Polymorphic variants in the vitamin D pathway genes and the risk of ovarian cancer among non-carriers of *BRCA1/BRCA2* mutations

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Abstract. Previous studies have produced inconsistent results regarding the contribution of single-nucleotide polymorphisms (SNPs) in the vitamin D receptor (*VDR*) gene to ovarian cancer (OC) in various ethnicities. Additionally, little has been established with regard to the role of SNPs located in the retinoid X receptor α (*RXRA*), vitamin D-binding protein [also know as group-specific component (*GC*)] and *VDR* genes in non-carriers of the breast cancer 1/2 early onset (*BRCA1/BRCA2*) gene mutations. All participating individuals in the present study were evaluated for *BRCA1* mutations (5382incC, C61G and 4153delA) with HybProbe assays, and for *BRCA2* mutation (5946delT) using high-resolution melting (HRM) analysis. The associations of 8 SNPs located in *RXRA*, *GC* and *VDR* were investigated in OC patients without the *BRCA1/BRCA2* mutations (n=245) and healthy controls (n=465). Genotyping of *RXRA* rs10881578 and rs10776909, and *GC* rs1155563 and rs2298849 SNPs was conducted by HRM analysis, while *RXRA* rs749759, *GC* rs7041, *VDR Bsm*I rs1544410 and *Fok*I rs2228570 genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism analysis. In addition, the gene-gene interactions among all tested SNPs were studied using the epistasis option in PLINK software. The lowest P-values of the trend test were identified for *VDR* rs1544410 and *GC* rs2298849 as P_{trend}=0.012 and P_{trend}=0.029, respectively. It was also found that, in the dominant inheritance model, *VDR Bsm*I contributed to an increased risk of OC [odds ratio (OR), 1.570; 95% confidence interval (CI), 1.136-2.171; P=0.006; P_{corr}=0.048]. The gene-gene interaction analysis indicated a significant interaction between *RXRA* rs749759 and *VDR Fok*I rs2228570 (OR for interaction, 1.687; χ^2=8.278; asymptotic P-value=0.004; P_{corr}=0.032). In conclusion, this study demonstrated that certain *VDR* and *RXRA* SNPs may be risk factors for OC in non-carriers of *BRCA1/BRCA2* mutations in the Polish population.

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Introduction

Ovarian cancer (OC) is a leading cause of mortality among gynecological carcinomas in Europe and the USA (1,2). Approximately 85% of all OC cases are sporadic, while 15% are associated with a family history of ovarian and other cancers linked to mutations in high-penetrance genes, such as *BRCA1/BRCA2*, mismatch repair genes or tumor protein p53 (*TP53*) (3,4). In addition to genetic factors, there are several other determinants that modulate the risk of OC development (3,4), including advancing age, exposure to chemicals and/or pollutants, oral contraceptive use, parity, breast-feeding period, lifestyle and diet (3,5). Other factors influencing the development of OC include exposure to sunlight and dietary intake of vitamin D precursors (6). The role of vitamin D in the homeostasis of calcium and bone health is well-established (7,8), and an increasing number of studies have investigated the involvement of vitamin D in numerous other aspects of health, including the growth of various cancers (9). The actions of the active form of vitamin D, 1,25-dihydroxyvitamin D3 [1,25$(OH)_2D_3$], in human bodies are mediated by several different proteins. These mainly include vitamin D-binding protein (VDBP), vitamin D receptor (VDR) and retinoid X receptor (RXR) (10-12). VDBP is a 56-58 kDa plasma α-globulin encoded by the group-specific component (*GC*) gene (10). This protein functions as a major blood plasma transporter protein for vitamin D and its metabolites (10). VDR forms heterodimers with RXR and binds to DNA to initiate a series of epigenetic events leading to chromatin rebuilding and initiation of transcription (11,12). Evidence has indicated that a *GC* single-nucleotide polymorphism (SNP) is associated with blood plasma vitamin D levels (13). Furthermore, the *VDR* gene also contains various SNPs, a number of which may alter 1,25$(OH)_2D_3$ action (8). It has recently been demonstrated that certain SNPs situated in the RXR-α (*RXRA)* gene play a role

in the development and recurrence of certain cancers (14,15). Therefore, in the present study, 8 SNPs in the *RXRA*, *GC* and *VDR* genes, situated in different blocks of linkage disequilibrium (LD), were selected in order to study whether these SNPs may be genetic risk factors for OC. These SNPs were studied in a group of healthy controls and OC patients who were non-carriers of the most common mutations of the *BRCA1*/*BRCA2* genes.

Materials and methods

Patients and controls. The patients included 245 women with histologically determined OC according to the International Federation of Gynecology and Obstetrics (FIGO) (16), who were diagnosed at the Clinic of Gynecological Surgery, Poznań University of Medical Sciences (Poznań, Poland) between January 2012 and April 2014. Histopathological classification, including the stage, grade and tumor type (Table I), was performed by an experienced pathologist. Patients and controls were Caucasian and from the Wielkopolska area of Poland. The controls were composed of 465 unrelated healthy female volunteers who were matched by age to the patients with cancer (Table I). Written informed consent was provided by all individuals involved in the study. The study procedures were approved by the Ethics Committee of Poznań University of Medical Sciences (Poznań, Poland).

Table I. Clinical characteristics of ovarian cancer patients and healthy controls.

Characteristic	Patients (n=245)	Controls (n=465)
Age, years; mean ± SD	58.9±9.6	57.0±6.2
Histological grade, n (%)		
G1	84 (34.3)	
G2	83 (33.9)	
G3	78 (31.8)	
Gx	0 (0.0)	
Clinical stage, n (%)		
I	93 (38.0)	
II	41 (16.7)	
III	82 (33.5)	
IV	29 (11.8)	
Histological type, n (%)		
Serous	79 (32.3)	
Mucinous	30 (12.2)	
Endometrioid	46 (18.8)	
Clear cell	25 (10.2)	
Brenne	0 (0.0)	
Mixed	23 (9.4)	
Solid	25 (10.2)	
Untyped carcinoma	17 (6.9)	

Genotyping. Genomic DNA was obtained from peripheral blood leukocytes by salt extraction. All participating individuals were tested for the three most common *BRCA1* mutations affecting the Polish population (5382incC, C61G and 4153delA) using the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany) with HybProbe probes and a LightCycler DNA Master HybProbe kit (Roche, Indianapolis, IN, USA). Information on HybProbe probe sequences is available upon request. In addition, they were tested for the presence of the most common *BRCA2* mutation (5946delT) using high-resolution melting (HRM) analysis (Table II) using the LightCycler 480 system and 5x HOT FIREPol® EvaGreen® HRM Mix (containing HOT FIREPol DNA polymerase, 5x EvaGreen HRM buffer, 12.5 mM $MgCl_2$, dNTPs, EvaGreen® dye, bovine serum albumin and no ROX dye) (Solis BioDyne, Tartu, Estonia). The reaction system (10 μl) contained 1X Hot Fire Pol EvaGreen HRM Mix, 0.2 pmol/μl of each primer and 2 ng/ml DNA template. Primer sequences and conditions for HRM analysis are presented in Table II. Polymerase chain reaction (PCR) was performed under the following conditions: Initial denaturation step at 95˚C for 15 min, followed by 50 cycles at 95˚C for 10 sec and 60˚C for 10 sec, with a final elongation step at 72˚C for 15 sec. Amplified DNA fragments were then subjected to HRM; the temperature was increased from 80-95˚C in 0.1˚C/2 sec increments. The DNA samples were subsequently genotyped for 8 SNPs in *RXRA*, *GC* and *VDR* (Table II). SNPs were selected with the use of the genome browsers of the International HapMap Consortium (http://www.hapmap.org/index.html.en), University of California, Santa Cruz (http://genome.ucsc.edu), and dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). SNPs were selected according to functional significance, location in district LD blocks, and minor allele frequency (MAF) >0.1 in the Caucasian population. Genotyping of the *GC* rs1155563 and rs2298849, and *RXRA* rs10881578 and rs10776909 SNPs was conducted by HRM using the LightCycler 480 system and 5x Hot Fire Pol EvaGreen HRM Mix (Solis BioDyne). The PCR program and the final concentrations of reagents for HRM reactions are presented above. Primer sequences and conditions for HRM analyses including primer-dependent annealing temperature, PCR product length and melting range are presented in Table II. Genotyping of the *GC* rs7041, *VDR* *Bsm*I rs1544410 and *Fok*I rs2228570, and *RXRA* rs749759 SNPs was performed by PCR followed by restriction fragment length polymorphism (RFLP) analysis with the appropriate restriction enzymes (Fermentas, Vilnius, Lithuania), according to the manufacturer's instructions. Primer sequences and conditions for PCR-RFLP analyses and restriction fragment length are presented in Table II. Genotyping quality was evaluated by repeated genotyping of 15% randomly selected samples.

Statistical analysis. For each SNP, the Hardy-Weinberg equilibrium (HWE) was assessed by Pearson's goodness-of-fit χ^2 statistic. The differences in the allele and genotype frequencies between cases and controls were determined using standard χ^2 or Fisher's exact tests. The odds ratio (OR) and associated 95% confidence interval (CI) were also calculated. Data were analyzed under recessive and dominant inheritance models. For the additive inheritance model, SNPs were tested for association with OC using the Cochran-Armitage trend test. To adjust for the multiple testing, a Bonferroni correction

Table II. HRM and RFLP conditions for the identification of polymorphisms genotyped in the data set.

Gene	rs ID	Location	SNP function[a]	MAF[b]	Alleles	Primers for PCR amplification (5'-3')	Annealing temperature (˚C)	PCR product length (bp)	HRM analysis	RFLP analysis	
									Melting temperature range (˚C)	RE	Restriction fragment length (bp)
RXRA	rs10881578	Chr9:137232535	Intron	0.30	A/G	F: TCTTGAGCAATGCCAGCAG R: CCACAGCTCACACATCCAATC	60.6	75	80-90		
	rs10776909	Chr9:137288746	Intron	0.18	C/T	F: CAGCCTGTGGCCTGCTCA R: AACCTCCGGCCCTTGGAG	60.6	95	82-92		
	rs749759	Chr9:137324652	Intron	0.24	G/A	F: ATAGGGCTTGCCTGCCTAGA R: CTCCACCATAGCCCAAGTGA	62.6	382		*Bst*XI	A=382 G=243+139
GC	rs7041	Chr4:72618334	Missense p.Asp432Glu	0.42	G/T	F: GGAGGTGAGTTTATGGAACAGC R: GGCATTAAGCTGGTATGAGGTC	66.3	493		*Hae*III	T=493 G=414+79
	rs1155563	Chr4:72643488	Intron	0.30	C/T	F: GGTTATTCTAAGACTGTGCTCTTGC R: ATGTGTTCTCACTGTTCGACTCC	63.0	116	71-78		
	rs2298849	Chr4:72648851	Intron	0.22	C/T	F: TCCACTGGCAAAACACATTAC R: GGGACATCTGCATTTATCCTG	60.6	118	73-83		
VDR	rs1544410	Chr12:48239835	Intron	0.34	A/G (B/b)	F: GGAGACACAGATAAGGAAATAC R: CCGCAAGAAACCTCAAATAACA	60.6	248		*Fsp*I	A (B)=248 G (b)=175+73
	rs2228570	Chr12:48272895	Missense p.Met51Thr	0.42	C/T (F/f)	F: GCACTGACTCTGGCTCTGAC R: ACCCTCCTGCTCCTGTGGCT	72.5	341		*Fok*I	C (F)=341 T (f)=282+59
BRCA2	rs80359550 c.5946delT	Chr13:32914438	Frameshift p.Ser1982Argfs	NA	-/T	F: TCACCTTGTGATGTTAGTTTGGA R: CACTTGTCTTGCGTTTTGTAATG	60.6	162	80-95		

[a]According to dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). [b]Minor allele frequency (MAF) calculated from the control samples. *GC* gene encodes vitamin D-binding protein. RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; HRM, high-resolution melting; RE, restriction enzyme; *RXRA*, retinoid X receptor α; *VDR*, vitamin D receptor; *BRCA2*, breast cancer 2, early onset; Chr, chromosome; F, forward; R, reverse.

Table III. Association of polymorphic variants of *RXRA*, *GC* and *VDR* genes with the risk of ovarian cancer.

Gene	rsID	Alleles[a]	MAF[b]	Genotypes (DD/Dd/dd)[c], n		P_{trend}-value	$P_{genotypic}$-value	$P_{allelic}$-value	Dominant model[d]		Recessive model[e]	
				Cases	Controls				OR (95% CI)	P-value	OR (95% CI)	P-value
RXRA	rs10881578	A/G	0.30	130/100/15	230/194/41	0.209	0.383	0.212	0.866 (0.635-1.180)	0.362	0.674 (0.365-1.245)	0.205
RXRA	rs10776909	C/T	0.18	173/62/10	311/139/15	0.505	0.396	0.500	0.841 (0.601-1.176)	0.310	1.277 (0.565-2.886)	0.556
RXRA	rs749759	A/G	0.24	139/92/14	271/168/26	0.726	0.924	0.726	1.065 (0.779-1.457)	0.692	1.023 (0.524-1.998)	0.946
GC	rs7041	G/T	0.42	88/115/42	155/230/80	0.630	0.770	0.630	0.892 (0.645-1.234)	0.490	0.996 (0.661-1.501)	0.984
GC	rs1155563	C/T	0.30	115/115/15	219/211/35	0.798	0.765	0.808	1.006 (0.738-1.372)	0.968	0.801 (0.429-1.498)	0.487
GC	rs2298849	C/T	0.22	166/72/7	275/172/18	0.029	0.079	0.033	0.689 (0.497-0.954)	0.025	0.730 (0.301-1.774)	0.486
VDR	rs1544410	A/G (B/b)	0.34	80/134/31	201/216/48	0.012	0.023	0.016	1.570 (1.136-2.171)	0.006	1.258 (0.778-2.036)	0.348
VDR	rs2228570	C/T (F/f)	0.42	74/113/58	158/227/80	0.068	0.111	0.064	1.189 (0.852-1.660)	0.308	1.493 (1.020-2.184)	0.038

Statistic [a]Underline denotes the minor allele in the control samples. [b]Minor allele frequency (MAF) calculated from the control samples. [c,d] represents the minor allele in the control samples. [d]dd+Dd vs. DD (d is the minor allele). [e]dd vs. Dd+DD (d is the minor allele). *GC* gene encodes vitamin D-binding protein. *RXRA*, retinoid X receptor α; *VDR*, vitamin D receptor; OR, odds ratio; CI, confidence interval.

Table IV. Results of haplotype analysis of the *RXRA*, *GC* and *VDR* genes in patients with ovarian cancer.

Polymorphisms	χ^2	Global P-value
RXRA[a]		
rs10881578, rs10776909	2.820	0.420
rs10776909, rs749759	4.874	0.181
rs10881578, rs10776909, rs749759	7.862	0.345
GC[b]		
rs7041, rs1155563	0.843	0.839
rs1155563, rs2298849	4.871	0.181
rs7041, rs1155563, rs2298849	7.956	0.336
VDR[c]		
rs1544410, rs2228570	9.345	0.025

[a]Empirical 5% quantile of the best P-value, 0.01066; [b]empirical 5% quantile of the best P-value, 0.01146; [c]empirical 5% quantile of the best P-value, 0.01984. *GC* gene encodes vitamin D-binding protein. *RXRA*, retinoid X receptor α; *VDR*, vitamin D receptor.

was employed. Haplotype analysis was performed using the UNPHASED 3.1. program (https://sites.google.com/site/fdudbridge/software/unphased-3-1) with the following analysis options: All window sizes, full model and uncertain haplotype. Haplotypes with a frequency <0.01 were set to zero. The P-values for global tests of haplotype distribution between cases and controls were determined. Statistical significance was assessed using the 1,000-fold permutation test. The gene-gene interactions among all tested SNPs were analyzed using the logistic regression and epistasis option in PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/). PLINK creates a model based on allele dosage for each SNP and considers allelic by allelic epistasis. To all significant associations, the Bonferroni correction considering the number of tested SNPs was applied.

Results

Association of RXRA, GC and VDR SNPs with development of OC. The prevalence of *RXRA*, *GC* and *VDR* genotypes did not exhibit deviation from HWE between patients and control groups (P>0.05). The number of each genotype, OR and 95% CI evaluation for the 8 *RXRA*, *GC* and *VDR* SNPs are listed in Table III. The lowest P-values of the trend test were found for *VDR Bsm*I rs1544410 and *GC* rs2298849 in women with OC (P_{trend}=0.012 and P_{trend}=0.029, respectively). The statistical significance threshold for multiple testing determined by correction of SNP number was P=0.00625. Therefore, it was found that, in a dominant inheritance model, *VDR Bsm*I contributes to increased risk of OC (OR, 1.570; 95% CI, 1.136-2.171; P=0.006). However, none of the other *RXRA*, *GC* and *VDR* polymorphisms demonstrated a significant contribution to OC either in dominant or recessive inheritance models (Table III).

Association of RXRA, GC and VDR haplotypes with development of OC. Haplotype analysis of the studied *RXRA*, *GC*

Table V. Results of gene-gene interaction analysis.

SNP 1		SNP 2		OR for interaction	χ^2	Asymptotic P-value
Gene	Identifier	Gene	Identifier			
GC	rs7041	*GC*	rs1155563	1.184	0.903	0.342
GC	rs7041	*GC*	rs2298849	1.129	0.370	0.543
GC	rs7041	*RXRA*	rs10881578	0.930	0.172	0.678
GC	rs7041	*RXRA*	rs10776909	1.241	1.152	0.283
GC	rs7041	*RXRA*	rs749759	1.097	0.274	0.601
GC	rs7041	*VDR*	rs1544410	0.808	1.575	0.210
GC	rs7041	*VDR*	rs2228570	1.257	2.254	0.133
GC	rs1155563	*GC*	rs2298849	0.826	0.539	0.463
GC	rs1155563	*RXRA*	rs10881578	1.186	0.781	0.377
GC	rs1155563	*RXRA*	rs10776909	1.743	5.764	0.016
GC	rs1155563	*RXRA*	rs749759	1.328	1.850	0.174
GC	rs1155563	*VDR*	rs1544410	0.890	0.353	0.553
GC	rs1155563	*VDR*	rs2228570	1.413	3.867	0.049
GC	rs2298849	*RXRA*	rs10881578	1.042	0.037	0.848
GC	rs2298849	*RXRA*	rs10776909	0.823	0.582	0.446
GC	rs2298849	*RXRA*	rs749759	0.821	0.757	0.384
GC	rs2298849	*VDR*	rs1544410	0.882	0.341	0.559
GC	rs2298849	*VDR*	rs2228570	1.156	0.551	0.458
RXRA	rs10881578	*RXRA*	rs10776909	0.823	0.783	0.376
RXRA	rs10881578	*RXRA*	rs749759	0.897	0.316	0.574
RXRA	rs10881578	*VDR*	rs1544410	1.322	2.132	0.144
RXRA	rs10881578	*VDR*	rs2228570	0.939	0.131	0.717
RXRA	rs10776909	*RXRA*	rs749759	1.463	3.100	0.078
RXRA	rs10776909	*VDR*	rs1544410	1.187	0.707	0.400
RXRA	rs10776909	*VDR*	rs2228570	1.681	6.678	0.010
RXRA	rs749759	*VDR*	rs1544410	1.088	0.213	0.644
RXRA	**rs749759**	***VDR***	**rs2228570**	**1.687**	**8.278**	**0.004**
VDR	rs1544410	*VDR*	rs2228570	1.206	1.292	0.256

Statistically significant results are highlighted in bold (P<0.00625). *GC* gene encodes vitamin D-binding protein. SNP, single-nucleotide polymorphism; *RXRA*, retinoid X receptor α; *VDR*, vitamin D receptor; OR, odds ratio.

and *VDR* SNPs did not indicate any contribution of SNP combinations to the risk of OC (Table IV). In OC patients, the lowest global P-value, P=0.025, was observed for haplotypes composed of the *VDR* rs1544410 and rs2228570 SNPs (Table V). However, these results did not reach significance when permutations were used to generate empirical P-values. The empirical 5% quantile of the best P-value following 1,000 permutations was 0.01066 for *RXRA*, 0.01146 for *GC* and 0.01984 for *VDR*.

Analysis of gene-gene interactions among the RXRA, GC and VDR polymorphisms. The gene-gene interactions among all tested SNPs conducted by the logistic regression and epistasis option in PLINK software demonstrated a significant interaction between *RXRA* rs749759 and *VDR* rs2228570, amounting to an OR for interaction of 1.687, χ^2=8.278, asymptotic P-value=0.004 and Bonferroni correction (P_{corr})=0.032 (Table V). In addition to this finding, an asymptotic P-value of <0.05 was observed for the following combinations: *RXRA* rs10776909 and *VDR* rs2228570 (OR, 1.681; χ^2=6.678; P=0.010; P_{corr}=0.08); *GC* rs1155563 and *RXRA* rs10776909 (OR, 1.743; χ^2=5.764; P=0.016; P_{corr}=0.128); and *GC* rs1155563 and *VDR* rs2228570 (OR, 1.413; χ^2=3.867; P=0.049; P_{corr}=0.392) (Table V). However, these P-values did not remain statistically significant after Bonferroni correction.

Discussion

Adequate $1,25(OH)_2D_3$ levels seem to be involved in the prevention of many diseases, including cardiovascular, musculoskeletal, autoimmune and infectious disorders, diabetes mellitus, infertility and others (17). The particularly significant protective role of vitamin D has been demonstrated in the context of the development and progression of various malignancies (18). Inadequate plasma levels of vitamin D have been associated with poor prognosis or development of head and neck cancers, thyroid, lung, liver, breast, gastric and colon cancers (19-25).

Reduced vitamin D levels have also been observed in patients with OC compared with general population, and low vitamin D levels have been associated with an increased risk of developing certain histological OC subtypes, such as borderline and mucinous (26,27). Recently, Walentowicz-Sadlecka *et al* (28) reported that low levels of $25(OH)D_3$, a pre-hormonal form of vitamin D, are accompanied by a reduced survival rate in patients with OC.

The antitumor activity of $1,25(OH)_2D_3$ can be mediated by microRNA specific for the telomerase transcript in OC and in other human cancer types (29). It has been demonstrated that $1,25(OH)_2D_3$ also inhibits proliferation of adrenocortical and pancreatic cancer cells and suppresses hepatocellular carcinoma development by reducing inflammatory cytokine production *in vivo* (30-32). In addition, vitamin D inhibits motility, invasion and metastasis of squamous cell carcinoma and suppresses breast and prostate cancer progression in murine models (33,34). Furthermore, the vitamin D analog EB1089 was found to trigger apoptosis of gastric cancer cells, and in preclinical studies exerted an anti-proliferative effect on human OC xenografts in murine models (35,36).

VDRs have been identified in various types of malignant cells (37). This implies that the anticancer action of $1,25(OH)_2D_3$ may be mediated by the levels of VDR, and also by VDBP and RXRA levels, which further suggests that SNPs situated in genes encoding these proteins may contribute to OC development.

In the present study, a significant contribution of the *VDR Bsm*I SNP to OC was observed in the Polish population. This result confirmed our previous studies, which demonstrated a moderate association of the *Bsm*I *VDR* B gene variant with OC (38). The meta-analysis by Qin *et al* (39) implicated the *Bsm*I SNP as a moderate risk factor for OC in the European population. In contrast to these findings, there was no association of the *Bsm*I polymorphism with OC in a number of other studies, which included a Caucasian population and cohorts from Massachusetts and New Hampshire in the USA (40,41). Three meta-analyses also did not confirm *Bsm*I SNP as a risk factor of OC in Caucasian, North American, Asian and overall populations (42-44).

In the present study, no significant difference was identified in the prevalence of the *Fok*I SNP between OC patients and controls. These observations are in agreement with those of Clendenen *et al* (40), who did not observe the *Fok*I SNP to be a risk factor for OC in Caucasian women. By contrast, other studies identified the *Fok*I polymorphism as a risk factor for OC in Massachusetts and New Hampshire, Indian, Caucasian, Japanese and overall populations (41-43,45-48).

The distinct influence of the *Bsm*I and *Fok*I SNPs on OC risk in various ethnicities may result from exposure of the studied groups to various environmental factors, the size of these groups and their genetic background. The possible role of *Bsm*I and *Fok*I polymorphisms on the action of VDR have been demonstrated elsewhere (8,49-51). The *Bsm*I SNP may alter the length of the polyadenylate sequence within the 3'-untranslated region of the *VDR* gene (8). Furthermore, Luo *et al* (49) demonstrated that the *Bsm*I SNP was responsible for the significantly lowered *VDR* mRNA levels in patients bearing the A (B) allele as compared to bearers of the GG (bb) genotype. The *Fok*I polymorphism results in the creation of two protein variants; longer VDR, encoded by the changed allele form (ATG) (f), has an additional three amino acids and is 1.7 times less efficient than the shorter, common allele form (ACG) (F) (50). In addition, Monticielo *et al* (51) demonstrated significantly increased vitamin D levels in individuals possessing the TT (ff) genotype versus carriers of the CC (FF) genotype of the *Fok*I SNP. Recently, Larcombe *et al* (13) demonstrated high frequency of VDR f allele associated with a downregulation of the Th1 immune response.

In the current study, no associations were observed between OC and the SNPs *GC* rs1155563 and rs7041, and *RXRA* rs10881578, rs10776909 and rs749759. To date, certain polymorphisms situated in *GC* have been reported to be associated with vitamin D metabolite levels in blood plasma (13,52). The *GC* rs7041 SNP was associated with high concentrations of VDBP in blood plasma and a high binding affinity to $25(OH)D_3$ in a Canadian cohort (13). In addition, the *GC* (436K) alleles (rs4588) (Fig. 1B) were associated with lower $25(OH)D_3$ concentrations in young Canadian adults of East Asian and European ancestry (52).

However, in the present study, a significant interaction was identified between the *RXRA* rs749759 and *Fok*I rs2228570 SNPs. Previous studies have proposed that *RXRA* rs7861779 and rs12004589 SNPs may be used as markers for colorectal cancer (53). Haplotype CGGGCA (rs1805352, rs3132297, rs3132296, rs3118529, rs3118536 and rs7861779) within linkage blocks of *RXRA* are associated with a reduced risk of metachronous neoplasia in the proximal colon (5). The *RXRA* haplotype, situated 3' of the coding sequence (rs748964 and rs3118523) increased the risk of renal carcinoma among carriers with the (CG) haplotype compared to the (GA) common haplotype (54). The *RXRA* SNPs (rs10881583, rs881658, rs11185659, rs881657 and rs7864987) were linked to poor disease-free survival in patients with breast cancer (15). Furthermore, head and neck squamous cell carcinoma patients possessing the *RXRA* SNP rs3118570 exhibited an increased risk of developing a second primary tumor or recurrence (55).

In conclusion, the current study confirmed that the *VDR Bsm*I SNP is risk factor for OC in non-carriers of the *BRCA1*/*BRCA2* mutations in the Polish population. Furthermore, a significant interaction between the *RXRA* rs749759 and *VDR Fok*I rs2228570 SNPs in these studied groups was identified. However, the results of this study must be verified other independent cohorts.

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