Null Results in Brief

No Association between Progesterone Receptor Gene +331G/A Polymorphism and Endometrial Cancer

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Introduction

The major hypothesis proposed to explain endometrial cancer risk is the unopposed estrogen hypothesis which states that elevated levels of plasma estrogens not counterbalanced by high levels of plasma progesterone increase risk of endometrial cancer (1-3).

The action of progesterone on the endometrium is mediated by the progesterone receptor (hPR) that exists in two different forms (hPR-A and hPR-B), structurally similar except for their NH2 terminus (164 amino acids longer in hPR-B), which are the result of the transcription from two alternative promoters of the hPR gene and are expressed at approximately the same level in the endometrium. The isomorf hPR-B is a transcriptional activator and stimulates epithelial cell growth of endometrium, whereas hPR-A is transcriptionally inactive and inhibits both the estrogen-induced and hPR-B-dependent proliferation of endometrium (4, 5).

Recently, in vitro studies by de Vivo et al. (6) showed that a single nucleotide polymorphism, +331G/A (rs10895068), in the promoter region of the progesterone receptor gene (PGR) increases transcription of the hPR-B form, relative to the hPR-A form.

De Vivo et al. also showed a positive association of this polymorphism with the risk of developing endometrial cancer cases in a case-control study of 187 endometrial cancer cases and 397 matched controls nested within the Nurses’ Health Study cohort (odds ratio, 1.90; 95% confidence interval, 1.10-3.29 for +331A carriers versus noncarriers). Other studies have addressed the relationship of the +331A variant with of cancers of the ovary (7, 8) or breast (9-13) with variable results.

With the aim to confirm de Vivo et al.’s findings, we typed the +331G/A polymorphism in a case-control study of 275 endometrial cancer patients and 314 control subjects.

Materials and Methods

Study Population. Women were recruited from 12 Swedish counties between February 1996 and December 1997 (14). Women were eligible if they were born in Sweden, had no prior hysterectomy, and had no previous history of cancer. Endometrial cancer cases were histopathologically confirmed. Population controls who were resident in the study area were randomly selected from continuously updated population register and frequency matched to cases by 5-year age group.

Blood samples from cases women were drawn at the hospital departments before surgery or any cancer treatment and from controls at a primary health care unit or at home.

The Ethical Committee, Uppsala University and the Ethical Committee, Karolinska Institutet, Stockholm, Sweden approved the study design. Only patients who gave informed consent were included in the study.

Laboratory Analysis. Leukocyte genomic DNA was extracted from whole blood (EDTA) according to standard procedures. Genotyping was done by the 5’ nucle ASE assay (Ta man), using locked nucleic acid chemistry, under conditions described elsewhere (15). Taqman primers and probes were designed and synthesized by Proligo (Paris, France). Their sequences are as follows: PCR primers, GAATGGGCTG-TACCGAG, GGCACTTGAGTGGCTGC; Taqman probes (a plus sign indicates the presence of locked nucleic acid-modified bases), FAM-ACGCGG+C+TCT+CT+T+TATCT, HEX-ACGCG+GC+TCT+TT+T+TATCT.

The order of DNAs from cases and controls was randomized on PCR plates to assure that an equal proportion of cases and controls could be analyzed simultaneously and duplicate genotyping done for 10% of the total series for quality control.

Laboratory personnel was blinded to case-control status of samples. Genotyping call rate was 93.4%, and concordance rate of duplicated samples was 99.8%.

Statistical Analyses. A t test was used to test for differences in mean age and body mass index of cases and controls. Odds ratios were estimated using unconditional logistic regression models. The effect for age was examined by including an additional regression term into the logistic regression models. Association analyses were done under a dominant mode of inheritance effect, in accordance with the previous report of (6); disease risk was compared between subjects carrying at least one copy of the rare allele and those who had

### Table 1. Association of PGR polymorphism +331G/A with endometrial cancer risk: odds ratio (95% confidence interval)

|          | Cases | Controls | Crude | Adjusted for age |
|----------|-------|----------|-------|------------------|
| Noncarriers of +331A allele | 255   | 289      | 1.00  | 1.00             |
| Carriers of +331A allele     | 20    | 25       | 0.91 (0.49-1.67) | 1.04 (0.56-1.93) |
Compared with control subjects, endometrial cancer cases were using hormone replacement therapy. About 47% of the cases and 41% of the controls were nonusers (Table 2). Approximately 9% of the cases and 6% of the controls were never users, and we did not find any interaction with hormone replacement therapy (users versus nonusers) were evaluated by including appropriate interaction term in the model. All statistical analyses were done using the SAS software package, version 9.1 (SAS Institute, Cary, NC).

### Results and Conclusion

Compared with control subjects, endometrial cancer cases were older (median = 66.7 versus 64.0, \( P_{\text{test}} = 0.001 \)) and had a greater body mass index (27.3 versus 25.4, \( P_{\text{test}} = 0.004 \)). Approximately 9% of the cases and 6% of the controls were premenopausal. About 47% of the cases and 41% of the controls were using hormone replacement therapy. Seven percent of the cases and 8% of the controls carried the +331A allele.

Contrary to De Vivo et al., we found no association of +331G/A single nucleotide polymorphism with endometrial cancer risk (odds ratio for carriers versus noncarriers, 0.91; 95% confidence interval, 0.49-1.67; Table 1). Adjustment for age had no effect on these estimates. In the study by de Vivo et al., the estimated risk for carriers with body mass index over 28 kg/m² was particularly elevated compared with lean noncarrier women (odds ratio, 4.71; 95% confidence interval, 1.87-11.87), indicating an interaction between adiposity and +331G/A genotype (\( P = 0.006 \)). Our results, however, did not confirm this finding either (Table 2). We did not observe a stronger association among hormone replacement therapy users compared with never users, and we did not find any interaction with hormone replacement therapy use (Table 2).

This is the first study that attempts to confirm previous findings of De Vivo et al. of an increase risk of endometrial cancer for +331A carriers. Our results do not support an association of the +331A allele with endometrial cancer risk. The present study is of traditional case-control design, whereas that by de Vivo et al. was a case-control study nested within a cohort. Theoretically, a traditional case-control design could increase the chances of control selection bias. However, we believe that such selection bias is very unlikely to occur with respect to carrier status of a gene.

In our study, the +331A allele carrier frequency is a bit lower compared with the frequency reported by De Vivo et al. (8% versus 11%). Because of the small number of +331A homozygotes (one case and three controls), we could not explore a recessive effect of the allele. One strength of the present study is the great homogeneity of the population. One of the eligibility criteria of the study was that women were born and resident in Sweden and >98% of the participants are White Caucasians of Scandinavian origin. Thus, bias from population stratification is very unlikely.

In conclusion, our results do not confirm previous findings of De Vivo et al. of an association between endometrial cancer risk and +331G/A polymorphism.

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