Impact of Two Functional Progesterone Receptor Polymorphisms (PRP): +331G/A and PROGINS on the Cancer Risks in Familial Breast/Ovarian Cancer

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Abstract: Background: More than half of the families with breast and/or ovarian cancer (BC/OC) have no *BRCA1* or *BRCA2* mutation, moreover the broad lifetime risks reported within families with a BRCA1/2 mutation suggest other genes are also responsible.

Objective: Assess the prevalence, gene-gene and phenotype-genotype associations of two functional progesterone receptor polymorphisms (PRP), *PROGINS* and +331G/A, in familial BC/OC.

Methods: DNA samples from 318 randomly selected probands tested for *BRCA1/2* mutations were genotyped for the *PRP* and *CHEK2*1100delC* variant.

Results: *BRCA1* was associated with BC at young age, p=0.002; *+331A* marginally with OC, p=0.07, and *PROGINS* with male BC, p=0.04. Homozygous *+331A/A* co-segregated with *BRCA2* variants more frequently than expected by chance alone. Co-occurrence of *+331A* with a *BRCA1BRCA2* mutation was associated with multiple BC events compared to *+331A* or *BRCA1/BRCA2* alone, p=0.02.

Conclusions: The PRP are risk factors for familial BC/OC, and *+331A* allele is a gene modifier of *BRCA1* and *BRCA2*.

INTRODUCTION

More than 50% of the families with BC and OC do not have mutations in the *BRCA1* and *BRCA2* genes [1-4]. So far, linkage analyses have failed in identifying new high penetrant genetic risk-factors responsible for the familial aggregation of those malignancies. It is hypothesized that the genetic basis in the remaining cases is highly heterogeneous [3-6] and that genes involved are low penetrant such as the *CHEK2*1100delC* variant [7, 8]. In addition, within families with a mutation in *BRCA1* or *BRCA2*, several lines of evidence suggest that additional genetic risk-factors/modifiers are present. For example, no current explanation exists for the fact that: i) in families with the same *BRCA1* or *BRCA2* mutation, some individuals develop BC, some OC, and others develop either both cancers or none; and ii) for the broad life-time risks for breast and OC reported among carriers of the same *BRCA1* or *BRCA2* mutation (Stanford Comprehensive Cancer Center statistics; http://cancer.stanford.edu/). Recent evidence for genetic modifiers have been provided by Smith and colleagues, who showed that women who tested negative for the familial *BRCA1*/*BRCA2* mutation do have an increased risk for BC compared to the general population [9].

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Despite the fact that the DNA-repair action of BRCA1 and BRCA2 proteins is ubiquitous across the human tissues, it is noteworthy that their inactivation leads predominantly to cancer of the breast and ovaries. In these tissues, cell homeostasis is mainly controlled by steroid hormones (estrogen and progesterone) and their respective receptors. Furthermore, it has recently been shown that a progesterone antagonist prevents *BRCA1*-mediated breast tumorigenesis in a mouse-model [10]. It is therefore plausible that genetic variants modifying the action of steroid hormone receptors might modify, alone or in combination with other risk-factors, the risk for BC and OC.

The progesterone receptor (PR) has two isoforms, PRA and PRB, which mediate all major responses to progesterone such as proliferation in the breast (mediated mainly through PRB [11]), and apoptosis in the ovarian epithelium (through PRA [12]).

Two functional *PR* gene polymorphisms (*PROGINS* and +331G/A) have been identified. *PROGINS* is a haplotype of three genetic variations in complete linkage disequilibrium: G3432T (Val660Leu substitution), C3764T (silent, His770His), and a HS-1/PV Alu insertion in intron G. *PROGINS* has a frequency of 0.12-0.16 among Caucasians [13-15] and reduces the activity of both PRA and PRB [16]. In case of the +331G/A promoter polymorphism [13], the rare allele *+331A* (with a frequency of 0.06-0.08 among

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Table 1. List of BRCA1 and BRCA2 Sequence Variants

Type of variant	Exon number	Genetic change	Protein change	probands
BRCA1				
Trunc. Mut.	1-2	del. exons1/ 2	DELETION	1
UV	11	1377 g>t	Asp420Tyr	1
UV	11	1998 g>a	Val627Ile	1
Trunc. Mut.	11	2057 del-8	STOP CODON 669	1
Trunc. Mut.	11	2312del5	STOP CODON 737	2
UV	11	2596 c>a	Thr826Lys	1
Trunc. Mut.	11	2841 g>t	STOP CODON 908	2
Trunc. Mut.	11	3109 ins-aa	STOP CODON 1000	1
UV	11	3867 g>a	Glu1250Lys	1
Trunc. Mut.	13	IVS12-1643del3835	Frameshift	1
Trunc. Mut.	14	4510 del 3 ins-tt	STOP CODON 1465	1
Trunc. Mut.	22	5464 g>a	STOP CODON 1782	2
Trunc. Mut.	24	5622 c>t	STOP CODON 1835	1
BRCA2				
Trunc. Mut.	5	697 del-aa	STOP CODON 182	1
Trunc. Mut.	6	IVS 6+1g>t	Frameshift	1
UV	10	1628 a>g	Lys467Arg	1
UV	11	3482 a>g	His1085Arg	1
Trunc. Mut.	11	3827 del-g	STOP CODON 1208	2
UV	11	3910 a>g	Asn1228Asp	1
Trunc. Mut.	11	5441 del-ctta	STOP CODON 1739	1
Trunc. Mut.	11	6024del-ta	STOP CODON 1943	1
Trunc. Mut.	11	6714 del-acaa	STOP CODON 2166	1
UV	12	IVS12-3 t>c	in frame del. (32 aa)	1
Trunc. Mut.	16	7962 del-6 ins-9	STOP CODON 2579	1
Trunc. Mut.	17	IVS16-1 g>t	Frameshift	1
UV	21	8967 c>g	Asp2913Glu	1
Trunc. Mut.	23	9345 g>a (alt. spl.)	EXON 23 DEL.	1
Trunc. Mut.	18	8286 del-t	STOP CODON 2693	1

UV: unclassified variant; Trunc. Mut.: protein truncating mutation; del.: deletion; ins: insertion; alt. spl.: alternative splicing; IVS: splice junction variant.

Caucasians) increases the relative PRB/PRA expression compared to *+331G*. Both polymorphisms have been shown to modify the risk for breast [2, 17-19] and OC [14, 15, 17, 20, 21] in several case-control studies. However these associations were not confirmed by other studies [13, 17, 22-26]. The role of these *PR* variants in the pathogenesis of familial BC and OC is largely unknown.

The aim of our study is to evaluate the role of *+331A* and *PROGINS* as risk-factors for familial BC and OC, alone or in combination with sequence variants in the *BRCA1* and *BRCA2* genes or with *CHEK2*1100delC*. We studied the prevalence, the genotype-tumor phenotype associations and the gene-gene interactions in a population of randomly selected probands who were tested for *BRCA1* and *BRCA2* mutations because of a family history of BC and OC.

MATERIALS AND METHODOLOGY

Study Population

Three-hundreds and eighteen (318) probands (men and women) were randomly selected from a DNA bank of pa-

tients that had been tested for *BRCA1* and *BRCA2* mutations because of a personal/family history of BC and/or OC at our Department of Clinical Genetics. All probands originated from the Southern region of the Netherlands (Limburg and Brabant) and had been counseled at our Cancer Genetics Service. The study was approved by the Medical Ethics Committee of the hospital. From the 318 individuals, 29 were already deceased at the time of the study, and 191 gave written informed consent to retrieve clinical and family data from their medical records. This group (220 individuals) will be indicated as CF-group (Clinical Features), to distinguish it from the whole population (n=318). Among the CF-group, 12 women had not been diagnosed with any cancer but had been tested because none of the affected relatives was still alive.

Genetic Analysis

BRCA1 and BRCA2: DNA extraction and genetic analysis of *BRCA1* and *BRCA2* mutations have been already described [27]. Mutation screening included the whole coding sequence plus splice junctions of the *BRCA1* and *BRCA2* genes. The term 'sequence variants' in the present study refers to both genetic unclassified variants (UVs) and truncating mutations (Table **1**). In addition, a number of rare polymorphisms previously classified as UVs found amongst the probands are also listed in Table **1**. Results of *BRCA1* and *BRCA2* screening were revealed only after the genotyping for the other three genetic markers was completed.

Progesterone receptor (PR) polymorphisms: PROGINS (*A1/A2*; Reference SNP Cluster Report: rs1042838[1] and rs1042839[2] and NCBI Z49816[3]) and +331G/A (rs10895068) detection was performed by restriction fragment length polymorphism analysis [15]. Analyses were performed twice to reduce the risk of miss-genotyping. With respect to the *PROGINS* polymorphism, only the V660L substitution in exon 4 (G3432T) was genotyped and used to assess the presence or the absence of the *PROGINS* allele. *A1* refers to the most common allele, *A2* to *PROGINS*.

*CHEK2*1100delC*: was detected by PCR, followed by DNA sequence analysis. Exon 10 of the *CHEK2* gene was amplified using specific primers: 5' GCAAGTTCAACAT-TATTCCCTTTT (forward) and 5' ATCACCTCCTAC-CAGCCTGTGC (reverse). The PCR reaction was performed using the Taq polymerase (Invitrogen, Life Technologies, Inc., Carlsbad, CA) as recommended by the manufacturer with 33 ng of genomic DNA. PCR conditions consisted of 6 touch-down cycles (annealing temperature from 64°C to 60.5°C) and 72°C/45 sec of elongation, followed by 30 extra cycles (94°C/30 sec, 60.5°C/30 sec and 72°C/45 sec). The PCR products (245bp) were sequenced using the BigDye Terminator kit 1.1 (Applied Biosystems, Warrington Cheshire, UK) and the ABI3100 sequencer.

Statistical Analysis

Genetic clustering: expected numbers of probands in which more than one genetic variant co-occurred were estimated based on the frequency of each variant in the study population. Expected number and observed numbers were compared using a log-linear model.

Gene-phenotype correlations: were assessed by Fisher's exact test in the CF-group.

RESULTS

I. Genotype Distribution

The present study includes 318 randomly selected probands tested for *BRCA1* and *BRCA2* mutations because of a family history of breast or OC. Table **2** shows the allele frequencies of each of the genetic parameters investigated. The two *PR* polymorphisms and the *CHEK2*1100delC* variant were in Hardy-Weinberg equilibrium (not shown).

Table 2. Prevalence of *PROGINS* (*A2*), *+331A*, *CHEK2*1100delC* Variant, *BRCA1* and *BRCA2* Sequence Variants

***PROGINS* (n=296) [a]**	**n**	**(%)**
A1/A1	222	(75.0)
A1/A2	68	(23.0)
A2/A2	6	(2.0)
all. frequency	A1	0.86
all. frequency	A2	0.14
***+331G/A* (n=298) [a]**	**n**	**(%)**
G/G	264	(88.6)
G/A	32	(10.7)
A/A	2	(0.7)
all. frequency	331G	0.94
all. frequency	331A	0.06
***CHEK2* (n=278) [a]**	**n**	**(%)**
wild-type/wild-type	267	(96.0)
**1100delC*/wild-type	11	(4.0)
**1100delC/*1100delC*	0	(0.0)
all. frequency	wild-type	0.98
all. frequency	*1100delC	0.02
***BRCA1/BRCA2* (n=318) [a]**	**n**	**(%)**
wild-type	262	(82.4)
BRCA1 variant	21	(6.6)
BRCA2 variant	35	(11.0)

a: number of individuals successfully screened for the indicated genetic factor.

Two-hundred-seventy-eight (278) individuals were successfully screened for all the genetic factors. Fig. **1** displays their genotypic distribution. None of the patients who carried a genetic variant in the *BRCA1* or *BRCA2* gene, carried also the *CHEK2*1100delC* variant. On the contrary, *PROGINS* and *+331A* were present both among *BRCA1* and *BRCA2* sequence variant carriers as in the *BRCA1* and *BRCA2* wild-type group Fig. **1**. In particular, the co-occurrence of homozygous *+331A/A* and *BRCA2* mutations was significantly higher than expected by chance alone (observed number = 1,

[1]rs1042838 refers to the G3432T in exon 4, Val660Leu. Nucleotide position is based on cDNA counting, NCBI X51730.
[2]rs1042839 refers to the C3764T in exon 5 His770His.
[3]NCBI Z49816 corresponds the HS-1/PV Alu insertion in intron G.
NCBI Reference SNP Cluster Report: http://www.ncbi.nlm.nih.gov/entrez

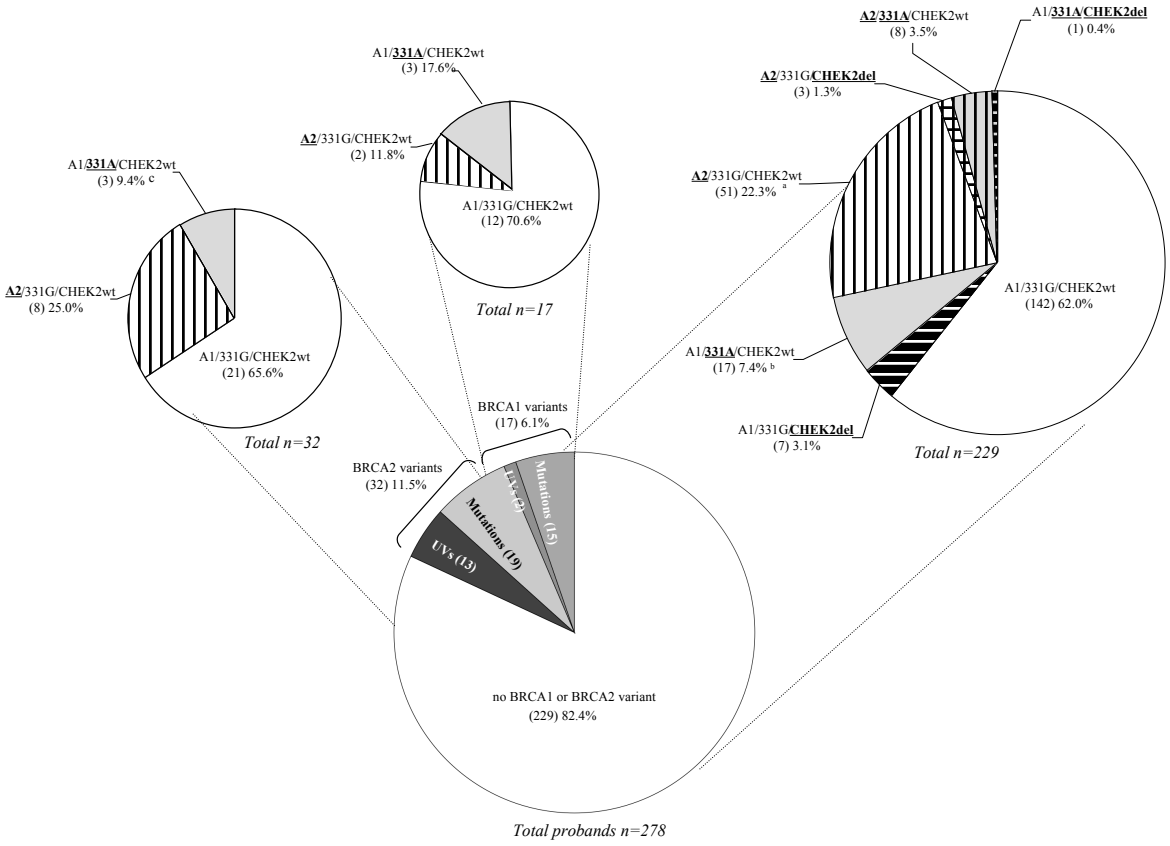

Fig. (1). Genotype distribution and co-occurrence of more than one rare genetic variant in the population.

Genotype distribution is represented in I) the whole population (circle at the bottom); II) carriers of a sequence variant in the *BRCA1* gene (above middle); III) carriers of a sequence variant in the *BRCA2* gene (left), and IV) *BRCA1* and *BRCA2* wild-type carriers (circle at the right).

Unless specified in the footnotes, probands were heterozygous for the genetic variant under consideration.

a: Five of 51 probands were *A2/A2* and 46 were *A1/A2*.

b: Sixteen of the 17 probands were *+331G/A* and one was *+331A/A*.

c: Two of the three probands were *+331G/A* and one was *+331A/A*.

expected number = 0.078, ratio (obs/exp) = 12.8, 95% CI=1.8-91.0; log-linear model). The only other case with a homozygous +331A/A observed in this series did show a rare polymorphism in the *BRCA2* gene: Lys2950Asn.

II. Clinical Characteristics

Clinical information was available from 220 patients (i.e. CF group). The majority of the CF-group (187/220; 6 men and 181 women) had been diagnosed with BC. From these, 21 women had primary BC twice (ipsi- or contralateral) and 24 were diagnosed with epithelial OC. Three of these 24 women also developed BC. Twelve women had not been diagnosed with any cancer. The inclusion criteria for *BRCA1* and *BRCA2* genetic diagnosis (indication for DNA analysis) and additional clinical features are also described in Table **3**.

The 220 probands belonged to 214 independent families including 3949 first and second-degree family members [2087 (52.8%) female, 1721 (43.6%) male and 148 (3.6%) of unknown gender]. Over 95% of the families (204/214) presented at least one case of BC (maximum 6 cases; average age at diagnose: 51.1±9.9) and about half of the families (109/214) had three or more relatives with BC (including the proband). A total of 564 relatives with BC were recorded. Twenty-nine families (0.7%) presented at least one patient with two primary breast cancers.

About one quarter of the families (51/214, 23.8%) had at least one case of OC and 19 families (9.0%) had two or more cases of OC (maximum 4 cases; average age at diagnose: 53.7±13.6). A total of 78 relatives having OC were recorded. Cancers at sites other than breast and ovaries were seen in half of the families: colon cancer (70 cases), lung cancer (n=27), prostate cancer (n=19), gastric cancer (n=15), skin cancer (n=14), brain tumors (n=12), pancreatic cancer (n=11), ORL cancer (n=11), cervical cancer (n=5), leukemia (n=5), bladder cancer (n=4), other cancers (n=47).

III. Genotype Phenotype Correlations (Table 4)

III-a. PROGINS PR Polymorphism

The *PROGINS* allele (*A1/A2* or *A2/A2*) was significantly more frequent among male compared to female probands,

Table 3. Clinical Characteristics of the Probands (CF-Group, n=220)

	n (%)
Breast cancer	
Yes	187 [a] (85.0)
No	33 (15.0)
Age of breast cancer onset (mean ± SD)	47.3 ± 11.1[b]
Breast cancer twice	
Yes	21 (11.2)
No	166 (88.8)
Age of second breast cancer onset (mean ± SD)	51.0 ± 9.9
Ovarian cancer	
Yes	24 (11.2)
No	190 (88.8)
Age of ovarian cancer onset (mean ± SD)	52.4 ± 15.1
Histology of ovarian cancer	
I-1 Epithelial serous	8 (33.3)
I-2 Epithelial mucinous	6 (25.0)
I-3 Epithelial endometrioid	1 (4.2)
I-4 Epithelial Brenner	1 (4.2)
Unknown	8 (33.3)
Other cancer (no breast and ovary) [c]	
Yes	12 (5.5)
No	208 (94.5)
Indication for DNA analysis	
I. One first degree relative diagnosed with:	
I.a. breast cancer below 35 years	10 (5.3)
I.b. bilateral breast cancer. First is premenopausal	4 (2.1)
I.c. male breast cancer	3 (1.6)
II. Two first or second degree relatives diagnosed with:	
II.a. breast or ovarian cancer before menopause	50 (26.7)
II.b. ovarian cancer (only first degree)	3 (1.6)
III. Three or more patients in the family	111 (59.4)
IV. Pre-symptomatic [d]	1 (0.5)
V. Unknown	5 (2.7)

[a]breast cancer patients include 6 men.
[b]the average age of breast cancer in man onset was 59.0 ± 6.6.
[c]other sites of cancer onset were colon (n=2), brain (n=3), melanoma (n=4), pancreas (n=1), (mesothelioma in peritoneum, n=1) and one ORL cancer. All women presenting cancer at sites other than breast or ovary were also diagnosed with breast (n=11) or ovarian (n=1) cancer.
[d]presymptomatic indicates that the variant in *BRCA1* or *BRCA2* was already known in the family.

both in the whole group (n=220; p=0.047) and among patients diagnosed with BC (n=167; p=0.04). Further, patients carrying the *PROGINS* allele did not differ significantly with respect to any of the other investigated clinical or histological features.

III-b. +331G/A PR Polymorphism

Patients carrying the *+331A* allele were diagnosed more frequently with two primary BC and with OC than non-carriers. These differences were of borderline significance (p=0.09 and 0.07, respectively; Table **4**).

*III-c. CHEK2*1100delC Variant*

All *CHEK2*1100delC* carriers were women with BC, none of them had OC (Table **4**). Further, no other clinical or histological features investigated was influenced by the presence of this variant.

III-d. BRCA1 Mutations

Patients carrying a mutation in the *BRCA1* gene were significantly younger when BC was diagnosed (p=0.002). When also *BRCA1* UVs were considered, results did not change (p=0.028; results not shown).

III-e. BRCA2 Mutations

Patients carrying a mutation on the *BRCA2* gene did not differ from patients being *BRCA2* wild-type with respect to any of the clinical characteristics investigated.

IV. Gene-Gene Interactions

To evaluate the effect of co-occurrence of genetic risk factors we looked for patients having unfavorable clinical characteristics, such as occurrence of both BC and OC, BC twice, BC below the age of 40 years, or OC before the menopause and compared their frequency with the numbers of genetic risk alleles: none, one or two. The following combinations of genetic risk factors were investigated: *+331A* and *BRCA1/2*; *PROGINS* and *BRCA1/2*; *+331A* and *PROGINS*; *CHEK2*1100delC* and *+331A*; and *CHEK2*1100delC* and *PROGINS*. Only the combination: *+331A* and *BRCA1/2* resulted in significant differences in the distribution of patients having unfavourable features (p=0.02; Table **5**). Notably, no patient carrying both *+331A* and a mutation in the *BRCA1* or *BRCA2* gene was seen in the average feature group, whereas all three patients carrying both *+331A* and a *BRCA1* or *BRCA2* mutation belonged to the group of patients with unfavorable clinical features. One of those women was *+331A/A* homozygous and carried a mutation in the *BRCA2* gene, the other two women were *+331G/A* heterozygous and carried one *BRCA1* mutation each. All those three women were diagnosed twice with BC.

DISCUSSION

Some case-control studies have suggested that *PROGINS* is protective against BC [2] but a risk-factor for OC [14, 15, 17], whereas the *+331A* allele appeared to be a risk-factor both for BC [19] and for OC [15, 21]. Other authors, however, did not find any association [13, 17, 22-26, 28]. So far, the study of Runnebaum and coworkers [20] is the only one that investigates the effect of one of the *PR* polymorphisms (*PROGINS*) on BC and OC risk among *BRCA1* and *BRCA2* carriers and reports that *PROGINS* increases the risk for OC

Table 4. Clinical Characteristics of the Genotypes

	PROGINS							*+331G/A*						
	A1/A1		*A1/A2*		*A2/A2*			*G/G*		*G/A*		*A/A*		
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	***p value***	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**	***p value***
Gender														
Male	2	(40.0)	2	(40.0)	1	(20.0)	*0.0474*	6	(100)	0	(0.0)	0	(0.0)	*1.0000*
Female	143	(74.1)	46	(23.8)	4	(2.1)		172	(88.7)	20	(10.3)	2	(1.0)	
BC														
Yes	123	(73.7)	41	(24.6)	3	(1.8)	*0.3150*	153	(90.5)	15	(8.9)	1	(0.6)	*0.1092*
No	22	(71.0)	7	(22.6)	2	(6.5)		25	(80.6)	5	(16.1)	1	(3.2)	
Gender BC														
Male	2	(40.0)	2	(40.0)	1	(20.0)	*0.0374*	6	(100)	0	(0.0)	0	(0.0)	*1.0000*
Female	121	(74.7)	39	(24.1)	2	(1.2)		147	(90.2)	15	(9.2)	1	(0.6)	
Age BC														
</=40	35	(77.8)	10	(22.2)	0	(0.0)	*0.6802*	41	(93.2)	3	(6.8)	0	(0.0)	*0.8236*
>40	88	(72.1)	31	(25.4)	3	(2.5)		112	(89.6)	12	(9.6)	1	(0.8)	
Two BCs														
Yes	14	(73.7)	5	(26.3)	0	(0.0)	*1.0000*	16	(84.2)	2	(10.5)	1	(5.3)	*0.0887*
No	109	(73.6)	36	(24.3)	3	(2.0)		137	(91.3)	13	(8.7)	0	(0.0)	
OC														
Yes	16	(72.7)	6	(27.3)	0	(0.0)	*0.8725*	17	(77.3)	4	(18.2)	1	(4.5)	*0.0736*
No	127	(74.3)	40	(23.4)	4	(2.3)		155	(90.1)	16	(9.3)	1	(0.6)	

	CHEK2*1100delC					**BRCA1**					**BRCA2**				
	Wild type		**Variant**			**Wild type**		**Variant**			**Wild type**		**Variant**		
	n	**(%)**	**n**	**(%)**	***p value***	**n**	**(%)**	**n**	**(%)**	***p value***	**n**	**(%)**	**n**	**(%)**	***p value***
Gender															
Male	5	(100)	0	(0.0)	*1.0000*	6	(100)	0	(0.0)	*1.0000*	5	(83.3)	1	(16.7)	*0.2676*
Female	170	(95.5)	8	(4.5)		202	(94.4)	12	(5.6)		204	(95.3)	10	(4.7)	
BC															
Yes	148	(94.9)	8	(5.1)	*0.6066*	178	(95.2)	9	(4.8)	*0.3959*	178	(95.2)	9	(4.8)	*0.6721*
No	27	(100)	0	(0.0)		30	(90.9)	3	(9.1)		31	(93.9)	2	(6.1)	
BC Gender															
Male	5	(100)	0	(0.0)	*1.0000*	6	(100)	0	(0.0)	*1.0000*	5	(83.3)	1	(16.7)	*0.2592*
Female	143	(94.7)	8	(5.3)		172	(95.0)	9	(5.0)		173	(95.6)	8	(4.4)	
Age BC															
</=40	34	(89.5)	4	(10.5)	*0.2048*	43	(86.0)	7	(14.0)	*0.0016*	49	(98.0)	1	(2.0)	*0.4485*
>40	114	(96.6)	4	(3.4)		135	(98.5)	2	(1.5)		129	(94.2)	8	(5.8)	
Two BCs															
Yes	19	(89.5)	2	(10.5)	*0.2520*	20	(90.9)	2	(9.1)	*0.2862*	21	(95.5)	1	(4.5)	*1.0000*
No	137	(95.6)	6	(4.4)		158	(95.8)	7	(4.2)		157	(95.2)	8	(4.8)	
OC															
Yes	21	(100)	0	(0.0)	*0.5985*	21	(87.5)	3	(12.5)	*0.1258*	23	(95.8)	1	(4.2)	*1.0000*
No	157	(94.9)	8	(5.1)		181	(95.3)	9	(4.7)		181	(95.3)	9	(4.7)	

Table 5. Gene-Gene Interactions

	AVERAGE FEATURES			ADVERSE FEATURES (1)			
	Total	n	(%)	*Total*	n	(%)	*p-value*
	116			73			*0.0213*
No variant (i.e. 331G/G and BRCA1&2 wild type)		98	(64.5)		54	(35.5)	
+331A		11	(68.8)		5	(31.3)	
BRCA1 or BRCA2 mutation		7	(38.9)		11	(61.1)	
+331A and BRCA1 or BRCA2 mutation		0	(0.0)		3	(100)	

(1) the group of patients with unfavorable clinical features defined as: BC below 40 years (n = 39), OC before 55 years (n = 20), two BCs (n = 20) or BC and OC (n = 2) in the same person.

in *BRCA1*/2 mutation carriers who were never exposed to oral contraceptives.

In the present study, women carrying the *PROGINS* allele did not differ in the frequency of developing BC or OC compared to non-carriers. Interestingly, the *PROGINS* allele was significantly more frequent among men than among women diagnosed with BC. Breast cancer in men is a rare disease whose etiology is largely unknown [8]. Though, it appears that steroid hormone imbalances are important epidemiological risk-factors also in male BC and more than 90% of breast tumors in man are PR positive [29]. The number of male patients with BC in our study (n=6) was too small to draw any definitive conclusion, therefore, larger epidemiological studies are needed to confirm the role of *PROGINS* in male BC.

We observed a trend for an increased risk of OC and BC appearing twice among women carrying the *+331A* allele. The fact that those features were not significantly higher among *BRCA1* and *BRCA2* carriers may be due to insufficient statistical power, but may also reflect that *+331A* allele is a stronger predictor for both OC and multiple BC than *BRCA1* and *BRCA2*.

We found evidence that suggest that the *+331A* allele in combination with *BRCA1* or *BRCA2* mutations have a synergistic effect. Firstly, the number of probands being homozygous *+331A/A* and carrying a mutation in the BRCA2 gene was significantly higher than in case they segregated independently. Interestingly the only two cases in this series who were homozygous *+331A/A* appeared to have either a mutation or a rare polymorphism in *BRCA2*. This additionally suggests that some rare polymorphisms are likely to be functional and to affect cancer risks. Secondly, a synergistic effect is further corroborated by the fact that all the patients carrying both *+331A* allele and a mutation in *BRCA1* or *BRCA2* belonged to the group with specially unfavorable clinical features, and had multiple BC events in particular. A molecular explanation of this synergistic effect for BR risk can be due to the fact that the *+331A* allele, as a result of the increased PRB/PRA expression increases cell proliferation in the breast epithelium (mediated by PRB) in response to progesterone [11, 12]. This, in combination with a defective DNA-repair machinery (due to a *BRCA1* or *BRCA2* variants), will facilitate the accumulation of cells with damaged DNA and, in turn, their malignant transformation.

CONCLUSION

In conclusion, we provide evidence that the *PR +331A* allele is a risk factor for familial BC/OC and acts as gene modifier in families with a *BRCA1* or *BRCA2* sequence variant. If independently confirmed by others, genetic screening of the *+331G/A* polymorphism should be considered together with *BRCA1* and *BRCA2* mutation detection for an individual risk assessment of the probands and their relatives. Whether *PROGINS* will result into a prevalent risk factor for male BC, should be assessed in series including a larger number of men with BC.

LIST OF ABBREVIATIONS

CI = 95% confidence interval

UV = unclassified variant

CHEK2 = cell-cycle-checkpoint kinase 2

PROGINS = progesterone receptor intron G insertion

CF population = clinical features population

PRP = progesterone receptor polymophisms

BC = breast cancer

OC = ovarian cancer

ACKNOWLEDGEMENTS

Ms. R. Brandao was supported by the Gulbenkian Foundation (Portugal). This study was supported by funds from the Maastricht University Hospital. We thank all patients involved in this study for their co-operation.

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Received: June 05, 2007 Revised: June 29, 2007 Accepted: July 03, 2007