Progesterone Receptor Promoter *+331A* Polymorphism is Associated with a Reduced Risk of Endometrioid and Clear Cell Ovarian Cancers

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Abstract

Objective: **The progestagenic milieu of pregnancy and oral contraceptive use is protective against epithelial ovarian cancer. A functional single nucleotide polymorphism in the promoter of the progesterone receptor (*+331A*) alters the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. In this study, we sought to determine whether this polymorphism affects ovarian cancer risk.**

Methods: **The *+331G/A* polymorphism was genotyped in a population-based, case-control study from North Carolina that included 942 Caucasian subjects (438 cases, 504 controls) and in a confirmatory group from Australia (535 cases, 298 controls). Logistic regression analysis was used to calculate age-adjusted odds ratios (OR).**

Results: **There was a suggestion of a protective effect of the *+331A* allele (AA or GA) against ovarian cancer in the North Carolina study [OR, 0.72; 95% confidence interval (95% CI), 0.47-1.10]. Examination of genotype frequencies by histologic type revealed that this was due to a decreased risk of endometrioid and clear cell cancers (OR, 0.30; 95% CI, 0.09-0.97). Similarly, in the Australian study, there was a nonsignificant decrease in the risk of ovarian cancer among those with the *+331A* allele (OR, 0.83; 95% CI, 0.51-1.35) that was strongest in the endometrioid/clear cell group (OR, 0.60; 95% CI, 0.24-1.44). In the combined U.S.-Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline), the *+331A* allele was significantly associated with protection against this subset of ovarian cancers (OR, 0.46; 95% CI, 0.23-0.92). Preliminary evidence of a protective effect of the *+331A* allele against endometriosis was also noted in control subjects (OR, 0.19; 95% CI, 0.03-1.38).**

Conclusions: **These findings suggest that the *+331G/A* progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid/clear cell ovarian cancer.** (Cancer Epidemiol Biomarkers Prev 2004;13(12):2141–7)

Introduction

Epidemiologic studies have shown that both pregnancy and use of oral contraceptives dramatically reduce ovarian cancer incidence (1). Reduction in numbers of lifetime ovulations due to pregnancy or oral contraceptive use may decrease risk by reducing gonadotropin levels, oxidative stress, DNA replication errors, and inclusion cyst formation in the ovarian epithelium. In addition, whereas estrogens and androgens have been shown to increase ovarian cancer risk, both pregnancy and oral contraceptive use are characterized by a protective progestagenic hormonal milieu (1, 2). We have previously reported that oral contraceptives with high progestin potency were associated with a greater ovarian cancer risk reduction than those with low progestin potency (3). In addition, we have shown that progestins may reduce ovarian cancer risk by stimulating the apoptosis of genetically damaged ovarian epithelial cells that otherwise might eventually evolve a fully transformed phenotype (4, 5). This may account for the observation that the protective effect of pregnancy and oral contraceptives is far greater than the extent to which lifetime ovulatory cycles are reduced (1).

In view of the protective effect of progestins against ovarian cancer, progesterone receptor variants with altered biological activity may affect ovarian cancer susceptibility. A German group reported that an insertion polymorphism in intron G of the progesterone receptor was associated with a 2.1-fold increased ovarian cancer risk (6, 7). It was subsequently shown that this intronic *Alu*I insertion is in linkage disequilibrium with

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polymorphisms in exons 4 and 5. However, several subsequent studies have failed to confirm an association between these polymorphisms and ovarian cancer risk (8-12). In addition, there is little published evidence that this complex of polymorphisms, termed PROGINS, alters progesterone receptor function.

More recently, sequencing of the progesterone receptor gene has revealed several additional polymorphisms, including one in the promoter region (*+331G/A*; ref. 13). The *+331A* allele creates a unique transcriptional start site that favors the production of progesterone receptor B (PR-B) isoform over progesterone receptor A (PR-A; ref. 13). The PR-A and PR-B isoforms are ligand-dependent members of the nuclear receptor family that are structurally identical, except for an additional 164 amino acids at the NH_2 terminus of PR-B, but their actions are distinct (14, 15). The full-length PR-B functions as a transcriptional activator, and in the tissues where it is expressed, it is a mediator of various responses, including the proliferative response to estrogen or the combination of estrogen and progesterone (16). PR-A is a transcriptionally inactive dominant-negative repressor of steroid hormone transcription activity that is thought to oppose estrogen-induced proliferation. An association has been reported between the *+331A* allele and increased susceptibility to endometrial (13) and breast cancers (17). It was postulated that up-regulation of PR-B in carriers of the *+331A* allele might enhance formation of these cancers due to an increased proliferative response.

We used a case-control study design to explore whether the *+331G/A* polymorphism in the progesterone receptor promoter affects susceptibility to various histologic types of ovarian cancer in North Carolina. A second, independent, case-control study from Australia was examined to confirm associations seen in the North Carolina study.

Materials and Methods

Subjects

North Carolina Ovarian Cancer Study. Primary ovarian cancer cases enrolled in the study were identified through the North Carolina Central Cancer Registry, a statewide, population-based tumor registry, using rapid case ascertainment. Eligibility criteria for ovarian cancer cases include diagnosis since January 1, 1999, ages 20 to 74 years at diagnosis, no prior history of ovarian cancer, and residence in a 48-county area of North Carolina. Physician permission was obtained before an eligible case was contacted. The diagnosis of epithelial ovarian cancer (borderline or invasive) was confirmed by the study pathologist. The response rate among eligible cases was 82%. Nonresponders were classified as patient refusal (6.7%), inability to locate the patient (4.0%), physician refusal (3.5%), death (2.6%), or debilitating illness (1.6%). Population-based controls were identified from the same 48-county region as the cases and were frequency matched to the ovarian cancer cases based on race (Black and non-Black) and age (5-year age categories) using list-assisted random digit dialing. Potential controls were screened for eligibility and were required to have at least one intact ovary and no prior diagnosis of ovarian cancer. Seventy-three percent of controls identified by random digit dialing who passed the eligibility screening agreed to be contacted and were sent additional study information. Among those sent additional study information, the response rate was 68%. The study protocol was approved by the Duke University Medical Center Institutional Review Board and the human subjects committees at the North Carolina Central Cancer Registry and each of the hospitals where cases were identified. Trained nurse interviewers obtained written informed consent from study subjects at the time of the interview, usually in the home of the study subject. A 90-minute questionnaire was given to obtain information on known and suspected ovarian cancer risk factors including family history of cancer in first- and second-degree relatives, menstrual characteristics, pregnancy and breast-feeding history, hormone use, and lifestyle characteristics such as smoking, alcohol consumption, physical activity, and occupational history. A life events calendar, including marriage and education, was used to improve recall. Additionally, anthropometric descriptors (height, weight, waist, and hip circumference) were measured and blood samples (30 mL) were collected. Germ line DNA was extracted using PureGene DNA isolation reagents according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN). Analysis of data from the North Carolina study was limited to Whites. Data from 81 African American controls and 67 cases were excluded because of the low frequency of the polymorphism. Data were collected from 16 non-Black, non-Caucasian cases and 10 controls but were excluded because of the significant racial diversity and small size of this group.

Australian Study. Details of cases and controls included in the Australian study have been described previously (18). Briefly, the case sample consisted of 553 women with primary epithelial ovarian cancer ascertained as incident case subjects as part of a large population-based, case-control study from major gynecologic-oncology treatment centers in New South Wales, Victoria, and Queensland from 1990 to 1993 (n = 363) and from the Royal Brisbane Hospital, Queensland from 1985 to 1996 (n = 190). Histopathologic information regarding tumor behavior (low malignant potential or invasive), histology, stage, and grade was available for all women; information on potential or known ovarian cancer risk factors was ascertained by detailed questionnaire for the subset of cases in the population-based study and included age, ethnicity, country of birth, parity, oral contraceptive use, tubal ligation, hysterectomy, and age at menarche. Limited information ascertained from hospital records was also available for the Royal Brisbane Hospital patients and included age, ethnicity, and country of birth. Because blood samples were not collected from controls who participated in the ovarian cancer case-control study, an additional group of women, selected based on date-of-birth distribution to best match cases, were included in the analyses. The control sample consisted of 300 adult female unrelated monozygotic twins (one per pair), ages 30 to 90 years, recruited through the volunteer Australian Twin Registry for the Semistructured Assessment for the Genetics of Alcoholism study. This study reported participation rates of ~70% for monozygotic female twins and recruited individuals nationally from major cities in the eastern states of Australia. Limited information

ascertained by detailed questionnaires as part of the Semi-structured Assessment for the Genetics of Alcoholism study was available for these women to assess confounding and included age, ethnicity, country of birth, parity, and age at menarche. More than 90% of case and control subject groups were of northern European descent, and all subjects were from major cities in the eastern Australian states. Approvals were obtained from the ethics committees of the University of Melbourne, New South Wales Cancer Council, Anti-Cancer Council of Victoria, and Queensland Institute of Medical Research in Australia. Written informed consent was obtained from each participant. DNA isolation methods have been detailed elsewhere (18). Fourteen Australian cases ages <30 years were excluded from this analysis because no controls were ages <30 years. Additionally, four cases and two controls were excluded because they did not have *+331G/A* polymorphism results. Thus, the Australian sample used for this analysis consisted of 535 cases and 298 controls.

Genotyping of *+331G/A* Polymorphism. Allelic discrimination was done using the MGB primer/probe Taqman assay on the ABI Prism 7700 system. Details of the methods are described in the following sections.

North Carolina Study. Each 20 μL PCR reaction contained 18 pmol of forward primer 5′-CACGAGTTTGATGCCAGAGAAA-3′, 18 pmol of reverse primer 5′-GCGACGGCAATTTAGTGACA-3′, 4 pmol of G-allele probe (VIC)-CGGCTCcTTTATC-(MGBNFQ)-3′, 4 pmol of A-allele probe (FAM)-CGGCTCtTTTATCTC-(MGBNFQ)-3′ (200 nmol/L), 10 μL of 2× Taqman Universal Master Mix without AmpErase UNG (Applied Biosystems, Foster City, CA), and 25 ng of extracted leukocyte DNA. Cycling conditions were 95°C for 10 minutes followed by 40 cycles of 92°C for 15 seconds and 60°C for 60 seconds. Allelic discrimination was done in 96-plate format in the ABI Prism 7700 and analyzed using the ABI Prism 7700 software. Some samples in the North Carolina ovarian cancer study were subjected to sequencing to confirm results obtained using the Taqman assay. To do this, a 50 μL PCR reaction was done using forward primer 5′-AACTCAGCGAGGGACTGAGA-3′ and reverse primer 5′-GAGGACTGGAGACGCAGAGT-3′, 0.5 ng/μL genomic DNA, 0.5 nmol/L forward primer, 0.5 nmol/L reverse primer, 0.2 mmol/L deoxynucleotide triphosphate, 1.5 mmol/L $MgCl_2$ (Applied Biosystems), 1× Applied Biosystems PCR buffer, and 0.025 units/μL AmpliTaq Gold DNA polymerase (Applied Biosystems). PCR conditions consisted of an initial denaturing step at 95°C for 12 minutes, 32 cycles of 94°C for 60 seconds, 55.0°C for 60 seconds, and 72°C for 3 minutes, and an extension step at 72°C for 10 minutes. Samples were held at 4.0°C until they were purified using QIAquick 96 vacuum filter plates (Qiagen, Germantown, MD) and finally eluted in 150 μL of 10 mmol Tris-HCl (pH 8.5). A sequencing reaction was done using 1 μL of purified product and 4.4 pmol of unlabeled forward primer in a BigDye Terminator Cycle Sequencing Reaction as described by the supplier (Applied Biosystems). Samples were analyzed on the ABI 3100 system and sequences determined using GeneScan software (Applied Biosystems).

Australian Study. Genotyping was done with Taqman methodology using identical probes as the North Carolina study. For detection and sequence confirmation of positive controls, a 381-bp product was amplified using the forward primer 5′-GTACGGAGCCAGCAGAAGTC-3′ and reverse primer 5′-ATCCTGTCGTCAGGGGAACT-3′. Denaturing high-performance liquid chromatography (Helix System, Varian Chromatography Systems, Walnut Creek, CA) was used to identify heterozygous GA individuals at 62°C recommended by the MELT program (http://insertion.stanford.edu/melt.html). Genotypes were confirmed by sequencing. Heterozygous GA PCR product was subcloned using the pGEM-T system to obtain G and A clones to use as control standards for the SDS allelic discrimination assay. The 15 μL PCR reaction contained 900 nmol/L of forward primer 5′-GCGACGGCAATTTAGTGACA-3′, 900 nmol/L of reverse primer 5′-TGCACGAGTTTGATGCCAGA-3′ (giving a 68-bp product), 150 nmol/L of A-allele probe, 200 nmol/L of G-allele probe, 1× Platinum Quantitative PCR SuperMix UDG (including passive reference ROX dye, Invitrogen, Melbourne, Victoria, Australia), and 15 ng of genomic or control sample that had been dried in 96-well plates. PCR was done using the ABI 7700 SDS PCR machine for 2 minutes at 50°C and 2 minutes at 95°C followed by 45 two-step cycles of 15 seconds at 92°C and 1 minute at 60°C.

Statistical Analysis. The genotype data were tested for Hardy-Weinberg equilibrium using the χ^2 goodness-of-fit test. Multivariate unconditional logistic regression models, adjusted for age, were used to estimate odds ratio (OR) and 95% confidence interval (95% CI) for the association between polymorphism and epithelial ovarian cancer for all cases as well as for various disease categories. Potential confounders including menopausal status, tubal ligation, oral contraceptive use, body mass index, family history of breast or ovarian cancer in first- and second-degree relatives, and parity were individually adjusted for in the North Carolina data to determine if they changed the crude OR by ≥10%. Analysis stratified by each of these factors was also conducted to assess potential effect modification. We found no evidence of confounding by these factors and therefore felt it appropriate to combine the Australian and North Carolina data despite limited epidemiologic data in the Australian sample. The Breslow-Day χ^2 test was used to assess homogeneity of the results from the two study populations. Analyses involving the combined data set were based on a reanalysis of the raw data and were adjusted for study as well as age. All calculations were done using SAS 8.0 (SAS Institute, Inc., Cary, NC).

Results

The demographic features, epidemiologic risk factors, and pathologic characteristics of cases and controls in the North Carolina (Caucasians only) and Australian studies are shown in Table 1. Of note, the median ages of the cases and controls in both North Carolina and Australian studies are similar. Caucasian women with ovarian cancer in North Carolina were more likely to have used oral contraceptives compared with Australian women with ovarian cancer (67% and 49%, respectively). Invasive ovarian cancer cases comprised 77% of the North Carolina cases compared with 84% of the Australian cases. The *+331G/A* single nucleotide polymorphism in the promoter of the progesterone receptor initially was genotyped in samples from the North

Table 1. Demographics and pathologic characteristics of cases and controls

	North Carolina Study		Australian Study	
	Cases (n = 438), n (%)	Controls (n = 504), n (%)	Cases (n = 535), n (%)	Controls (n = 298), n (%)
Age*				
Median (range)	55 (20-74)	53 (20-75)	59 (30-95)	50 (30-94)
Menopause status				
Premenopausal/perimenopausal	166 (38)	204 (40)		
Postmenopausal	272 (62)	300 (60)		
Parity*				
0	93 (21)	68 (13)	71 (20)	38 (13)
1	73 (17)	72 (14)	51 (15)	20 (7)
2	146 (33)	210 (42)	103 (30)	75 (25)
≥3	126 (29)	154 (31)	123 (35)	160 (55)
Oral contraceptive use*				
Yes	294 (67)	349 (69)	169 (49)	
No	144 (33)	155 (31)	179 (51)	
Tumor behavior				
Borderline	102 (23)		87 (16)	
Invasive	336 (77)		448 (84)	
Tumor stage †				
1	160 (37)		166 (31)	
2	33 (8)		42 (8)	
3	224 (52)		276 (52)	
4	14 (3)		43 (8)	
Tumor histology				
Serous	270 (62)		318 (59)	
Endometrioid	56 (13)		63 (12)	
Mucinous	49 (11)		61 (11)	
Mixed cell	1 (0)		36 (7)	
Clear cell	23 (5)		32 (6)	
Other	39 (9)		25 (5)	

NOTE: Fourteen Australian cases ages <30 years were excluded from the entire analysis because no controls were ages <30 years.
*Parity use not known for 187 Australian cases and 298 Australian controls. Oral contraceptive use not known for 187 Australian cases and 5 Australian controls.
†Stage not known for eight Australian and seven NC cases.

Carolina Ovarian Cancer study using a Taqman assay. In 91 samples in which there was some ambiguity regarding the genotype using the Taqman assay, DNA sequencing was done for confirmation, and in all cases, the original genotypes were confirmed. The *+331A* allele was found in 59 of 504 (11.7%) Caucasian controls and the distribution of genotypes was in Hardy-Weinberg equilibrium (P = 0.53). Among individuals who reported their race to be African American, only 1 of 81 (1.2%) controls and 0 of 67 with ovarian cancer carried the *+331A* allele. In view of the rarity of the *+331A* allele in African Americans, these subjects were excluded from analyses of the association with ovarian cancer risk.

There were very few *+331A* homozygotes and these were combined with GA heterozygotes in calculating crude and age-adjusted ORs (Table 2). In the North

Table 2. Association between *+331G/A* polymorphism and risk of invasive and borderline epithelial ovarian tumors

Genotype	Borderline and invasive cases, n (%)	Controls, n (%)	OR* 95% CI	Invasive cases, n (%)	Controls, n (%)	OR* 95% CI
North Carolina study						
	n = 438	n = 504		n = 336	n = 504	
GG	400 (91.3)	445 (88.3)	1.00 (reference)	307 (91.4)	445 (88.3)	1.00 (reference)
AG	37 (8.4)	58 (11.5)		28 (8.3)	58 (11.5)	
AA	1 (0.2)	1 (0.2)		1 (0.3)	1 (0.2)	
AG/AA	38 (8.7)	59 (11.7)	0.72 (0.47-1.10)	29 (8.6)	59 (11.7)	0.72 (0.45-1.15)
Australian study						
	n = 535	n = 298		n = 448	n = 298	
GG	483 (90.3)	266 (89.3)	1.00 (reference)	407 (90.8)	266 (89.3)	1.00 (reference)
AG	48 (9.0)	30 (10.1)		37 (8.3)	30 (10.1)	
AA	4 (0.7)	2 (0.7)		4 (0.9)	2 (0.7)	
AG/AA	52 (9.7)	32 (10.7)	0.83 (0.51-1.35)	41 (9.2)	32 (10.7)	0.76 (0.46-1.27)

NOTE: Fourteen Australian cases ages <30 years were excluded from the entire analysis because no controls were ages <30 years.
*ORs adjusted for age. For combined data, ORs are adjusted for the study as well.

Carolina sample, there was a suggestion that the *+331A* allele was associated with a modest reduction in risk of both borderline tumors and invasive ovarian cancers (OR, 0.72; 95% CI, 0.47-1.10). Samples from the Australian study were genotyped independently and 10.7% of controls were found to carry the *+331A* allele. The distribution of genotypes in controls was found to be in Hardy-Weinberg equilibrium (P = 0.27). Although not statistically significant, a similar inverse association with invasive ovarian cancer risk was observed (OR, 0.83; 95% CI, 0.51-1.35; Table 2). Excluding the borderline ovarian cancers revealed little change in the point estimates of the association between the *+331A* allele and ovarian cancer for either North Carolina or Australian comparisons (Table 2).

Analyses by histologic subtype for the North Carolina and Australian studies are presented in Table 3. A modest, nonsignificant decreased risk was observed in the North Carolina study among carriers of the *+331A* allele for the common serous histologic type (OR, 0.80; 95% CI, 0.49-1.29), but there was a striking decreased risk of endometrioid cancers (OR, 0.43; 95% CI, 0.13-1.40). Because endometrioid and clear cell ovarian tumors are thought to have a common etiology due to their association with endometriosis (19), these cases were combined to examine the overall association with the *+331A* allele of the progesterone receptor promoter polymorphism (OR, 0.30; 95% CI, 0.09-0.97). No consistent effect was observed between the *+331A* allele and mucinous ovarian cancers. These relationships according to histologic subtype were not modified by age, parity, history of oral contraceptive use, body mass index, or family history of breast/ovarian cancer.

In the Australian data, the protective effect of the *+331A* allele was most pronounced in endometrioid cancers (OR, 0.51; 95% CI, 0.17-1.53). The OR (95% CI) for the combined endometrioid and clear cell group was 0.60 (0.25-1.44). The Breslow-Day χ^2 test was indicative of homogeneity between the North Carolina and the Australian studies with respect to the association between the *+331A* allele and risk of ovarian cancer overall (P = 0.58) as well as endometrioid and clear cell ovarian cancer (P = 0.24). Pooling data from both North Carolina and Australian studies and controlling for study site, the age-adjusted OR (95% CI) for the association between the *+331A* allele and endometrioid/clear cell cancers combined (n = 174; 166 invasive, 8 borderline) was 0.46 (0.23-0.92).

Table 3. Association between progesterone receptor polymorphism and risk of invasive and borderline epithelial ovarian tumors by histologic type and study

	GG	AG	AA	AG/AA (%)	OR* (95% CI)
North Carolina study					
Controls	445	58	1	59 (11.7)	1.00 (reference)
Serous	244	26	0	26 (9.6)	0.81 (0.50-1.32)
Mucinous	44	5	0	5 (10.2)	0.80 (0.30-2.14)
Endometrioid	53	3	0	3 (5.4)	0.43 (0.13-1.40)
Clear cell	23	0	0	0 (0.0)†	
Endometrioid/ clear cell	76	3	0	3 (3.8)	0.30 (0.09-0.97)
Mixed	1	0	0	0 (0.0)†	
Other	35	3	1	4 (10.3)	0.86 (0.29-2.51)
Australian study					
Controls	266	30	2	32 (10.7)	1.00 (reference)
Serous	285	31	2	33 (10.4)	0.89 (0.52-1.52)
Mucinous	55	6	0	6 (9.8)	0.91 (0.36-2.27)
Endometrioid	59	3	1	4 (6.3)	0.51 (0.17-1.53)
Clear cell	29	3	0	3 (9.4)	0.83 (0.24-2.92)
Endometrioid/ clear cell	88	6	1	7 (7.4)	0.60 (0.25-1.44)
Mixed	32	3	1	4 (11.1)	1.01 (0.32-3.17)
Other	23	2	0	2 (8.0)	0.73 (0.15-3.44)

*ORs are according to genotype (AG/GG) compared with the reference group genotype (GG) and are adjusted for age and corresponding study.
†Sample size too small to calculate.

Associations between the *+331A* allele and endometriosis were examined in the North Carolina study because endometriosis is known to increase the risk of endometrioid and clear cell ovarian cancers (19). The rate of self-reported endometriosis was 12.6% in cases and 7.5% in controls, similar to other reports in the literature (19). Endometriosis was associated with an increased risk of ovarian cancer (OR, 1.76; 95% CI, 1.14-2.72). This was mostly attributable to an increased risk of endometrioid/clear cell cases (OR, 3.87; 95% CI, 2.09-7.17; non–endometrioid/clear cell cases OR, 1.36; 95% CI, 0.84-2.20). Preliminary evidence of a protective effect of the *+331A* allele of the progesterone receptor polymorphism against endometriosis was also noted in control subjects (OR, 0.19; 95% CI, 0.03-1.38).

Discussion

Epidemiologic studies have long suggested that heredity plays a role in ovarian cancer predisposition (20). Two high-penetrance ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*, have been identified, defects that increase ovarian cancer risk dramatically (21, 22). It is estimated that up to ~10% of ovarian cancers are attributable to inherited mutations in *BRCA1* and *BRCA2* (22), but <0.5% of individuals in most populations carry these mutations. Although other high-penetrance genes may exist, low-penetrance polymorphisms are likely to contribute to the burden of ovarian cancers classified as sporadic. The PROGINS polymorphism in the progesterone receptor was initially reported to increase ovarian cancer risk (6, 7), but this finding was not confirmed by subsequent studies, including the North Carolina Ovarian Cancer study (8-12). The potential for false-positive results in association studies is now widely accepted, and confirmation in independent populations is now deemed critical prior to concluding that a true association exists (23).

A functional polymorphism in the progesterone receptor promoter (*+331A*) that favors production of PR-B is carried by ~11% of the Caucasian population (13). The group that described this polymorphism has reported associations between the *+331A* allele and increased risks of endometrial cancer (OR, 1.9; 95% CI, 1.10-3.29; ref. 13) and breast cancer (OR, 1.33; 95% CI, 1.01-1.74; ref. 17). The most striking increased risks were observed in obese women (endometrial cancer OR, 4.71; breast cancer OR, 2.30), suggesting an interaction between the polymorphism and the endogenous hormonal milieu. Because there were few rare allele homozygotes, these associations were based on a model in which heterozygotes were pooled with rare allele

homozygotes. It was postulated that the rare allele of this polymorphism may increase endometrial and breast cancer risks by enhancing PR-B-mediated proliferation in response to estrogen.

In the population-based North Carolina Ovarian Cancer Study, risk analyses were confined to Caucasian subjects because of the rarity of *+331A* allele in African American women. Among Caucasian women, we observed a weak protective effect of the *+331A* allele against ovarian cancer (borderline and invasive). Histologic subtype analysis revealed that there was a weak, nonsignificant decrease in risk of serous cancers, which are the most common subtype, whereas a stronger decreased risk for endometrioid cancers was observed. This association became even stronger and statistically significant after combining endometrioid and clear cell cancers, with about a two-thirds reduction in risk (OR, 0.30; 95% CI, 0.09-0.97) in carriers of the *+331A* allele, although the 95% CIs are wide suggesting the instability of the estimate. In view of the potential for false-positive results in association studies of genetic polymorphisms, we sought to confirm our findings in the Australian study. The frequency of the *+331A* allele among Caucasian controls varied by <1% between Australian and North Carolina studies and controls reported in the Nurses' Health Study (13, 17). The Australian study was not a population-based, case-control study and fewer data were available regarding risk factors. Nevertheless, the results of the Australian study were similar to those of the North Carolina study, with a modest overall protective effect that was most pronounced for endometrioid cancers (OR, 0.51; 95% CI, 0.17-1.53). Age was not associated with genotype and adjusting for age had minimal effect on the ORs reported in this article.

Serous and endometrioid/clear cell ovarian cancers share many of the same risk factors, such as parity and oral contraceptive use, but there is evidence to suggest that differences exist in their etiology, molecular pathogenesis, and clinical behavior. For example, there are differences between these histologic subtypes with respect to behavior (borderline versus invasive) and stage that likely reflect etiologic heterogeneity. In addition, mutations in *BRCA1* and *BRCA2* predispose primarily to serous cancers (24), which arise from epithelial cells that line the ovarian surface or underlying inclusion cysts. In contrast, it is thought that some, if not all, endometrioid and clear cell cancers arise from deposits of ovarian endometriosis (19). Coexistent endometriosis is commonly noted in women with ovarian endometrioid/clear cell cancers, and a strong association between endometriosis and these cancers has been reported in epidemiologic studies. Because endometriosis is likely to be underdiagnosed, the relationship between endometriosis and clear cell/endometrioid ovarian cancers may be stronger than noted in case-control studies.

The finding that the *+331A* allele was associated with a decreased risk of endometrioid and clear cell ovarian cancers was somewhat unexpected in view of prior reports of an increased risk of endometrial and breast cancers in carriers of the *+331A* allele (13, 17). However, these three diseases differ with respect to associated risk factors and predisposing hormonal milieu. Endometriosis is associated with endometrioid and clear cell ovarian cancers (19) but does not increase endometrial or breast cancer risk. In contrast, oral contraceptives are protective against all histologic types of epithelial ovarian cancer as well as endometrial cancers (1) but may increase breast cancer risk (25). In view of these significant differences in etiology, it is not surprising that predisposition to these cancers is affected differentially by the progesterone receptor promoter polymorphism.

PR-A and PR-B are both expressed in the ovarian (26), endometrial (27), and breast epithelium (28), and the relative expression of the isoforms is frequently altered during malignant transformation. In the present study, the *+331A* allele of the progesterone receptor promoter polymorphism was protective against endometrioid and clear cell ovarian cancers. We also observed preliminary evidence that this polymorphism may protect against endometriosis, the precursor of many of these cancers. Endometriotic implants have been shown to express only the PR-A isoform (27), and it has been suggested that the absence of PR-B may account for the lack of appropriate cycling of these glands. In normal cycling endometrium, PR-A expression is predominant during the proliferative phase, whereas a shift toward PR-B occurs with differentiation in the early secretory phase (29). Because the *+331A* allele of the progesterone receptor promoter polymorphism favors production of the PR-B isoform, it is possible that this might prevent the PR-A/PR-B imbalance in endometriotic implants and protect against the growth and spread of endometriosis to the extent that it becomes clinically apparent. The reduced risk of endometrioid and clear cell cancers in women with the *+331A* allele might be attributable to a lower likelihood of carriers developing more extensive endometriosis, which serves as a precursor for these cancers. In contrast to the pathogenic model proposed for endometriosis in which the *+331A* allele counters an abnormal imbalance in the PR-A/PR-B ratio in normal breast and endometrial tissues, the polymorphism may create an imbalance that enhances both the proliferative response to estrogen and cancer risk.

The literature is fraught with false-positive association studies of genetic susceptibility polymorphisms, but several features mitigate the likelihood of this in the present study. First, the known protective benefit of progestins against ovarian cancer provides a preexisting biological plausibility for the observed association. In addition, the finding that the *+331A* allele is protective against both endometrioid/clear cell cancers and their precursor lesion (endometriosis) is also supportive. Confirmation of the positive association obtained in the North Carolina study by the Australian study also represents an additional critical validation step. Finally, unlike many polymorphisms that lack known functional significance, the *+331A* allele is known to increase transcription of PR-B *in vitro* (13).

Despite the agreement between North Carolina and Australian data, the 95% CIs of the latter study are relatively wide. Furthermore, the control subjects in the Australian study were not collected in the context of an ovarian cancer study. However, allele frequencies in the Australian controls were similar to those seen in Caucasian controls in the North Carolina study. Another limitation of this study is that the number of cases of the less common histologic types was relatively modest,

limiting the power to draw definitive conclusions. Additional studies are needed to confirm the protective effect of the *+331A* allele against endometrioid and clear cell ovarian cancers.

In summary, the *+331A* allele of the progesterone receptor promoter polymorphism is carried by about one in nine Caucasian women and is associated with a decrease in risk of endometrioid and clear cell ovarian cancers. We also obtained preliminary evidence in support of a protective effect against endometriosis. These findings suggest that the *+331G/A* progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the growth of endometriosis and its subsequent transformation into endometrioid/clear cell cancers. This study provides evidence for the existence of low-penetrance ovarian cancer susceptibility polymorphisms. If multiple polymorphisms are identified that either increase or decrease the risk of various histologic types of ovarian cancer, this might be used in the future for risk stratification that would facilitate screening and prevention strategies.

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