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Tagging SNPs in the Excision Repair Cross-Complementing Group 4 (*ERCC4*) Gene Increased Risk of Cervical Squamous Cell Carcinoma (CSCC) and Modulate the Disease Outcome

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Abstract

Background: Given the important role of *ERCC4* gene in multiple DNA repair systems, we hypothesized that genetic variations within this gene may be a cervical squamous cell carcinoma (CSCC) risk and disease modulatory factor.

Methods: In population-based, case-control association study including 143 CSCC patients and 207 healthy women, two *ERCC4* tagSNPs were studied.

Results: A significant protective effect against CSCC was observed assuming a dominant model in case of *ERCC4*rs3136176 ([AA]+[AT]vs.[TT]: p=0.04,OR=0.43), and genotype [AA] strongly protects against poorly (G3) differentiated CSCC ($p_{correced}$=0.008,OR=0.15) and significantly increased the disease remission rate (p=0.05,OR=0.48).

A statistically significant increase frequency of *ERCC4*rs1799798 [A] allele was seen in patients with well differentiated (G1) CSCC (p=0.02, OR=2.40). Contrary, an opposite trend was observed when G1 was compared with G2 (moderately differentiated) CSCC (p=0.06). Furthermore, *ERCC4*rs1799798 [A] allele tended to be increased in patients with carcinoma planoepitheliale keratodes (Cpk) (p=0.07).

Haplotype *ERCC4*rs3136176[A]/ERCC4rs1799798[G] significantly decreased risk of G1 as well as G3 CSCC (p=0.02,OR=0.50, and p=0.017,OR=0.42, respectively) and only tended to decrease risk of CSCC (p=0.07,OR=0.758) as well as carcinoma planoepitheliale akeratodes (Cpa) (p=0.059,OR=0.71).

In contrast haplotype AA significantly increased risk of G1 CSCC and risk of Cpk (p=0.01, OR=2.51, and p=0.049, OR=1.96, respectively), whereas haplotype TG increased risk of G3 CSCC (p=0.037, OR=2.17).

The overall survival rates showed similar mean survival rates according to patients' genotypes at both studied SNPs.

Conclusion: The above findings consistently suggested that genetic variants in *ERCC4* gene may play significant role in CSCC pathophysiology.

Keywords: ERCC4; Genetic variation; Cervical squamous cell carcimona (CSCC); Molecular marker

Introduction

Cervical squamous cell carcinoma (CSCC) is the third most common cancer diagnosed in women worldwide, with a disproportionate share of the mortality associated with this disease occurring in developing countries. Worldwide, approximately 500,000 new cases are diagnosed each year, with a higher rate of incidence among women of lower socioeconomic status, especially in developing countries [1]. It is well known that cancer progression is associated with persistent high-risk human papillomaviruses (HPV) infection and with deregulated viral gene expression, which leads to excessive cell proliferation, deficient DNA repair, and the accumulation of genetic damage in the infected cell [2]. Dozens of genes are involved in DNA damage repair to maintain genomic stability through different pathways, including direct repair, base excision repair, nucleotide

excision repair (NER), mismatch repair and double-strand break repair [3,4].

NER, one of the most versatile DNA repair systems, is responsible for repairing damage caused by many exogenous mutagens. The NER pathway consists of two distinct molecular processes: one - the Global Genome NER (GG-NER), which repairs DNA lesions independently of their localization in the genome, and second - the Transcription-Coupled NER (TC-NER) pathway, which is activated by lesions in DNA regions involved in transcription. In both cases, the unfolding of the double DNA helix is assured by the helicases XPD (polarity 3'-5') and XPB (polarity 5'-3') [5]. This process renders the lesion accessible to the endonuclease XPG, which, in conjunction with the helicase XPA, recognizes and verifies the presence of the DNA lesion and subsequently performs the incision of the damaged nucleotide in the 3' edge of the lesion. In a third critical step, the endonuclease XPF (ERCC4), in association with ERCC1, removes the damaged nucleotide from the 5' edge of the damaged chain and deliberates a fragment of 24–32 bases [6,7]. Finally, a complex of DNA polymerases and ligases are recruited in order to perform the synthesis of missing complementary nucleotides and restore normal nucleotide sequence in the damaged chain [6,7].

Besides NER, ERCC4 is also uniquely involved in recombination of DNA inter-strand crosslinking repair (ICL), and takes part in double strand break repair because of its unique function in damage site recognition [8,9].

So, since *ERCC4* is involved in both mentioned above repair systems its role in the cancer pathogenesis is postulated. It has been noted that expression level of *ERCC4* correlated with risk, progression, cisplatine resistance and clinical course of human cancers, including squamous cell carcinoma of the head and neck (SCCHN) [10-16]. Additionally, it is also hypothesized that XPF has potential to guide next-generation personalized cancer therapy since expression level of XPF in squamous cell carcinoma of the head and neck (SCCHN) tumors correlates with clinical response to DNA damaging agents [10]. Moreover, other study showed that the relative expression level of XPF was the only independent risk factor for SCCHN from simultaneously studied other proteins (i.e. ERCC1, XPA, XPC, XPD, and XPG) and may be a crucial rate-limiting factor in DNA repair [11].

The genetic alterations of this gene may affect the function of their proteins and lead to diseases or cancers. In addition to expression studies, polymorphism analysis of DNA repair genes, including *ERCC4* gene, can determine whether germline allelic variants are linked to cancer susceptibility and response to DNA damaging therapy.

The *ERCC4* gene is mapped to chromosome 16p13.12, ~ 42 kb in size, consists of 11 exons and 10 introns (Figure 1) and a total of 580 single nucleotide polymorphisms (SNPs) in human *ERCC4* have been reported according to the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?chooseRs=all&go=Go&locusId=2072).

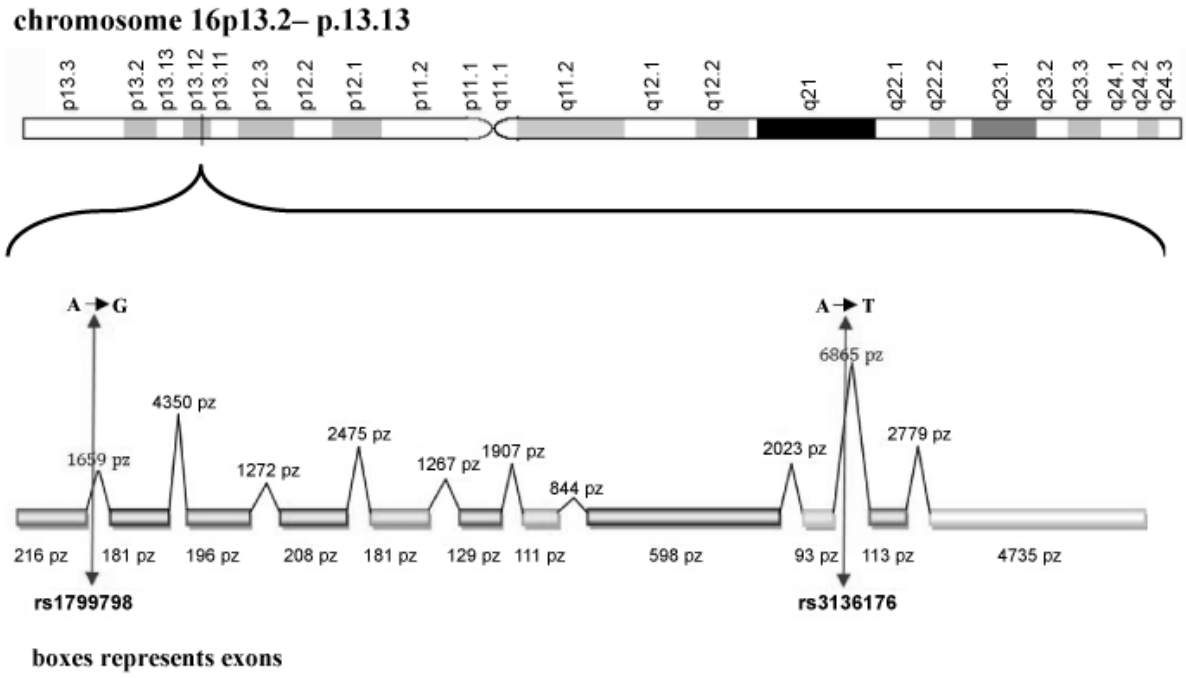

Figure 1. Schematic structure of *ERCC4* gene with selected tagSNPs location within gene with chromosome location.

So far, genetic variation within *ERCC4* gene has been studied in breast [17-24], endometrial [25], lung [26,27], melanoma [28], pancreatic [29], laryngeal [30,31], bladder [32-34], colorectal [35,36] and head and neck [37-39] cancer with the inconsistent results but in cervical squamous cell carcinoma it will be the first study.

In this case–control study of cervical squamous cell carcinoma, we examined potential associations between *ERCC4* gene, involved in the NER pathway and in ICL, and risk of CSCC, as well as potential modification of disease outcome. Thus, we performed a population-based case-control study to evaluate the associations between two tagSNPs in *ERCC4* gene: rs3136176 and rs1799798 and risk of CSCC in Polish women using data from a case–control study of CSCC, as well as we looking for possible disease-modifying associations.

Subjects and Methods

Study population

A total of 143 Caucasian patients with cervical cancer were studied. Patients were treated at Department of Oncology and Gynecological Oncology Clinic, Wroclaw University of Medicine. All cases of cervical cancer were histologically defined as cervical squamous cell carcinoma (CSCC), of which 23 cases were well differentiated (G1), 89 cases moderately (G2), and 16 poorly differentiated (G3) [40]. Stage of the disease was classified according the International Federation of Gynecology and Obstetrics (FIGO) [41]. Classification resulted as follows: stage I: 23 patients; stage II: 49 patients; stage III: 53 patients; and stage IV: 9 patients. In three patients there was no stage description.

Blood samples were taken from patients with CSCC before radiotherapy.

According to tumor stage and extent, radiotherapy alone or combined with concurrent chemotherapy was administered. All patients were treated by external beam radiotherapy (conformal planning); most of them also received brachytherapy (LDR or HDR). A total of 128 patients were treated with curative intent, and 15 received palliative treatment. The patients were evaluated for tumor response: after radical treatment complete regression was achieved in 77 patients and in 18 cases, only partial regression was seen. In 33 patients disease progression was seen.

The control group consisted of 207 healthy, cancer-free women at the time of recruitment. All participants were Polish Caucasians.

Informed consent was obtained from each individual. The study was approved by the local ethics committee.

Genotyping/determination of polymorphisms: Amplification of genomic DNA

Genomic DNA was prepared from peripheral white blood cells using the QIAamp® DNA Blood Mini Kit (Qiagen, Germany) as described previously by Pawlak *et al.* [42].

Single-nucleotide polymorphisms (SNPs) were selected across preselected cancer-related genes, choosing Tag SNPs with use the LD TAG SNP Selection platform on website of National Institutes of Environmental Health Sciences for Europeans (http://snpinfo.niehs.nih.gov/snptag.htm).It uses a refined greedy algorithm originally implemented in software TAGster [43] for LD tag SNP selection. LD is measured by r^2 or composite linkage disequilibrium (CLD).

The *ERCC4*rs3136176 and *ERCC4*rs1799798 SNPs were genotyped with the Allelic discrimination (AD) technique with use the TaqMan® SNP Genotyping Assays (made to Order, C___3285107_10 and C___7487495_20, respectively). In AD assay a unique pair of fluorescent dye detectors is used (two TaqMan® MGB probes that target a SNP site) and the change in fluorescence of the dyes associated with the probes are measured.

All the assays were validated and predesigned. Reaction components and amplification parameters were based on manufacturer's instructions. The ABI Prism® 7300 (Life Technologies) sequence-detection system was used for amplification for TaqMan SNP genotyping assay plates. SDS version 2.1 (Life Technologies) software was used for data acquisition and analysis. The same software was used for the allelic discrimination-analysis module.

Plate genomic control DNA samples and non-template controls (water) were included for each reaction. The TaqMan SNP genotyping assay was controlled (25% of randomly chosen samples from both groups) to check for genotyping accuracy. Identical genotypes were identified in all repeated samples. The ID sample subjects during genotyping were not known.

Statistical analyses

Evaluation of the Hardy-Weinberg equilibrium (HWE) was performed for the whole studied groups by comparing the observed and expected frequencies of genotypes using χ^2 analysis. The χ^2 test was used to compare categorical data between patients with CSCC and controls. When at least one cell value was not more than 5, the Yates' correction was applied to the χ^2-value. Differences were considered as statistically significant if the p value was <0.05.

Because of the multiple comparisons of the allele, genotype, and haplotype frequencies, Bonferroni multiple adjustments were employed to the level of significance. The haplotype frequencies for pairs of alleles were estimated using the SHEsis program (http://analysis.bio-x.cn/myAnalysis.php) [44,45].

The log-rank test was used to assess the effect of each polymorphic variable on disease-free survival from the date of starting therapy to the date of last follow-up. Analysis was cured on the group of patients radically treated with the stage of the disease II and III. A total of 70 patients were assessed in this analysis; 15 manifested as having well-differentiated tumors (G1), 48 moderately differentiated (G2), and seven poorly differentiated CSCC (G3). Survival curves were estimated by the Kaplan-Meier method and a two-sided *p* value of <0.05 from a log-rank test was considered as statistically significant difference. Log-rank tests were performed with STATISTICA10.0 software (StatSoft, Crakow, Poland).

Results

All *ERCC4* genotype distributions in the controls as well as in CSCC patients were consistent with those expected from the Hardy–Weinberg equilibrium model (all $p>0.05$, Table 1).

SNPs in the ERCC4 gene

Although no association was found when comparing the genotype in both studied polymorphic sites, a formally significant protective effect was observed assuming a dominant model in case of *ERCC4*rs3136176 SNP ([AA] + [AT] vs. [TT]: p= 0.04, OR= 0.43, 95% CI:0.19 – 0.99, Table 1). Moreover, when the rs3136176 [AA] homozygote was used as the reference group, [TT] genotype tended to increased risk of disease (p=0.06, OR=2.23, 95% CI: 0.95–5.22, Table 1).

	CSCC patients n=143 n (%)	**Control group** n=207 n (%)	**p**$^{total\#}$	**p**	**OR**	**95%CI**
rs3136176						
Genotype						
[AA]	78 (54.5)	116 (56.0)	ns[1]	referent		
[AT]	50 (35.0)	81 (39.2)		0.71	0.92	0.58 – 1.45
[TT]	15 (10.5)	10 (4.8)		0.06	2.23	0.95 – 5.22
Allele						
[A]	206 (72.0)	313 (75.6)		0.29	0.83	0.59 – 1.17
[T]	80 (28.0)	101 (24.4)			1.20	0.86 – 1.69
Dominant model						
[AA] + [AT] vs. [TT]	128 (89.5)	197 (95.2)		0.04	0.43	0.19 – 0.99
	15 (10.5)	10 (4.8)			2.31	1.01 – 5.30
Recessive model						
[TT] + [AT] vs. [AA]	65 (45.5)	91 (44.0)		0.78	1.06	0.69 – 1.63
	78 (54.5)	116 (56.0)			0.94	0.61 – 1.45
rs1799798						
Genotype						
[GG]	107 (74.8)	162 (78.3)	ns[2]	referent		
[GA]	32 (22.4)	42 (20.3)		0.59	1.15	0.69 – 1.94
[AA]	4 (2.8)	3 (1.4)		0.59*	2.02	0.44 – 9.20
Allele						
[G]	246 (86.0)	366 (88.4)		0.35	0.81	0.51 – 1.26
[A]	40 (14.0)	48 (11.6)			1.24	0.79 – 1.94
Dominant model						
[GG] + [GA] vs. [AA]	137 (97.3)	207 (98.6)		0.59	0.50	0.11 – 2.25
	4 (2.8)	3 (1.4)			2.01	0.44 – 9.14
Recessive model						
[AA] + [GA] vs. [GG]	36 (25.2)	45 (21.7)		0.45	1.21	0.73 – 2.00
	107 (74.8)	162 (78.3)			0.83	0.50 – 1.36

OR – Odds Ratio
95%CI – 95% Confidence Intervals
^– p-value after Bonferroni correction
* – p-value after Yate's correction
# – global p-value for genotype

rs3136176	rs1799798
[1] - χ^2= 4.217333, df= 2, p= 0.121508 $HWE_{CSCC\ patients}$: χ^2= 2.502273, p= 0.113745	[2] - χ^2= 1.072568, df= 2, p= 584951 $HWE_{CSCC\ patients}$: χ^2= 0.699073, p= 0.403135

HWE $_{controls}$: χ^2= 0.764352, p= 0.382012	HWE $_{controls}$: χ^2= 0.021731, p= 0.882812

Table 1: *ERCC4*rs3136176 and *ERCC4*rs1799798 genotype, allele and phenotype frequencies in cervical squamous cell carcinoma (CSCC) patients and controls.

In our group of healthy cancer-free women and CSCC patients both studied SNPs were in tight LD (control group: D'= 0.820, CSCC patients: D'= 0.998).

Haplotype evaluation of studied polymorphic sites was performed and the frequencies of the haplotypes did not differ in the patients and in the controls (the global p value after the Bonferroni correction was 0.18) notwithstanding a trend toward decreased frequency of *ERCC4*rs3136176[A]/*ERCC4*rs1799798[G] haplotype was noted in CSCC patients as compared with healthy women (p=0.068, χ^2= 3.314, OR=0.750, 95% CI: 0.550 – 1.023, Table 2).

	CSCC patients	Control group	χ^2	p	OR	95%CI
ERCC4rs3136176 / ERCC4rs1799798						
A A	39.98 (0.140)	45.89 (0.111)	1.262	0.261335	1.296	0.823 – 2.041
A G	166.02 (0.580)	267.11 (0.645)	3.314	0.068698	0.750	0.550 – 1.023
T G	79.98 (0.280)	98.89 (0.239)	1.388	0.238713	1.229	0.872 – 1.732
T A	0.02 (0.000)	2.11 (0.005)	–	–	–	–
Total control= 414.0, total case= 286.0 $\chi2_{Global}$= 3.395831, df= 2 (frequency <0.03 in both control & case has been dropped), p= 0.183065						

Table 2. Haplotype (ERCC4rs3136176 / ERCC4rs1799798) frequencies in cervical squamous cell carcinoma (CSCC) patients and controls.

ERCC4 gene polymorphisms and clinical data in CSCC

The features of the *ERCC4* genes polymorphisms according to the patients clinicopathologic characteristics, namely, the grade, stage of disease, response to treatment and progression-free survival was analyzed.

The association between *ERCC4* SNPs and susceptibility to CSCC varied in relation to histologic grade of tumor. The distribution of *ERCC4*rs3136176 genotypes significantly varies between CSCC patients and healthy women ($p_{after\ Bonferroni\ correction}$= 0.01, χ^2= 10.269320, Table 3). Particularly, when the rs3136176[AA] homozygote was used as the reference group, the [TT] genotype was associated with a significantly increased risk of poorly (G3) differentiated CSCC ($p_{after\ Yate's\ correction}$= 0.01, OR= 6.57, 95% CI:1.64 – 26.33, Table 3). In a dominant model ([AA] + [AT] vs. [TT]) this association was much stronger ($p_{after\ Yate's\ correction}$ = 0.008, OR=6.57, 95% CI: 1.79 – 24.01, Table 3).

	Control group (n= 207) n (%)	G1 (n= 23) n (%)	G2 (n= 89) n (%)	G3 (n= 16) n (%)		p_{total}	p	OR	95%CI
rs3136176									
Genotype									
[AA]	116 (56.0)	13 (56.5)	47 (52.8)	7 (43.8)		ns[1]	referent		
[AT]	81 (39.1)	7 (30.4)	34 (38.2)	5 (31.2)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control	ns[2] 0.01[3] ns[4] ns[5] ns[6]	0.57 0.71 0.77 0.57 1.00 0.77	1.34 1.33 0.99 0.76 0.99 1.00	0.49 – 3.72 0.31 – 5.77 0.29 – 3.38 0.29 – 1.98 0.58 – 1.67 0.31 – 3.26
[TT]	10 (4.8)	3 (13.1)	8 (9.0)	4 (25.0)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control		1.00 0.56 0.20 0.35	0.74 2.48 3.36 2.65	0.17 – 3.18 0.43 – 14.34 0.80 – 14.16 0.65 – 10.89

					G2 vs. control G3 vs. control		0.18 0.01*	1.96 6.57	0.73 – 5.27 1.64 – 26.33
Allele									
[A]	313 (75.6)	33 (71.7)	128 (71.9)	19 (59.4)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		1.00 0.25 0.15 0.56 0.34 0.04	1.01 0.58 0.57 0.82 0.83 0.47	0.49 – 2.07 0.22 – 1.49 0.26 – 1.24 0.42 – 1.62 0.56 – 1.23 0.23 – 0.99
[T]	101 (24.4)	13 (28.3)	50 (28.1)	13 (40.6)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		1.00 0.25 0.15 0.56 0.34 0.04	0.99 1.74 1.75 1.22 1.21 2.12	0.48 – 2.04 0.67 – 4.51 0.81 – 3.81 0.62 – 2.41 0.81 – 1.80 1.01 – 4.45
Dominant model									
[AA]+[AT] vs. [TT]	197 (95.2)	20 (84.9)	81 (91.0)	12 (75.0)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.85* 0.59* 0.15* 0.25* 0.17 0.008*	1.52 2.22 0.30 0.34 0.51 0.15	0.37 – 6.25 0.42 – 11.68 0.08 – 1.14 0.09 – 1.33 0.20 – 1.35 0.04 – 0.56
	10 (4.8)	3 (13.1)	8 (9.0)	4 (25.0)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control	0.85* 0.59* 0.15* 0.25* 0.17 0.008*	0.66 0.45 3.38 2.96 1.95 6.57	0.16 – 2.71 0.09 – 2.37 0.88 – 12.95 0.75 – 11.63 0.74 – 5.11 1.79 – 24.01	
Recessive model									
[TT]+[AT] vs. [AA]	91 (44.0)	10 (43.5)	42 (47.2)	9 (56.2)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.75 0.43 0.50 1.00 0.61 0.34	1.16 1.67 1.44 0.98 1.14 1.64	0.46 – 2.93 0.46 – 6.05 0.49 – 4.20 0.41 – 2.34 0.69 – 1.88 0.59 – 4.57
	116 (56.0)	13 (56.5)	47 (52.8)	7 (43.8)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control	0.75 0.43 0.50 1.00 0.61 0.34	0.86 0.60 0.70 1.02 0.88 0.61	0.34 – 2.17 0.17 – 2.17 0.24 – 2.03 0.43 – 2.43	

					G2 vs. control G3 vs. control				0.53 – 1.45 0.22 – 1.70	
rs1799798										
Genotype										
[GG]	162 (78.3)	14 (60.9)	68 (76.4)	11 (68.8)			0.067	referent		
[GA]	42 (20.3)	7 (30.4)	19 (21.3)	5 (31.2)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control	ns[8] ns[9] ns[10] ns[11] ns[12]	0.27 0.83* 0.62* 0.18 0.81 0.49*	0.56 1.10 1.63 1.93 1.08 1.75	0.20 – 1.58 0.27 – 4.43 0.50 – 5.26 0.73 – 5.08 0.59 – 1.99 0.58 – 5.32
[AA]	3 (1.4)	2 (8.7)	2 (2.2)	0 (0.0)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.32* 0.64* 0.63* 0.09* 1.00* 0.45*	0.21 – – 7.71 1.59 –	0.03 – 1.59 – – 1.19 – 50.09 0.26 – 9.72 –
Allele										
[G]	366 (88.4)	35 (76.1)	155 (90.1)	27 (84.4)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.06 0.54* 1.00* 0.02 0.65 0.69*	2.12 1.70 0.80 0.42 1.13 0.71	0.95 – 4.75 0.53 – 5.47 0.28 – 2.29 0.20 – 0.88 0.67 – 1.93 0.26 – 1.93
[A]	48 (11.6)	11 (23.9)	23 (9.9)	5 (15.6)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.06 0.54* 1.00* 0.02 0.65 0.69*	0.47 0.59 1.25 2.40 0.88 1.41	0.21 – 1.06 0.18 – 1.90 0.44 – 3.57 1.14 – 5.03 0.52 – 1.50 0.52 – 3.84
Dominant model										
[GG] + [GA] vs. [AA]	204 (98.6)	21 (91.3)	87 (97.8)	16 (100.0)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.39* 0.22* 0.15* 0.13* 1.00* 0.52*	4.14 – – 0.15 0.64 –	0.55 – 31.14 – – 0.02 – 0.98 0.11 – 3.90 –
	3 (1.4)	2 (8.7)	2 (2.2)	0 (0.0)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control	0.39* 0.22* 0.15* 0.13* 1.00* 0.52*		0.24 – – 6.48 1.56 –	0.03 – 1.81 – – 1.02 – 40.97 0.26 – 9.52	

					G2 vs. control G3 vs. control				–	
Recessive model										
[AA] + [GA] vs. [GG]	45 (21.7)	9 (39.1)	21 (23.6)	5 (31.2)		G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control		0.13 0.87* 0.73* 0.06 0.72 0.57	0.48 0.71 1.47 2.31 1.11 1.64	0.18 – 1.27 0.18 – 2.72 0.46 – 4.72 0.94 – 5.69 0.62 – 2.01 0.54 – 4.95
	162 (78.3)	14 (60.9)	68 (76.4)	11 (68.8)	G1 vs. G2 G1 vs. G3 G2 vs. G3 G1 vs. control G2 vs. control G3 vs. control	0.13 0.87* 0.73* 0.06 0.72 0.57		2.08 1.41 0.68 0.43 0.90 0.61	0.79 – 5.49 0.37 – 5.45 0.21 – 2.18 0.18 – 1.06 0.50 – 1.62 0.20 – 1.85	

^– p-value after Bonferroni correction

* – p-value after Yate's correction

#– global p-value for genotype

Bold indicates a statistically significant differences

rs3136176	rs1799798
[1] – G1 vs. controls: χ^2= 2.880038, df= 2, p= 0.236923	[7] – G1 vs. controls: χ^2= 6.818182, df= 2, p= 0.033148
2 – G2 vs. controls: χ^2= 1.901089, df= 2, p= 0.386531	[8] – G2 vs. controls: χ^2= 0.296027, df= 2, p= 0.862423
3 – G3 vs. controls: χ^2= 10.269320, df= 2, p= 0.005920	[9] – G3 vs. controls: χ^2= 1.251555, df= 2, p= 0.534887
4 – G1 vs. G3: χ^2= 1.053727, df= 2, p= 0.590487	[10] – G1 vs. G2: χ^2= 3.380476, df= 2, p= 0.184581
5 – G1 vs. G3: χ^2= 1.053727, df= 2, p= 0.590487	[11] – G1 vs. G3: χ^2= 1.484755, df= 2, p= 0.476033
6 – G3 vs. controls: χ^2= 4.111672, df= 2, p= 0.042641	[12] – G2 vs. G3: χ^2= 1.046887, df= 2, p= 0.592509

Table 3: The *ERCC4*rs3136176 and *ERCC4*rs1799798 alleles and genotypes frequencies in patients with cervical squamous cell carcinoma (CSCC) well (G1), moderately (G2) and poorly (G3) differentiated and healthy women.

In case of SNP rs1799798, when the [GG] genotype was used as reference group, we observed a trend toward increased risk of well differentiated (G1) CSCC in case of patients with [AA] genotype ($p_{after\ Yate's\ correction}$= 0.09, OR= 7.71, 95% CI: 1.19 – 50.09, Table 3). Stronger, but not statistically significant, association was seen when the recessive model ([AA] + [GA] *vs.* [GG]) was conducted (p= 0.06, OR= 2.31, 95% CI:0.94 – 5.69, Table 3).

We then investigated associations between the *ERCC4* haplotypes (reconstructed in the order of rs3136176 and rs1799798). Haplotype AA significantly increased risk of G1 CSCC (p=0.01, OR=2.507, 95% CI: 1.191 – 5.274, Table 4). The increased risk of G3 CSCC was associated with haplotype TG (p=0.037, OR= 2.166, 95% CI: 1.032 – 4.543, Table 4), whereas haplotype AG decreased the risk of G1 as well as G3 CSCC (p=0.02, OR=0.497, 95% CI: 0.269–0.917 and p=0.017, OR= 0.422, 95% CI: 0.204 – 0.872, respectively, Table 4).

	Control group	G1	G2	G3		χ^2	p	OR	95%CI

*ERCC4***rs3136176** / *ERCC4***rs1799798**									
A A	45.89 (0.111)	11.00 (0.239)	22.98 (0.129)	5.00 (0.156)	Control vs. G1	6.204	0.012770	2.507	1.191 – 5.274
					Control vs. G2	0.378	0.538724	1.183	0.693 – 2.019
					Control vs. G3	0.588	0.443270	1.477	0.542 – 4.024
					G1 vs. G2	3.437	0.063750	2.120	0.946 – 4.750
					G1 vs. G3	0.795	0.795	0.795	0.183 – 1.899
					G2 vs. G3	0.173	0.677755	1.249	0.437 – 3.569
A G	267.11 (0.645)	22.00 (0.478)	105.02 (0.590)	14.00 (0.438)	Control vs. G1	5.153	0.023232	0.497	0.269 – 0.917
					Control vs. G2	1.823	0.176953	0.780	0.544 – 1.119
					Control vs. G3	5.691	0.017071	0.422	0.204 – 0.872
					G1 vs. G2	1.861	0.172506	0.637	0.332 – 1.221
					G1 vs. G3	0.126	0.722583	0.849	0.343 – 2.102
					G2 vs. G3	2.570	0.108888	0.540	0.253 – 1.155
T G	98.89 (0.239)	13.00 (0.283)	49.98 (0.281)	13.00 (0.406	Control vs. G1	0.405	0.524449	1.247	0.631 – 2.462
					Control vs. G2	1.093	0.295801	1.236	0.831 – 1.839
					Control vs. G3	4.348	0.037069	2.166	1.032 – 4.543
					G1 vs. G2	0.001	0.980831	1.009	0.491 – 2.073
					G1 vs. G3	1.298	0.254545	1.737	0.669 – 4.508
					G2 vs. G3	2.032	0.154011	1.752	0.805 – 3.813
T A	2.11 (0.005)	0.00 (0.000)	0.02 (0.000)	0.00 (0.000))	Control vs. G1	–	–	–	–
					Control vs. G2	–	–	–	–
					Control vs. G3	–	–	–	–
					G1 vs. G2	–	–	–	–
					G1 vs. G3	–	–	–	–
					G2 vs. G3	–	–	–	–
Global result: total Control= 414.0, total G1= 46.0, total G2= 178.0, total G3= 32.0									
Control vs. G1: χ^2_{Global}= 7.638241, df= 2 (frequency <0.03 in both control & G1 has been dropped), $p_{Fisher's}$= 0.022011, $p_{Pearson's}$= 0.021947									
Control vs. G2: χ^2_{Global}= 1.823813, df= 2 (frequency <0.03 in both control & G2 has been dropped), $p_{Fisher's}$= 0.401824, $p_{Pearson's}$= 0.401758									
Control vs. G3: χ^2_{Global}= 5.859575, df= 2 (frequency <0.03 in both control & G3 has been dropped), $p_{Fisher's}$= 0.053500, $p_{Pearson's}$= 0.053408									
G1 vs. G2: χ^2_{Global}= 3.721821, df= 2 (frequency <0.03 in both G1 & G2 has been dropped), $p_{Fisher's}$= 0.155639, $p_{Pearson's}$= 0.155531									
G1 vs. G3: : χ^2_{Global}= 1.565432, df= 2 (frequency <0.03 in both G1 & G3 has been dropped), $p_{Fisher's}$= 0.457219, $p_{Pearson's}$= 0.457219									
G2 vs. G3: χ^2_{Global}= 2.685530, df= 2 (frequency <0.03 in both G2 & G3 has been dropped), $p_{Fisher's}$= 0.261217, $p_{Pearson's}$= 0.261123									

Table 4: *ERCC4*rs3136176 and *ERCC4*rs1799798 haplotype frequencies in patients with cervical squamous cell carcinoma (CSCC) well (G1), moderately (G2) and poorly (G3) differentiated and healthy women.

Stratification by histological subtypes of CSCC showed that the rs3136176 may be associated with carcinoma planoepitheliale akeratodes (Cpa) (Table 5). When the [AA] genotype was used as reference group, we observed a significant increased frequency of [TT] genotype in patients with Cpa as compared to healthy women ($p_{after\ Yate's\ correction}$= 0.03, OR= 2.71, 95% CI: 1.08 – 6.82, Table 5). Similar association was seen when the dominant model ([AA] + [GA] vs. [GG]) was conducted (p= 0.02, OR= 2.74, 95% CI: 1.12 – 6.72, Table 5).

	Control group n= 207 n (%)	**Cpk** n= 33 n (%)	**Cpa** n= 90 n (%)		p_{total}	**p**	**OR**	**95%CI**
rs3136176								
Genotype								
[AA]	116 (56.0)	19 (57.6)	47 (52.2)		ns[1]	referent		

[AT]	81 (39.2)	12 (36.4)	32 (35.6)	Cpk vs. Cpa Control vs. Cpk Control vs.Cpa	ns[2] ns[3]	0.86 0.80 1.00	0.93 0.90 0.98	0.40 – 2.17 0.42 – 1.97 0.57 – 1.66
[TT]	10 (4.8)	3 (6.1)	11 (12.2)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control		0.82* 0.64* 0.03	1.48 1.83 2.71	0.37 – 5.91 0.46 – 7.27 1.08 – 6.82
Allele								
[A]	313 (75.6)	50 (75.8)	126 (70.0)	Cpk vs. Cpa Control vs.Cpk Control vs.Cpa		0.38 1.00 0.17	0.75 1.02 0.76	0.39 – 1.43 0.56 – 1.87 0.52 – 1.13
[T]	101 (24.4)	16 (24.2)	54 (30.0)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control		0.38 1.00 0.17	1.34 0.98 1.31	0.70 – 2.56 0.53 – 1.79 0.89 – 1.94
Dominant model								
[AA] + [AT] vs. [TT]	197 (95.2)	31 (93.9)	79 (87.8)	Cpk vs. Cpa Control vs.Cpk Control vs.Cpa		0.83 0.59 0.02	0.70 0.52 0.36	0.18 – 2.66 0.14 – 2.01 0.15 – 0.89
	10 (4.8)	3 (6.1)	11 (12.2)	Cpk vs. Cpa Control vs.Cpk Control vs.Cpa		0.83 0.59 0.02	1.44 1.91 2.74	0.38 – 5.51 0.50 – 7.32 1.12 – 6.72
Recessive model								
[TT] + [AT] vs. [AA]	91 (44.0)	15 (42.4)	43 (47.8)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control		0.72 0.87 0.60	1.16 0.99 1.14	0.52 – 2.45 0.48 – 2.06 0.70 – 1.88
	116 (56.0)	19 (57.6)	47 (52.2)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control		0.72 0.87 0.60	0.86 1.01 0.87	0.39 – 1.91 0.49 – 2.09 0.53 – 1.44
rs1799798								
Genotype								
[GG]	162 (78.3)	22 (66.7)	68 (75.6)		ns[1]	referent		
[GA]	42 (20.3)	9 (27.3)	20 (22.2)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control	ns[2] ns[3]	0.48 0.29 0.68	0.72 1.58 1.13	0.29 – 1.81 0.68 – 3.68 0.62 – 2.07
[AA]	3 (1.4)	2 (6.1)	2 (2.2)	Cpk vs. Cpa Cpk vs. Control Cpa vs. Control		0.57* 0.24* 1.00*	0.32 4.91 1.59	0.04 – 2.43 0.78 – 31.03 0.26 – 9.72
Allele								
[G]	366 (88.4)	53 (80.3)	156 (86.7)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.22 0.07 0.55	1.59 0.53 0.85	0.76 – 3.35 0.27 – 1.05 0.50 – 1.44
[A]	48 (11.6)	13 (19.7)	24 (13.3)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.22 0.07 0.55	0.63 1.87 1.17	0.30 – 1.32 0.95 – 3.68 0.69 – 1.98

Dominant model								
[GG] + [GA] vs. [AA]	204 (98.6)	31 (93.9)	88 (97.8)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.62* 0.29* 0.63*	2.84 0.23 0.65	0.38 – 21.02 0.04 – 1.42 0.11 – 3.94
	3 (1.4)	2 (6.1)	2 (2.2)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.62* 0.29* 0.63*	0.35 4.39 1.55	0.05 – 2.61 0.71 – 27.31 0.25 – 9.41
Recessive model								
[AA] + [GA] vs. [GG]	45 (21.7)	11 (33.3)	22 (24.4)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.32 0.14 0.61	0.65 1.80 0.86	0.27 – 1.54 0.81 – 3.99 0.48 – 1.54
	162 (78.3)	22 (66.7)	68 (75.6)	Cpk vs. Cpa Cpk vs. control Cpa vs. Control		0.32 0.14 0.61	1.55 0.56 1.16	0.65 – 3.69 0.25 – 1.23 0.65 – 2.09

^– p-value after Bonferroni correction

* – p-value after Yate's correction; #– global p-value for genotype;

rs3136176

[1] – Cpk vs. Cpa: χ^2= 1.000745, df= 2, p= 0.606335

[2] – Cpk vs. controls: χ^2= 0.154262, df= 2, p= 0.925770

[3] – Cpa vs. controls: χ^2= 5.223751, df= 2, p= 0.073497

rs1799798

[4] – Cpk vs. Cpa: χ^2= 1.615913, df= 2, p= 0.445826

[5] – Cpk vs. controls: χ^2= 4.057297, df= 2, p= 0.131621

[6] – Cpa vs. controls: χ^2= 0.394092, df= 2, p= 0.821159

Table 5: ERCC4rs3136176 and ERCC4rs1799798 alleles and genotypes frequencies in cervical squamous cell carcinoma (CSCC): carcinoma planoepitheliale keratodes (Cpk), and carcinoma planoepitheliale aceratodes (Cpa) and healthy women.

rs1799798 SNP was not associated with histological subtypes of CSCC (Table 5), but we noted a trend toward different distribution of allele at this SNP between healthy women and patients with carcinoma planoepitheliale keratodes (Cpk) (p=0.07, Table 5).

In case of the *ERCC4* haplotypes we noted a significant increased risk of Cpk when the AA haplotype was present (p=0.049, OR=1.956, 95% CI: 0.991 – 3.861, Table 6). In contrast, the haplotype AG tended to decreased risk of carcinoma planoepitheliale akeratodes (Cpa) (p= 0.059, OR= 0.709, 95% CI:0.496 – 1.014, Table 6).

	Control group	Cpk	Cpa		χ2	p	OR	95%CI
ERCC4rs3136176 / ERCC4rs1799798								
A A	45.89 (0.111)	13.00 (0.197)	23.99 (0.133)	Cpk vs. Cpa Cpk vs. Control group Cpa vs. Control group	1.534 3.854 0.575	0.215556 0.049650 0.448350	0.627 1.956 1.226	0.298 – 1.319 0.991 – 3.861 0.723 – 2.080
A G	267.11 (0.645)	37.00 (0.561)	102.01 (0.567)	Cpk vs. Cpa Cpk vs. Control group Cpa vs. Control group	0.007 1.899 3.564	0.931129 0.168200 0.059054	1.025 0.692 0.709	0.581 – 1.810 0.408 – 1.171 0.496 – 1.014
T G	98.89 (0.239)	16.00 (0.242)	53.99 (0.300)	Cpk vs. Cpa Cpk vs. Control group Cpa vs. Control group	0.785 0.002 2.343	0.375562 0.967137 0.125836	1.339 1.013 1.356	0.701 – 2.557 0.552 – 1.858 0.917 – 2.005

T A	2.11 (0.005)	0.00 (0.000)	0.01 (0.000)	Cpk vs. Cpa Cpk vs. Control group Cpa vs. Control group	– – –	– – –	– – –	– – –
Total control group= 414.0, total Cpk= 66.0, total Cpa=180.0 Cpk vs. Cpa: $\chi 2_{Global}$= 1.868017, df= 2 (frequency <0.03 in both Cpk & Cpa has been dropped), p= 0.392975 Cpk vs. control group: $\chi 2_{Global}$= 4.070642, df= 2 (frequency <0.03 in both Cpk & control group has been dropped), p= 0.130639 Cpa vs. control group: $\chi 2_{Global}$= 3.586152, df= 2 (frequency <0.03 in both Cpa & control group has been dropped), p= 0.166447								

Table 6: *ERCC4*rs3136176 and *ERCC4*rs1799798 haplotype in cervical squamous cell carcinoma (CSCC): carcinoma planoepitheliale keratodes (Cpk), and carcinoma planoepitheliale aceratodes (Cpa) and healthy women.

We also found a link between genetic variants at rs3136176 SNP and the effect of treatment (Table 7). When the recessive model ([TT] + [AT] vs. [AA]) was applied we observed that presence of [T] allele (genotype [TT]+ [AT]) significantly decreased the disease remission rate (p=0.05, OR=2.28, 95% CI: 0.99 – 5.26, Table 7). Additionally, when the [AA] genotype was used as reference group, the same effect was observed for a heterozygous patients (p=0.035, OR=2.57, 95% CI: 1.06 – 6.27). The second studied SNP was not associated with remission rate in our group of patients, but it is noteworthy that [AA] genotype not occurred in patients with disease progression (Table 7). We also did not found any association between particular haplotype and remission rate (Table 8).

	CR (n= 77) n (%)	**PD** (n= 33) n (%)	p_{total}#	**p**	**OR**	**95%CI**
rs3136176						
Genotype						
[AA] [AT] [TT]	46 (59.7) 22 (28.6) 9 (11.7)	13 (39.4) 16 (48.5) 4 (12.1)	ns[1]	referent 0.035 0.76	 2.51 0.64	 1.06 – 6.27 0.17 – 2.40
Allele						
[A] [T]	114 (74.0) 40 (26.0)	42 (63.6) 24 (36.4)		0.12	0.61 1.63	0.33 – 1.14 0.88 – 3.02
Dominant model						
[AA] + [AT] vs. [TT]	68 (88.3) 9 (11.7)	29 (87.9) 4 (12.1)		0.80*	0.96 1.00	0.27 – 3.33 0.30 – 3.66
Recessive model						
[TT] + [AT] vs. [AA]	31 (40.3) 46 (59.7)	20 (60.6) 13 (39.4)		0.05	2.28 0.48	0.99 – 5.26 0.19 – 1.00
rs1799798						
Genotype						
[GG] [GA] [AA]	56 (72.7) 17 (22.1) 4 (5.2)	26 (78.8) 7 (21.2) 0 (0.0)	ns[2]	referent 0.81 0.43	 0.89 –	 0.33 – 2.40 –
Allele						
[G]	129 (83.8)	59 (89.4)		0.28	1.63	0.67 – 3.99

[A]	25 (16.2)	7 (10.6)			0.61	0.25 – 1.50
Dominant model						
[GG] + [GA] vs. [AA]	73 (94.8) 4 (5.2)	33 (100.0) 0 (0.0)		0.61*	– –	– –
Recessive model						
[AA] + [GA] vs. [GG]	21 (27.3) 56 (72.7)	7 (21.2) 26 (78.8)		0.50	0.72 1.39	0.27 – 1.90 0.53 – 3.69

^– p-value after Bonferroni correction

* – p-value after Yate's correction

– global p-value for genotype

1 - $\chi 2$= 4.438183, df= 2, p= 0.108815

2 - $\chi 2$= 1.836043, df= 2, p= 0.399375

Table 7: *ERCC4*rs3136176 and *ERCC4*rs1799798 genotype and allele frequencies in squamous cell cervical cancer (CSCC) patients with disease remission (CR) and disease progression (PD).

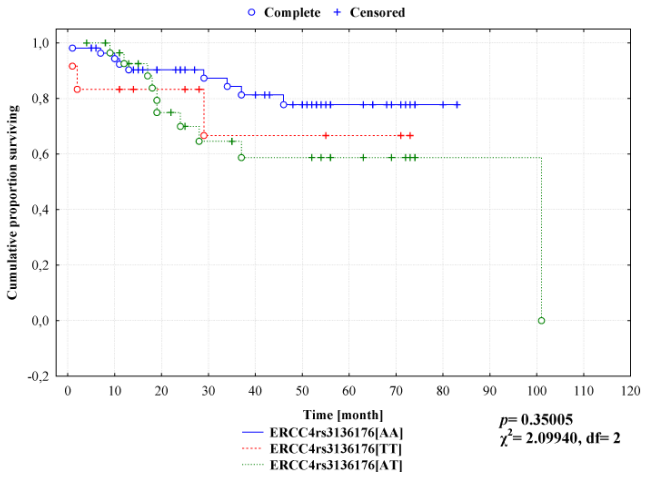

Figure 2. Cumulative proportion surviving in radically treated CSCC patient with the stage of the disease II and III stratified by *ERCC4*rs3136176 genotypes.

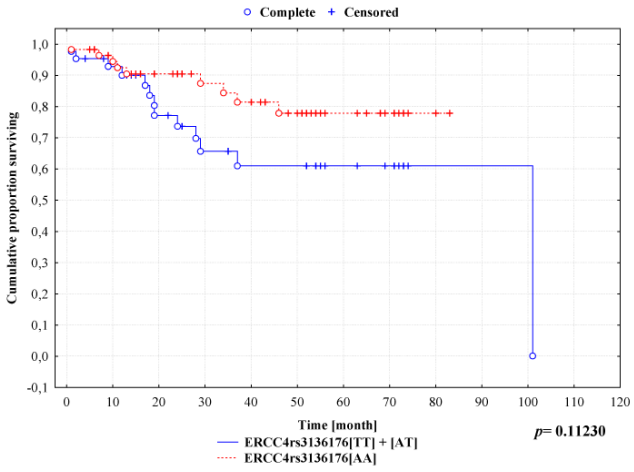

Figure 3. Cumulative proportion surviving in radically treated CSCC patient with the stage of the disease II and III stratified by *ERCC4*rs3136176 genotypes in recessive model.

To evaluate the impact of the *ERCC4* gene polymorphisms on the progression-free survival the Kaplan-Meier analysis was done. The median follow-up was 48 months for the patients after radical treatment. The actuarial 48-month progression-free survival was 74.5% ± 6.4% for whole group of patients after radical treatment. Concerning the overall survival rates found using Kaplan–Meier method and log-rank test, we observed similar mean survival rates according to patients' genotypes at both studied SNPs (Figures 2-4).

Discussion

Given ascending proof that HPV infection is a major risk factor for cervical cancer [46], host genetic variations controlling cell division and maintenance of genome integrity may determine the risk of developing cervical cancer [47-49]. DNA repair systems act to maintain genome integrity in the face of replication errors, environmental insults, and the cumulative effects of age and an accumulation of damaged DNA are one of the way to cancer development. Sufficient DNA repair activity ensures the stability and fidelity of the genome when exposed to carcinogens in the process of cell growth and differentiation [50], whereas instability in the genome in cancer patients may indicate a possible involvement of defective

DNA repairing capacity [51]. Genes encoding proteins involved in the DNA repair systems are consider as a potential cancer risk factors. Especially, genetic variations within those genes, which may lead to abnormal function and/or expression of encoded proteins, are in special interests. Increasing studies have revealed that polymorphisms in the *ERCC4* gene, interacting with environmental or other genetic factors, have association with many types of cancers [17-39].

	CR	PD	χ^2	p	OR	95%CI
ERCC4rs3136176 / ERCC4rs1799798						
A A	6.98(0.106)	25.00(0.162)	1.186	0.276164	0.611	0.250 – 1.493
A G	35.02(0.531)	89.00(0.578)	0.420	0.516996	0.826	0.462 – 1.474
T A	0.02(0.000)	0.00(0.000)	–	–	–	–
T G	23.98(0.363)	40.00(0.260)	2.411	0.120473	1.628	0.878 – 3.018
Total remission= 154.0, total progression= 66.0 $\chi 2_{Global}$= 2.906389, df= 2 (frequency <0.03 in both CR & PD has been dropped), p= 0.233822						

Table 8. *ERCC4*rs3136176 and *ERCC4*rs1799798 haplotype frequencies squamous cell cervical cancer (CSCC) patients with disease remission (CR) and disease progression (PD).

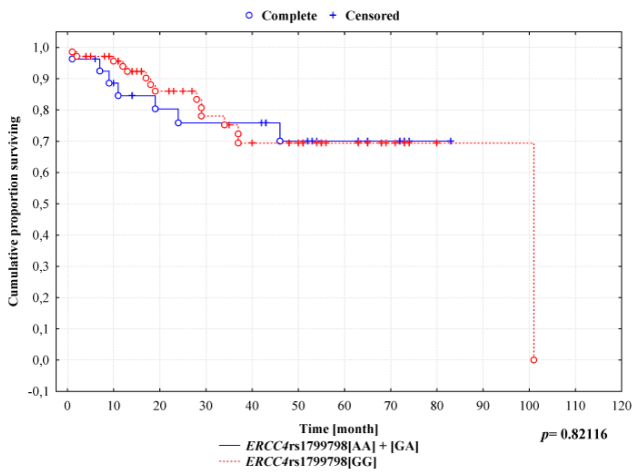

Figure 4. Cumulative proportion surviving in radically treated CSCC patient with the stage of the disease II and III stratified by *ERCC4*rs1799798 genotypes in recessive model.

This study indicates that variant types of DNA repair gene play partial roles in modifying individual susceptibility to cervical cancer. The polymorphic markers that we chose have been selected across preselected cancer-related genes and in Europeans identified as tagSNPs. In our work selected tagSNPs not only were risk factor of cervical squamous cell carcinoma but also modulates the course of disease. First proof for association of *ERCC4* tagSNPs with CSCC was achieved from univariate analysis. In particular, observed disease-protective effect of *ERCC4*rs3136176 tagSNP is described for the first time in case of cervical squamous cell carcinoma. Moreover, detailed analysis showed that presence of [A] allele (genotype [AA] + [AT]) not only protects against CSCC, but also promote the disease remission and protects against development of poorly (G3) differentiated CSCC, which is linked with worse clinical outcome. Altogether this indicates that adenine at rs3136176 within intron 9 of *ERCC4* gene may be considered as a protective factor against CSCC development. Moreover, is also associated with good prognosis which may be informative factor for prognostic factor for CSCC patients. The second studied by us tagSNP rs1799798 has a weaker impact of overall CSCC as well as the clinical forecast and only tended to be associated with grade of tumor with the best prognosis: (G1) and with carcinoma planoepitheliale keratodes (Cpk) histologic subtype of tumor which is linked with better prognosis.

When we entertain the whole gene, effects of polymorphisms are best represented by their haplotypes and its analysis increases the power to detect disease associations because of higher heterozygosity and tighter linkage disequilibrium with disease-causing mutations. In addition, haplotype analysis offers the advantage of not assuming that any of the genotyped polymorphisms is functional; rather, it allows for the possibility of an ungenotyped functional variant to be in linkage disequilibrium with the genotyped polymorphisms [52].

Our research indicate that haplotype *ERCC4*rs3136176[A]/*ERCC4*rs1799798[G] significantly decreased risk of G1 CSCC as well as G3 CSCC and only tended to decrease risk of CSCC as well as carcinoma planoepitheliale akeratodes (Cpa). In contrast haplotype AA significantly increased risk of G1 CSCC and risk of Cpk, whereas haplotype TG increased risk of G3 CSCC. Meanwhile, chosen tagSNPs are not a predictor factor in case of CSCC since the overall survival rates showed similar mean survival rates according to patients' genotypes at both studied SNPs.

Altogether this may indicate that *ERCC4* tagSNPs are a CSCC risk as well as prognostic but not a predictor factor.

A few studies have investigated the associations between *ERCC4* SNPs in cancers [17-36], but still there is no information in literature data applying *ERCC4* in cervical squamous cell carcinoma. Only two studies analyzing relationship between *ERCC4* and squamous cell carcinoma (SCC) but in case of head and neck cancer [35,36]. The obtained by Yu et al. [35] results indicated that some SNPs within *ERCC4* gene are associated with SCCHN (i.e. rs3136038, rs2276466). The second available research, examining several DNA repair genes including *ERCC4*, did not reveal any association of studied *ERCC4* SNP (rs1799801) with SCCHN [36]. To the best of the authors' knowledge, none of investigators studied the associations between

ERCC4 genetic variants and cancers examining the rs3136176 in case of cancer associations. Our important result is the first in worldwide literature data.

Till now only three studies analyzing the rs1799798 SNP in cancers [26,31,37]. Shao et al. [26] did not reveal any association of this SNP in lung cancer. Similarly Michiels et al. [33] also did not found any association of rs1799798 with bladder cancer. This SNP also did not influenced risk of squamous cell carcinoma of the head and neck [37]. Identified by us weak association of rs1799798 with CSCC has not been previously reported for any cancers and the functional significance of the variants is unknown. Thus, they need to be confirmed in future studies.

Perhaps, this phenomenon could be associated with the different pattern of *ERCC4* expression in CSCC patients and potential influence of its level by studied SNPs. rs3136176 and rs1799798 tagSNPs are located within intron 9 and intron 1, respectively, and it is well known that SNPs mapped to introns most likely will not yield altered amino acid sequences, but they may still alter gene regulation in terms of transcription, splicing, and transcript turnover rates. To the best of author's knowledge the functional impact of those SPNs are still not known. So far, there is no literature data analyzing the *ERCC4* expression in SCC in cervix as well as no information about the link between gene variation and expression pattern of *ERCC4.* However, existed data indicate a significant role of ERCC4 in next-generation personalized cancer therapy [10] and may be a crucial rate-limiting factor in DNA repair [11], but the final explanation of our observation needs further studies.

Moreover, because of the low number of patients in subgroups, our results achieved for association between studied polymorphisms and grade of differentiation need to be interpreted with caution [53,54].

Concluding, to reveal the genetic susceptibility to cervical cancer, it is necessary to identify genetic risk markers that can predict the development of cervical cancer and modulate the disease outcome. Our population-based, case-control association study may indicate that *ERCC4* tagSNPs are a CSCC risk as well as prognostic but not a predictor factors.

Acknowledgments

Conflicts of interest

The authors declare no conflicts of interest.

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