

Review

The role of androgens and polymorphisms in the androgen receptor in the epidemiology of breast cancer

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Published: 2 April 2003

Breast Cancer Res 2003, **5**:164-173 (DOI 10.1186/bcr593)

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Abstract

Testosterone binds to the androgen receptor in target tissue to mediate its effects. Variations in testosterone levels and androgen receptor activity may play a role in the etiology of breast cancer. Here, we review the epidemiologic evidence linking endogenous testosterone to breast cancer risk. Paradoxically, results from observational studies that have examined polymorphisms in the androgen receptor suggest that the low-activity androgen receptor increases breast cancer risk. We review the quality of this evidence and conclude with a discussion of how the androgen receptor and testosterone results coincide.

Keywords: androgen receptor, breast cancer, CAG polymorphism, testosterone

Introduction

The role of androgens in breast cancer etiology has been a subject of both curiosity and confusion. It is still unclear by which mechanisms testosterone exerts its activity in the female breast, and whether the effects are predominantly proliferative or anti-proliferative on breast cells at physiologic levels. In the present review we evaluate the results from epidemiologic studies on the role of circulating testosterone and a functional polymorphism in the androgen receptor (AR) in breast cancer. We also highlight some of the epidemiologic challenges in addressing these questions.

Sources of endogenous testosterone

There are two main sources of androgens in women. Testosterone is produced directly by the ovary and by conversion of the adrenal androgens dehydroepiandrosterone and dehydroepiandrosterone-sulfate into androstenedione, and then further to testosterone in peripheral tissue [1]. In premenopausal women, approximately 25% of circulating testosterone is secreted directly from the adrenal gland and 25% from the ovary, whereas the remaining 50% is produced by peripheral conversion of androstenedione [2]. Testosterone levels vary over the

menstrual cycle with peak levels mid-cycle, and diurnally with highest levels in the early morning [3].

Testosterone and androstenedione are produced by the interstitial cells of the ovarian stroma and may continue to respond to gonadotropins and produce testosterone after the menopause [4]. In normal postmenopausal women the ovarian vein has been observed to have higher concentrations of testosterone than is found in peripheral blood; bilateral oophorectomy results in reductions in testosterone levels by as much as 50% [5].

Several smaller cross-sectional studies have found lower testosterone levels in postmenopausal than premenopausal women [6–8] or lower levels in perimenopausal than premenopausal women [2]. Large longitudinal studies that have followed women through the menopausal transition have observed either no significant change in testosterone [2,9] or a 15% decrease in both testosterone and androstenedione at menopause [10]. In one study of women aged 50–89 years testosterone levels were lowest at the time of the menopause, whereas women older than 70 years or more than 20 years postmenopause had levels approximating those of premenopausal women [11].

In summary, there is increasing evidence that the ovary continues to produce androstenedione and testosterone in healthy postmenopausal women. Levels may either remain the same or decrease slightly at menopause. However, women with bilateral oophorectomy may be androgen deficient.

Testosterone and breast cancer risk

Prospective studies that examined the association between testosterone and breast cancer

Data from eight prospective cohort studies have been reported on the association between endogenous testosterone levels and breast cancer risk using testosterone measured from blood samples gathered at baseline from postmenopausal women [12–19]. Six of these studies were nested case–control studies [12,14–18]; one was a case–cohort study [19] and one a full cohort study [13]. Only one of these studies reported results for premenopausal women [12].

Six of the eight studies reported a statistically significant increase in postmenopausal breast cancer risk with increasing levels of endogenous testosterone [14–19]. A recently conducted pooled analysis [20] of these eight prospective studies estimated that the relative risk for breast cancer in women whose levels of testosterone were in the top quintile as compared with women in the bottom quintile was 2.22 (95% confidence interval 1.59–3.10). A statistically significant dose–response relationship was also observed (P for trend <0.001) [20]. Two of the studies also reported statistically significantly increasing breast cancer risk with increasing levels of free testosterone [15,19], a measure of bioavailable testosterone.

The study of premenopausal women [12] found no statistically significant differences between cases and noncases in mean levels in either premenopausal or postmenopausal women, but the sample size was small (premenopausal women: 17 cases, 67 controls; postmenopausal women: 22 cases, 88 controls).

Can the observed association between testosterone levels and breast cancer risk be due to bias?

Effects of measurement biases

One limitation of the studies reviewed is that serum testosterone may not be the ideal measure of testosterone. Total testosterone includes both free testosterone and bound testosterone. Furthermore, serum levels do not take into account the peripheral conversion of precursor androgens into testosterone in the breast tissue itself. The effect of this measurement error is most likely to be nondifferential, therefore biasing results toward the null.

Measurement biases due to use of a single hormone measurement or degradation of hormones in stored specimens over time would most likely be nondifferential,

resulting in attenuated estimates of disease risk. All of the existing prospective studies (Table 1) analyzed testosterone measured from only one blood draw, which might not be representative of the cumulative exposure to testosterone; however, given the prospective design of the studies, any inaccuracy in measurement would probably bias risk estimates toward the null.

The consideration of time of day that blood was drawn and fasting status can help to avoid the biases due to using a single hormone measurement. To avoid this bias, three of the studies either matched with respect to time of blood draw [14,18] or restricted individuals to having their blood drawn in the morning [13]. Because the effect of these sporadic variations would be to bias the results toward the null, this may help to explain one of the null associations observed [12].

The studies that observed an association between testosterone and breast cancer risk attempted to reduce measurement bias due to degradation by matching cases to controls on the date of blood draw [14–18] and storage conditions such as sample location/shelf in the freezer [15]. This was not done in the studies reporting no association between testosterone and breast cancer risk [12,13] or in one of the positive studies [19].

Laboratory assay variation would also most likely be nondifferential because cases and controls were analyzed concurrently in these studies. The intra-assay and inter-assay coefficients of variation in these studies were rather good, ranging from 4% to 14%. However, the coefficient of variation was not reported in one of the null studies [12].

Thus, although there may be some attenuation in effect estimates in all of the studies, it is not clear whether measurement bias due to degradation can explain the discrepancies between the two null studies and the positive studies.

Temporal Bias

If breast cancer development increases testosterone levels, then studies that included individuals diagnosed shortly after baseline hormone measurement may have artificially elevated estimates of the risk for breast cancer associated with testosterone levels. Two of the positive studies and both of the null studies excluded those who were diagnosed 6–24 months after baseline [12–14,17]. However, the study with the most conservative cut-point of 24 months [14] reported a significant positive association between testosterone levels and breast cancer risk. Thus, although it is possible that temporal bias played a role in the four positive studies with no exclusions, this latter study suggests that temporal bias cannot explain the association between testosterone and breast cancer risk.

Table 1

The relative risk for breast cancer associated with testosterone levels in postmenopausal women: results from prospective studies

Author, year [reference]	Cohort	Age (years) ¹	Sample size	Matching	Adjusted for	Exposure	Cases	Controls	Categories ²	RR ³	95% CI	P	Covariate adj-RR	95% CI	P
Wysocki, 1987 [12]	Washington County, MD, USA	36-90	39 Cases, 155 controls	Race, age, time since last menstrual period	Matching variables only	T	39	155	N/A	N/A	N/A	NS	N/A	N/A	NS
Garland, 1992 [13]	Rancho Bernardo, CA	50-79	15 Cases, 409 at risk		Age, BMI (tertiles), smoking at baseline (Y/N), other hormones ⁴	T	5	132	37-176	1.0					
Dorgan, 1996 [14]	Columbia, MO, USA	52-73	71 Cases, 133 controls	Exact age, date (±1 year) and time of blood draw (±2 hours)	Years since menopause, height, weight, parity, family history; matched analysis	T	9	32	<98	1.0			1.0		
Berrino, 1996 [15]	Study of Hormones and Diet in the Etiology of Breast Tumors, Italy	40-69	24 Cases, 87 controls	Recruitment center, recruitment date, daylight saving period at time of recruitment, location of freezer storage (freezer and level)	Age at menarche, age at first childbirth, number of births, age at menopause, weight, height, BMI, waist:hip ratio, other hormones, matched analysis	free T	24	87	<0.57	1.0	0.4-9.3	0.005	4.6	1.1-20.0	
Thomas, 1997 [16]	Guernsey, UK	Mean 59	61 Cases, 179 controls	Age (±2 years), date of blood	Age at menarche, parity, number of years postmenopausal, BMI, estradiol and SHBG; matched analysis	T	13	59	<210	1.00					
Zeleniuch-Jocquotte, 1997 [17]	NYU Women's Health Study, USA	49-65	85 Cases, 163 controls	Age at enrollment (±6 months), date of initial blood donation (±3 months), menopausal status	BMI, age at menarche, parity, age at first full-term pregnancy, age at menopause, family history, history of benign breast condition, history of oophorectomy, lifetime months of lactation, smoking; matched analysis	T	85	163	<210	1.0	1.0-5.6	<0.05			

Continued overleaf

Table 1

Continued

Author, year [reference]	Cohort	Age (years) ¹	Sample size	Matching	Adjusted for	Exposure	Cases	Controls	Categories ²	RR ³	95% CI	P	Covariate adj-RR	95% CI	P
Hankinson, 1998 [18]	Nurses Health Study, USA	46–69	147 Cases, 299 controls	Age (±2 years), month of collection, time of day that blood was drawn (±2 hours), fasting status	BMI (at age 18, quartiles), family history, age at menarche (quartiles), parity/age at 1st birth, age at menopause (quartiles); matched analysis	T	33 38 37 39	75 79 78 67	<160 160–220 230–310 >310	1.00 1.12 1.10 1.34		0.05	1.0 1.12 0.60–2.10 1.07 0.57–2.00 1.40 0.73–2.70		
Cauley, 1999 [9]	Study of Osteoporotic Fractures, USA	65–75	97 Cases, 250 controls		Age, BMI, age at menarche, first birth, and menopause, surgical menopause (Y/N), nulliparity (Y/N), family history, past estrogen use (Y/N) ⁴ , walking for exercise (Y/N), alcohol consumption(g/d quintiles)	T	10 23 33 31	56 65 58 64	<1.6 1.6–2.4 2.4–3.8 >3.8	1.0 1.7 3.5 2.5	0.7–4.2 1.5–8.2 1.1–6.0	0.01	1.0 2.2 0.7–7.1 6.4 2.1–19.6 3.3 1.1–10.3		
							10 25 30 32	57 61 68 60	<121 121–176 177–276 >276	1.0 2.1 3.0 2.8	0.9–5.0 1.3–6.7 1.2–6.5	0.01	1.0 2.2 0.7–7.1 5.5 1.8–17.0 3.6 1.1–11.7		0.008

¹Baseline age distribution. ²All units converted to pg/ml. ³Age adjusted. ⁴Analysis also conducted excluding past estrogen users. ⁵Adjusted for BMI and age only. ⁶Adjustment did not change results from the crude analysis. BMI, body mass index; CI, confidence interval; NS, not significant; NR, not reported; RR, relative risk; SHBG, sex hormone binding globulin; T, testosterone.

Effects of confounding

Lack of control for body mass index (BMI) or age at menopause could result in a positive bias away from the null. All of the studies that reported a significant association between testosterone levels and breast cancer risk included either BMI or height and weight as covariates in the statistical model. All but one of the null studies [13] considered either the amount of time menopausal [12,14,16] or age at menopause [15,17–19] as a covariate to control for the effects of menopause on testosterone levels. Thus, it is unlikely that confounding by these variables can explain the associations observed between testosterone levels and breast cancer risk.

Comparability/generalizability

All of the prospective studies that examined the testosterone–breast cancer association were conducted using cohorts from Caucasian populations. Only two studies [15,16] were conducted outside the USA, one in Italy [15] and one on the island of Guernsey [16]. It is therefore unlikely that differences in the populations studied can explain the discrepant results between studies. There are, as far as we know, no prospective data from nonwhite populations.

Studies of testosterone measured after diagnosis that examined the testosterone–breast cancer association

Several case–control studies published during the past 20 years have evaluated the association between testosterone levels and breast cancer risk. Comparisons with levels in control individuals have shown that breast cancer patients who were postmenopausal [21–25] and those who were premenopausal [26–28] had significantly elevated testosterone levels. Table 2 presents the odds ratios of breast cancer associated with categories of serum testosterone, whereas Table 3 presents results of studies that compared mean levels of testosterone. The measures of testosterone in cases were almost twice those of controls. Although these retrospective studies support the prospective study results showing an association between increased testosterone and increased breast cancer risk, these results may not be as readily interpretable as those from the prospective studies, given the possibility that the presence of cancer, or the treatment for it, might have increased testosterone levels.

Summary

Both retrospective and prospective studies have reported statistically significant associations between increased levels of testosterone and increased breast cancer risk. These associations are unlikely to be due to measurement biases, the influence of disease, or lack of adjustment for the confounding effects of BMI or age at menopause.

Table 2**The relative risk of breast cancer associated with testosterone levels: results from case-control studies**

Author, year [reference]	Population	Exposure	Cases	Controls	Categories ¹	OR	95% CI	<i>P</i>
Secreto, 1984 [27] ²	Premenopausal women in Milan	Serum T	13	47	<590	1.0		
			4	9	590–670	2.6	0.6–10.9	0.0004
			10	6	671+	10.2	2.6–40.0	
		Urinary T	9	35	<8.2	1.0		
			4	7	8.2–10.5	2.3	1.2–12.9	
			10	5	10.6+	8.4	2.1–33.6	0.002
Secreto, 1989 [28] ²	Women in Milan age 30–49 years	Serum T	31	51	<309	1.0		
			32	17	309+	3.4	1.6–7.3	0.05
		Urinary T	36	50	<7.6	1.0		
			24	16	7.6+	2.1	0.9–4.8	NS
Secreto, 1991 [24] ³	Postmenopausal women in Milan <69 years of age	Serum T	16	40	<146	1.0		
			16	40	146–212	1.2	0.5–3.0	
			18	38	213–275	1.5	0.6–3.7	
			25	32	>275	2.7	1.1–6.7	0.03
		Urinary T ⁴	11	43	<18	1.0		
			14	38	18–31	1.2	0.5–2.9	
			17	37	32–46	2.2	0.8–5.7	
			30	26	>46	4.7	1.8–12.1	0.001
		Serum DHT	15	37	<36	1.0		
			20	40	36–57	1.6	0.7–3.7	
16	38		58–82	1.3	0.5–3.1			
24	35		>82	2.0	0.8–5.0	NS		
Lipworth, 1996 [25] ⁵	Postmenopausal women from Sweden	Serum T ⁶	23	35	260	1.00		
			15	27	350	0.75	0.33–1.75	
			47	30	470	2.64	1.27–5.46	
			36	30	700	2.30	0.97–5.50	
								0.041

¹Serum T and DHT converted to pg/ml and urinary T converted to µg/24 hours. ²Age adjusted model. ³Adjusted for age, occupation and number of children. ⁴Units in pg/ml. ⁵Adjusted for age and residence. ⁶Categorized by quartile medians. CI, confidence interval; DHT, dihydrotestosterone; NS, not significant; OR, odds ratio; T, testosterone.

Table 3**Mean levels of testosterone in breast cancer cases and controls**

Author, year [reference]	Population	Exposure	Group	<i>n</i>	Mean ¹	SD	<i>P</i> ²
Secreto, 1983 [21]	Postmenopausal women	Serum T	Controls	30	310	110	
			Carcinoma	28	550	200	0.001
Secreto, 1983 [26]	Premenopausal women	Urinary T	Controls	22	6.25	3.48	
			Familiality	21	5.41	3.60	NS
			Hyperplasia	39	6.97	4.44	NS
			Carcinoma	18	11.3	6.78	0.01
Secreto, 1984 [27]	Premenopausal women	Serum T	Controls	55	470	160	
			Breast Hyperplasia	31	550	200	<0.05
			Breast Cancer	23	620	220	<0.005
Hill, 1985 [22]	Postmenopausal women	Serum T	Healthy Caucasian	43	NS		
			Healthy Japanese	59	NS		0.01
			Cases Japanese	33	NS ³		0.01
Adlercreutz, 1989 [23]	Postmenopausal women	Serum T	Vegetarians	10	172.80	86.40	
			Omnivores	9	233.28	66.24	<0.05
			Cases	8	319.68	132.48	<0.05

¹Serum T converted to pg/ml and urinary T converted to µg/24 hours. ²Comparisons with mean levels in controls using the *t*-test. ³Comparison with healthy Japanese group. SD, standard deviation; T, testosterone.

Androgen receptor, the AR-CAG repeat, and breast cancer risk

The main receptor for testosterone is the AR. A functional polymorphism in the *AR* gene has been examined in female breast cancer, and the literature is reviewed to shed light on the possible mechanisms by which testosterone may affect breast cancer risk.

Androgen receptor protein and breast cancer

The AR is expressed in the majority of breast cancers [29–35]. Several studies have been conducted to examine the effects of androgens on the growth of AR-positive breast cancer cell lines. These studies have reported both inhibitory [36,37] and stimulatory [38,39] effects. These divergent effects have been observed to be specific to the cell line under study [40].

To our knowledge, the only *in vivo* study of the effect of testosterone on breast cell proliferation was conducted in rats and showed that treatment with testosterone results in both tumor regression and a reduction in estrogen receptor expression [41]. However, it is unclear whether the testosterone levels used represent physiologic doses. No *in vivo* or epidemiologic studies have examined the association between serum or tissue testosterone levels and breast cell proliferation in tumors with varying degrees of AR expression.

In summary, the effects of androgens on breast cancer cell growth are still unclear. In contrast to the epidemiologic observation of a consistent association between serum testosterone levels and increasing breast cancer risk, *in vivo* studies reported an antiproliferative effect and *in vitro* studies reported both proliferative and antiproliferative effects.

The androgen receptor gene and a polymorphic CAG repeat

The AR is encoded by a single 90 kilobase gene on the X chromosome (Xq11-q12), which encodes a 11-kilobase mRNA transcript composed of eight exons [42–46]. Epidemiologic evidence for a role of the *AR* gene in breast cancer was first suggested by studies of male breast cancer patients. A mutation in *AR* in the DNA-binding domain resulting in an inability to bind androgens was first reported in a pair of brothers with breast cancer [47]. In a study of 13 male breast cancer patients, one was observed to carry a similar mutation [48]. In another small study of 11 male breast cancer patients [49], this mutation was not observed. These results suggested that the mutation may play a role in the development of breast cancer in some males.

Within the first exon of *AR* lies a polymorphic CAG repeat that encodes a polyglutamine tract of variable length. The normal size range of these repeats is between 6 and 39 repeats [50,51]. Between 40 and 66 repeats have

been observed in patients with a rare, neurodegenerative disorder called spinal and bulbar muscular atrophy [52], which is characterized by androgen insensitivity with gynecomastia, testicular atrophy, oligospermia, azoospermia, and elevated serum gonadotropins.

AR-CAG repeat length and androgen receptor activity

Several studies have observed an association between increasing *AR*-CAG repeat length and a linear decrease in AR transactivation activity [53–56]. Consistent with this, male carriers of the short *AR*-CAG repeat length are at increased risk for prostate cancer [51,57–62].

AR-CAG repeat length and breast cancer

The association between the length of the *AR*-CAG repeat polymorphism and breast cancer risk has been examined in several case-control studies (Table 4) [63–68]. The long *AR*-CAG repeat, which is representative of the less active AR, was associated with a statistically significant increase in breast cancer risk in a population of women from Quebec [66] and in a population of *BRCA1* mutation carriers [63]. Four additional studies [64,65,67,69] reported slightly increased risk for breast cancer associated with the long allele, but none of these findings were statistically significant. A study nested within the Nurses' Health Study cohort [68] found no increased breast cancer risk associated with the long AR allele overall, but an increased risk was observed when analyses were limited to those individuals with a first-degree family history of breast cancer (odds ratio 1.70, 95% confidence interval 1.2–2.4). Another trinucleotide repeat in the *AR*, a GGC repeat, has been observed to be associated with prostate cancer risk [59,60,62,70,71]. One of three studies that examined the GGC repeat length and breast cancer [65,67,69] found a significant association in women diagnosed before age 45 years [69], but no evidence of an interaction between the CAG and GGC repeat with breast cancer risk was observed.

The three studies that reported a significant association between long *AR*-CAG repeat and breast cancer risk [63,66,68] included both premenopausal and postmenopausal women. One study stratified with respect to menopausal status and found that the significant association with the long *AR*-CAG repeat was observed only in postmenopausal women (odds ratio 3.22, 95% confidence interval 1.54–6.75) and not in premenopausal women (odds ratio 1.03, 95% confidence interval 0.43–2.48). If this effect modification is true then it may explain, at least in part, the nonsignificant results in the studies restricted to women aged under 40 years [64].

Issues with the studies of AR-CAG repeat length and breast cancer risk

The gene for the *AR* lies on the X chromosome, and therefore women carry two alleles whereas men carry only a

Table 4

Relative risk for breast cancer associated with the AR-CAG repeat

Author, year [reference]	Population	AR genotype ¹	Long allele	Cases	Controls	Adjusted for	RR (95% CI)
Rebbeck, 1999 [63]	Multi-institutional study of <i>BRCA1</i> mutation carriers ascertained through families with a history of breast and/or ovarian cancer between 1978 and 1997	SS SL+LL	28+ repeats	19 146			1.81 (1.06–3.08) ²
Spurdle, 1999 [64]	Early onset breast cancer (<40 years) and age matched controls from Australia	SS SL LL	21+ repeats	78 189 101	71 138 75	Age, country of birth, state, education, marital status, number of live births, height, weight 1 year ago, age at menarche, oral contraceptive use, family history, estrogen receptor polymorphism, mother's country of birth, father's country of birth	1.4 (0.92–2.15) 1.4 (0.87–2.26)
Dunning, 1999 [65]	Cases from East Anglian region of the UK and random controls from the EPIC cohort	SS SL LL	23+ repeats	209 215 84	160 212 54		0.82 (0.62–1.09) 1.31 (0.87–1.97)
Ciguere, 2001 [66]	Incident cases from Quebec city and age and area of residency matched controls	SS ³ SL+LL	21+ repeats	17 238	61 400	Matched analysis	2.14 (1.22–3.73)
Kadouri, 2001 [67]	Affected and unaffected <i>BRCA1/2</i> carriers from two genetics clinics: one in Jerusalem, Israel, and the other in London, UK	SS SL+LL	28+ repeats				<i>BCRA1/2</i> carriers: 0.80 (0.44–1.46) Noncarriers: 1.27 (0.83–1.96)
Haiman, 2002 [68]	Cases and controls from the Nurses' Health Study and controls matched on year of birth, menopausal status, postmenopausal hormone use, and time of day, month, and fasting status at blood draw	SS SL+LL	22+ repeats	179 548	247 713	Age at menarche, parity, age at first birth, BMI at age 18 years, weight gain since age 8, benign breast disease, first degree family history, duration of postmenopausal hormone use; matched analysis	1.06 (0.83–1.35)
Suter, 2003 [69]	Cases (<45 years) identified through the Cancer Surveillance System of Western Washington and frequency-matched controls on 5-year age group and reference year	SS SL LL	22+ repeats	121 255 148	122 206 133	Age at reference and reference year	1.3 (0.9–1.8) 1.2 (0.8–1.7)

¹S, short allele; L, long allele. ²Analyses using 29 and 30 repeats as the cut-point produced progressively higher significant risk estimates and progressively earlier age of onset, no trend test.³Published odds ratio models the SS genotype as the high-risk allele. BMI, body mass index; CI, confidence interval; RR, relative risk.

single allele. In general, normal women are a mosaic, with one allele randomly expressed in each cell. A recent study [72] reported that 13% of young (27–45 years old) breast cancer cases exhibited preferential activation of one of the *AR* alleles as measured by genotyping of peripheral blood DNA, but there was no preference toward the allele with the longer or shorter CAG repeat. Analyses of the *AR* in women that only consider the length of the CAG repeat on one allele assume that this is the active allele in the breast tissue. Analyses that use the average of the CAG repeat lengths or the sum of the repeats consider the contributions of both alleles; however, if only one allele is preferentially expressed then this would result in misclassification. There is a high rate of heterozygosity in the *AR*-CAG repeat length, and therefore this is likely to be a major misclassification problem, which should bias the results toward the null. Genotyping methods can be optimized to detect better whether there is a preferentially active *AR* allele by either genotyping tumor tissue or serum DNA using methylation sensitive enzymes [72].

Summary

The studies conducted thus far suggest that the long *AR*-CAG repeat (less active *AR*) may be associated with increased breast cancer risk in women who are postmenopausal, have a first-degree family history of breast cancer or who have a known *BRCA1* mutation. The location of the *AR* gene on the X chromosome means that results from epidemiologic studies will be biased toward the null as long as we do not know which allele is expressed.

Discussion

If the long *AR*-CAG repeat (less active *AR*) is associated with increased breast cancer risk in postmenopausal women, then how do these results coincide with results showing that increased testosterone levels increase postmenopausal breast cancer risk?

One hypothesis to explain this apparent paradox is that the less active *AR* may be involved in a physiologic feedback associated with increased circulating testosterone. However, the only data available discount this hypothesis. Two studies have examined the association between the *AR*-CAG repeat length and circulating testosterone levels in normal women [68,73]. *AR*-CAG repeat length was inversely associated with testosterone levels. In other words, the less active *AR* was associated with lower circulating testosterone levels, and the results were statistically significant both in a study of premenopausal women [73] and in a study of postmenopausal women [68].

If the *AR* is not involved in a feedback mechanism to influence testosterone levels in postmenopausal women, then it is possible that the effect of testosterone on the breast epithelium does not act through binding to the *AR*. Testosterone may exert its effect on breast tissue through con-

version of testosterone to estrone, which is then aromatized into estradiol in adipose tissue, and the increased estradiol levels may result in increased breast cell proliferation and breast cancer risk.

Testosterone may also exert an indirect effect on breast cancer proliferation by sequestering sex hormone binding globulin, leaving more estradiol in the non-protein-bound state and able to act on breast tissue [25,74]. Approximately 66% of total testosterone is bound to sex hormone binding globulin, 31% is bound to albumin, and 2% is bound to cortisol binding protein [75]. Two of the studies suggesting an association between testosterone and breast cancer [16,17] reported that this association disappeared when adjusting for estradiol levels. However, in the pooled analysis [20] the significant association between testosterone and breast cancer risk remained after adjustment for estradiol [20].

Finally, it is possible that further studies will show that *AR*-CAG repeat length is not linked to breast cancer risk.

Conclusion

Prospectively conducted epidemiologic studies have found that increased levels of serum testosterone are associated with an increase in postmenopausal breast cancer risk. However, a number of questions remain. Several lines of evidence suggest a role of *AR* in breast cancer risk, and sparse epidemiologic data suggest that a long *AR*-CAG repeat yielding a less active *AR* may be associated with increased risk. There still remain a number of questions on how testosterone increases breast cancer risk. Although *in vitro* studies report both proliferative and antiproliferative effects of testosterone on the growth of various breast cancer cell lines, we still need to further understand under which *in vivo* circumstances does testosterone exert these effects. Finally, we do not know whether androgens affect breast cancer risk in premenopausal women. Further analyses of the role of *AR*-CAG repeat length and breast cancer using genotyping methods that assess which allele is the active *AR* allele are clearly needed. Additional data are also needed to help elucidate the apparent paradox between the *AR*-CAG repeat length, testosterone levels, and breast cancer risk.

Competing interests

None declared.

Acknowledgements

This research (EOL) was supported by funds from the California Breast Cancer Research Program, Grant Number 8GB-0010.

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