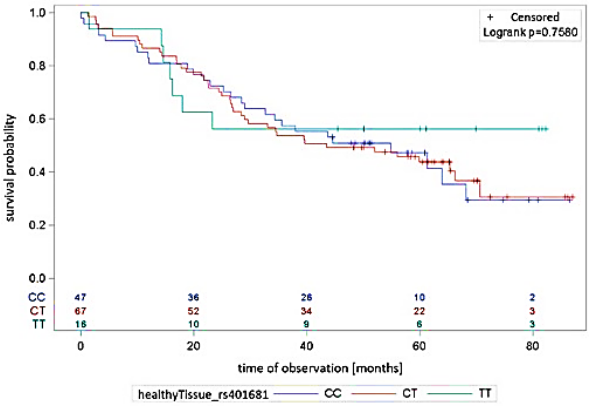

Fig. 5. Patient survival depending on rs401681 genotype in normal lung tissue; Kaplan-Meier curves.
Ryc. 5. Przeżycie chorych w zależności od genotypu polimorfizmu rs401681 w prawidłowej tkance płuc; krzywe Kaplana i Meiera.

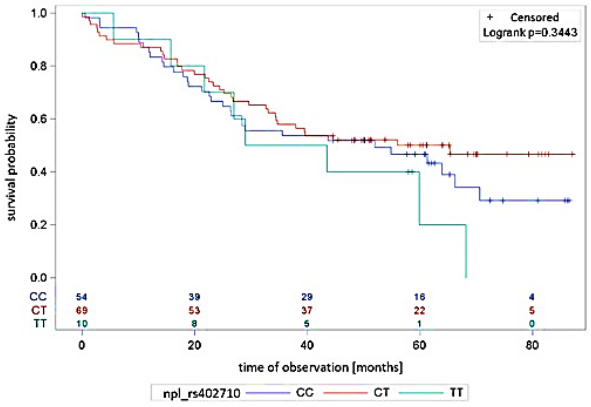

Fig. 6. Patient survival depending on rs402710 genotype in neoplastic lung tissue; Kaplan-Meier curves.
Ryc. 6. Przeżycie chorych zależnie od genotypu polimorfizmu rs402710 w tkance płuc zmienionej nowotworowo; krzywe Kaplana i Meiera.

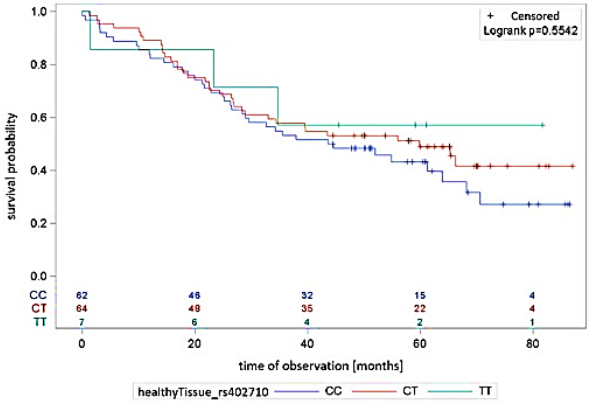

Fig. 7. Patient survival depending on rs402710 genotype in normal lung tissue; Kaplan-Meier curves.
Ryc. 7. Przeżycie chorych w zależności od genotypu polimorfizmu rs402710 w prawidłowej tkance płuc; krzywe Kaplana i Meiera.

Multivariate analysis

In the contrast to the genotype or allele distribution of the rs401681 and rs402710 polymorphisms of the *CLPTM1L* gene, as well as the patients' sex, smoking and family history, the patient's age at the time of diagnosis and carcinoma staging were the only factors influencing patient survival in multivariate analysis (not shown).

DISCUSSION

The recent data of 2022 indicates that *CLPTM1L* gene SNPs have a significant impact on the susceptibility to various cancers. Among them not only lung but also bladder, esophageal, pancreatic and skin cancers are described [12]. The high expression of the *CLPTM1L* gene was found to promote poor prognosis and increase the invasion, proliferation and migration of oral squamous cell carcinoma [16]. According to research, *CLPTM1L* seems to be the most often overexpressed antiapoptotic factor in lung tumors and is associated with DNA damage [17]. *CLPTM1L* overexpression can predict poor prognosis in patients with lung cancer [18]. The antiapoptotic feature of *CLPTM1L* is a potential mechanism of the invasion of human lung cancer cells and neoplasm susceptibility to resistance to chemotherapy [19]. However, Ni et al. [20] suggested that *CLPTM1L* may be important for maintaining cellular stability and that a loss of this function might result in increased chemosensitivity to cisplatin. It has been also demonstrated that *CLPTM1L* acts as a critical coactivator of estrogen receptor β (ERβ), which is involved in the progression of non-small cell lung carcinoma, among others, by inducing cancer cell radioresistance [21].

The implications of rs401681 and rs402710 *CLPTM1L* gene polymorphisms on carcinogenesis are not completely clarified. Rs401681, located in the intron of the *CLPTM1L* gene, can regulate its expression, while rs402710, located in a non-coding transcript exon variant, can affect the regulation of transcription that causes overexpression of the *CLPTM1L* gene. Both rs401681and rs402710 and also the entire coding of the *CLPTM1L* gene, as well as a promoter region of the *TERT* gene are regions of high linkage disequilibrium [22]. The linkage disequilibrium pattern is probably different in various populations, i.e. in Asia, Europe and Africa. As the *CLPTM1L* gene is located near the *TERT* gene, it could regulate telomerase reverse transcriptase expression, and by this way influence apoptosis [12]. Based on East Asian results, it is suggested that the region containing rs401681 and rs402710 could interact with a *TERT* promoter; hence, these SNPs could confer lung cancer risk by regulating *TERT* expression instead of *CLPTM1L* [23]. It is recognized that rs402710 may affect lung tissue tumorigenesis in vitro by blocking DNA damage--induced apoptosis via the enhanced accumulation of an antiapoptotic agents [19].

In Zhao et al. [24], a study performed among Caucasians and East Asians, significantly increased risks for lung malignancy were found for rs402710 and rs401681 in all the genetic models. In addition, it was identified that both these SNPs express significantly greater risks for adenocarcinoma and squamous cell carcinoma when stratified by the histological type of tumors. Furthermore, associations of these polymorphisms with lung carcinoma risk were observed both in current and former smokers. It is even compelling when considering the fact that the *CLPTM1L* – rs401681 (G > A) polymorphism was found to be significantly associated with decreased lung cancer risk, especially among European populations [25]. Also Tang et al. [26] reported that the *CLPTM1L* gene rs402710 (C > T) and rs401681 (C > T) polymorphisms are associated with decreased cancer risk. It is consistent with the protective association of

the above-mentioned *CLPTM1L* SNPs with lung carcinoma, which was reported by authors from Asia. Xun et al. [11] found that rs402710 and rs401681 were associated with a decreased lung cancer risk in the northwest Chinese Han population. They identified that the minor alleles of rs402710 in *CLPTM1L* were associated with a 0.76-fold decreased risk of lung cancer. In a study conducted by Chen et al. [27], the TT genotype was less frequent in patients with lung adenocarcinoma in both the rs402710 and rs401681 genotypes than in the controls. In 2013 Li et al. [22] performed a meta-analysis and tried to explain some conflicting data concerning the *TERT* and *CLPTM1L* relationship with cancers. They suggested that the *TERT-CLPTM1L* region may have different effects in different cancer types and differing data might be due to different allele frequencies in various ethnicities, as seen in HapMap. It could be also explained by the varying linkage disequilibrium (LD) of the studied SNPs with other potential or causal ones. They indicate that both the rs402710 and rs401681 influence an increased cancer risk but in different genetic models: rs402710 in heterozygous and homozygous, while rs401681 only in the homozygous variant. Furthermore, the functional mechanisms of the *CLPTM1L* gene polymorphism variants related to lung cancer risk are considered to be different in various ethnic groups. Considering the genetic changes underlying the neoplastic transformation of cells, changes in the distribution of genotypes and alleles of the studied polymorphisms in the cancerous and healthy lung tissue could be expected.

To our knowledge, to date there have been no studies concerning rs401681 and rs402710 polymorphisms in tissues of patients with lung adenocarcinoma. For this reason, we analysed the genotype and allele distribution of rs401681 and rs402710 in two separate tissue samples (obtained from a lung tumor and its healthy surroundings) of each person from the group of patients with histologically confirmed lung adenocarcinoma. We used an interesting and rare procedure to obtain DNA involving the use of paraffin-embedded lung tissue specimens stored in the archives of the Department of Pathomorphology. The genetic results were analysed in relation to gender, tobacco smoking, family history of malignancy and patient survival. Neither the genotype nor allele distribution of the studied SNPs established in the neoplastic and healthy lung tissue statistically differed in the whole study group. The same observations between the subgroups divided by gender, cigarette smoking or survival (deceased vs living at the time of observation) were noticed. No significant changes in the genotype or allele frequency were observed in the neoplasm tissue obtained from the women and men, smokers and no-smokers, dead and survivors, just as in the case of healthy tissue. An interesting observation was a marked tendency for differences in the rs401681 genotype distribution in the adenocarcinoma tissue between men and women (TT genotype), but it did not achieve the limit of statistical significance.

Based on literature data, our observations are no exception. It was demonstrated that the incidence of LOH is variable in different histological types of neoplasm, even if it concerns the same organ [8]. For example, the occurrence of LOH was reported in squamous neoplasms but not in adenocarcinoma in oral carcinoma [9].

In our study, the influence of the genotype or allele distribution on patient survival depicted in the Kaplan-Meier plots was not significant. The only negative risk factors revealed for survival were the patient's age at the time of diagnosis, the N category of TNM carcinoma classification and neoplasm staging (according to 8th ed. UICC criteria from 2018). This observation is not surprising. Our results do not confirm the initial assumptions of the study, but we are aware that it could be the effect of the small sample size. Another explanation for our observations is not reassuring. It is quite possible that genetic changes cross the tumor border and also affect tissue without histological pathologies, misdiagnosed as normal and healthy. The such a suspicion should be confirmed. The lack of a control group consisting of healthy people as well as DNA derived from the patient's blood sample complicate carrying out a comprehensive analysis and the formulation of reliable conclusions. All the above points to further work on this interesting topic.

CONCLUSIONS

The results obtained in this study seem to justify the following conclusions:

1. Both neoplastic and healthy lung tissue had a comparable distribution of both the genotype and allele of rs401681 and rs402710 of the *CLPTM1L* gene in patients with lung adenocarcinoma.
2. The patient's survival time was not dependent on the genotype of the studied SNPs either in the neoplastic or healthy lung tissue, while the patient's age at the time of diagnosis, the N category of TNM carcinoma classification and neoplasm staging (according to 8th ed. UICC criteria from 2018) were the only significant affecting factors.

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Conflict of interest

The authors declare that no competing interests exist.

Author's contribution

Study design – D. Czyżewski, B. Drozdzowska
Data collection – M. Rydel, D. Czyżewski, B. Drozdzowska
Data interpretation – J. Żywiec, K. Klimczyk, D. Czyżewski
Statistical analysis – J. Kasperczyk, J. Żywiec
Manuscript preparation – J. Żywiec
Literature research – K. Klimczyk, J. Żywiec, M. Rydel

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