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ORIGINAL ARTICLE

Progesterone Receptor (PGR) Gene Variants Associated with Breast Cancer and Associated Features: a Case-Control Study

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Abstract

Insofar as altered estrogen receptor-progesterone receptor (PR) expression contribute to breast cancer pathogenesis, previous studies examined the association of genetic variation in PR gene (*PGR*) with breast cancer, but with mixed outcome. We evaluated the association between *PGR* variants, and breast cancer and associated features. A retrospective case-control study involving 183 female breast cancer patients, and 222 control women. *PGR* genotyping was done by real-time PCR. Minor allele frequencies of rs1042838, rs590688, and rs10895068 *PGR* gene polymorphisms were significantly higher in breast cancer patients compared to controls. Patients carrying rs1042838 G/T, rs590688 C/C, and rs10895068 G/A genotypes had higher risk of breast cancer, while carriage of rs3740753 G/G genotype was associated with marginal reduction in breast cancer risk. In addition, carriage of rs1042839, rs3740753, and rs10895068 minor allele was associated with Her2 status, while rs3740753 and rs10895068 were associated with effective hormone replacement therapy. Furthermore, carriage of rs10895068 minor allele in breast cancer women were also associated with age at first pregnancy, hormone receptor (RH) status, and previous use of oral contraceptives. *PGR* haploview analysis documented moderate-strong linkage disequilibrium (non-random association of alleles at different loci) between 7 of the 8 tested *PGR* SNPs, thus allowing construction of 7-locus *PGR* haplotypes. Two haplotypes, ATG**C**CGA and **G**TG**C**CGA, both containing rs590688, were positively associated with breast cancer, thus assigning a breast cancer-susceptible nature to these haplotypes. *PGR* rs1042838, rs590688, and rs10895068, and ATGCCGA and **G**TG**C**CGA haplotypes are related with increased breast cancer susceptibility in Tunisian women.

Keywords Breast cancer · Haplotypes · Mutation · Progesterone · Progesterone receptor

Introduction

Breast cancer is the second leading cause of cancer-related deaths worldwide [1], and is a multifactorial disease which results from interaction between modifiable (environmental) and non-modifiable factors [2, 3]. The former includes breast-feeding and oral contraceptive use, while the latter includes age, early menarche, late menopause, ethnicity, and genetic factors [4–6]. Breast cancer has heterogeneous phenotype, and is subgrouped based on the pathobiology, survival, and the presence of identifiable risk factors [7, 8]. Clinically, the onset and progression of breast cancer is associated with sex hormone receptor status, particularly human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), and progesterone receptor (PGR) status characteristics [9], which depends on the age group the patient belongs to,

Progesterone is the major progestogen in humans, and a key intermediate in the formation of sex hormones and corticosteroids. Progesterone is involved in the regulation of the growth, development and maintenance of female reproductive tissues, and plays a key role in breast development. Progesterone mediates its effects by binding its inactive

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cytosolic receptor (PGR), resulting in its activation and nuclear translocation as steroid-receptor complex. Progesterone-PGR complex then binds progesterone response elements, leading to increased receptor phosphorylation, and altered transcription of progesterone-responsive genes [10]. PGR belongs to ligand-activated nuclear receptors superfamily, and its gene is located on chromosome 11q22–23, comprising eight exons and seven intons. PGR is expressed as co-expressed PGR-A and PGR-B isoforms [11], with distinct promoters and translational start sites. While the 94-kDa PGR-A acts as transcriptional repressor, the 114 kDa PGR-B is transcriptional activator [12, 13]. Balanced expression of both PGR isoforms is required for maintaining mammary gland function [14], and imbalance in PGR-A and PGR-B expression is associated with increased risk of breast cancer [15]. In this regard, it was shown that altered PGR-A and PGR-B balance distorts progesterone effects on breast cells, thus increasing breast cancer risk.

PGR is highly polymorphic, with more than 800 SNPs were identified throughout *PGR* gene. A number of studies reported associations between *PGR* gene variants and hormone-related disorders, including breast cancer [16–19]. While a number of studies reported moderate association of *PGR* SNPs, in particular V660 L (rs1042838) variant, with increased risk of breast cancer [17, 20], others reported no such association [19, 21], but reported positive association of breast cancer with polymorphisms in the estrogen receptor (ESR) *ESR1* [19, 21] and *ESR2* [19] genes. Using a case-control study design, here we investigate the association between *PGR* gene variants and breast cancer risk in Tunisian women, and evaluated the contribution of possible modifying factors on the effect of PGR on breast cancer risk.

Subjects and Methods

Study Subjects This was a retrospective case-control study, which was performed at the outpatient oncology service of Fattouma Bourguiba University Hospital (Monastir, Tunisia) in the period February 2014 to March 2016. Study subjects comprised 183 women with breast cancer (mean age 50.0 ± 12.0 years), and 222 cancer-free university and hospital employees, or volunteer women (mean age 40.9 ± 4.3 years), who served as controls. Controls reported no personal or family history of breast cancer, and were matched to cases according to self-declared ethnic origin. Breast cancer assessment was as per American Cancer Society (www.cancer.org) guidelines, which included mammography and breast biopsy testing for confirmation of breast cancer; all cases had these procedures done. We retrospectively collected demographic and clinical information from medical records, and by personal interview by the same interviewer, using a structured questionnaire. Detailed clinical information included patient age, age at diagnosis of primary breast cancer, menopausal status, metastatic disease at breast cancer presentation. In addition, detailed histology (disease stage and nuclear grade), sex hormone receptor (ER and PR) status, and primary treatment including surgery, radiation, chemotherapy, and/or endocrine therapy, were recorded for all patients. The study was approved by the ethics committee of UCH Fattouma Bourguiba in Tunisia and all participants provided informed consent.

***PGR* Genotyping** Peripheral venous blood was collected from study participants in EDTA vacutainer tubes for preparing DNA. Extraction of genomic DNA was performed using QIAamp DNA Blood Mini Kit (Qiagen, Inc., Manchester, UK). We selected eight haplotype tagging SNPs (htSNPs; representative SNPs in regions of high linkage disequilibrium) in *PGR* gene using SNPbrowser 4.0 (Applied Biosystems, Foster City, CA), which were previously reported to be linked with breast cancer, using NCBI Entrez Gene SNP Geneview. We included htSNPs which had a haplotype $R > 95\%$, and minor allele frequency (MAF) exceeding 2% in Caucasians. The tag SNPs selected were rs471767, rs578029, rs1042838, rs590688, rs3740753, rs10895068, rs608995, and rs1942836.

PGR genotyping was performed by VIC- and FAM-labelled allelic discrimination method, using assay-on-demand TaqMan assays, which were ordered from Applied Biosystems. The reaction was performed in 6 μl volume on StepOne Plus real-time PCR system, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping procedure; concordance was >99%. Apart from rs3740753, in which two genotyping failures among control participants (0.5%) were obtained, genotyping rate of the remaining SNPs was 100%. The homozygous major allele genotype (1/1) was used as reference (OR = 1.00) in calculation of the odds ratios (OR) associated with overall breast cancer risk.

Statistical Analysis Statistical analysis was done using SPSS version 23 (IBM; Armonk, NY). Continuous and categorical variables were presented as means (± SD), or as percent total. Differences in means were assessed by Student's *t*-test, and Pearson χ^2 test were used for assessing inter–group significance. Hardy–Weinberg equilibrium (HWE) was evaluated by Haploview (www.broad.mit.edu/mpg/haploview). Calculation of the power for detecting an association between *PGR* variants and breast cancer was done using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/cgi-bin/cc2k.cgi). The parameters used were 183 breast cancer patients and 222 control women, genotypic relative risk for heterozygote (1/2) and minor allele homozygous (2/2), and the MAF for breast cancer cases and controls for the eight tested SNPs, and assuming a 11.27 per

100,000 prevalence of breast cancer in Tunisia. Assuming these parameters, we calculated the overall power (86.7%) as the average power of the eight tested SNPs.

All analyses were performed under the assumption of additive genetic effect. We controlled for the following potential confounders in multivariate logistic regression analysis: previous/current use of oral contraceptives, parity, age at first live birth, age of menarche or menopause, and body-mass index (BMI). Haploview was used to check linkage disequilibrium (LD) between SNPs, beside their haplotype patterns. *PGR* haplotypes were reconstructed by the expectation maximization algorithm. Of the possible 256 haplotypes, only 10 were found to be common (frequency > 2%), and thus were included in further analysis. Taking the control group as reference, logistic regression analysis was used for determination of the odds ratios (OR) and 95% confidence intervals (95%CI) associated with breast cancer risk. Statistical significance was set at $P < 0.05$; statistically significant differences being designated as boldface in the tables.

Results

Study Subjects Table 1 shows the demographic and clinical characteristics of study participants. The prevalence of smoking, and history of smoking, breast-feeding, or oral contraceptive use were comparable between breast cancer patients and control women. Mean age at inclusion of study ($P < 0.001$), BMI ($P = 0.002$), menopause status ($P = 0.001$), and menarche ($P = 0.03$), were significantly different between breast cancer cases and control women, and thus were selected as the main covariates that were controlled for in subsequent analysis.

Association Studies Genotype distributions of the *PGR* variants rs471767, rs578029, rs1042838, rs3740753, rs10895068, rs1942836, but not rs590688 or rs608995 were in HWE among study subjects (Table 2). Minor allele frequencies (MAF) of the 8 tested *PGR* SNPs in breast cancer cases and control women are presented in Table 2. Higher MAF of rs1042838 ($P = 0.038$), rs590688 ($P = 0.009$), and rs10895068 ($P = 0.006$) was seen in breast cancer cases than in control women.

The distribution of *PGR* genotypes in women with breast cancer and control women are summarized in Table 3. Breast cancer patients carrying rs1042838 G/T [OR (95% CI) = 1.83 (1.13–2.97)], rs590688 C/C [OR (95% CI) = 1.85 (1.11–3.08)], and rs10895068 G/A [OR (95% CI) = 3.58 (1.38–9.29)] genotypes had a higher risk of breast cancer, while carriage of rs3740753 G/G genotype was associated with marginal reduction in breast cancer risk [OR (95% CI) = 0.27 (0.07–0.96)].

Table 1 Characteristics of Study Subjects

	Cases ($n = 183$)	Controls ($n = 222$)	P^c
Age (yr) [a]	50.0 ± 12.1	40.9 ± 4.3	<0.001
BMI (kg/m^2) [a]	28.6 ± 5.5	26.0 ± 5.8	0.002
Menarche (yr) [a]	12.2 ± 1.5	12.7 ± 1.3	0.03
Smoking [b]	4 (2.2)	7 (3.2)	0.76
Breast feeding [b]	126 (68.9)	146 (65.8)	0.51
Menopausal status [b]	82 (44.8)	51 (23.0)	0.001
Oral contraceptive use [b]	25 (13.7)	29 (13.1)	0.88
Histology			
Ductal	167 (91.3)	N/A	N/A
Lobular	4 (2.2)	N/A	N/A
Nodal status [b]			
N0	92 (50.3)	N/A	N/A
N1	65 (35.5)	N/A	N/A
N2	26 (14.2)	N/A	N/A
Positive RH Status [b]	42 (25.9)	N/A	N/A
Her-2 [b]	55 (34.0)	N/A	N/A
Management:			
Surgery [b]	112 (65.9)	N/A	N/A
Radiotherapy [b]	87 (51.5)	N/A	N/A
Hormone replacement [b]	71 (42.0)	N/A	N/A

RH hormone receptor, *N/A* not applicable

[a] Mean ± SD

[b] Number of subjects (percent total)

[c] Student t-test for continuous variables, Pearson χ^2 test for categorical variables

Influence of *PGR* SNPs on BC Features We analyzed the influence of *PGR* SNPs on menarche, hormone receptor (RH), Her2 status, responsiveness to hormone treatment, along with menopause status previous oral contraceptive use, and breastfeeding. Carriage of rs1042839 ($P = 0.01$), rs3740753 ($P = 0.04$), and rs10895068 ($P = 0.003$) minor allele was associated with Her2 status, while carriage of rs3740753 ($P = 0.009$) and rs10895068 ($P < 0.001$) were associated with effective hormone replacement therapy. Furthermore, carriage of rs10895068 minor allele in women with breast cancer was also associated with age at first pregnancy ($P = 0.04$), RH status ($P = 0.02$), and previous use of oral contraceptives ($P = 0.02$) (Table 4).

Haploview Analysis Varied LD was noted between the 8 tested *PGR* variants (Fig. 1). Extensive diversity in the haplotype assignment was seen, with common haplotype (>2% of total) seen in 10 of the identified SNPs, which captured 80.5% of all haplotypes. Increased frequency of haplotypes ATG**C**CGA ($P = 8.00 \times 10^{-4}$), and **G**TG**C**CGA ($P = 0.003$) was noted in women with breast cancer than in control women, thus assigning breast cancer-susceptible nature to these haplotypes

Table 2 *PGR* SNPs Analyzed

Number	SNP	Location [a]	Alleles	HWE	Cases [b]	Controls [b]	X[b]	*P*	*Pc* [c]	OR (95% CI)
1	rs471767	100,905,297	A:G	0.68	115 (0.31)	134 (0.31)	0.015	0.903	1.000	
2	rs578029	100,922,404	T:A	0.54	103 (0.28)	120 (0.28)	0.013	0.909	1.000	
3	rs1042838	100,933,412	G:T	0.07	62 (0.17)	51 (0.12)	4.297	0.038	0.226	1.52 (1.02–2.27)
4	rs590688	100,975,974	G:C	0.005	187 (0.51)	181 (0.42)	6.741	0.009	0.070	1.45 (1.09–1.92)
5	rs3740753	100,998,771	C:G	1.00	50 (0.14)	69 (0.16)	0.937	0.333	0.961	
6	rs10895068	101,000,214	G:A	1.00	17 (0.05)	6 (0.02)	7.504	0.006	0.047	3.46 (1.35–8.87)
7	rs608995	101,035,002	A:T	0.03	122 (0.33)	149 (0.35)	0.118	0.731	1.000	
8	rs1942836	101,178,616	T:C	0.49	73 (0.20)	84 (0.19)	0.031	0.859	1.000	

[a] Location based on HapMap build 37.3

[b] Number of alleles (frequency)

[c] Pc = corrected P (Bonferroni correction)

(Table 5). In contrast, reduced frequency of haplotype ATGGCGA ($P = 0.006$) was seen in women with breast cancer than in control women, thus assigning breast cancer-protective nature to this haplotype (Table 5).

Discussion

Several genetic variants in *PGR* gene were previously tested for their possible association with altered risk of breast cancer in several ethnic groups, often with inconclusive findings. This was due to the SNP selection, and also to limiting the analysis to the single SNP level. In this retrospective case-control study, we confirmed positive association between breast cancer and three *PGR* tagging SNPs (rs1042838/V660 L, rs590688, and rs10895068/+113G/A) among Tunisian Arab women. To minimize the possibility of racially heterogeneity as a possible confounder, we limited the selection of study subjects (cases and controls) to Tunisian Arabs. Limiting the origin of the study participants minimizes the possibility of population stratification bias, inherent in genetic association studies. In addition, as environmental and acquired factors may influence the outcome, we controlled for key variates throughout the analysis. However, this does not rule out the possible contribution of modifiable and non-modifiable factors not included in the panel of covariates that were not controlled for in the analysis.

MAF of rs1042838 and 10,895,068 is highest among Caucasians (21.4% and 5.9%), compared to Africans (1.9% and 1.1%), Asians (1.4% and 0.8%) and Hispanics (14.9% and 1.8%). In contrast, MAF of rs590688 is comparable between Caucasians (48.7%) and Africans (48.6%), but is markedly lower in Asians (22.5%) (www.ncbi.nlm.nih.gov/projects/SNP/snp). Of the tested SNPs, only rs1042838 showed significant association with breast cancer ($P = 0.038$), which was later lost after correcting for multiple comparisons

Table 3 *PGR* Genotype Frequencies in Cases and Controls

SNP	1 / 1			1 / 2			2 / 2		
	Cases	Controls	P	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
rs471767	85 (0.46) [a]	106 (0.49)	0.63	81 (0.44)	86 (0.40)	1.17 (0.77–1.78)	17 (0.09)	24 (0.11)	0.88 (0.45–1.75)
rs578029	95 (51.9)	115 (53.2)	0.92	73 (39.9)	82 (38.0)	1.08 (0.71–1.63)	15 (8.2)	19 (8.8)	0.96 (0.46–1.98)
rs1042839	127 (69.4)	172 (79.6)	0.04	50 (27.3)	37 (17.1)	1.83 (1.13–2.97)	6 (3.3)	7 (3.2)	1.16 (0.38–3.54)
rs590688	56 (30.6)	83 (38.4)	0.05	67 (36.6)	85 (39.4)	1.17 (0.73–1.86)	60 (32.8)	48 (22.2)	1.85 (1.11–3.08)
rs3740753	136 (74.3)	158 (73.8)	0.05	44 (24.0)	43 (20.1)	1.19 (0.74–1.92)	3 (1.6)	13 (6.1)	0.27 (0.07–0.96)
rs10895068	166 (90.7)	210 (97.2)	0.005	17 (9.3)	6 (2.8)	3.58 (1.38–9.29)	—	—	—
rs608995	88 (48.1)	101 (46.8)	0.95	68 (37.2)	81 (37.5)	0.96 (0.63–1.48)	27 (14.8)	34 (15.7)	0.91 (0.51–1.63)
rs1942836	118 (64.5)	142 (65.7)	0.94	57 (31.1)	64 (29.6)	1.07 (0.70–1.65)	8 (4.4)	10 (4.6)	0.96 (0.37–2.52)

Alleles were coded as "1" (major allele) and "2" (minor allele)

[a] Number of subjects (frequency)

Table 4 Correlation Studies

	rs1042839		rs590688		rs3740753		rs10895068	
	P	**β (SE)**	***P***	**β (SE)**	***P***	**β (SE)**	***P***	**β (SE)**
Menarche	0.84	0.06 (0.30)	0.51	−0.16 (0.25)	0.17	0.36 (0.27)	0.05	7.28 (6.14)
1st pregnancy	0.74	0.02 (0.07)	0.24	−0.07 (0.06)	0.66	−0.03 (0.07)	**0.04**	**7.15 (5.82)**
RH	0.23	−1.20 (0.99)	0.99	0.01 (0.77)	0.18	1.18 (0.89)	**0.02**	**18.89 (20.28)**
Her2	**0.01**	**−2.99 (1.40)**	0.16	−1.15 (0.83)	**0.04**	**1.92 (1.01)**	**0.003**	**28.53 (17.84)**
HRT	0.40	−1.57 (1.01)	0.26	1.66 (0.79)	**0.009**	**3.87 (0.98)**	**<0.001**	**46.36 (27.49)**
Post-menopausal	0.17	1.27 (0.96)	0.97	−0.03 (0.70)	0.24	−0.92 (0.80)	0.10	−9.55 (8.97)
Oral Contraceptives	**0.001**	**3.96 (1.52)**	0.21	1.15 (0.96)	0.33	−0.97 (1.00)	**0.02**	**14.39 (12.89)**
Breast feeding	0.65	−0.89 (1.99)	0.09	−2.86 (1.82)	0.63	−0.90 (1.85)	0.98	2.16 (2.50)
Obesity (BMI >30 kg/m^2)	0.32	−1.27 (1.28)	0.45	−0.36 (0.47)	0.76	0.46 (0.52)	0.81	−0.16 (0.36)

RH hormone receptor, *HRT* hormone replacement theapy

Boldface indicates significant correlations between *PGR* variant and specific parameter

(*Pc* = 0.226). This variant (PGR-12) is located in exon 4, and is functional involving V to L substitution in amino acid 660, which influences PGR transcript stability, and thus decrease progesterone responsiveness [22]. It should be noted that the magnitude of the estimated ORs are moderate [OR (95% CI) = 1.52 (1.02–2.27)], thus raising the possibility of chance finding.

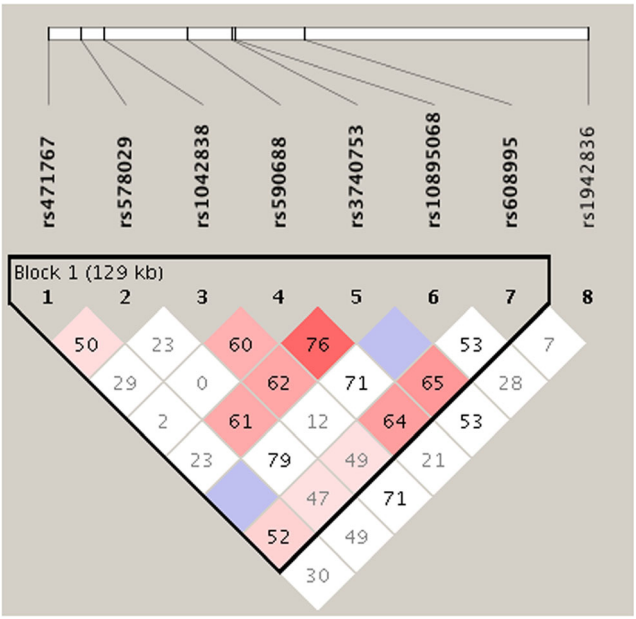

Fig. 1 Linkage disequilibrium (LD) map of *PGR* SNPs genotyped by Haploview. The positions of the SNPs (Build 37.3) are displayed above the Haploview output. The relative LD between any pair of SNPs is indicated by the color scheme, which represents LD relationships, which is based on D' values (normalized linkage disequilibrium measure or D) multiplied by 100; D' is calculated as D divided by the theoretical maximum for the observed allele frequencies. Values approaching zero indicate no LD, and those approaching 100 indicate complete LD. The square colored red represent varying degrees of LD < 1 and LOD (logarithm of odds) > 2 scores; darker shades indicating stronger LD

The association between rs1042838 and breast cancer seen in Tunisians was in agreement with Australian [20], Spanish [21], and UK [17] studies, but in apparent disagreement with US [16, 18, 23–25], Swedish [23], Australian [26], and Europeans [27], and others [23, 28]. These apparent discrepancies may be explained by bias in selection of breast cancer patients [24–26, 29], ethnic background [19, 21, 26, 29], heterogeneous background of cases and controls [18, 23], and in inclusion of control women with positive family history of breast cancer (23, 25, 29]. Given that our study was underpowered to detect OR less than 1.75, future study involving larger number of cases and controls is needed to confirm, or alternatively rule out the association of rs1042838 (V660 L) with breast cancer.

In addition to rs1042838, our study documented association between the intronic rs590688 and the 5'-UTR rs10895068 (+331G > A) *PGR* variants and breast cancer. The rs590688 is a common variant among Caucasians (48.7%) and Africans (48.6%), with markedly lower distribution in Asians (22.5%) (www.ncbi.nlm.nih.gov/projects/SNP/snp). Earlier studies demonstrated association of rs590688 with breast cancer in unselected African-American (*Pc* = 0.03) [16], but not in unselected European-American (*Pc* = 0.62) [16], or Western USA-Hawaii (Multi-ethnic Cohort Study; MEC) women [18]. However, analysis showed that rs608995 was associated with breast cancer in European-American women when subjects were subgrouped according to parity, menarche, combined hormone replacement therapy (CHRT), and obesity [16]. By comparison, rs590688 correlated with Her2 status and CHRT, but not with parity, menarche or obesity in our studied sample.

By interacting with GATA5 transcription factor, the +331A/G (rs10895068) *PGR* variant stimulates the production of the PR-B isoform in mammary cells. This in turn enhances the susceptibility to breast cancer [30]. Several studies

Table 5 Distribution of 7-Locus *PGR* Haplotypes in Cases and Controls

Haplotype [a]	Total [b]	Cases [b]	Controls [b]	X[b]	P	
ATGGCGA	0.263	0.217	0.303	7.58	0.006	0.63 (0.46–0.87)
ATGCCGT	0.112	0.105	0.117	0.29	0.590	
GAGGCGA	0.087	0.070	0.102	2.494	0.114	
GAGCCGA	0.075	0.079	0.071	0.193	0.661	
ATTCGGT	0.072	0.072	0.073	0.003	0.955	
GTGGCGA	0.049	0.041	0.055	0.816	0.366	
ATGGCGT	0.033	0.038	0.030	0.352	0.553	
ATGCCGA	0.03	0.051	0.011	11.217	8.00×10^{-4}	4.68 (1.73–12.65)
AAGGCGA	0.029	0.032	0.027	0.193	0.660	
AAGCCGT	0.028	0.021	0.033	1.05	0.305	
GTGCCGA	0.027	0.046	0.012	8.559	0.003	4.16 (1.52–11.39)

[a] Only haplotypes with frequencies >2.5 were included

[b] Haplotype frequency

examined the association between the functional rs10895068 (+331G > A) *PGR* variant and breast cancer, but with inconclusive result. This *PGR* variant was strongly associated with breast cancer in our studied group, and carriage of the (minor) A allele correlated with parity, RH, Her2 status, CHRT, and previous use of oral contraceptives. This was in agreement with an earlier US study on 1322 breast cancer and 1953 control women, which documented strong association of the +331G > A (rsrs10895068) *PGR* variant with breast cancer, particularly in obese subjects [30]. In contrast, lack of association of rs10895068 with breast cancer was reported for Australian [20], Dutch [22], UK [17], Western USA [24, 25] and Southern USA [18, 31] subjects, and a meta-analysis involving 10 studies comprising 13,702 breast cancer patients and 14,762 controls confirmed lack of association of rs10895068 with breast cancer, irrespective of the genetic model used [32].

While *PGR* rs3740753 variant was associated with breast cancer at the allele level. This was in disagreement with an earlier US (Southern California and Hawaii) study, which reported an association between rs3740753 and ovarian cancer, but not breast cancer [18]. In addition, haploview analysis demonstrated association of ATG**C**CGA and **G**TG***C***CGA haplotypes with breast cancer. The effect of each of these haplotypes on the risk of breast cancer is driven by the presence of rs590688, which is in strong LD with rs1042838 and rs3740753. Our results were in agreement with the result of a US study, which reported on the association of three haplotype blocks, all containing rs590688, with breast cancer risk in African American subjects [18].

In conclusion, our study provides evidence for positive (rs1042838, rs590688 and rs10895068) and negative (rs3740753) association of *PGR* SNPs, as well as ATG**C**CGA and **G**TG***C***CGA haplotypes with breast cancer. Our study has strengths, namely that cases and controls were ethnically matched, hence reducing the problems of differences in genetic background, and that covariates were controlled for in single SNP and haplotype analysis. However, our study has some limitations. The mean age of control group was lower than that of the study group, thereby prompting the speculation that some of the control subjects may eventually be diagnosed with breast cancer in the future. Another limitation relates to the study design (retrospective case-control study), thus prompting the speculation of cause-effect relationship. In addition, the study was based on a limited number of cases and controls, and was limited to Tunisian Arabs, thereby necessitating parallel studies on different ethnic groups. Follow up studies on other *PGR* variants, and populations of related and distant ethnic origin are needed to confirm (or alternatively rule out) the association of *PGR* variants and risk of breast cancer.

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Author Contributions RMG Specimen processing, drafting of manuscript. MAA Genotyping. BHE Genotyping. HHJ Data analysis. SZ Patient selection and referral. HB Patient selection and referral. FH Patient selection and referral. TM Control women selection and referral. WYA Project leader; finalizing analysis and manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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