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Association between estrogen receptor β polymorphisms and prostate cancer in a Slovak population

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Abstract. Sex steroid hormones have important roles in the function of the prostate; however, they may also serve as factors in the initiation and progression of carcinogenesis. Estrogens, acting through estrogen receptors, may significantly affect prostate cancer development and progression. The main aim of the present study was to analyze the association between the rs3020449, rs4986938 and rs1256049 polymorphisms in the promoter region of the estrogen receptor β (*ESR2*) gene and prostate cancer risk in the Slovak population. A total of 510 patients with prostate cancer and 184 healthy men were included in the present study. No association between the rs4986938 and rs1256049 polymorphisms and prostate cancer development and progression was revealed; however, there was a statistically significant association between the rs3020449 GG genotype [odds ratio (OR), 2.35; P=0.002] and the G allele (OR, 1.42; P=0.005) and a higher risk of prostate cancer development. The rs3020449 GG genotype was significantly associated with a higher risk of development of carcinoma with a Gleason score >7 (OR, 2.66; P=0.005), as well as with the development of carcinoma with pT3/pT4 (OR, 2.28; P=0.02). According to the results from the present study, the rs3020449 polymorphism, in the promoter region of *ESR2,* may be considered to have a role in the development and progression of prostate cancer in the Slovak population.

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Introduction

Prostate cancer is the third most common oncological disease in men, according to the incidence and mortality rates in Slovakia (1). Endogenous sex steroid hormones, along with environmental and dietary factors, and immune and inflammatory responses are involved in the pathogenesis of prostate cancer (2). Prostate cancer is an androgen-dependent tumor, which notably increases with age. However, there is consistent evidence that both total and bioavailable serum testosterone levels significantly decline with age (3). Circulating testosterone levels decline with age at a greater extent compared with that in circulating estradiol, resulting in an elevated ratio of estradiol to testosterone. The increased ratio might also indirectly reflect aromatase activity and a higher conversion of testosterone to estradiol at an older age (4). Estrogens play an important role in male sex hormone secretion, in the growth, differentiation and homeostasis of normal prostate tissue as well as in prostate carcinogenesis (5). Epidemiological studies have not confirmed the direct association between serum estrogen levels and prostate cancer risk (6-8), there is a possibility that intraprostatic estrogen milieu may play a more important role than circulating estrogen levels.

Estrogen action is commonly mediated by two receptors, estrogen receptor α (ERα) and estrogen receptor β (ERβ), which are encoded by separate genes (ESR1 and ESR2, respectively). Both receptors belong to nuclear receptors, acting as ligand-activated transcription factors. ERα and ERβ share high sequence homology, particularly in a DNA-binding domain, allowing both receptors to recognize the estrogen-responsive element on the DNA. Lower sequence homology has been described in the ligand-binding domain, suggesting that both receptors may have different specific ligands (9). Estrogen receptors activated by their ligands, act through two signaling mechanisms. The main mechanism includes diffusion of estrogens across the cell membrane and their binding to estrogen receptors. The receptors then dimerize and bind to estrogen responsible element sequences in the promoter region of the target genes and such affect gene transcription. The second mechanism is mediated

by membrane-localized estrogen receptors. The binding of steroid ligands leads to rapid signaling mediated by G protein activation. This includes the generation of cyclic nucleotides (cAMP and cGMP) and calcium efflux, which activates kinase cascades (10).

The exact role of estrogen receptors in prostate carcinogenesis requires further elucidation. It is hypothesized that the two types of estrogen receptors have different roles in prostate cancer. ERα is proposed to contribute to cellular proliferation, inflammation and has been found to be upregulated in malignant epithelial prostate tissue (11), while ERβ exhibits antiproliferative, anti-invasive and proapoptotic effects (12,13), and its expression declines during the development of prostate cancer (14).

The *ESR2* gene, encoding ERβ, is located on chromosome 14q23.1 (15) and is expressed in both stromal and epithelial cells of the prostate. The loss of *ESR2* expression may be considered as a risk factor for prostate cancer (16). The precise mechanism of how *ESR2* is regulated in prostate cancer cells is still unknown. Decreased *ESR2* expression may be caused by the methylation of CpG islands, located in the promoter region (17). The presence of polymorphisms in the coding regions of the gene may also affect gene expression levels or transcript stability. Among the most extensively studied polymorphisms in the *ESR2* gene are rs1256049 and rs4986938; however, the functional significance of both polymorphisms is still unknown. The rs1256049 is a synonymous variant located within the ligand binding domain in exon 5 (18). Meta-analysis has shown significant association between rs1256049 and prostate cancer in Caucasians, but not in overall population (19). The second polymorphism, rs4986938 represents a G>A transition in the 3'-untranslated region of exon 8 (20). It is hypothesized that the untranslated regions of genes are regulatory elements, controlling translation and may be a target for microRNAs (21). Numerous studies have investigated the association between the rs4986938 polymorphism and different types of cancer; it was found to be associated with breast (22,23) and prostate cancers (24). However, a meta-analysis conducted to investigate the association of rs4986938 and the overall risk of cancer found no significant associations (25). The polymorphism rs3020449 is located near the transcription start site of the promoter 0N of the *ESR2* gene. It is hypothesized that polymorphisms located in the promoter region could affect transcription factor binding and affect gene transcription (26). The association between rs3020449 and prostate cancer has not been investigated; however, it was found to be associated with other oncological diseases, such as endometrial (27), ovarian (28) and breast cancers (29).

The aim of the present study was to determine the *ESR2* expression levels in hyperplastic and malignant prostate tissues and analyze the possible association of three polymorphisms in the *ESR2* gene (rs3020449, rs4986938 and rs1256049) with prostate cancer development and progression.

Materials and methods

Study population. The case-control study included 510 patients with histologically verified prostate cancer and 184 healthy men. Tissue samples from 22 patients with prostate cancer and 12 patients with benign prostatic hyperplasia (BHP) were collected during routine surgery, placed into RNA stabilization solution and stored at -80˚C until further analysis. All patients were recruited at the Department of Urology, University Hospital Martin in Slovakia, between 2005 and 2019. Healthy volunteers were selected from men attending routine urological examination and were confirmed to have no history of cancer or any prostate disease. The present study was approved by the Ethics Committee of Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin and all men provided written informed consent to participate in the study. The clinical characteristics of the study groups are summarized in Table I.

Genotyping. Genomic DNA samples from the individuals were isolated from whole blood using The Wizard® Genomic DNA Purification kit (Promega Corporation) according to the manufacturer's protocol and stored at -20˚C until further analysis. The *ESR2* gene polymorphism, rs3020449 was analyzed using tetra-primer amplification refractory mutation system PCR approach (30) allowing allele-specific amplification using the following primers: IP1, 5'-GCATTGTCCTTT TTACATATTGTTAGGGTA-3'; IP2, 5'-AATTCTCAAGGA AATTTTAGCAAAGCC-3'; OP1, 5'-TAGATTTTGTCAAAC ACTTTTGGTGGAT-3'; OP2, 5'-CCAAATGATTAAGGA GAAATAACAGCAG-3'. The PCR Master mix contained 100 ng genomic DNA, 2.4 μl 5X HOT FIREPol® Blend Master Mix RTL (Solis BioDyne OÜ), 0.5 μl each primer and 6.6 μl nuclease-free water. The following thermocycling conditions were used: Initial denaturation at 95˚C for 15 min followed by 35 cycles at 95˚C for 20 sec, 56˚C for 50 sec and 72˚C for 1 min, with a final extension at 72˚C for 5 min. The PCR products were separated using 2% agarose gel electrophoresis. The allele-specific product size for rs3020449 was 231 or 193 bp for the A and G alleles, respectively).

The *ESR2* gene polymorphisms, rs4986938 and rs1256049 were determined using the PCR-restriction fragment length polymorphism method and the following primers: rs4986938 forward, 5'-GACCTGCTGCTGGAGATGCT-3' and reverse, 5'-AATGAGGGACCACACAGCA-3'; and rs1256049 forward, 5'-TCTTGCTTTCCCCAGGCTTT-3' and reverse, 5'-ACCTGT CCAGAACAAGATCT-3'. The PCR Master mix contained 100 ng genomic DNA, 6 μl Dream Taq Green PCR master mix (2X) (Thermo Fisher Scientific Inc.), 30 ng forward and reverse primers and nuclease-free water to a total volume of 12 μl. The following thermocycling conditions were used: Initial denaturation at 95 C for 5 min followed by 35 cycles at 95˚C for 20 sec, 58˚C for 50 sec for rs4986938 or 56˚C for 50 sec for rs1256049, and 72˚C for 1 min, with a final extension at 72˚C for 5 min. The PCR products of the rs4986938 polymorphism were digested with *Alu*I, which produced a 234 bp sized band for the GG genotype; 168 and 66 bp sized bands for the AA genotype and 234, 168 and 66 bp sized bands for the GA genotype. The PCR products of the rs1256049 polymorphism were digested with *Rsa*I, which produced a 156 bp sized band for the GG genotype; 110 and 46 bp sized bands for the AA genotype and 156, 110 and 46 bp sized bands for the GA genotype.

Gene expression analysis. Isolation of total RNA was performed using an AllPrep DNA/RNA/miRNA Universal kit (Qiagen GmbH) according to the manufacturer's protocol. For each sample, an equal quantity of RNA (1 μg) was used for reverse transcription into cDNA with a RT2 First Strand kit, following the standard protocol (Qiagen GmbH). Reverse transcription-quantitative

Table I. Characteristics of patients with prostate cancer and healthy subjects.

Characteristics	Healthy controls (n=184)	Prostate cancer (n=510)
Age, years		
Mean ± SD	57.61±10.39	67±8.26
PSA, ng/ml		
Median (25-75th percentile)	0.81 (0.49-1.60)	10.57 (5.84-28.76)
Gleason score		
≤7	NA	270
>7	NA	136
Mean ± SD	NA	7.28±1.25
Missing	NA	104
Pathological stage		
pT1/pT2	NA	88
pT3/pT4	NA	148
Missing	NA	274

NA, not applicable.

PCR analysis of the *ESR2* expression level was performed using Custom RT^2 Profiler PCR array (Qiagen GmbH). *GAPDH* and *actin* served as housekeeping genes.

Statistical analysis. Genotype frequencies were calculated for the patients with prostate cancer and the healthy controls. Observed genotype frequencies were tested for Hardy-Weinberg equilibrium in the control group. Dominant, codominant and recessive genetic models were evaluated. The comparison of the genotype distribution and association with selected clinical data was performed using a Fisher's exact test. Fisher's exact test, calculation of odds ratios and 95% confidence intervals (CIs) were performed using the StatsDirect statistical package (v2.7.0.2). The test for linkage disequilibrium of the selected polymorphisms was performed using Haploview 4.2 software.

The relative quantification method was used for the analysis of *ESR2* expression levels. The C_q values of the *ESR2* gene were compared with the average C_q values of the two housekeeping genes to obtain ΔC_q values. The fold-change was calculated as $2^{-\Delta\Delta Cq}$ (31). The data are represented in the figures as median ± IQR. The Mann-Whitney test was used for the comparison of the *ESR2* mRNA expression levels between patients with prostate cancer and with BHP. The Mann-Whitney test with Bonferroni correction was used for the comparison of the *ESR2* mRNA expression levels between rs3020449 genotypes. All P-values were derived from two-sided tests and P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using the StatsDirect statistical package (v2.7.0.2).

Results

Genotype analysis. The genotype frequencies of the three analyzed *ESR2* variants did not deviate from the Hardy-Weinberg equilibrium and their genotyping success rates were over 95%. The analyzed polymorphisms were not found to be in linkage disequilibrium, while the estimated R^2 values were 0.02, 0.02 and 0.12, respectively (Fig. 1). The distribution of the genotypes and alleles of the three analyzed *ESR2* polymorphisms in both the control group and in the patients with prostate cancer are summarized in Table II. Dominant, codominant and recessive genetic models were evaluated.

There was a statistically significant association between the rs3020449 GG genotype [odds ratio (OR), 2.35; 95% CI 1.31-4.36; P=0.002] compared with that in the AA genotype, as well as in the recessive model (OR, 2.20; 95% CI 1.23-3.92; P=0.002) and the higher risk of prostate cancer development. The frequency of the rs3020449 G allele (OR, 1.42; 95% CI 1.10-1.84; P=0.005) was also significantly higher in the patients with prostate cancer (Table II). The other two analyzed *ESR2* variants, rs4986938 and rs1256049, were not found to be associated with the risk of prostate cancer development.

To detect the possible associations between the *ESR2* polymorphisms and the selected clinical features, patients were stratified according to Gleason score (≤7 and >7), pathological T stage (pT1/pT2 and pT3/pT4) and prostate-specific antigen levels (<10 and ≥10 ng/ml). There was a statistically significant association between the rs3020449 GG genotype and a higher risk of development of carcinoma with a Gleason score ≤7 (OR, 1.97; 95% CI 1.09-3.86; P=0.029); however, a more significant association was observed in patients with Gleason score >7 (OR, 2.66; 95% CI 1.27-5.64; P=0.005). The rs3020449 G allele was significantly associated with development of carcinoma with a Gleason score >7 (OR, 1.53; 95% CI 1.09-2.13; P=0.01) (Table III).

After stratification of the patients with prostate cancer according to pathological T stage, a significant association between the rs3020449 GG genotype (OR, 2.28; 95% CI 1.10-4.76; P=0.02), as well as with the G allele (OR, 1.39; 95% CI 1.00-1.93; P=0.04) and the development of carcinoma with pT3/pT4 was detected. In the group of patients with pT1/pT2, there was no significant association with increased risk. The results are summarized in Table IV. The rs3020449

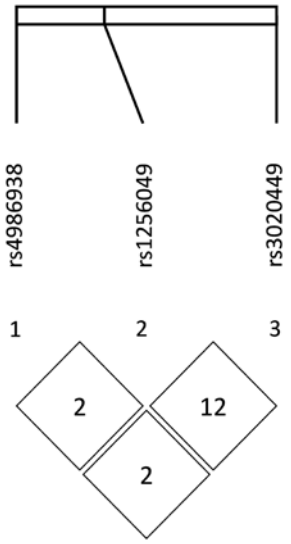

Figure 1. R^2 plot of analyzed *ESR2* gene variants estimated by Haploview 4.2. *ESR2*, estrogen receptor β. The analyzed polymorphisms were not found to be in linkage disequilibrium, according to the estimated R^2 values.

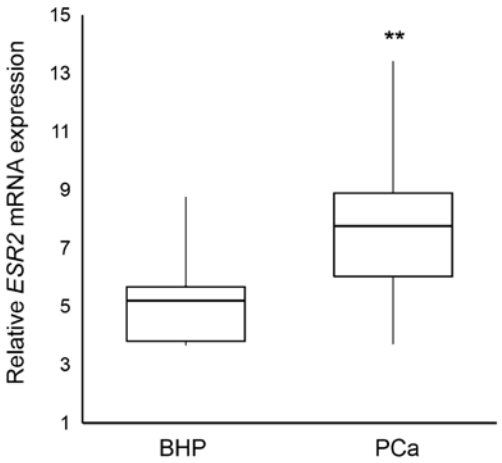

Figure 2. Relative *ESR2* mRNA expression levels in patients with BHP and prostate cancer. The boxes define the first quartile, median and trird quartile values. The lines define minimum and maximum values. Mann-Whitney test was used for the comparison between groups. **P<0.01. BHP, benign prostatic hyperplasia; PCa, prostate cancer; *ESR2*, estrogen receptor β.

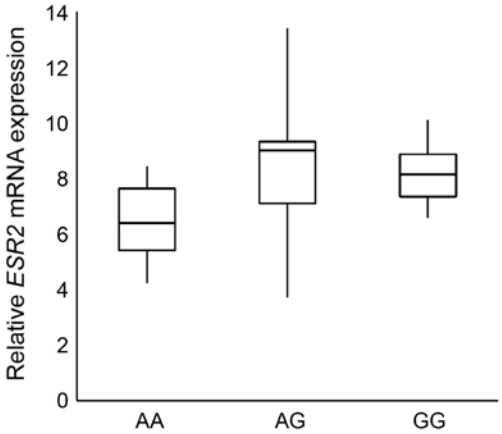

Figure 3. Relative *ESR2* mRNA expression levels in patients with prostate cancer with the different rs3020449 genotypes. The boxes define the first quartile, median and trird quartile values. The lines define minimum and maximum values. *ESR2*, estrogen receptor β.

GG genotype was significantly associated with a higher risk of prostate cancer development in both groups of patients with PSA <10 ng/ml (OR, 2.24; 95% CI 1.19-4.26; P=0.01), as well as with PSA≥10 ng/ml (OR, 2.10; 95% CI 1.12-4.00; P=0.02) (Table V). There was no association between the rs4986938 and rs1256049 variants and Gleason score, pathological T stage and PSA levels in patients with prostate cancer (Tables III-V).

Expression levels of ESR2 mRNA. The relative *ESR2* mRNA expression levels were found to be significantly higher in BHP tissues compared with that in prostate cancer tissues (P=0.002) (Fig. 2). It was found that *ESR2* mRNA expression levels were 5.47-fold higher in BHP tissues compared with that in prostate cancer tissues. Analysis of relative *ESR2* mRNA expression levels in patients with prostate cancer with different rs3020449 genotypes revealed that the rs3020449 GG genotype had 3.38-fold lower *ESR2* expression levels compared with that in patients with the AA genotype (P=0.04) (Fig. 3); however the result was not statistically significant after Bonferroni correction for multiple comparisons.

Discussion

Prostate cancer is a heterogenous disease, with numerous factors contributing to its development and progression. The prostate is a hormone-dependent tissue, and estrogens are the targets of research. The aim of the present study was to evaluate the association between three *ESR2* polymorphisms and the increased risk of prostate cancer, and to determine the relative *ESR2* mRNA expression levels in hyperplastic and malignant prostate tissues. There was only a limited number of tissue samples; however, significantly higher relative *ESR2* mRNA expression levels were found in BHP tissues compared with that in prostate cancer tissues. Several publications have reported the decrease or loss of ERβ protein expression during prostate cancer progression using immunohistochemical staining (32,33). Latil *et al* (34) also reported a decrease in ERβ mRNA expression levels in the majority of prostate tumors compared to that in normal tissue. Pasquali *et al* (35) described that the loss of the ERβ protein may promote cell proliferation and possibly carcinogenesis. On the other hand, some reports also suggest a negative role of ERβ protein expression levels in prostate cancer prognosis (36,37). Grindstad *et al* (38) found that the ERβ protein expression levels were associated with reduced time to biochemical failure. Opposing observations obtained by different research groups might be partially explained by the existence of different ERβ isoforms. The wild-type ERβ1 inhibits proliferation, has tumor-suppressing effects and is lost during prostate cancer progression. Its splice variant, ERβ2, increases proliferation, therefore is oncogenic and is expressed in advanced prostate cancer (39).

One of the factors with the potential to affect the expression levels of the *ESR2* gene are single nucleotide polymorphisms. In the present study, there was no association between the rs4986938 and rs1256049 polymorphisms and the risk of prostate cancer development and progression.

Table II. Distribution of the *ESR2* genotypes and alleles and their association with the risk of prostate cancer.

			Controls vs. Prostate cancer	
Genotype	Healthy controls, n	Prostate cancer, n	OR (95% CI)	P-value
ESR2 rs3020449				
Codominant model				
AA	75	173	1.00 (ref.)	
AG	90	234	1.13 (0.77-1.65)	0.58
GG	19	103	2.35 (1.31-4.36)	0.002[a]
Dominant model				
AA	75	173	1.00 (ref.)	
AG + GG	109	337	1.34 (0.93-1.92)	0.11
Recessive model				
AA + AG	165	407	1.00 (ref.)	
GG	19	103	2.20 (1.29-3.92)	0.002[a]
Allele				
A	240	580	1.00 (ref.)	
G	128	440	1.42 (1.10-1.84)	0.005[a]
ESR2 rs1256049				
Codominant model				
GG	166	460	1.00 (ref.)	
GA	18	47	0.94 (0.52-1.78)	0.88
AA	0	3	NA	NA
Dominant model				
GG	166	460	1.00 (ref.)	
GA + AA	18	50	1.00 (0.56-1.88)	1.00
Allele				
G	350	967	1.00 (ref.)	
A	18	53	1.07 (0.60-1.96)	0.89
ESR2 rs4986938				
Codominant model				
GG	88	228	1.00 (ref.)	
GA	77	229	1.15 (0.79-1.67)	0.47
AA	19	49	1.00 (0.54-1.89)	>0.99
Dominant model				
GG	88	228	1.00 (ref.)	
GA + AA	96	278	1.12 (0.79-1.59)	0.55
Recessive model				
GG + GA	165	457	1.00 (ref.)	
AA	19	49	0.93 (0.52-1.73)	0.77
Allele				
G	253	685	1.00 (ref.)	
A	115	327	1.05 (0.81-1.37)	0.74

[a]Statistically significant results (P<0.05). NA, not applicable; *ESR2*, estrogen receptor β.

The majority of published studies have also not confirmed an association between the rs4986938 and rs1256049 polymorphisms in the Caucasian population (40-44) or in mixed populations (45-47) and prostate cancer risk. On contrary, there are some studies that have described a significant association between the rs4986938 and rs1256049 polymorphisms and increased risk of prostate cancer development in Iranian (48) and Caucasian populations (49). On the other hand, a Japanese study discovered that both the rs4986938 and rs1256049 polymorphisms were significantly associated with a decreased risk of prostate cancer (24). As a result of the conflicting results of the published studies, Li *et al* (25) conducted a meta-analysis, in which they found no evidence of an association between the rs4986938 polymorphism and

Table III. Association between the *ESR2* genotypes and alleles and Gleason score in prostate cancer patients.

	Gleason score ≤7			Gleason score >7		
Genotype	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value
ESR2 rs3020449						
Codominant model						
AA	100	1.00 (ref.)		43	1.00 (ref.)	
AG	120	1.00 (0.65-1.53)	1.00	64	1.24 (0.74-2.10)	0.45
GG	50	1.97 (1.04-3.84)	0.029[a]	29	2.66 (1.27-5.64)	0.005[a]
Dominant model						
AA	100	1.00 (ref.)		43		
AG + GG	170	1.17 (0.78-1.75)	0.43	93	1.49 (0.91-2.44)	0.10
Recessive model						
AA + AG	220	1.00 (ref.)		107	1.00 (ref.)	
GG	50	1.97 (1.09-3.68)	0.02[a]	29	2.35 (1.20-4.67)	0.007[a]
Allele						
A	320	1.00 (ref.)		150	1.00 (ref.)	
G	220	1.29 (0.97-1.71)	0.07	122	1.53 (1.09-2.13)	0.01[a]
ESR2 rs1256049						
Codominant model						
GG	242	1.00 (ref.)		122	1.00 (ref.)	
GA	28	1.06 (0.55-2.12)	0.88	11	0.83 (0.34-1.94)	0.70
AA	0	NA	NA	3	NA	NA
Dominant model						
GG	242	1.00 (ref.)		122	1.00 (ref.)	
GA + AA	28	1.06 (0.55-2.12)	0.88	14	1.05 (0.47-2.35)	1.00
Allele						
G	512	1.00 (ref.)		255	1.00 (ref.)	
A	28	1.06 (0.56-2.07)	0.88	17	1.30 (0.61-2.72)	0.49
ESR2 rs4986938						
Codominant model						
GG	118	1.00 (ref.)		66	1.00 (ref.)	
GA	125	1.21 (0.80-1.83)	0.36	54	0.93 (0.57-1.54)	0.81
AA	26	1.02 (0.51-2.08)	1.00	13	0.91 (0.38-2.11)	0.85
Dominant model						
GG	118	1.00 (ref.)		66	1.00 (ref.)	
GA + AA	151	1.17 (0.79-1.74)	0.44	67	0.93 (0.58-1.49)	0.82
Recessive model						
GG + GA	243	1.00 (ref.)		120	1.00 (ref.)	
AA	26	0.93 (0.48-1.84)	0.87	13	0.94 (0.41-2.10)	1.00
Allele						
G	361	1.00 (ref.)		186	1.00 (ref.)	
A	177	1.08 (0.80-1.45)	0.61	80	0.94 (0.66-1.35)	0.79

[a]Statistically significant results (P<0.05). NA, not applicable; *ESR2*, estrogen receptor β.

prostate cancer risk, while a meta-analysis into the association between the rs1256049 and prostate cancer revealed a significant association in the Caucasian population, but not in the overall population (19).

The promoter region of the *ESR2* gene is complex and has not been fully described; however, it is hypothesized that polymorphisms in this region could affect the binding of enhancers or repressors to regulate gene transcription (50). To the best of our knowledge, this is the first study evaluating the association between the rs3020449 polymorphism and the risk of prostate cancer. A significant association between the rs3020449 polymorphism and a higher risk of prostate cancer development and progression was found. The functional impact of this polymorphism is unknown. Decrease in *ESR2* mRNA

Table IV. Distribution of the *ESR2* genotypes and alleles in patients stratified according to the pathological stage.

	pT1/pT2			pT3/pT4		
Genotype	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value
ESR2 rs3020449						
Codominant model						
AA	35	1.00 (ref.)		52	1.00 (ref.)	
AG	37	0.88 (0.49-1.59)	0.67	66	1.06 (0.64-1.75)	0.90
GG	16	1.80 (0.77-4.20)	0.16	30	2.28 (1.10-4.76)	0.02[a]
Dominant model						
AA	35	1.00 (ref.)		52	1.00 (ref.)	
AG + GG	53	1.04 (0.60-1.81)	0.90	96	1.27 (0.79-2.04)	0.31
Recessive model						
AA + AG	72	1.00 (ref.)		118	1.00 (ref.)	
GG	16	1.93 (0.87-4.21)	0.08	30	2.21 (1.14-4.36)	0.01[a]
Allele						
A	107	1.00 (ref.)		170	1.00 (ref.)	
G	69	1.21 (0.82-1.78)	0.34	126	1.39 (1.00-1.93)	0.04[a]
ESR2 rs1256049						
Codominant model						
GG	82	1.00 (ref.)		133	1.00 (ref.)	
GA	7	0.79 (0.27-2.08)	0.66	15	1.04 (0.47-2.28)	1.00
AA	0	NA	NA	3	NA	NA
Dominant model						
GG	82	1.00 (ref.)		133	1.00 (ref.)	
GA + AA	7	0.79 (0.27-2.08)	0.66	18	1.25 (0.59-2.65)	0.60
Allele						
G	171	1.00 (ref.)		281	1.00 (ref.)	
A	7	0.79 (0.28-2.05)	0.83	21	1.45 (0.72-2.95)	0.32
ESR2 rs4986938						
Codominant model						
GG	40	1.00 (ref.)		74	1.00 (ref.)	
GA	42	1.20 (0.68-2.11)	0.59	57	0.83 (0.51-1.36)	0.48
AA	7	0.81 (0.27-2.22)	0.82	16	1.00 (0.45-2.22)	1.00
Dominant model						
GG	40	1.00 (ref.)		74	1.00 (ref.)	
GA + AA	49	1.12 (0.66-1.93)	0.70	73	0.90 (0.57-1.42)	0.66
Recessive model						
GG + GA	82	1.00 (ref.)		131	1.00 (ref.)	
AA	7	0.74 (0.25-1.94)	0.66	16	1.06 (0.49-2.27)	1.00
Allele						
G	122	1.00 (ref.)		205	1.00 (ref.)	
A	56	1.01 (0.67-1.51)	1.00	89	0.96 (0.68-1.35)	0.80

[a]Statistically significant results (P<0.05). NA, not applicable; *ESR2*, estrogen receptor β.

expression levels were found in patients with prostate cancer and the rs3020449 GG genotype compared with that in the AA genotype; however, results were not statistically significant after Bonferroni correction. The potential limitation of presented study is the lack of survival analysis.

There are a limited number of studies that have analyzed the rs3020449 polymorphism with other diseases. Lattrich *et al* (27) found that rs3020449 was not associated with the development of endometrial cancer. The polymorphism was also found to be associated with the progression of ovarian cancer, as it was more frequent in patients with FIGO staged III + IV (28). On the other hand, it was not found to be associated with uterine fibroids (51). With respect to breast cancer, some studies have found no association of

Table V. Distribution of the *ESR2* genotypes and alleles in patients stratified according to the PSA levels.

	PSA <10 ng/ml			PSA ≥10 ng/ml		
Genotype	n	OR (95% CI)	P-value	n	OR (95% CI)	P-value
ESR2 rs3020449						
Codominant model						
AA	74	1.00 (ref.)		77	1.00 (ref.)	
AG	91	1.02 (0.66-1.58)	0.91	104	1.13 (0.73-1.72)	0.59
GG	42	2.24 (1.19-4.26)	0.01[a]	41	2.10 (1.12-4.00)	0.02[a]
Dominant model						
AA	74	1.00 (ref.)		77	1.00 (ref.)	
AG + GG	133	1.24 (0.82-1.90)	0.31	145	1.30 (0.86-1.94)	0.21
Recessive model						
AA + AG	165	1.00 (ref.)		181	1.00 (ref.)	
GG	42	2.21 (1.24-4.03)	0.007[a]	41	1.97 (1.10-3.58	0.02[a]
Allele						
A	239	1.00 (ref.)		258	1.00 (ref.)	
G	175	1.37 (1.03-1.84)	0.03[a]	186	1.35 (1.02-1.80)	0.04[a]
ESR2 rs1256049						
Codominant model						
GG	186	1.00 (ref.)		198	1.00 (ref.)	
GA	21	1.04 (0.53-2.05)	0.91	21	0.98 (0.50-1.92)	0.95
AA	0	NA	NA	3	NA	NA
Dominant model						
GG	186	1.00 (ref.)		198	1.00 (ref.)	
GA + AA	21	1.04 (0.53-2.05)	0.91	24	1.12 (0.59-2.16)	0.74
Allele						
G	393	1.00 (ref.)		420	1.00 (ref.)	
A	21	1.04 (0.54-2.01)	0.91	24	1.11 (0.59-2.11)	0.75
ESR2 rs4986938						
Codominant model						
GG	87	1.00 (ref.)		107	1.00 (ref.)	
GA	99	1.30 (0.85-1.98)	0.22	90	0.96 (0.63-1.46)	0.85
AA	21	1.12 (0.56-2.25)	0.75	21	0.91 0.46-1.82)	0.78
Dominant model						
GG	87	1.00 (ref.)		107	1.00 (ref.)	
GA + AA	120	1.26 (0.85-1.89)	0.25	111	0.95 (0.64-1.41)	0.80
Recessive model						
GG + GA	186	1.00 (ref.)		197	1.00 (ref.)	
AA	21	0.98 (0.51-1.91)	0.95	21	0.93 (0.48-1.80)	0.82
Allele						
G	273	1.00 (ref.)		304	1.00 (ref.)	
A	141	1.14 (0.84-1.53)	0.41	132	0.96 (0.71-1.29)	0.77

[a]Statistically significant results (P<0.05). NA, not applicable; *ESR2*, estrogen receptor β.

rs3020449 (52,53). On the other hand, Dai *et al* (29) described an association between rs3020449 and increased risk of breast cancer, as well as with tumor size and histological grade.

There are no published studies revealing an association between the rs3020449 polymorphism and prostate cancer risk; however, there are studies describing an association between other promoter polymorphisms in *ESR2* and prostate cancer. The National Cancer Institute's Breast and Prostate Cancer Cohort Consortium study reported an overall increased risk in prostate cancer and advanced stage with the rs3020450 (45). This polymorphism was found to be in complete linkage disequilibrium with rs2987983, which authors of a Swedish study found to be associated with prostate cancer risk and

suggested that the genetic variation in the promoter region of *ESR2* may play a part in the etiology of prostate cancer (54). Holt *et al* (42) reported an association between the rs1952586 polymorphism and the risk for higher Gleason score tumors.

In summary, the rs3020449 polymorphism in the *ESR2* gene markedly contributed to a higher prostate cancer risk in the Slovak population. Analysis of this polymorphism could also provide information regarding the prognosis of the disease, as it was significantly associated with the development of high-grade carcinomas (Gleason score >7) and tumors with pT3/pT4. The significance of the presented study underlines the fact that the rs3020449 was not found to be in linkage disequilibrium with polymorphisms previously studied with prostate cancer (26). Therefore, it is not likely that the association found in the present study was due to linkage of rs3020449 with previously reported polymorphisms. The functional impact of this polymorphism on the *ESR2* gene is still unknown. Analysis of relative *ESR2* mRNA expression levels revealed that patients with the rs3020449 GG genotype had tendency to have lower *ESR2* expression levels compared with those with the AA genotype. There might also be considerable differences in the genotype frequencies between populations, therefore the results are valid for Slovak and related populations; however, confirmation is required for populations with a different ethnic origin.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Author's contributions

JJ, MKS, HD, MH, DE, JK and DD contributed to the study conception and design. Material preparation and data collection were performed by JK, DE and MH. Analysis was performed by JJ, MKS and HD. The first draft of the manuscript was written by JJ and all authors commented on previous versions of the manuscript. JJ, DD and MKS confirmed the authenticity of all raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Slovakia (approval number: EK 43/2018) and was performed in accordance with ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the study.

Patient consent to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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