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Advances in Brief

Progesterone Receptor Gene Polymorphism Is Associated with Decreased Risk for Breast Cancer by Age 50[1]

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Abstract

In a population-based case-control study for breast cancer before the age of 51 years, 554 cases and 559 age-matched controls were genotyped for the polymorphic progesterone receptor allele *PROGINS*. Breast cancer risk was decreased in women carrying the *PROGINS* allele. The odds ratio adjusted for age and study region was 0.76 [95% confidence interval (CI), 0.58–1.00]. Compared with wild-type A1/A1 homozygotes, the odds ratio for A1/A2 heterozygotes and A2/A2 homozygotes was 0.82 (95% CI, 0.62–1.08) and 0.27 (95% CI, 0.10–0.74), respectively, suggesting a gene dosage effect of the A2 allele. There was suggestive evidence for a differential effect by menopausal status ($P = 0.07$) and by family history of breast cancer ($P = 0.15$).

Introduction

The HPR[4] gene is located on chromosome 11q22–23 and belongs to the steroid-thyroid-retinoic acid receptor superfamily of transcription factors (1). HPR exists in two isoforms, HPR-A and HPR-B, transcribed from the HPR gene by alternative initiation. HPR-A represses estrogen receptor gene activation and gene activation by HPR-B. Rowe *et al.* (2) identified a 306-bp insertion of the PV/HS-1 Alu subfamily in intron 7 of the HPR gene. Kieback *et al.* (3) demonstrated that the Alu insertion identifies an allele, named *PROGINS*, which is in linkage disequilibrium with a silent mutation in exon 5 and a mutation in exon 4, causing an amino acid change in the hinge region of the receptor. Four hospital-based case-control studies have been published that analyzed the association between this polymorphism and breast or ovarian cancer risk in patient populations with different ethnic backgrounds without analyses regarding age and menopausal status (4–7). It is unclear whether the mutant allele alters the risk for breast or ovarian cancer. We used a population-based case-control study to determine whether *PROGINS* was associated with breast cancer in a German Caucasian population under the age of 51 years.

Materials and Methods

Study Population. We assessed the relationship between *PROGINS* and breast cancer risk in a population-based genetic epidemiological case-control study in Germany designed to quantify the effect of relevant reproductive and life-style risk factors as well as genetic factors (8). The study was carried out in two geographical areas, covering a population of about 1.3 million in the Rhein-Neckar-Odenwald study region and a population of about 0.9 million in the Freiburg study region. Cases and controls were restricted to women who could speak and read German and who were residents of the study region. Patients had to be <51 years of age when first diagnosed with either *in situ* or invasive primary breast cancer between January 1, 1992 and December 31, 1995 in the Rhein-Neckar-Odenwald region and between January 1, 1993 and December 31, 1995 in the Freiburg region. Complete ascertainment of incident breast cancer patients was carried out at all of the hospitals in the study region (40 hospitals in total), with periodic checks with the pathology institutes serving these hospitals. Patients were either approached in the hospital before first discharge or invited to participate in the study by a letter signed by the attending physician after discharge. Clinical and pathological characteristics of the diagnosed breast cancers were abstracted from hospital records, and pathology reports were requested if possible. After approval for the study was given by all of the communities involved, the population registries of the two study regions provided a sample of 11,000 female residents between 20 and 75 years of age that was generated by systematic sampling. From this list, two controls matched by age and study region were randomly selected for each case and invited by letter to participate in the study. Controls up to 52 years of age at interview were eligible for inclusion. Reasons for nonparticipation were refusal of physicians to allow contact, health problems, nonresponse, and refusal of participation. Overall, 70% of eligible cases and 61% of eligible population controls participated by completing the study questionnaire.

Using a self-administered risk factor questionnaire, detailed information was collected from cases and controls on demographic factors; anthropometric measures; menstrual, reproductive, and breastfeeding histories; use of contraceptives and exogenous hormones; medical and screening histories; family history of cancer; selected occupational exposures; smoking history; and alcohol consumption. In addition, subjects were asked to give a blood sample of 20 ml. All information on case and control exposures was truncated at the date of diagnosis for cases and at the date of completion of the risk factor questionnaire for controls.

For this analysis, we included only cases and controls who were Germans (defined as having at least one parent of German nationality; 91% of cases and 96% of controls) and from whom both questionnaire data and DNA from a blood sample were available by mid-1997. Overall, 3% of the participants refused to provide a blood sample. One control per case (usually the first recruited control, unless a blood sample was not available) was chosen. A total of 577 cases and 579 controls were included in the molecular genetic analysis. Due to the failure of genotyping in 18 cases and 25 controls, the following analysis is based on 559 cases and 554 controls. The mean ages of cases and controls were 42.9 and 42.8 years, respectively.

Genotyping *PROGINS*. Genomic DNA was extracted from the EDTA blood samples using Blood & Cell Culture DNA kits as described by manufacturer (Qiagen GmbH, Hilden, Germany). The analysis of *PROGINS* was based on the PCR amplification of a fragment encompassing the 306-bp insertion polymorphism in intron 7 using the following primers: (*a*) sense primer OL-334, 5′-GCCTCTAAAATGAAAGGCAGAAAGC-3′; and (*b*) an-

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[4] The abbreviations used are: HPR, human progesterone receptor; OR, odds ratio; CI, confidence interval.

tisense primer OL-335, Cy5′-GCGCGTATTTTCTTGCTAAATGTCTG-3′ (Cy5′-labeled). All PCRs were carried out in 25-μl aliquots containing about 30 μg of genomic DNA, 5 pmol of each primer, 1× reaction buffer, 50 μM deoxynucleotide triphosphates, and 0.25 unit of Taq polymerase. The amplification was for 30 cycles, each consisting of 1 min of denaturing at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C. An initial denaturation step of 3 min at 94°C and a final extension at 72°C for 5 min were used. The A1 allele of HPR was defined as the absence of the insertion, as described in a previous publication (2). The PCR products were run directly on a high-resolution Hydrolink gel (AT Biochem, Malvern, PA) and processed by Fragment Manager software in the automated sequencer A.L.F. Express (Pharmacia Uppsala, Sweden). As expected, the A1 allele appeared as a 175-bp fragment, and the A2 allele, *PROGINS*, appeared as a 481-bp fragment.

Statistical Methods. The observed distribution of the *PROGINS* genotypes among women with and without breast cancer was used to calculate the frequency of the A2 allele. The allele frequency of A2 in cases and controls was compared, and the difference was assessed by χ^2 test. Yates' correction was used to identify deviations from the expected Hardy-Weinberg distribution of the genotypes among cases and controls. We assessed the association between the risk of breast cancer and the *PROGINS* genotypes as well as the reproductive factors using a multiple unconditional logistic regression model to obtain maximum likelihood estimates for the OR and 95% CIs. The statistical software package SAS release 6.12 was used (SAS Institute, Cary, NC). First-order interactions between risk factors (age at menarche, parity, breastfeeding, menopausal status, and family history) and the *PROGINS* genotypes were estimated under the standard multiplicative model.

Results

Association of *PROGINS* with Breast Cancer Risk. Overall, the *PROGINS* A2 allele frequency of 0.12 among cases was significantly lower than the frequency of 0.16 among controls ($P = 0.01$; Table 1). The observed allele frequencies did not deviate from the expected Hardy-Weinberg distribution.

In case-control analysis, A2 allele was found to be associated with a decreased OR of 0.76 for breast cancer (Table 2) when the analysis was based on the presence or absence of the A2 allele. The OR decreased with increasing number of A2 alleles when the analysis was performed according to three categories, with a $P < 0.01$ for trend (Table 2). The ORs for the homozygous and heterozygous A2 genotypes were 0.27 (95% CI, 0.10–0.74) and 0.82 (95% CI, 0.62–1.08), respectively, suggesting a dose effect of the A2 allele. The risk estimates did not change after adjusting for age, study region, and reproductive risk factors such as age at menarche, total duration of breastfeeding, menopausal status, and family history.

Table 1 *Genotypes and allele frequencies*

		Genotype				
Group	*N*	1-1	1-2	2-2	A2 allele frequency[a] (95% CI)	χ^2HW[b]
Cases	559	426	128	5	0.12 (0.10–0.14)	0.56
Controls	554	393	144	17	0.16 (0.14–0.18)	0.84

[a] χ^2 test of A2 allele frequencies in cases *versus* controls ($P = 0.01$).
[b] P for χ^2 test of deviation from Hardy-Weinberg proportions ($df = 2$).

Table 2 *Case-control analysis of the PROGINS allele as a risk factor for breast cancer*[a]

	Cases		Controls			
Genotype	*N*	%	*N*	%	OR	95% CI
A1/A1	426	76.2	393	70.9	1.00	
A1/A2	128	22.9	144	26.0	0.82	0.62–1.08
A2/A2	5	0.9	17	3.1	0.27	0.10–0.74
					(trend test: $P < 0.01$)	
A1/A1	426	76.2	393	70.9	1.00	
A1/A2 or A2/A2	133	23.8	161	29.1	0.76	0.58–1.00

[a] Adjustment for age and study region did not change the risk estimates.

Table 3 *Analysis of interaction between the PROGINS allele and established risk factors for breast cancer*[a]

	OR			
Variable	A1/A1	A1/A2	A2/A2	*P* for interaction
Age at menarche (yrs)				
≤12	1.0	0.85	0.72	0.32
≥13	0.98	0.64	0.54	
Parity				
≤2	1.0	0.71	0.50	0.57
≥3	0.65	0.56	0.40	
Duration of breastfeeding (mo)	0.98	0.71	0.48	0.35
Menopausal status				
Premenopausal/unknown[b]	1.0	0.69	0.47	0.07
Postmenopausal	0.65	1.13	0.77	
Family history of breast cancer (first-degree relative)				
No	1.0	0.69	0.48	0.15
Yes	2.22	3.19	2.20	

[a] Adjusted for all variables in the table.
[b] Unknown status includes women <51 years who had a hysterectomy but not a bilateral ovariectomy.

Interaction between *PROGINS* and Other Risk Factors for Breast Cancer. Because it has been suggested that *PROGINS* may affect ligand and hormone binding properties and hence hormonal regulation (3), we explored the effects of interaction between the *PROGINS* genotype and reproductive risk factors for breast cancer. The distribution of cases and controls for the different categories of risk factors considered was as follows: (*a*) age at menarche (12 or less years, 214 cases and 199 controls; 13 or more years, 345 cases and 355 controls); and (*b*) parity (two or less births, 501 cases and 464 controls; three or more births, 58 cases and 89 controls). For the categories such as age at menarche, parity, and duration of breastfeeding (in months), there was a decrease in risk with increasing copies of the A2 allele. There was no interaction between the *PROGINS* genotype and age at menarche ($P = 0.32$), parity ($P = 0.57$), and duration of breastfeeding ($P = 0.35$) on disease risk.

For the analysis of effect modification by menopausal status, women who were under the age of 51 years and who had undergone hysterectomy but not bilateral ovariectomy were considered to be of unknown menopausal status (89 cases and 74 controls). We were left with a small group of 35 cases and 39 controls who were postmenopausal, and 435 cases and 441 controls who were premenopausal. *PROGINS* A2 allele was associated with a reduced risk for premenopausal breast cancer but was not associated with postmenopausal breast cancer, thus giving weak evidence for differential effects of the *PROGINS* genotype on disease risk by menopausal status (Table 3). A statistical test of interaction, however, did not yield a significant result ($P = 0.07$ for interaction).

We also examined the data for effect modification by family history of breast cancer. Seventy cases and 27 controls had a first-degree family member with breast cancer, whereas 489 cases and 527 controls had no family history. The protective effect of the *PROGINS* A2 allele was evident among women without a first-degree family history of breast cancer, but not among women with such a first-degree family history. However, the association with the A2 allele was not significantly modified by a positive family history ($P = 0.15$ for interaction).

Discussion

This is the first population-based age-matched case-control study to our knowledge to examine the possible role of *PROGINS* in the development of breast cancer. We found a reduced risk of breast cancer in women by the age of 50 years who carry at least one A2 allele and a statistically significant trend of decrease in risk with an increase in the number of A2 alleles.

PROGINS has been suggested to show an association with ovarian and breast cancer. An increased frequency of the A2 allele was first reported in a group of 67 patients with ovarian cancer that was pooled from 26 German and 41 Irish patients (6). The observed difference between cases and controls, however, was predominantly due to a low frequency of the *PROGINS* allele among the 101 German control subjects. This association has not been confirmed in other studies (4, 5). Clearly, larger studies with well-characterized study populations are needed to clarify the effect of *PROGINS* on ovarian cancer risk. An association of *PROGINS* with breast cancer was suggested in a study reporting a significant ($P > 0.05$) deviation from Hardy-Weinberg equilibrium in the genotype distribution of 187 Irish breast cancer patients (7). There was a deficit of breast cancer patients homozygous for the A2 allele, which is compatible with our observation of a decreased risk of breast cancer associated with the A2 allele. The association of *PROGINS* with breast cancer was further examined in two studies in North America (68 patients and 101 hospital controls) and in the south of England (292 patients and 220 healthy volunteers; Refs. 4 and 5). The allele frequency of *PROGINS* was slightly lower in the North American Caucasian breast cancer patients compared with the hospital controls, but the difference was not statistically significant (4). No difference between cases and controls was observed in the English study (5).

The previous studies have not used a well-defined patient population and appropriately selected controls. Population controls with similar genetic background are generally preferable when one wishes to detect a possible (statistically significant) deviation of the distribution of allele frequencies among the cases compared to the "expected" distribution and thus provide evidence for an association of the respective genetic variants with the disease of interest. Therefore, it may not be surprising that the previously reported results are inconsistent. Furthermore, no information on cases and controls was given by any of the above studies with respect to age at diagnosis, menopause status, family history, and other variables that could have made an impact on the results. The design of our study reduces many possible biases and issues of comparability of the case and control groups that may arise in other study designs. Our finding of a reduced risk of breast cancer in women with the A2 allele is strengthened by the observation of an allelic dosage effect of the A2 allele. Our data provide suggestive evidence that the protective effect of the polymorphic progesterone receptor allele is confined to premenopausal women.

The *HPR-A* form of the progesterone receptor has been shown to repress estrogen receptor activation and the transcriptional activity of the HPR-B form. The polymorphic *HPR-A PROGINS* allele has been shown to have increased transcriptional activity and increased stability (3). *HPR-A PROGINS* could thereby repress estrogen receptor activation more efficiently and contribute to estrogen-related tumor promotion in the mammary gland of premenopausal women. Ongoing functional studies on *PROGINS* should provide more information on how the polymorphic progesterone receptor modifies the risk for breast cancer on the molecular level.

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References

1. Rousseau Merck, M. F., Misrahi, M., Loosfelt, H., Milgrom, E., and Berger, R. Localization of the human progesterone receptor gene to chromosome 11q22–q23. Hum. Genet., *77:* 280–282, 1987.
2. Rowe, S. M., Coughlan, S. J., McKenna, N. J., Garrett, E., Kieback, D. G., Carney, D. N., and Headon, D. R. Ovarian carcinoma-associated TaqI restriction fragment length polymorphism in intron G of the progesterone receptor gene is due to an Alu sequence insertion. Cancer Res., *55:* 2743–2745, 1995.
3. Kieback, D. G., Tong, X-W., Weigel, N. L., and Agoulnik, I. U. A genetic mutation in the progesterone receptor (PROGINS) leads to an increased risk of non-familial breast and ovarian cancer causing inadequate control of estrogen receptor driven proliferation. J. Soc. Gynecol. Investig., *5:* 40a, 1998.
4. Lancaster, J. M., Berchuck, A., Carney, M. E., Wiseman, R., and Taylor, J. A. Progesterone receptor gene polymorphism and risk for breast and ovarian cancer. Br. J. Cancer, *78:* 277, 1998.
5. Manolitsas, T. P., Englefield, P., Eccles, D. M., and Campbell, I. G. No association of a 306-bp insertion polymorphism in the progesterone receptor gene with ovarian and breast cancer. Br. J. Cancer, *75:* 1398–1399, 1997.
6. McKenna, N. J., Kieback, D. G., Carney, D. N., Fanning, M., McLinden, J., and Headon, D. R. A germline TaqI restriction fragment length polymorphism in the progesterone receptor gene in ovarian carcinoma. Br. J. Cancer, *71:* 451–455, 1995.
7. Garrett, E., Rowe, S. M., Coughlan, S. J., Horan, R., McLinden, J., Carney, D. N., Fanning, M., Kieback, D. G., and Headon, D. R. Mendelian inheritance of a Taq I restriction fragment length polymorphism due to an insertion in the human progesterone receptor gene and its allelic imbalance in breast cancer. Cancer Res. Ther. Control, *4:* 217–222, 1995.
8. Chang-Claude, J., Eby, N., Kiechle, M., Bastert, G., and Becher, H. Breastfeeding and breast cancer risk by age 50 among women in Germany. Cancer Causes Control, in press, 2000.