No association between androgen or vitamin D receptor gene polymorphisms and risk of breast cancer


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Endogenous hormone exposure is known to alter breast cancer susceptibility and genes responsive to such hormones are plausible candidates for predisposition genes. We have examined polymorphisms in genes for two members of the nuclear receptor superfamily which are expressed in breast tissue and known to moderate rates of cell proliferation in a case–control association study: the androgen receptor (AR) and the vitamin D receptor (VDR). We have used two series of Caucasian female breast cancer cases, one incident and one prevalent, and compared both with two sets of matched controls from the East Anglian region of Britain. Since the results are similar in the two series we have combined them. The AR poly[Gly]n and poly[Gln]n tracts were genotyped in a total of 508 female breast cancer cases and 426 controls. The VDR TaqI polymorphism was analysed in 951 cases and 627 controls drawn from the same population series. There were no significant differences between cases and controls for either the AR or VDR polymorphisms. Compared with individuals with two short alleles (<22 repeats) of the AR poly[Gly]n tract, the odds ratios and 95% confidence intervals (95% CI) for individuals with one or two alleles were 0.82 (95% CI 0.62–1.09) and 1.31 (95% CI 0.87–1.97), respectively. Heterozygotes and homozygotes for the VDR TaqI cutting site had odds ratios of 1.01 (95% CI 0.81–1.27) and 0.97 (95% CI 0.71–1.32), respectively. None of the AR or VDR polymorphisms investigated has a major effect on risk of breast cancer in the British population.

Introduction

Breast cancer is a common disease with major public health implications. Rare highly penetrant mutations in genes such as BRCA1, BRCA2 and TP53 explain <5% of total incidence. It is likely that other susceptibility genes exist: the first degree relatives of breast cancer cases have a 2-fold increase over the general population risk and only ~15% of this excess can be explained by known genes (1). These presently unidentified genes are likely to include commoner low penetrance predisposition alleles that could in principle explain a much greater proportion of total disease incidence than rare mutations of higher penetrance. To date there is substantial evidence for only one such locus, the HRAS1 VNTR (variable number of tandem repeats). This locus has a number of rare alleles, with a combined population frequency of 6%, which are associated with a 2-fold increased relative risk of breast cancer (2). Despite this small increase in risk, these alleles are together calculated to account for ~9% of total breast cancer incidence.

Breast cancer risk is known to be strongly related to endogenous hormone exposure and genes responsive to such hormones are therefore plausible candidates for being susceptibility genes. The androgen receptor (AR) and vitamin D receptor (VDR) are both members of the nuclear receptor superfamily which regulate the action of hormone-responsive genes. They function by binding hormone, androgens or 1,25-dihydroxyvitamin D, respectively, and then transactivating their respective hormone-responsive genes via hormone response elements in the promoters of the downstream genes. Both receptors are expressed in breast tissue and are known to moderate rates of cell division.

Missense mutations in the AR have been shown to cause partial androgen insensitivity (leading to feminization, including gynaecomastia) together with familial male breast cancer in a few families (3,4). Two of the amino acid substitutions implicated, Arg607Gln and Arg608Lys in the DNA-binding domain, reduce the affinity of these mutant ARs for their response elements and thus their transactivation efficiencies (5). The AR is expressed in female breast epithelium and androgens binding to these receptors inhibit breast cell proliferation (6,7), although AR status in breast tumours is not so strongly correlated with prognosis as oestrogen receptor and progesterone receptor status (8).

Exon 1 of the AR encodes two expressed polymorphic repeats, a poly[Gly]n and a poly[Gln]n tract. Expansion of the poly[Gly]n tract beyond the normal range of 11–35 repeats causes X-linked spinal-bulbar muscular atrophy (Kennedy’s disease) and frequently also mild androgen insensitivity (9). The length of the poly[Gln]n tract has been shown to be inversely related to transactivation efficiency and rate of sperm production (10,11). Recently, some studies have suggested an association of the poly[Gln]n tract with differences in prostate cancer risk: the shorter, more activating, repeat lengths are associated with a mildly increased (1.5- to 2.0-fold) risk of prostate cancer or an earlier age of onset and possibly also an increased risk of metastasis (12–16). The poly[Gly]n tract has also been investigated (17), but shows no significant differences between prostate cases and controls. Elhaji et al. (18) have also compared the poly[Gln]n tract in 80 breast tumour DNA samples with control germline DNA. They found longer repeat lengths in tumours than in the controls but their study design was unable to differentiate between somatic mutation in the tumour and the patient’s germline genotype. Rebbeck et al. (19) have studied this polymorphism in carriers of BRCA1 mutations, who are thus at very high risk of developing breast cancer.
cancer, and they have reported an earlier age of onset of cancer in individuals with >28 Gln (less activating) repeat lengths. Finally, Wu et al. (20) have very recently reported that 81 male breast cancer cases have a mean of 28 ± 3 repeats compared with 22 ± 3 in 73 normal male controls.

The VDR is expressed in breast cell lines. The hormonal form of vitamin D (1,25-dihydroxyvitamin D) has antiproliferative effects on breast tumour cells (21). There are numerous reported polymorphisms in the VDR gene: three, detectable with the enzymes BsmI, Apal and TaqI, located in intron 8 and exon 9, and others in the 3′ UTR (22). Three large studies all show these to be in strong linkage disequilibrium in Caucasians, such that there are only two common haplotypes defined by the presence or absence of the TaqI restriction site (22–24). It has been suggested that these haplotypes are associated with differences in bone mineral density and risk of osteoporosis (22), however, the evidence for this is controversial (25). The polymorphisms used are considered to be neutral markers and the functional VDR variant has not yet been identified. Polymorphisms of the VDR gene have also been examined in prostate cancer association studies (16,24,26) and there is some evidence that certain genotypes may be associated with altered risk, in particular Caucasians homozygous for the presence of the TaqI site may have a reduced risk of prostate cancer (24). Reports on Japanese and African-American populations have also suggested associations between certain VDR genotypes and breast cancer risk (27,28).

Here we have investigated this association in the British population.

Materials and methods

Population series

All patients and controls in this study were Caucasian females from the East Anglian region of the UK. Two separate series (Strata) were used to overcome the need to increase thresholds of statistical significance when carrying out multiple tests. The selection criteria for the two series differed slightly and the sets of results were initially examined individually and only combined if found to be similar.

The first series were a prospectively ascertained group of 288 incident patients attending the Addenbrooke’s Hospital (East Anglia) for treatment between 1992 and 1995 and diagnosed below age 71 years (mean 52.5 ± 12.8, range 28.6–70.8 years). These were compared with a group of 288 randomly selected, anonymous controls taken from the EPIC study (29), a population-based cohort study of diet and health (mean age 58.9 ± 9.2, range 44.7–75.6 years). This cohort contains ~25 000 individuals resident in Norfolk (East Anglia) recruited from 1992 to 1996.

The second series were a retrospectively ascertained group of patients identified through the East Anglian Cancer Registry, as part of the Anglian Breast Cancer Study, comprising all patients diagnosed below age 55 years since 1991 and still alive in 1996 (mean 46.6 ± 5.7, range 25.0–54.9 years). These were compared with a second group of 384 random controls also from the EPIC cohort (mean age 55.6 ± 8.1, range 39.9–69.9 years).

A 9 ml EDTA whole blood sample was taken from each study subject for DNA extraction. DNA was extracted consecutively as the blood samples arrived in the laboratory. At the time of the AR gene analysis DNA was available from a total of 508 cases and 426 controls. By the time of the AR13 gene analysis DNA was available from a further 443 cases and 338 controls. DNA was extracted consecutively as the blood samples arrived in the laboratory. At the time of the AR gene analysis DNA was available from a total of 508 cases and 426 controls. By the time of the AR gene analysis DNA was available from a further 443 cases and 338 controls. DNA was extracted consecutively as the blood samples arrived in the laboratory. At the time of the AR gene analysis DNA was available from a total of 508 cases and 426 controls. By the time of the AR gene analysis DNA was available from a further 443 cases and 338 controls.

DNA analysis

Androgen receptor: Separate PCR reactions were carried out across the two polymorphic loci on each DNA sample analysed. The primers for the poly[Gly] tract were AR13 (act etc ttc aec gec gaa gaa gge) and AR16 (atc agg tgt ggt gaa gtc get ttc e). AR16 was labelled with either the fluorescent dye JOE (PE Applied Biosystems, Warrington, UK) for cases or FAM for controls. PCR was carried out using AmpliTaq Gold™ (PE Applied Biosystems) according to the manufacturer’s recommended conditions and using a primer annealing temperature of 57°C in a buffer containing 3:1 deaza-GTP:GTP to lower the melting temperature of this GC-rich template. Fragments in the range 156–213 bp were obtained. The primers for the poly[Gln] tract were ARa (acc agg tag ctc gtg ggg cet etc etc tga tgg ge) and ARb (ecu gag cgt ggg cga aat gat gca ecu aag ecc cgg). ARb was also labelled with either JOE for cases or FAM for controls. PCR was carried out using AmpliTaq Gold™ according to the manufacturer’s recommended conditions using a primer annealing temperature of 55°C to give fragments in the size range 205–299 bp. A multiplex of both PCR products from a single sample case and both from a single control together with GS-500 ROX size marker and loading buffer (PE Applied Biosystems) was made and loaded into a single lane of Sequagel-6 matrix (National Diagnostics, Hull, UK) and detected on a model 373 sequencer (PE Applied Biosystems). The pairing of case and control individuals in a single lane overcame the discrepancies in sizing across the gels; in addition a homozygous sample of known size was loaded onto each gel to overcome sizing differences between gels. Band sizes were analysed using Genotyper™ software (PE Applied Biosystems).

Vitamin D receptor: A 400 bp PCR across the boundary of intron 8 and exon 9 of the VDR gene was carried out using primers B8F (cag age atg gac agg gag caa g) and B9R (tgg atc atc tgc gta tag age agg) (24) using Red-Hot DNA Polymerase (Advanced Biotechnologies, Epsom, UK) according to the manufacturer’s recommended conditions, using a primers annealing temperature of 47°C. PCR products were digested with TaqI enzyme (New England Biolabs, Hitchin, UK) to give fragments of 300 + 100 bp in the presence of the polymorphic cutting site. Digested fragments were separated on 4% NuSieve GTG agarose (Flowgen, Lichfield, UK).

Statistical methods

Associations between polymorphisms and breast cancer risk were analysed by logistic regression using the program S-Plus. Cases and controls were genotyped in two groups: Stratum A (288 cases and 288 controls) and Stratum B (up to 672 cases and 384 controls) and all analyses allowed for strata as a covariate.

The effects of the AR repeats were first assessed by testing for a trend in breast cancer risk with repeat length by fitting a parameter for repeat length (averaged over the two chromosomes) in logistic regression. We also treated the AR repeats as a di-allelic marker by dividing into long and short repeat lengths using values that have been used in other studies (12,13,19,20).

The linkage disequilibrium parameter (Δ) between the AR polymorphisms was calculated using maximum likelihood haplotype frequencies, using the AR allele length divisions suggested by Irvine et al. (30).

Results

Androgen receptor

DNA from series 1 and 2 was genotyped at the poly[Gly]13 and poly[Gln]16 tracts. There are no significant differences between the results obtained for the two different series (data not shown). The combined results (from a total of 508 cases and 426 controls) are shown in Figure 1. The poly[Gly]13 allele distribution has a range of 4–23 repeats with 17 repeats as the most common allele, while the poly[Gln]16 tract approximates to a normal distribution with a range of 10–39 and a mode of 23 repeats. Inspection of the individual allele frequencies reveals no differences between cases and controls greater than would be expected by chance.

The poly[Gln]16 alleles were observed in 129 different genotype classes (data not shown, but are available from the corresponding author on request). There is no trend for longer genotypes of either tract (expressed as mean length of an individual’s two alleles) to be associated with either increasing or decreasing risk: for the poly[Gly]13 tract the mean genotype is 13.8 repeats in controls and 14.0 in cases and the OR is 1.07 (95% CI 0.89–1.48) for each additional repeat carried, whilst for the poly[Gln]16 tract the mean genotype is 23.5 repeats in both cases and controls [OR = 0.99 (95% CI 0.93–1.04) per repeat]. Since poly[Gln]16 tract lengths have been shown to be inversely related to AR transactivation efficiencies (10,11), we have specifically looked at the genotypes of this tract and have considered genotypes involving alleles at each end of the poly[Gln]16 range. These results are shown in Table I. Neither heterozygous carriers nor homozygotes for alleles
<22 repeats or >29 repeats show significant differences in breast cancer risk. In series 2 there is some suggestion that the heterozygous carriers of an allele <22 repeats may be protected from breast cancer \([OR = 0.67 (95\% \text{ CI } 0.46–0.98)]\), but this result becomes insignificant when the control genotypes are corrected for Hardy–Weinberg equilibrium.

In contrast to a previous report on a very small sample of males (30), there is no evidence for linkage disequilibrium
between these two loci: $\Delta = -0.09$ in controls and 0.017 in cases and neither of these values reaches statistical significance.

**Vitamin D receptor**

In total 951 cases and 627 controls were genotyped for the VDR \textit{TaqI} restriction fragment length polymorphism (RFLP) and the genotype distributions are shown in Table II. Again, the results from the two case–control series are very similar. The rarer \textit{t} allele (presence of the \textit{TaqI} cutting site) has a frequency of 0.40 in both cases and controls. We observed no significant differences between cases and controls in any genotype class [OR (\textit{TT}) = 1.01 (95% CI 0.81–1.270, OR (\textit{tt}) = 0.97 (95% CI 0.71–1.32)].

**Discussion**

From these large, population-based case–control studies we see no evidence that common alleles of either the AR or VDR loci have an effect on risk of breast cancer in the general East Anglian, British population.

Our findings on the poly[Gln]n tract in the AR gene are in agreement with those of Spurdle et al. (31). We find no evidence for female carriers of tracts with $>28$ Gln to have an increased risk of breast cancer. This became a plausible hypothesis in the light of two other findings: first, that female carriers of such long repeats had an earlier onset of breast cancer if they were also BRCA1 mutation carriers (19); second, that male carriers are also predisposed to breast cancer (20).

Both these reports indicated that longer repeats, demonstrated to have reduced transactivation efficiency (11) may confer an increased risk of breast cancer amongst particular subsets of high risk of individuals, but we see no similar effect on general breast cancer risk. Several reports have additionally suggested that short AR glutamine repeats, with increased transactivation efficiency, are associated with a mildly increased risk of prostate cancer (12–16). Again, we see no similar effect on female breast cancer risk [OR (\textit{sl}) = 0.82 (95% CI 0.62–1.09), OR (\textit{ss}) = 1.31 (95% CI 0.87–1.97)].

We also found no effect of the VDR \textit{TaqI} RFLP on breast cancer risk in our population sample. We can exclude substantial effects of this locus since the 95% upper confidence limit for relative risk is 1.32 and the corresponding upper confidence limit for population attributable risk (PAR) is 0.17 [PAR = 0.029 (95% CI 0–0.17)]. This is in contrast to the previously suggested association with the \textit{BsmI} RFLP in the Japanese population (28): using 60 cases and 120 controls this study reported that the \textit{bb} genotype (which is equivalent to the \textit{TT} genotype described here; 24) confers an OR for breast cancer of 3.90 (95% CI 1.63–9.30).

The \textit{TaqI} RFLP of the VDR gene has been shown to be in very strong linkage disequilibrium with the other reported VDR polymorphisms in three large series of Caucasians (22–24). It is therefore likely that we can exclude all known VDR haplotypes from having a substantial effect on breast cancer risk in the British population.

We do not have the statistical power, in a study of this size, to carry out meaningful interaction studies, since neither gene shows any major effect on breast cancer risk and the AR gene has so many different genotype classes, and so these have not been attempted here.

We cannot exclude the possibility that different, as yet undescribed, polymorphisms in the VDR or AR are associated with breast cancer risk. However, it seems likely that other genes involved in response to endogenous hormones will be more important than these in determining common breast cancer risk.

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### References


