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<p>13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)</p> <p>This project assessed the association between breast cancer risk and polymorphisms of the androgen receptor (AR) and vitamin D receptor (VDR) genes among subjects in a population-based case-control study in Sweden. From this study population, breast cancer cases and control women have been randomly selected for genomic DNA analysis. The collection of blood or tissue specimens has been funded from other sources; this award was for the measurement of the AR and VDR polymorphisms on 300 cases and 300 controls who never used HRT, and a similar number of cases and controls who used HRT for 4 years of more.</p> <p>We obtained blood or tissue samples from 1569 breast cancer cases, and obtained blood samples from 1524 controls. Genotyping was successful for well over 95% of these. Statistical analyses are complete, and a draft manuscript has been prepared. We found no association between breast cancer risk and the polymorphisms investigated.</p>				
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Introduction

Steroid hormones clearly play an important role in breast cancer carcinogenesis, but the role of androgens is unclear. In vitro studies suggest that androgens inhibit breast cell growth (1), but human studies have reported higher circulating androgen levels in breast cancer cases than in controls (2). Androgens exert their effect through a ligand-activated nuclear receptor, the androgen receptor (AR), which is genetically polymorphic with a trinucleotide (CAG) repeat in exon 1 of the gene. This is a functional polymorphism: short repeats confer more efficient transactivation than longer repeats(3,4). Thus the short CAG repeat has been associated with an increased risk of prostate cancer (5,6), and the long repeat with male infertility (7,8). Investigations of the relation of the polymorphisms to breast cancer risk have generally shown short repeats to be associated with a decreased (9,10) or unaltered risk (11,12) but these alleles seem to be associated with decreased survival (13).

1 α ,25-dihydroxyvitamin D (Vitamin D), is another steroid hormone involved in cell growth and differentiation (14,15). Vitamin D exerts its action through the vitamin D receptor (VDR). There are several polymorphisms in the 3' untranslated region of VDR (all in linkage disequilibrium) that are of unclear functional significance (16,17) but that nevertheless have been associated with prostate cancer (18-20), and with osteoporosis (21,22). Some have also shown associations between polymorphisms in this region and breast cancer risk (23-25), while others have shown no association (11,26,27).

To clarify the effects of androgen and vitamin D signaling on breast cancer risk, we studied the AR CAG microsatellite and a poly-A microsatellite in the 3' UTR of VDR in relation to breast cancer in a large case-control study in a relatively genetically homogenous population. A total of 3879 cases and 3527 controls took part in the questionnaire phase of the study, providing data regarding use of exogenous hormones and other life style factors. From this study population, breast cancer cases and control women were randomly selected for genomic DNA analysis. The collection of blood or tissue specimens for DNA was funded from other sources; this award was for the measurement of the AR and VDR on 300 cases and 300 controls who never used HRT, and 300 cases and 300 controls who used HRT for 4 years of more.

Body of Progress Report

Under previous funding from other sources, all questionnaire data have been obtained and organized at the time the work supported by this grant began.

Administrative and Informatics Preliminaries:

The grantee organization, Dartmouth College, maintained a subcontract with the Karolinska Institutet for the collaborative work. In turn, the Karolinska Institutet had a relationship with investigators at Uppsala University in Uppsala, Sweden, where the molecular analysis were conducted. To facilitate the research, a tracking database for subject recruitment and specimen accrual was built.

Recontacting study subjects to obtain germline DNA for analyses:

This work was funded by other awards from the National Institutes of Health and from the Army Medical Research and Materiel Command Breast Cancer Research Program. We attempted to obtain blood or tissue sample from 1801 breast cancer patients, selected from the 3879 cases initially enrolled in the study. We have been successful in obtaining a source of DNA for 87.1% (1569) of the selected cases. 1322 (73.4% of those sampled) donated a blood sample, and we were able to access a tissue sample from an additional 247 cases (13.7%) who had died or declined to donate a blood sample. Eighty patients (4.4%) refused to participate in this phase of the study, and we were unable to obtain samples from 152 others due to other reasons (see table 1 below).

We attempted to obtain blood samples from 1712 control women (without previous breast cancer), selected from the 3527 control women initially enrolled in the study. (Since these subjects had not had breast cancer, there was no ready tissue source of DNA for them.) We have obtained blood samples from 1272 control women (74.3%). 349 control subjects (20.4%) declined to participate, and 91 (5.3%) had died before we could contact them. Taking advantage of an on-going endometrial cancer study in the same area, we were able to recruit an additional 252 controls as well.

Table 1. Participation frequencies among breast cancer cases and controls.

	Number selected	Donated Blood (%)	Allowed use of Tissue %	Refused (%)	No specimen for other reason ¹ (%)	Total participation, %
Cases	1801	1322 (73.4)	247 (13.7)	80 (4.4)	152 (8.4)	87.1
Controls	1712	1272 (74.3)	--	349 (20.4)	91 (5.3)	74.2

¹ Other reasons for non-participation (cases): Declined blood draw and died before contact regarding use of tissue, blood sample lost, tissue sample could not be obtained, and subject could not be traced. Other reasons for non-participation (controls): Subject died before contact.

Laboratory analysis

This Army award supported the analysis of a subgroup of these subjects (600 cases and 600 controls); analysis for the others has been supported by other sources (NIH and another USAMRMC award).

After being entered into the administrative tracking system, all blood/tissue specimens were sent to the laboratory in Uppsala, Sweden. DNA was extracted from virtually all the blood samples, and from the overwhelming majority of tissue samples. The table below details our high success rate in extracting and amplifying the DNA from the specimens.

Table 2. Frequency of successful sample genotyping

	Cases	Controls
Androgen receptor	98.3%	99.1%
Vitamin D receptor	96.3 %	99.1%

Analysis and Reporting

Statistical analysis is complete, and a draft manuscript has been prepared (see Appendix). The main findings are summarized in the table below. There was no substantial association between breast cancer risk and any of the polymorphisms studied.

Table 3. Overall estimates of odds ratios and confidence intervals

	Homozygous long			Genotype ¹ Heterozygous			Homozygous short		
	Cases/controls	OR ²	CI	Cases/controls	OR ²	CI	Cases/control s	OR ²	CI
Androgen receptor									
All cancers	438/435	1	Ref	718/729	1.0	0.8-1.1	386/347	1.1	0.9-1.3
Ductal	324/435	1	Ref	534/729	1.0	0.8-1.2	280/347	1.1	0.9-1.3
Lobular	61/435	1	Ref	82/729	0.8	0.6-1.2	39/347	0.8	0.5-1.2
Vitamin D receptor									
All Cancers	573/558	1	Ref	677/687	1.0	0.8-1.1	261/266	1.0	0.8-1.2
Ductal	424/558	1	Ref	500/687	1.0	0.8-1.1	191/266	0.9	0.8-1.2
Lobular	66/558	1	Ref	84/687	1.0	0.7-1.4	30/266	1.0	0.6-1.6

¹The AR alleles are: L – 22 repeats or longer, S – Less than 22 repeats. The VDR alleles are: L – 19 repeats or longer, S – Less than 19 repeats.

²The logistic regression model contained only genotype. Long-term users of menopausal hormone users and women with diabetes mellitus were over-sampled, thus the logistic regression models were conditional on age group and sampling scheme.

We anticipate completing the relevant manuscripts by April 1, 2003.

Training

The project has played a role in the training of two investigators. The data generated will be part of the Ph.D. thesis of Ms. Sara Wedren, and the work is also part of the activities for the post-doctoral fellowship of Dr. Elisabete Weiderpass.

Key Research Accomplishments

- completion of organizational prerequisites
- construction of a detailed administrative database
- successful recruitment of subjects to the molecular epidemiology study
- extraction and amplification of DNA from the specimens
- completion of laboratory analyses
- completion of statistical analyses of the epidemiological and laboratory data
- preparation of a draft manuscript

Reportable Outcomes

a) Manuscripts, abstracts, presentations:

The post-doctoral student working in the project (Elisabete Weiderpass, Karolinska Institutet, Stockholm) presented two posters describing the project at the 'Era of Hope' conference (Atlanta, Georgia, June, 2000).

The investigators have prepared a draft manuscript summarizing the association between breast cancer risk and polymorphisms in the androgen and vitamin D receptors

b) Patents and licenses applied for and/or issue

None

c) Degrees obtained that are supported by this award:

The Ph.D. thesis work of a graduate student in Cancer Epidemiology, Ms. Sara Wedren (Karolinska Institutet, Stockholm, Sweden), is partially drawn from this project. She is expected to defend her thesis, thus obtaining her Ph.D. degree, during the current academic year.

d) Development of cell lines, tissue or serum repositories:

A biological bank containing DNA samples from breast cancer patients and control women has been created and will be maintained using financial resources obtained elsewhere. The creation and maintenance of the biological bank follows the Swedish law for storage of biological samples for scientific proposals.

e) Informatics such as databases and animal models, etc:

- An administrative database containing information about study subjects has been created at the Karolinska Institutet;
- A database containing questionnaire information from breast cancer case patients and control women was created at the Karolinska Institutet, and the questionnaire information checked and corrected for typing errors and logical inconsistencies.
- Programs (using SAS programs) allowing calculation of time of use of different sorts of postmenopausal hormones were developed and tested at the Karolinska Institutet.
- Databases containing results from the laboratory analyses were created in Uppsala, Sweden, where the laboratory analyses were performed. These files were transferred to the Karolinska Institutet at the completion of the laboratory analysis.

f) Funding applied for based on work supported by this award:

A post-doctoral fellowship in Cancer Epidemiology was granted from the Swedish Cancer Society to Dr. Elisabete Weiderpass (Karolinska Institutet, Stockholm, Sweden) to work in this project. This fellowship partially supported her salary from January to December 2000.

g) Employment or research opportunities applied for and/or received based on experience/training supported by this award:

The graduate student working on the project, Ms. Sara Wedren, obtained a training fellowship from the Karolinska Hospital, to complete her medical training. She qualified for this fellowship program in part because of her experience in this project.

The post-doctoral fellow working on the project, Dr. Elisabete Weiderpass, was appointed Docent in Cancer Epidemiology at the Karolinska Institutet, Sweden, in September 2000. She qualified for this position in part because of the experience she acquired working in the project.

Conclusions

The project aims have been successfully completed. We found no association between breast cancer risk and polymorphisms of the androgen or vitamin D receptors.

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Appendix: Manuscript describing principal study findings:

Associations Between Androgen and Vitamin D Receptor Microsatellites and Postmenopausal Breast Cancer

Sara Wedrén, Cecilia Magnusson, Håkan Melhus, Andreas Kindmark, Fredrik Stiger, Maria Branting, Ingemar Persson, John Baron, Elisabete Weiderpass

Associations Between Androgen and Vitamin D Receptor Microsatellites and Postmenopausal Breast Cancer

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Abstract

We investigated the association between polymorphism in the androgen receptor gene and the vitamin D receptor gene and breast cancer risk in a large population-based case-control study performed in the genetically relatively homogenous Swedish population. We determined both androgen receptor (*AR*) and vitamin D receptor (*VDR*) genotype in 1,502 women with invasive breast cancer and in 1,510 control women and calculated odds ratios (OR) and 95 percent confidence intervals (CI) from logistic regression models. There were no associations between *AR* or *VDR* microsatellite lengths and breast cancer when we used defined "long" and "short" alleles using *a priori* criteria. However, there was statistically significant interaction between *VDR* genotype and parity in their association with breast cancer, such that women with two short alleles had a decreased risk for breast cancer that was not further influenced by increasing parity. In contrast, homozygosity for the long allele was associated to a more advanced clinical stage at diagnosis. In exploratory overall analyses where cut-offs between long and short alleles were determined *a posteriori*, we found that women with two <20 *AR* CAG repeats had an increased risk for breast cancer, OR 1.67 (CI 1.17-2.38) compared to those with two alleles ≥ 20 repeats long. Those with two *VDR* alleles with <21 poly-A were also at an increased risk, OR 1.26 (CI 1.04-1.51). Our data do not support major roles for *AR* or *VDR* polymorphism as breast cancer risk factors. However, we did find an interaction between *VDR* genotype and parity that remains to be corroborated and biologically explicated. (Word count: 250)

Introduction

The role of androgen stimulation in breast carcinogenesis is unclear. While *in vitro* experiments indicate that androgens inhibit breast cell growth (Orthman, 2002; Ferro, 2002), human studies have reported higher circulating androgen levels in breast cancer cases than in controls (The Endogenous Hormones and Breast Cancer Collaborative Group, 2002). Androgens exert their effect through a ligand-activated nuclear receptor, the androgen receptor (AR), which is genetically polymorphic, with a trinucleotide (CAG) repeat length variant in exon 1 of the gene. This polymorphism affects the function of the receptor such that short repeats confer more efficient transactivation than longer repeats (Knoke, 1999; Beilin, 2000; Irvine, 2000). In line with these findings, the short CAG repeat has been associated with an increased risk of prostate cancer (Mononen, 2002; Hsing, 2002; Ekman, 1999), and the long repeat with male infertility (Eckardstein, 2002; Kukuvtis, 2002; Dowsing, 1999). Investigations of the relation of the polymorphisms to breast cancer risk have generally shown short repeats to be associated with a decreased (Guiguère, 2001; Haiman, 2002) or unaltered risk (Dunning, 1999; Spurdle, 1999) but these alleles seem to be associated with decreased survival (Yu, 2000; Elhaji, 2001).

1 α ,25-dihydroxyvitamin D (Vitamin D), is another steroid hormone involved in cell growth and differentiation (Escaleira, 1993; Zhao, 2000). Vitamin D exerts its action via a specific receptor, the vitamin D receptor (VDR). There are several polymorphisms in the 3' untranslated region of VDR (all in linkage disequilibrium) that are of unclear functional significance (Yamagata, 1999; Durrin, 1999; Morrison, 1994) but that nevertheless have been associated with prostate cancer (Ingles, 1997; Correa-Cerro, 1999), and to osteoporosis (Morrison, 1994; Feskanish, 1996; Sainz, 1997; Giguère, 2000). Some have also shown associations between polymorphisms in this region and breast cancer risk (Hou, 2002; Bretherton-Watt D, 2001; Ingles, 2000; Curran, 1999), while others have shown no association (Newcomb, 2002; Dunning, 1999; Lundin, 1999; Ruggiero, 1998).

To clarify the effects of androgen and vitamin D signaling on breast cancer risk, we studied the AR CAG microsatellite and a poly-A microsatellite in the 3' UTR of VDR in relation to breast cancer in a large case-control study in a relatively genetically homogenous population.

Subjects and Methods

Parent study

We conducted a nation-wide population-based case-control study of all incident cases of primary breast cancer among women 50 and 74 years of age resident in Sweden between October 1993 and March 1995 (Magnusson et al. 1999). Breast cancer patients were identified at diagnosis through a notification system organized within the six Swedish regional cancer registries, to which reporting of all malignant tumors is mandatory. Control women were randomly selected from the Swedish Population Registry, containing updated information on current address, date of death, emigration and national registration number (NRN, uniquely identifying each resident) for the entire Swedish population. Control women were chosen to match the expected age distribution among the cases in 5-year age groups.

Cases were approached by their respective physicians and asked to participate in the study. After patient consent, the study secretariat was notified of the potential subject's identity and address, and a questionnaire was mailed, asking for detailed information regarding intake of menopausal hormones and oral contraceptives, weight, height, reproductive history, medical history, and other lifestyle factors. Controls were directly contacted with the questionnaire. Approximately 84 percent of eligible cases (n=3,345) and 82 percent of controls (n=2,999) subsequently returned a questionnaire. Another 455 controls who failed to return the questionnaire were interviewed by phone. Further, around 50 percent of the cases and controls were contacted by phone to complete information missing in their mailed responses. Findings from the study have been presented in several publications (Magnusson et al. 1998; Magnusson et al. 1998; Magnusson et al. 1999; Magnusson et al. 1999; Moradi et al. 2000; Terry et al. 2001). Information regarding tumor type, size, stage and receptor status has been collected from medical records in an ongoing follow-up study of all cases.

Molecular study

We randomly selected 1,500 women with invasive breast cancer and 1,500 controls (frequency-matched by age) among postmenopausal participants without any previous malignancy (except in-situ cervix carcinoma or non-melanoma skin cancer) in the parent study. In order to increase statistical power in subgroup analyses, we also selected all remaining eligible women who had taken menopausal hormone treatment (either medium potency estrogen treatment only or medium potency estrogen in combination with progestin) for at least 4 years (191 cases and 108 controls) and all women with self-reported diabetes mellitus (104 cases and 110 controls). In total, 1,801 cases and 1,712 controls were selected. Some controls from the parent study that were not selected for this analyses donated blood for a parallel study regarding endometrial cancer. As these women originated from the same source population and fulfilled all inclusion criteria, 252 of these controls were included in the analysis presented here.

Collection of biological samples

We contacted all selected living women by mail to request cooperation with the molecular study; those who agreed to participate and gave informed consent received a blood sampling kit by mail. Whole blood samples were drawn at a primary health care facility close to the woman's home and sent to us by standard mail. A majority of the samples arrived at our department within 1 day of blood draw. All blood samples were immediately stored at -20° C. Breast cancer cases who declined to donate a blood sample were asked to permit to our use of archived paraffin embedded tissue samples taken at breast cancer surgery. We also attempted to retrieve archived tissue samples from all deceased breast cancer cases. Samples were coded upon arrival and transferred to the laboratories without any information about the donor. We obtained blood samples or archived tissue samples for 1,322 and 247 breast cancer patients, respectively, and blood samples from 1,272 control women, yielding participation rates of 87 percent for the selected cases and 74 percent for controls.

DNA extraction

We isolated DNA from 3 ml whole blood using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. From non-

malignant cells in paraffin embedded tissue, we extracted DNA using a standard phenol/chloroform/isoamylalcohol protocol (Isola et al. 1994).

Genetic analyses

We amplified fragments corresponding to the CAG-repeat in the AR gene and the poly-A in the VDR gene by polymerase chain reaction (PCR) using the following primers: 5' – AGA GGC CGC GAG CGC AGC ACC TC – 3' (AR, forward), 5' – GCT GTG AAG GTT GCT GTT CCT CAT – 3' (AR, reverse), 5' – GTG TAG TGA AAA GGA CAC CGG A – 3' (VDR, forward), 5' – GAC AGA GGA GGG CGT GAC TC – 3' (VDR, reverse). A “touch-down”-PCR reaction was used, in which both reactions were simultaneously run in an ABI Prism 877 Integrated Thermal Cycler robot (PE Applied Biosystems). We used AmpliTaq Gold® kits and standard reagents (Applied Biosystems, Foster City, CA, USA). The amplification profile consisted of denaturation at 95° for 10 minutes, followed by 36 cycles of denaturation at 96° for 30 seconds, annealing at 59° to 57° for 40 seconds and elongation at 72° for 60 seconds, and final extension at 72°. The annealing temperature was 59° in the first three cycles, 58° in the following 12, and 57° in the last 21 cycles. We set up separate PCR reactions for samples that could not be amplified in the touch-down reaction. These reactions were performed on a Gene Amp PCR system 9700 (Perkin Elmer Co, Norwalk, CT, USA) programmed for denaturation at 96° for 10 minutes, followed by 36 cycles of denaturation at 96° for 30 seconds, annealing at 55° or 56° for 40 seconds, elongation at 72° for 60 seconds, and final extension at 72° for 7 minutes. The amplification products were read on a Genescan run ABI 377 DNA gel-slab electrophoresis sequencer (Perkin Elmer Co, Norwalk, CT, USA) with a TAMRA-labelled internal length standard (Genescan-500 TAMRA, Applied Biosystems,). We used Genotyper software to determine the genotypes (Genotyper vers 2.0, Perkin Elmer Corporation).

Genotyping results

We were able to successfully genotype 1 542 breast cancer cases for the AR microsatellite and 1 511 cases for the VDR microsatellite and 1 260 controls for both polymorphisms. With 251 additional genotyped controls from the parallel endometrial cancer study, the total number of controls included was 1 511.

Statistical analyses

We determined whether AR and VDR genotype frequencies were in Hardy-Weinberg equilibrium using standard χ^2 -statistics. Based on a priori decisions, we dichotomized AR CAG at the median repeat length among controls (22 repeats) and dichotomized the VDR poly-A repeats between the two peaks of the bimodal distribution of repeat lengths among controls (18 repeats). Since there is no clear evidence that there are natural cutoffs in the microsatellites with regard to function, we also used a posteriori determined cutoffs in a secondary exploratory analysis. We related the association between genotype and known or suspected risk factors for breast cancer among controls by χ^2 -statistics. We calculated odds ratios (OR) and 95 percent confidence intervals (CI) from conditional logistic regression models using maximum likelihood methods. Models were conditioned on age group and on sampling category to ensure that age-associated non-participation or over-sampling of long-term menopausal hormone users and women with diabetes mellitus did not introduce bias. We introduced known or suspected breast cancer risk factor co-variates into the model to detect any change in the genotype risk estimate as a sign of

confounding and/or presence in the causal pathway. The following variables were considered: age at menarche (continuous), age at first full-term birth (continuous), parity (continuous or 0/1/2/>2), use of combined oral contraceptives (ever/never), smoking (ever more than one year/never or less than one year), alcohol consumption (continuous), age at menopause (continuous), body mass index (BMI, continuous), height (continuous), weight gain during adult life (continuous or -25 to 0 kgs/0.5 to 11.5 kgs/ 12 to 110 kgs), family history of breast cancer (yes/no), history of benign breast disease (yes/no), use of menopausal hormone treatment (<1 year/1-4 years/>4 years), and intake of brassica vegetables (continuous or <1/1-3/>3 times per week) and fatty fish (servings week, continuous). Co-variables with independent associations with breast cancer risk after adjustment for the other co-variables were kept in the model unless they changed the genotype parameter estimates appreciably.

We investigated interactions between *AR* or *VDR* genotype and duration of menopausal hormone use, BMI, weight gain, parity, diabetes mellitus, and family history of breast cancer by performing separate analyses over strata of these exposures. Formal tests for interaction were performed by comparing models containing interaction terms with models containing only main effects, using likelihood ratio tests. We did not adjust our analyses for multiple comparisons.

We performed all analyses using SAS system PHREG procedure (Release 8.02, SAS institute Inc. Cary, NC, USA).

Results

Selected characteristics of the breast cancer cases and controls (Table 1) largely followed expected patterns of known epidemiological breast cancer risk factors. On average, cases had fewer children, were older at first birth, heavier, and more often had a family history of breast cancer. We found 30 alleles of the *AR* microsatellite that were (range 6-43 CAG repeats) approximately normally distributed (Fig 1). In the *VDR* microsatellite locus, we found 21 alleles that were bimodally distributed (Fig 2) (range 7-34). Among controls, there were no associations between *AR* genotype and other known or suspected breast cancer risk factors. *VDR* genotype, however, was associated with age at first full-term birth ($p=0.02$) and somatotypes at ages 7 and 18 ($p=0.07$ and $p=0.05$, respectively). The *VDR* SS genotype was more common among women who had their first child above the age of 30 and the *VDR* LL genotype was more common among women who had a lean body build during childhood and adolescence (data not shown).

There was no difference in the mean repeat length between cases and controls for the *AR* ($p=0.22$) or the *VDR* microsatellite ($p=0.96$) and no association between mean repeat length and age at breast cancer diagnosis (data not shown). When modeled with logistic regression, neither *AR* nor *VDR* genotype had any significant influence on breast cancer risk overall (Table 2). The same was true when breast cancers were subclassified into ductal and lobular types. In subgroup analyses, we found only one statistically significant interaction, namely one between *VDR* genotype and parity. It appeared as if all those with *VDR* SS genotype had a decreased risk for breast cancer compared to nulliparous women with LL genotype and that no additional protection was afforded by increasing parity (Table 3). There was no indication for an interaction between *AR* and *VDR* genotypes on breast cancer risk (Table 4).

In secondary exploratory analyses, we grouped alleles to maximize the contrasts between cases and controls (Table 5). With these cut-offs between long and short groups of alleles, carrying

two short (<20) AR CAG repeats or two short (<21) VDR poly-A was associated with increased breast cancer risks overall, OR 1.67 (CI 1.17-2.38) and OR 1.26 (1.04-1.51), respectively. While the overall association was noticeable also in subgroup analyses, no indications of interaction emerged using the *a posteriori* cut-offs and the VDR-parity interaction was weakened (data not shown).

There was no association between AR genotype and histological type, tumor size, or clinical stage at diagnosis (data not shown). AR genotype and estrogen receptor status in the tumor were not associated among the 65% cases for whom this information was available. VDR genotype was associated with stage at diagnosis ($p=0.05$). Subjects who were homozygous for the short allele were over-represented among stage 1 tumors and those who were homozygous for the long allele were over-represented in stage 4 cancers. VDR genotype was not associated with any of the other clinical characteristics (data not shown).

Discussion

We show that AR and VDR repeat polymorphism has very little influence on the risk for postmenopausal breast cancer.

Our study was large and it was performed in a relatively genetically homogenous population. The latter limits the potential for confounding due to varying genotype frequencies in populations with different base-line breast cancer risks, i.e. population stratification. It was population based and there are no convincing reasons to believe that differential participation associated to genotype would operate to cause selection bias. We had extensive information about other breast cancer risk factors, which enabled us to evaluate effect modification and confounding. The genotyping methods that we used are well established and widely trusted. The laboratory personnel were blinded to case-control status and could thus not have scored genotypes systematically with regard to genotype.

The role of androgens in the development of breast cancer is disputed. While *in vitro* experiments show that androgen stimulation inhibits the stimulatory effect of estrogens in breast epithelium (ref), epidemiological studies indicate that high circulating levels of androgens confer an increased breast cancer risk (The Endogenous Hormones and Breast Cancer Collaborative Group, JNCI, 2002). Androgens are precursors for estrogens, which may explain the latter finding. However, treatment with menopausal hormone preparations that contain androgen derived gestagens in combination with medium potency estrogens confer a higher breast cancer risk compared to preparations that contain only estrogen or those with progesterone derived gestagens (ref).

Several studies have established that long CAG repeats causes reduced AR transactivation (Knoke, 1999; Irvine, 2000). Consistent with this pattern, there is also fairly consistent evidence for an increased risk for prostate cancer with short CAG-repeats, i.e. high enhanced androgen signaling, and an increased risk for male infertility with long repeats, i.e. attenuated androgen signaling. Since AR is located on the X chromosome, men have only one copy of the AR gene. Women on the other hand have two copies of the gene and one of them is inactivated, most likely in a random fashion, at least with regard to CAG length (own unpublished observations). Unmeasured X inactivation status may be one explanation to the divergent results regarding the

influence of AR CAG repeat length on diseases and conditions in women. In a recent investigation, short CAG repeats were associated with an earlier age at menarche (Comings, 2002) while Westberg and colleagues found that short repeats were associated with higher androgen levels in women (Westberg, 2001). There are no established mechanistic models for how androgens would influence age at menarche, but in light of the increased risk for breast cancer with high circulating androgen levels (The Endogenous Hormones and Breast Cancer Collaborative Group, JNCI, 2002) these two studies are supported by our present results.

In contrast to our findings, some previous studies have shown long CAG to be associated to increased sporadic breast cancer risk (Haiman, 2002; Elhaji, 2001; Giguère, 2001) or increased risk among BRCA1 mutation carriers (Rebeck, 1999). Other studies reported no association (Spurdle, 1999; Dunning, 1999), or no modifying effect on BRCA1/2 mutation penetrance (Kadouri, 2001) or on age at presentation (Given, 2000; Menin, 2001). However, there is one report that breast cancers in women with shorter CAG are of higher grade and confer a shorter survival (Yu, 2000).

Vitamin D is involved in cell growth and differentiation (Escalera, 1993; Zhao, 2000) and appears to have antiproliferative effects (Colston, 2002). Although VDR is expressed in normal as well as malignant breast tissue (Friedrich, 2002) the functional significance of genetic variants of the receptor is unresolved (Yamagata, 1999; Durrin, 1999). Previous studies of the receptor gene variants and breast cancer risk have been conflicting, certain showing association (Hou, 2002; Bretherton-Watt D, 2001; Ingles, 2000; Curran, 1999), and others showing no association (Newcomb, 2002; Dunning, 1999; Lundin, 1999; Ruggiero, 1998). The ambiguous state of knowledge in conjunction with our present results point to that VDR polymorphism has no overall influence on the risk for breast cancer. The interaction between VDR genotype and parity present in our data has not previously been described. Bearing in mind the number of comparisons in this study, it may well represent a chance finding. On the other hand, both parity and vitamin D are assumed to influence the breast in a pro-differentiating manner and thus there is a basis for further hypotheses regarding their interaction.

Our data do not support major roles for AR or VDR polymorphism as breast cancer risk factors. However, we did find an interaction between VDR genotype and parity that remains to be corroborated and biologically explicated.

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Table 1 Selected characteristics for breast cancer cases and controls successfully genotyped for AR and VDR microsatellites.

	Cases/controls ¹	Cases	Controls
		MEANS (SD)	
Age (years)	1502/1510	63.3 (6.5)	63.2 (6.4)
Age at menarche (years)	1364/1382	13.5 (1.4)	13.5 (1.4)
Age at menopause (years)	1492/1497	50.4 (3.5)	50.1 (4.0)
Parity	1502/1510	1.8 (1.2)	2.2 (1.3)
Age at first full term birth (years)	1278/1364	25.4 (4.9)	24.8 (4.7)
Weight gain during adult life (kg)	1236/1189	13.3 (11.1)	11.8 (11.2)
Body mass index (kg/m ²)	1493/1489	25.8 (4.1)	25.5 (4.2)
Height (cm)	1497/1500	164.3 (5.8)	163.7 (5.5)
Alcohol intake (g/week)	1342/1268	2.5 (4.3)	2.3 (4.0)
Brassica intake (times/week)	1501/1397	3.5 (3.7)	3.9 (4.0)
Fatty fish intake (times/week)	1501/1397	1.0 (1.1)	1.1 (1.2)
		PERCENTAGES	
Duration of menopausal hormone use (yrs) ²	1491/1485		
	0	67	73
	<4	13	13
	>4	20	15
Oral contraceptive use	1444/1447	32	35
1 st degree family history of breast cancer	1466/1374	16	9
Previous benign breast disease	1502/1510	14	10
Smoker	1502/1510	43	43
Diabetes mellitus	1500/1396	9	8
Androgen receptor genotype ³	1502/1510		
Homozygous ≥ 22 repeats		28	28
Heterozygous		47	48
Homozygous < 22 repeats		25	23
Vitamin D receptor genotype ⁴	1502/1510		
Homozygous ≥ 19 repeats		38	37
Heterozygous		45	46
Homozygous < 19 repeats		17	18

¹Number of cases and controls for whom information was available

²Note that long-term users (≥ 4years) are over sampled both among cases and among controls, i.e. the proportion of users in our sample is not representative of the Swedish population.

³Pχ² comparing genotype distribution between cases and controls=0.44

⁴Pχ² comparing genotype distribution between cases and controls=0.87

Table 2. Overall estimates of odds ratios and confidence intervals

	Homozygous long			Genotype ¹			Homozygous short		
	Cases/controls	OR ²	CI	Heterozygous			Cases/controls		
				Cases/controls	OR ²	CI	OR ²	CI	
Androgen receptor	438/435	1	Ref	718/729	1.0	0.8-1.1	386/347	1.1	0.9-1.3
Ductal (n=1138)	324/435	1	Ref	534/729	1.0	0.8-1.2	280/347	1.1	0.9-1.3
Lobular (n=182)	61/435	1	Ref	82/729	0.8	0.6-1.2	39/347	0.8	0.5-1.2
Vitamin D receptor	573/558	1	Ref	677/687	1.0	0.8-1.1	261/266	1.0	0.8-1.2
Ductal (n=1115)	424/558	1	Ref	500/687	1.0	0.8-1.1	191/266	0.9	0.8-1.2
Lobular (n=180)	66/558	1	Ref	84/687	1.0	0.7-1.4	30/266	1.0	0.6-1.6

¹The AR alleles are: L - 22 repeats or longer, S - Less than 22 repeats. The VDR alleles are: L - 19 repeats or longer, S - Less than 19 repeats.

²The logistic regression model contained only genotype. Long-term users of menopausal hormone users and women with diabetes mellitus were over-sampled, thus the logistic regression models were conditional on age group and sampling scheme.

Table 3 Stratified analyses using a priori cut-offs

Genotype ¹	LL		LS		SS		p for interaction
	Cases/controls	OR CI	Cases/controls	OR ² CI	Cases/controls	OR ² CI	
Menopausal hormone treatment							
Never any kind	285/312	1 Ref	478/527	1.0 0.8-1.2	258/243	1.1 0.9-1.5	
0 to 4 years any kind	61/55	1 Ref	91/81	1.0 0.6-1.6	51/45	1.1 0.6-1.8	p=0.34 ³
At least 4 years any kind	86/61	1 Ref	144/104	1.0 0.6-1.5	74/51	1.0 0.6-1.6	
0 to 4 years combined E+P	45/41	1 Ref	69/64	1.0 0.6-1.7	27/37	0.7 0.4-1.3	p=0.36 ³
At least 4 years combined E+P	51/33	1 Ref	102/69	1.0 0.6-1.7	58/34	1.1 0.6-2.0	
0 to 4 years estrogen only	21/21	1 Ref	27/37	0.8 0.4-1.7	29/14	2.0 0.8-4.9	p=0.30 ³
At least 4 years estrogen only	36/23	1 Ref	40/32	0.8 0.4-1.7	19/17	0.8 0.3-1.8	
Parity							
Nulliparous	72/44	1 Ref	100/56	1.1 0.6-1.7	54/46	0.7 0.4-1.2	
1 childbirth	92/75	1 Ref	156/130	1.0 0.7-1.4	88/65	1.1 0.7-1.7	
2 childbirths	158/165	1 Ref	294/289	1.0 0.8-1.4	133/120	1.1 0.8-1.5	
More than 2 childbirths	116/151	1 Ref	168/254	0.9 0.6-1.2	111/116	1.3 0.9-1.8	
Nulliparous		1 Ref		1.1 0.7-1.8		0.7 0.4-1.2	p=0.26
1 childbirth		0.7 0.5-1.2		0.7 0.5-1.1		0.8 0.5-1.3	
2 childbirths		0.6 0.4-0.9		0.6 0.4-0.9		0.6 0.4-1.0	
More than 2 childbirths		0.5 0.3-0.6		0.4 0.3-0.6		0.6 0.4-0.9	
Body mass index (kg/m ²)							
<25	205/209	1 Ref	349/358	1.0 0.8-1.3	186/174	1.1 0.8-1.4	
25-<28	109/132	1 Ref	176/205	1.1 0.8-1.5	100/91	1.3 0.9-2.0	
>28	124/87	1 Ref	186/159	0.8 0.6-1.1	98/75	0.9 0.6-1.4	
<25		1 Ref		1.0 0.8-1.3		1.1 0.8-1.4	p=0.66
25-<28		0.9 0.6-1.2		0.9 0.7-1.2		1.1 0.8-1.6	
>28		1.5 1.0-2.0		1.2 0.9-1.6		1.3 0.9-1.9	
Diabetes mellitus							
No	411/378	1 Ref	645/621	1.0 0.8-1.1	345/291	1.1 0.9-1.3	p=0.65 ³
Yes	27/26	1 Ref	73/52	1.3 0.7-2.5	39/29	1.3 0.6-2.6	
Weight gain							

0 or less	35/44	1	Ref	55/70	0.9	0.5-1.7	30/37	1.0	0.5-2.0	
0-11.5 kgs	129/141	1	Ref	219/215	1.1	0.8-1.5	129/105	1.3	0.9-1.9	
12 or more	199/152	1	Ref	324/296	0.8	0.6-1.1	153/130	0.9	0.7-1.2	
0 or less		1	Ref		1.0	0.6-1.7		1.0	0.5-2.0	p=0.56
0-11.5 kgs		1.2	0.7-1.9		1.3	0.8-2.0		1.5	0.9-2.5	
12 or more		1.6	1.0-2.7		1.4	0.8-2.1		1.5	0.9-2.4	
1 st degree family history										
No	362/359	1	Ref	578/597	1.0	0.8-1.2	319/291	1.1	0.9-1.3	
Yes	67/41	1	Ref	119/61	1.1	0.7-1.9	60/26	1.4	0.7-2.5	
Menopausal hormone treatment										
Never any kind	390/401	1	Ref	447/487	0.9	0.8-1.1	164/193	0.9	0.7-1.1	
0 to 4 years any kind	70/73	1	Ref	91/87	1.1	0.7-1.7	40/28	1.6	0.9-2.9	p=0.19 ³
At least 4 years any kind	107/79	1	Ref	134/103	1.0	0.6-1.4	57/35	1.1	0.7-1.9	
0 to 4 years combined E+P	43/55	1	Ref	67/66	1.3	0.8-2.2	30/21	1.9	0.9-3.8	p=0.26 ³
At least 4 years combined E+P	80/53	1	Ref	88/61	0.9	0.6-1.5	40/23	1.1	0.6-2.0	
0 to 4 years estrogen only	26/29	1	Ref	35/31	1.3	0.6-2.8	15/13	1.6	0.6-4.1	p=0.72 ³
At least 4 years estrogen only	27/24	1	Ref	48/39	1.2	0.6-2.3	17/9	1.6	0.6-4.3	
Parity										
Nulliparous	75/46	1	Ref	118/67	1.1	0.7-1.8	30/34	0.5	0.3-0.9	
1 childbirth	147/100	1	Ref	134/117	0.8	0.5-1.1	47/53	0.6	0.4-1.0	
2 childbirths	198/222	1	Ref	267/257	1.2	0.9-1.5	112/95	1.4	1.0-1.9	
More than 2 childbirths	153/190	1	Ref	158/246	0.8	0.6-1.1	72/84	1.0	0.7-1.5	
Nulliparous		1	Ref		1.1	0.7-1.7		0.5	0.3-0.9	p=0.006
1 childbirth		0.9	0.6-1.4		0.7	0.4-1.1		0.5	0.3-0.9	
2 childbirths		0.5	0.3-0.8		0.6	0.4-0.9		0.7	0.4-1.1	
More than 2 childbirths		0.5	0.3-0.7		0.4	0.2-0.6		0.5	0.3-0.8	
Body mass index (kg/m ²)										
<25	287/272	1	Ref	321/334	0.9	0.7-1.2	121/135	0.9	0.6-1.2	
25-<28	138/167	1	Ref	159/195	1.0	0.7-1.3	79/66	1.4	1.0-2.1	
>28	147/111	1	Ref	190/146	1.0	0.7-1.4	60/64	0.7	0.4-1.0	

Diabetes mellitus	<25	1	Ref	0.9	0.7-1.1	0.8	0.6-1.1	p=0.07
	25-<28	0.8	0.6-1.1	0.8	0.6-1.0	1.2	0.8-1.7	
	>28	1.3	0.9-1.7	1.2	0.9-1.6	0.9	0.6-1.3	
Diabetes mellitus	No	1	Ref	0.9	0.8-1.1	0.9	0.7-1.1	p=0.72 ³
	Yes	1	Ref	1.1	0.6-2.0	1.5	0.7-3.1	
Weight gain	0 or less	1	Ref	0.9	0.5-1.6	0.8	0.4-1.5	p=0.46
	0-11.5 kgs	1	Ref	0.8	0.6-1.0	0.8	0.6-1.2	
	12 or more	1	Ref	1.0	0.8-1.3	1.2	0.8-1.6	
1 st degree family history	0 or less	1	Ref	0.9	0.5-1.6	0.9	0.4-1.6	p=0.46
	0-11.5 kgs	1.4	0.9-2.2	1.1	0.7-1.7	1.1	0.7-1.8	
	12 or more	1.3	0.8-2.0	1.3	0.8-2.0	1.5	0.9-2.4	
1 st degree family history	No	1	Ref	0.9	0.8-1.1	0.9	0.7-1.1	p=0.54
	Yes	1	Ref	1.1	0.7-1.8	1.3	0.7-2.3	
Diabetes mellitus	No	1	Ref	0.9	0.7-1.1	0.9	0.7-1.1	p=0.54
	Yes	1.6	1.1-2.4	1.8	1.3-2.6	2.1	1.2-3.6	

¹The AR alleles are: L - 22 repeats or longer, S - Less than 22 repeats. The VDR alleles are: L - 19 repeats or longer, S - Less than 19 repeats.

²The logistic regression model contained only genotype. Long-term users of menopausal hormone users and women with diabetes mellitus were over-sampled, thus the logistic regression models were conditional on age group and sampling scheme.

³P for interaction, in the case of menopausal hormone use and diabetes mellitus, was calculated on 4 or 2 degrees of freedom, respectively, since the main effect of the co-variate cannot be estimated due to over-sampling.

Table 4 Interaction between AR and VDR microsatellites using *a priori* cut-offs

	<u>Androgen receptor genotype</u>	Homozygous \geq 22 repeats	Heterozygous	Homozygous <22 repeats
<u>Vitamin D receptor genotype</u>	Homozygous >18 repeats	1 Ref	1.1 (0.9-1.5)	1.1 (0.8-1.6)
	Heterozygous	1.1 (0.8-1.5)	1.0 (0.7-1.2)	1.2 (0.9-1.6)
	Homozygous \leq 18 repeats	0.9 (0.6-1.4)	1.1 (0.7-1.5)	1.1 (0.8-1.7)

p for interaction =0.50

Table 5 Overall estimates of odds ratios and confidence intervals using exploratory *a posteriori* cut-offs

Genotype ¹	LL			LS			SS		
	Cases/controls	OR ²	CI	Cases/controls	OR ²	CI	Cases/controls	OR ²	CI
Androgen receptor (best cutoff at 20)	1020/1031	1	Ref	404/382	1.1	0.9-1.3	85/52	1.7	1.2-2.4
<i>Ductal AR n=1115</i> <i>(best cutoff at 20)</i>	759/1031	1	Ref	296/382	1.1	0.9-1.3	60/52	1.6	1.1-2.3
<i>Lobular AR n=177</i> <i>(best cutoff at 20)</i>	128/1031	1	Ref	45/382	1.0	0.7-1.4	4/52	0.6	0.2-1.6
Vitamin D receptor (cutoff at median, 21)	458/507	1	Ref	569/573	1.1	0.9-1.3	484/431	1.2	1.0-1.5
<i>Ductal VDR n=1115</i> <i>(best cutoff at 21)</i>	340/507	1	Ref	422/573	1.1	0.9-1.3	353/431	1.2	1.0-1.5
<i>Lobular VDR n=175</i> <i>(best cutoff at 21)</i>	53/507	1	Ref	73/573	1.2	0.8-1.7	54/431	1.2	0.8-1.8

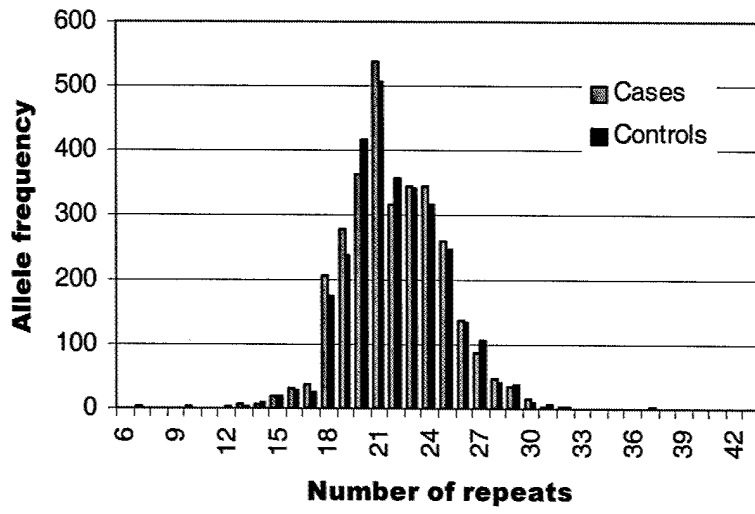


Figure 1 Distribution of AR CAG repeat alleles by case-control status.