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SELECTED ESTROGEN RECEPTOR 1 AND ANDROGEN RECEPTOR GENE POLYMORPHISMS IN RELATION TO RISK OF BREAST CANCER AND FIBROCYSTIC BREAST CONDITIONS AMONG CHINESE WOMEN

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Abstract

Background—Polymorphisms in sex hormone receptor-encoding genes may alter the activity of sex hormone receptors and thereby affect susceptibility to breast cancer and related outcomes.

Methods—In a case-control study of women from Shanghai, China, we examined the risk of breast cancer and fibrocystic breast conditions associated with the *ESR1* *PvuII* (rs2234693) and *XbaI* (rs9340799) and *AR* CAG repeat ((CAG)_n) and GGC repeat ((GGC)_n) polymorphisms among 614 women with breast cancer, 467 women with fibrocystic conditions, and 879 women without breast disease. We also evaluated whether risk differed by the presence/absence of

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proliferative changes (in the extratumoral epithelium or fibrocystic lesion), menopausal status, or body mass index (BMI). Age-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using logistic regression.

Results—Only associations with AR (CAG)_n and (GGC)_n genotypes were detected. Allocating AR (CAG)_n genotypes into six categories, with the (CAG)_{22–24}/(CAG)_{22–24} genotype category designated as the reference group, the (CAG)_{>24}/(CAG)_{>24} genotype category was associated with an increased risk of fibrocystic breast conditions (OR, 1.8; 95% CI, 1.1–3.0). Relative to the AR (GGC)₁₇/(GGC)₁₇ genotype, the (GGC)₁₇/(GGC)₁₄ genotype was associated with elevated risks of incident breast cancer (OR, 2.6; 95% CI, 1.3–5.4) and fibrocystic conditions (OR, 2.3; 95% CI, 1.1–4.5). Results did not differ according to proliferation status, menopausal status, or BMI.

Conclusion—Although these data lend support for a link between AR variation and breast disease development, given the low frequency of the putative risk-conferring genotypes and other constraints, further confirmation of our results is needed.

Keywords

Estrogen receptor alpha; androgen receptor; genetic polymorphism; breast neoplasm; fibrocystic breast disease; Chinese

The biological effects of sex steroid hormones are mediated in part by their binding to nuclear receptors in various tissues, including the mammary gland. Once bound to sex hormones, these receptors, functioning as transcription factors, attach to hormone-response elements in the promoter region of target genes and recruit coregulatory proteins, which together influence the rate of target gene expression [1]. Polymorphic variation in sex hormone receptor-encoding genes may therefore alter the activity of the receptor molecules, and in turn, susceptibility to breast cancer and related conditions.

In the estrogen receptor 1 (*ESR1*) gene (chromosome 6q25.1), which encodes for ERα, two single nucleotide polymorphisms (SNPs) located in intron 1 have been commonly studied in relation to breast cancer risk: a T/C substitution at –397 bp (also known as *ESR1 PvuII* or rs2234693) and an A/G substitution at –351 bp (also known as *ESR1 XbaI* or rs9340799) upstream from exon 2. The results from most studies of Caucasian women have been statistically null [2–11]. In comparison, those from studies of Asian women have been conflicting [12–16]. A recent meta-analysis ([17]; including 11 studies for rs2234693 and 10 studies for rs9340799) suggests that the rs2234693 CT/CC (versus TT) genotypes may confer a marginal reduction in breast cancer risk (OR, 0.95; 95% CI: 0.89–1.00), although less so when stratifying by ethnicity (Caucasian: OR, 0.97; 95% CI: 0.91–1.03; Asian: OR, 0.92; 95% CI: 0.79–1.06). Whether similar results would be observed after accounting for several reports of null associations not considered in this meta-analysis [11,14,16,18], however, is unclear.

Within the first exon of the androgen receptor (AR) gene (chromosome Xq11–q12) lie two microsatellite polymorphisms, one with a CAG repeat [(CAG)_n] and one with a GGN repeat [(GGN)_n], both also posited to influence breast cancer risk [1]. Nested within the (GGN)_n sequence is a polymorphic GGC repeat [(GGC)_n]. The AR (CAG)_n and (GGN)_n polymorphisms encode for the polyglutamine and polyglycine tracts, respectively, which flank a ligand-independent transcription activating domain and modulate the transactivation activity of the AR protein [19]. Studies examining these AR repeat polymorphisms in relation to breast cancer risk, the majority of which have been conducted in Caucasian populations, have been inconclusive. Some [20–25] but not all studies [6,11,26–37], including those limited to *BRCA1* or *BRCA1/2* mutation carriers [23,28,33,37], have found an excess risk of breast cancer associated with longer (CAG)_n alleles. Although the results

of a recent meta-analysis [38] indicate that carrying at least one (versus no) ≥ 22 CAG allele may be instead linked to reduced breast cancer risk (OR, 0.86; 95% CI: 0.67–1.10), several studies were not evaluated [11,21,22,24,28,32], including the two largest showing evidence of no association [11,32]. Of the studies examining breast cancer risk associated with the *AR* (GGN)_n or (GGC)_n polymorphism [25,27,28,30,39], two have found an association between longer (GGC)_n alleles and reduced risk of early-onset breast cancer [30,39].

Relatively little investigation has been conducted on the potential association of sex hormone receptor gene polymorphisms with fibrocystic breast conditions. Although the etiology of these benign conditions, which encompass non-proliferative breast disease, proliferative disease without atypia, and atypical hyperplasia, is not well understood, women who develop proliferative breast lesions have a greater risk of breast cancer than women who develop non-proliferative breast lesions [40–42]. Limited data also suggest that *ESR1* and *AR* polymorphisms may influence the progression of benign breast disease to breast cancer [43,44]. Therefore, investigating whether *ESR1* and *AR* loci are related to fibrocystic breast changes, especially those exhibiting proliferative features, may assist in further elucidating the origins of breast cancer.

In this report, we present the results of our case-control study examining the relationship of selected *ESR1* and *AR* polymorphisms with the risk of breast cancer and fibrocystic breast conditions in Chinese women.

MATERIALS AND METHODS

Study Design and Population

As described previously [45], the study population includes female members of a randomized trial of regular breast self-examination and breast cancer mortality conducted in Shanghai, China [46,47], who participated in ancillary case-control studies of benign and malignant breast conditions conducted between September 1995 and July 2000. Eligible cases were women diagnosed by breast biopsy with a fibrocystic breast condition from September 1995 to July 2000 or with breast cancer from November 1989 to July 2000. Among those women diagnosed from September 1995 to July 2000, 551 (89% of the 622 eligible) cases with fibrocystic breast conditions and 376 (88% of the 426 eligible) cases with breast cancer were interviewed in person, of whom 470 and 323 provided a blood sample, respectively. The recruitment of women diagnosed with breast cancer prior to September 1995 was performed retrospectively, with preferential selection of cases under age 45 and a similar number of older cases. Among the 830 earlier diagnosed (or prevalent) breast cancer cases, 153 of the 270 cases under age 45 and 147 of the 560 cases ages 45 and older were interviewed and provided a blood sample. The breast cancer cases in this study were generally comparable to breast cancer cases in the entire cohort, respectively, in terms of risk factors ascertained at baseline, including parity (94.1% vs. 93.8%), age at first birth (for ≥ 30 years: 26.3% vs. 21.2%), breastfeeding history (83.4% vs. 84.4%), prior history of a breast lump (6.5% vs. 7.4%), and family history of breast cancer (4.0% vs. 3.5%).

Eligible controls were women selected randomly from the trial cohort who had not received a breast biopsy. For each case diagnosed between September 1995 and August 1997, two controls were selected by matching on age and menstrual status. For each diagnosed between September 1997 and July 2000, controls were frequency matched on age (in 5-year groups) and the hospital affiliation of their factories at baseline. All controls had not been previously diagnosed with breast cancer at the time of interview. Of the 1,071 (75% of the eligible) controls interviewed, 911 provided a blood sample.

Informed consent was obtained from each participant prior to interview. The study protocol was approved by the Institutional Review Office of the Fred Hutchinson Cancer Research Center; the Station for Prevention and Treatment of Cancer of the Shanghai Textile Industry Bureau in accordance with an assurance filed with the Office for Human Research Protections of the U.S. Department of Health and Human Services; and the Human Genetic Resources Administration Office of China.

Histologic Classification of Breast Tissue

A single pathologist provided an independent histologic diagnosis of every case. Also for cases diagnosed after September 1995, this pathologist classified benign changes, either in the lesion of women with fibrocystic conditions alone or in the extratumoral epithelium of women with breast cancer, as non-proliferative or proliferative when sufficient mammary gland tissue (at least five scanning power fields, excluding areas of fibroadenoma) was available for microscopic review. The classification scheme used was developed by Stalsberg and co-workers [48]. The procedures implemented to assure reliable histologic classification have been described previously [49]. Any form of atypical hyperplasia, moderate or florid ductal hyperplasia, or moderate or predominant sclerosing adenosis without atypia were classified as proliferative changes; mild or no ductal hyperplasia and mild or no sclerosing adenosis without atypia were classified as non-proliferative changes. Proliferation status was determined for 338 (72%) of the 470 fibrocystic cases and for 243 (75%) of the 323 cancer cases diagnosed after September 1995.

Genotyping

DNA extraction—Genomic DNA was extracted from buffy coat samples using a salt-precipitation [50] and/or a phenol-chloroform method [51]. In addition, genomic DNA was extracted from whole blood using the QIAamp® DNA Blood Midi Kit (Qiagen, Alameda, CA), if available, when the quantity of DNA isolated from buffy coat was insufficient for genotyping and when the genotyping of *AR* and other microsatellite polymorphisms indicated possible sample contamination. DNA concentration was quantified by UV spectrophotometry.

***ESR1* polymorphisms**—SNaPshot™ assays (Applied Biosystems (ABI), Foster City, California) were used to determine *ESR1* rs2234693 and rs9340799 genotypes, following methods similar to Modugno et al. [52]. The SNPs were co-amplified with the forward and reverse primers 5'-ATC-CAG-GGT-TAT-GTG-GCA-ATG-AC-3' and 5'-ACC-CTG-GCG-TCG-ATT-ATC-TGA-3'. Probes for the rs2234693 and rs9340799 SNPs were 5'-(GACT)₇-GGG-AAA-CAG-AGA-CAA-AGC-ATA-AAA-C-3' and 5'-(GATC)₁₆-GAC-CAA-TGC-TCA-TCC-CAA-CTC-3', respectively.

***AR* polymorphisms**—The number of CAG repeats was determined by amplification in a single PCR using the secondary primers (2A/2B) described by Tsai *et al.* [53]. The forward and reverse primers were 5'-AGA-GGC-CGC-GAG-CGC-AGC-ACC-TC-3' (labeled with NED) and 5'-GCT-GTG-AAG-GTT-GCT-GTT-CCT-CAT-3'. The number of GGC repeats was determined by nested PCR amplification using primers similar to those described by Hsing *et al.* [54] for the primary reaction and by Irvine *et al.* [55] for the secondary reaction. The forward and reverse primers were 5'-ACC-CTC-AGC-CGC-CGC-TTC-3' and 5'-CTG-GGA-TAG-GGC-ACT-CTG-CTC-AC-3' for the initial reaction and 5'-ACT-CTC-TTC-ACA-GCC-GAA-GAA-GGC-3' (labeled with PET) and 5'-ATC-AGG-TGC-GGT-GAA-GTC-GCT-TTC-C-3' for the secondary reaction.

Quality control—In each batch, three in-house genomic DNA samples of known genotype and a reaction without DNA template were included as positive and negative controls,

respectively. For a given assay, the same set of positive controls was run in each batch. For the *ESR1* assays, positive controls included a homozygote for the major allele, a heterozygote, and a homozygote for the minor allele. For the *AR* assays, representative clones with confirmed CAG and GGC sequences served as references for correct allele sizing. Laboratory personnel were blinded to the case-control status of all samples.

Possible contamination was evaluated in the process of genotyping all samples for *AR* and other microsatellite polymorphisms. Those samples that appeared to be contaminated, even after repeating the microsatellite assays, were further evaluated by comparing the genotype results between paired buffy coat and whole blood samples whenever possible. Samples were labeled as “contaminated” if (a) genotyping results of paired samples were discordant or (b) paired samples were not available for such assessment.

Statistical Analysis

Analyses were based on a total of 614 women with breast cancer, 467 women with fibrocystic breast conditions, and 879 women without clinical breast disease, after excluding 43 women (8 with breast cancer, 3 with fibrocystic breast conditions, and 32 without clinical breast disease) due to possible sample contamination and one additional woman with breast cancer due to a lack of sufficient genomic DNA. Using logistic regression, age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the relative risk of breast cancer and fibrocystic breast conditions associated with the genotypes of each polymorphism. To ensure analyses of the *AR* (CAG)_n polymorphism were based on categories of sufficient size, the following six genotype categories were defined according to the number of CAG repeats (n) in each *AR* allele: (CAG)_{22–24}/(CAG)_{22–24}, (CAG)_{22–24}/(CAG)_{<22}, (CAG)_{22–24}/(CAG)_{>24}, (CAG)_{<22}/(CAG)_{<22}, (CAG)_{<22}/(CAG)_{>24}, and (CAG)_{>24}/(CAG)_{>24}. These cutpoints were chosen to approximate the 25th and 75th percentiles of the *AR* (CAG)_n allele distribution among controls and allow direct comparison of the results of our study to others, the majority of which have allocated *AR* (CAG)_n genotypes into two categories, <22 (i.e., (CAG)_{<22}/(CAG)_{<22}) and ≥22 (i.e., one or more (CAG)_{≥22} alleles). Since roughly 47% of all *AR* (CAG)_n alleles among controls were comprised of 22 to 24 CAG repeats, the category of (CAG)_{22–24}/(CAG)_{22–24} was designated as the reference group. For the *AR* (GGC)_n polymorphism, risk estimates were calculated for each genotype with an observed frequency of >1%, relative to the major homozygous genotype (i.e., (GGC)₁₇/(GGC)₁₇); those genotypes with an observed frequency of <1% were combined into a single category. Multivariable-adjusted ORs and 95% CIs have not been presented, since further adjustment for recognized risk factors of breast cancer, including parity, age at first birth, breastfeeding history, prior history of a breast lump, and family history of breast cancer, did not materially alter our results.

For the subset of women with sufficient non-malignant breast tissue available for histologic classification, the aforementioned analyses were repeated, stratifying on proliferation status. Subgroup analyses were also conducted to evaluate whether the risk of breast cancer and fibrocystic breast conditions associated with *ESR1* and *AR* genotypes differed by menopausal status (premenopausal, postmenopausal) or BMI (<23.0 kg/m², ≥23.0 kg/m²). The presence of effect modification by menopausal status and BMI was assessed using the likelihood ratio test, comparing nested models with and without a cross-product term for SNP genotype and the exposure of interest. No formal adjustment for multiple comparisons was performed. All analyses were performed using Stata[®] 9 (StataCorp, College Station, TX).

RESULTS

Genotype data quality measures

Of the positive control samples, 96.7% (87 of 90) were successfully genotyped for the *ESR1* rs2234693 and rs9340799 polymorphisms, 94.2% (65 of 69) were successfully genotyped for the *AR* (CAG)_n polymorphism, and 100% (70 of 70) were successfully genotyped for the *AR* (GGC)_n polymorphism. Genotyping accuracy for the positive control samples (after excluding those few samples of undetermined genotype) was 98.9% for the *ESR1* rs2234693 polymorphism and 100% for the other three polymorphisms studied. For the samples of study participants, genotyping success for each polymorphism exceeded 99%. None of the *ESR1* and *AR* genotype frequency distributions among controls deviated from Hardy-Weinberg equilibrium.

Population characteristics

Age-adjusted frequencies of selected characteristics by case-control status are presented in Table 1. Frequency distributions were adjusted to the age distribution of the controls, given age differences by case status. Relative to the controls, the cases with breast cancer were more likely to be nulliparous, postmenopausal, and have a higher BMI, whereas the cases with fibrocystic breast conditions were more likely to be premenopausal. Also, both sets of cases were more likely to experience menarche earlier, have a prior history of breast lumps, and have a family history of breast cancer than controls.

ESR1

Similar *ESR1* allele frequencies were observed for cases with breast cancer, cases with fibrocystic breast conditions, and controls. Accordingly, the age-adjusted risk estimates for breast cancer and fibrocystic breast conditions associated with the *ESR1* rs2234693 and rs9340799 genotypes, when examined separately and jointly, were close to unity (Table 2). There were no marked differences in risk for either disease outcome by proliferation status, menopausal status, or BMI, although some stratum-specific odds ratio estimates were based on small numbers (Supplemental Tables 1 and 2). These inferences remained unchanged after excluding the prevalent cases of breast cancer from the analysis.

AR

The *AR* (CAG)_n and (GGC)_n allele frequency distributions by case-control status are presented in Figure 1. Alleles of the *AR* (CAG)_n polymorphism ranged from 6 to 41 CAG repeats in length, with the most common allele spanning 22 CAG repeats. Alleles of the *AR* (GGC)_n polymorphism ranged from 3 to 21 GGC repeats in length. The most common alleles contained 13, 16, 17, or 18 GGC repeats, together accounting for >94% of all alleles genotyped.

Common genotypes of both *AR* (CAG)_n and (GGC)_n polymorphisms were related to breast cancer risk (Table 3). Specifically, we observed a modest increase in breast cancer risk associated with the (CAG)_{22–24}/(CAG)_{>24} genotype category (age-adjusted odds ratio (OR), 1.4; 95% confidence interval (CI): 1.0, 1.9), but not the (CAG)_{>24}/(CAG)_{>24} genotype category (OR 1.2; 95% CI: 0.73–1.9), relative to the (CAG)_{22–24}/(CAG)_{22–24} genotype category. We also found an elevated OR for breast cancer associated with the (GGC)₁₇/(GGC)₁₄ genotype, relative to the (GGC)₁₇/(GGC)₁₇ genotype (OR 1.9; 95% CI: 0.98–3.7). Excluding the prevalent breast cancer cases from the analysis, the ORs associated with the (CAG)_{22–24}/(CAG)_{>24} and (CAG)_{>24}/(CAG)_{>24} genotype categories were similar; the OR associated with the (GGC)₁₇/(GGC)₁₄ genotype was greater in magnitude (OR, 2.6; 95% CI: 1.3–5.4). Breast cancer risk estimates did not differ by proliferation status or BMI (Supplemental Table 3). There was suggestive evidence that associations of the (CAG)_{22–24}/

(CAG)_{>24} genotype category and the (GGC)₁₇/(GGC)₁₄ genotype with breast cancer risk may be stronger among postmenopausal than premenopausal women (Supplemental Table 3).

The (CAG)_{>24}/(CAG)_{>24} genotype category, relative to the (CAG)₂₂₋₂₄/(CAG)₂₂₋₂₄ genotype category, was associated with an increased risk of fibrocystic breast conditions (Table 3; OR 1.8; 95% CI: 1.1–3.0). The (GGC)₁₇/(GGC)₁₄ genotype was also positively associated with the risk of fibrocystic breast conditions (OR 2.3, 95% CI: 1.1–4.5), although there were only 20 cases of fibrocystic breast conditions and 16 controls with this genotype. These associations generally persisted irrespective of proliferation status, menopausal status, or BMI (Supplemental Table 4).

DISCUSSION

Given that estrogens and androgens, which act via binding to their receptor proteins, are critical in female breast development, interindividual differences in susceptibility to breast cancer and fibrocystic breast conditions may arise from *ESR1* and *AR* gene variation. Assessing the risk of breast cancer and fibrocystic breast conditions associated with selected *ESR1* and *AR* polymorphisms in Chinese women, we found that risks for both outcomes were associated with the *AR* polymorphisms only. Due to the modest sample size and lack of hormone receptor status data, however, we cannot exclude the possibility of *ESR1* rs2234693 and rs9340799 associations that are small in magnitude, stronger in certain subgroups, or exclusive to specific tumor subtypes. Also with only four polymorphisms examined, we acknowledge that associations could exist with unmeasured polymorphisms in either gene and that the associations observed may be explained instead by polymorphisms in strong linkage with the *AR* microsatellite polymorphisms.

Of the studies conducted in China [12,14,15,18], the earliest and largest study (1069 cases, 1166 controls) found a 40% increase in breast cancer risk associated with the *ESR1* rs2234693 CT/TT (versus CC) genotype [12]. While this finding has not been confirmed in subsequent studies, the frequency of the T allele among their controls (60.3%) was similar to ours (61.8%) and those of the other Shanghai studies [range: 59.0% [18] to 61.7% [15]]. There has been one report of a link between rs9340799 and breast cancer from a hospital-based study of 138 cases and 140 controls in Beijing, in which the GA/GG (versus AA) genotype was associated with reduced risk [14]. The frequency of the rs9340799 G allele, however, was higher for the controls in this study (28.9%) than all those performed in Shanghai [range: 18.4% [18] to 25.9% [12]], including ours (19.2%), suggesting that this finding could have arisen from an overestimate of the expected allele frequency in the controls rather than a deficiency in the cases.

We found that *ESR1* rs2234693 and rs9340799 genotypes were unrelated to the risk of fibrocystic breast conditions, irrespective of proliferation status. To date, only Gallicchio *et al.* have investigated the role of estrogen receptor gene polymorphisms in the progression from benign to malignant breast disease [43]. In their study of 1,438 Caucasian women with benign breast disease, subsequent breast cancer risk was marginally associated with one of the six *ESR1* variants and one of the four *ESR2* variants examined. Taken together, it remains unclear whether *ESR1* polymorphisms contribute to the development of fibrocystic breast lesions or their progression to breast cancer.

The role of *ESR1* variation in breast cancer etiology merits further investigation, considering recent evidence suggesting that polymorphisms located upstream and within *ESR1* alter breast cancer susceptibility in Chinese women. In a multi-stage genome-wide association study, an association with breast cancer risk was identified for rs2046210, a SNP situated 29

kb upstream of the first untranslated exon and 180 kb upstream of the transcription start site of the first exon of *ESR1* [56]. In a hospital-based case-control study of breast cancer, associations were detected for several other *ESR1* SNPs in intron 1 – rs3778609, rs12665044, and rs827421 – all located in the same haplotype block [57]. The SNP exhibiting the strongest association with breast cancer risk, rs3778609, was also associated with surrogate measures of lifetime estrogen exposure, including ages at menarche and first full-term pregnancy, and with clinical phenotypes, including ER α tumor expression and tumor grade/stage [57]. Neither the SNPs that we examined (rs2234693 and rs9340799), nor rs2046210, rs12665044, and rs827421, are in strong linkage disequilibrium (LD) with one another (pairwise $r^2 \leq 0.80$ in HapMap CHB samples); the extent of LD with rs3778609 is unknown, since it is not in the HapMap database.

Our data offers some support to the hypothesis that a woman's risk for developing fibrocystic breast conditions and breast cancer is influenced by *AR* CAG and GGC repeat length. Relative to the carriage of two 22–24 CAG repeat alleles, the carriage of two >24 CAG repeat alleles was associated with an 84% increase in the risk of fibrocystic breast conditions. Greater than two-fold increased risks for fibrocystic breast conditions and incident breast cancer were associated with the (GGC)₁₇/(GGC)₁₄ genotype, relative to the (GGC)₁₇/(GGC)₁₇ genotype, with the association for breast cancer somewhat more pronounced in postmenopausal women. Our study was not well powered to detect subgroup differences in risk, however, especially for the putative risk-bearing *AR* genotypes, which were relatively uncommon in the study population. Also with the number of comparisons made, these associations may have arisen simply by chance and thus warrant confirmation in other studies.

Drawing firm conclusions from our results, in conjunction with those of prior association studies of *AR* polymorphisms and breast cancer, is challenging for additional reasons. To our knowledge, the present study is the first to examine both *AR* (CAG)_n and (GGC)_n polymorphisms in relation to breast cancer risk among Chinese women, as well as the first to examine these polymorphisms in relation to the risk of fibrocystic breast conditions. It is also the first to allocate *AR* (CAG)_n genotypes into finer categories and to evaluate individual *AR* (GGC)_n genotypes. In previous analyses, *AR* CAG and GGC alleles have been broadly classified as either “short” or “long” according to some designated number of repeats. When applying this less specific classification of *AR* (CAG)_n and (GGC)_n genotypes to our data, namely with long alleles defined as ≥ 22 CAG repeats and ≥ 17 GGC repeats, no associations were observed. The age-adjusted ORs (and 95% CIs) for breast cancer and fibrocystic breast conditions associated with the carriage of one or more (vs. no) long CAG alleles were 1.04 (0.71–1.51) and 1.13 (0.74–1.74), respectively, and those associated with the carriage of one or more (vs. no) long GGC alleles were 1.26 (0.66–2.39) and 1.07 (0.54–2.13), respectively. If *AR* CAG or GGC alleles of only specific lengths influence breast cancer risk, then the methods used previously to analyze *AR* (CAG)_n and (GGC)_n genotype data may have been too insensitive, thereby precluding associations from being consistently detected across studies.

Since the *AR* gene is located on the X chromosome, there is potentially added misclassification owing to skewed X chromosome inactivation. Normally, of the two X chromosomes present in female mammalian cells, one is inactivated at random during embryogenesis, resulting in about 50% of cells expressing the maternally inherited X chromosome and 50% of cells expressing the paternally inherited X chromosome. Skewed X chromosome inactivation, or the preferential expression of one X chromosome in at least 90% of cells, however, is a putative risk factor for both familial and sporadic breast cancer [58,59]. Approximately 90% of women are heterozygous for the *AR* gene due to variation in CAG and GGC repeat length [19]. Therefore, without data on which X chromosome is

preferentially expressed, the extent to which the results of this study and others are biased is unclear.

Furthermore, the biologic mechanisms by which *AR* variation may affect androgen production and sensitivity to result in the malignant transformation of breast tissue are not well understood. A shorter *AR* CAG repeat length has been linked to greater *AR* transactivation activity [60] and also increased circulating testosterone among healthy women in some [61,62] but not all [29,39] studies. The positive association that we found between the carriage of two >24 CAG repeat alleles and breast disease thus appears to conflict with published evidence linking greater circulating testosterone to increased breast cancer risk [63]. In the only known study to examine *AR* GGN or GGC length in relation to androgen levels in women, longer GGC length (≥ 17) has been associated with lower testosterone levels in users, but not non-users, of oral contraceptives [39]. Although specifically observed in healthy, premenopausal women from high-risk breast cancer families, this inverse relation between GGC length and testosterone level, if true and generalizable, could explain our noted increases in risk of fibrocystic breast conditions and breast cancer for the (GGC)₁₇/(GGC)₁₄ to (GGC)₁₇/(GGC)₁₇ genotype comparison.

With the majority of our breast cancer cases and a subset of our cases with fibrocystic breast conditions and controls previously genotyped for *BRCA1* and *BRCA2* mutations [64], we investigated whether the risk of breast cancer or fibrocystic breast conditions associated with the selected *ESR1* and *AR* polymorphisms might differ by *BRCA1/2* mutation status in our study population. These exploratory analyses did not yield any noteworthy findings, but given the relatively low prevalence of any *BRCA1* or *BRCA2* mutations (as specified in ref [30], breast cancer: 19.0%; fibrocystic breast: 15.6%; controls: 15.9%), the statistical power to detect any such risk differences was limited.

In summary, our data suggest that susceptibility to developing fibrocystic breast conditions and breast cancer is linked to the number of *AR* CAG and GGC repeats, but not *ESR1* rs2234693 or rs9340799 genotype, among Chinese women. Until additional studies can fully assess the relationship between *AR* repeat length polymorphisms and breast disease in the context of X chromosome inactivation and clarify the relationship between *AR* variation and androgen sensitivity in women, however, our findings should be interpreted cautiously.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AR	androgen receptor
BMI	body mass index

BSE	breast self-examination
CI	confidence interval
ER	estrogen receptor
ESR1	estrogen receptor 1
OR	odds ratio
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism

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Figure 1.
AR CAG and GGC repeat allele frequency distributions by case-control status

Table 1

Selected characteristics^a for controls and cases of breast cancer and fibrocystic breast conditions

Characteristic ^b	Controls (n=879)		Breast Cancer (n=614)		Fibrocystic Breast Conditions (n=467)	
	n	%	n	%	n	%
Age (years)						
<40	12	1.4	77	12.5	62	13.3
40-44	376	42.8	169	27.5	200	42.8
45-49	171	19.4	97	15.8	123	26.3
50-54	64	7.3	40	6.5	22	4.7
55-59	38	4.3	47	7.7	12	2.6
60-64	106	12.1	99	16.1	15	3.2
≥65	112	12.7	85	13.8	33	7.1
Age at menarche (years)						
<13	140	15.9	104	17.0	89	16.8
14	176	20.0	118	20.7	109	23.1
15	161	18.3	134	21.8	93	20.1
16	185	21.1	116	20.0	77	15.6
≥17	216	24.6	139	20.5	97	24.5
Number of live births						
Nulliparous	36	4.1	37	6.2	23	4.8
1	560	64.0	318	57.3	362	64.0
2	110	12.6	105	16.2	37	11.3
≥3	169	19.3	152	20.3	40	19.9
Age at first birth (years)						
Nulliparous	36	4.1	37	6.2	23	4.8
<25	236	27.0	183	26.4	79	27.4
25-29	476	54.4	285	48.8	284	53.9
≥30	127	14.5	105	18.5	75	13.9
Age at menopause (years)						
Pre-menopausal	529	60.2	293	53.1	375	64.3

Characteristic ^b	Cases					
	Controls (n=879)		Breast Cancer (n=614)		Fibrocystic Breast Conditions (n=467)	
	n	%	n	%	n	%
<45	61	6.9	64	10.9	20	5.6
45-49	127	14.4	117	17.7	35	12.6
≥50	162	18.4	140	18.3	37	17.4
Body mass index (kg/m ²)						
<23.0	385	43.8	236	38.2	247	46.6
≥23.0	494	56.2	376	61.8	218	53.4
Prior breast lumps						
Never	828	96.6	548	93.4	395	89.4
Ever	29	3.4	38	6.6	50	10.6
First degree relative with breast cancer						
No	864	98.3	586	95.8	448	96.5
Yes	15	1.7	28	4.2	19	3.5

^aFor all characteristics except age, the direct age-adjusted percentages based on the age distribution of controls are presented for cases of breast cancer and fibrocystic conditions

^bData missing for age at menarche (n=6), number of live births (n=11), age at first birth (n=14), BMI (n=4), and prior breast lumps (n=72)

Table 2
Age-adjusted ORs and 95% CIs for breast cancer and fibrocystic breast conditions associated with *ESR1 PvuII* and *XbaI* genotypes

Genotype	Controls						Cases					
	Controls			Breast Cancer			Breast Cancer			Fibrocystic Breast Conditions		
	n	%		n	%	OR (95% CI)	n	%	OR (95% CI)	n	%	OR (95% CI)
<i>ESR1 PvuII</i> (rs2234693)												
TT	327	(37.4)		229	(37.4)	1.00 (reference)	180	(38.6)	1.00 (reference)			
CT or CC	547	(62.6)		383	(62.6)	1.00 (0.81–1.24)	286	(61.4)	0.98 (0.77–1.24)			
CT	427	(48.9)		290	(47.4)	0.97 (0.77–1.21)	220	(47.2)	0.96 (0.75–1.23)			
CC	120	(13.7)		93	(15.2)	1.10 (0.80–1.52)	66	(14.2)	1.03 (0.72–1.48)			
<i>ESR1 XbaI</i> (rs9340799)												
AA	569	(65.0)		395	(64.3)	1.00 (reference)	300	(64.4)	1.00 (reference)			
AG or GG	307	(35.0)		219	(35.7)	1.03 (0.83–1.27)	166	(35.6)	1.05 (0.82–1.33)			
AG	277	(31.6)		197	(32.1)	1.02 (0.82–1.28)	147	(31.5)	1.02 (0.80–1.31)			
GG	30	(3.4)		22	(3.6)	1.04 (0.59–1.84)	19	(4.1)	1.27 (0.69–2.33)			
<i>ESR1 PvuII</i> <i>ESR1 XbaI</i>												
TT AA	327	(37.4)		229	(37.4)	1.00 (reference)	180	(38.6)	1.00 (reference)			
CT AA	207	(23.7)		143	(23.4)	0.99 (0.75–1.29)	107	(23.0)	0.96 (0.71–1.29)			
CC AA	33	(3.8)		22	(3.6)	0.95 (0.54–1.67)	13	(2.8)	0.76 (0.39–1.51)			
CT AG	220	(25.2)		147	(24.0)	0.95 (0.73–1.25)	113	(24.2)	0.97 (0.72–1.30)			
CC AG	57	(6.5)		49	(8.0)	1.23 (0.81–1.86)	34	(7.3)	1.08 (0.67–1.74)			
CC GG	30	(3.4)		22	(3.6)	1.04 (0.58–1.84)	19	(4.1)	1.23 (0.66–2.28)			

Table 3

Age-adjusted ORs and 95% CIs for breast cancer and fibrocystic breast conditions associated with AR (CAG)_n and (GGC)_n genotypes

Genotype	Cases														
	Controls					Breast Cancer					Incident Breast Cancer Only				
	n	%	n	%	OR	95% CI	n	%	OR	95% CI	n	%	OR	95% CI	OR
<i>AR CAG repeat</i>															
22-24/22-24	201	(22.9)	121	(19.7)	1.00	(reference)	63	(19.7)	1.00	(reference)	96	(20.6)	1.00	(reference)	(reference)
22-24/<22	241	(27.4)	154	(25.1)	1.06	(0.78–1.43)	81	(25.4)	1.04	(0.71–1.53)	117	(25.1)	1.06	(0.76–1.49)	(0.76–1.49)
22-24/>24	184	(20.9)	157	(25.6)	1.41	(1.03–1.92)	85	(26.6)	1.46	(0.99–2.15)	108	(23.2)	1.22	(0.86–1.73)	(0.86–1.73)
<22/<22	74	(8.4)	50	(8.1)	1.12	(0.73–1.71)	25	(7.8)	1.07	(0.62–1.82)	35	(7.5)	1.00	(0.62–1.62)	(0.62–1.62)
<22/>24	125	(14.2)	94	(15.3)	1.25	(0.88–1.78)	46	(14.4)	1.21	(0.77–1.88)	65	(13.9)	1.06	(0.72–1.57)	(0.72–1.57)
>24/>24	54	(6.1)	38	(6.2)	1.16	(0.73–1.87)	19	(6.0)	1.12	(0.61–2.03)	45	(9.7)	1.84	(1.14–2.97)	(1.14–2.97)
<i>AR GGC repeat</i>															
17/17	505	(57.5)	365	(59.4)	1.00	(reference)	181	(56.7)	1.00	(reference)	267	(57.2)	1.00	(reference)	(reference)
17/18	92	(10.5)	54	(8.8)	0.81	(0.57–1.17)	32	(10.0)	0.97	(0.62–1.50)	51	(10.9)	1.06	(0.73–1.56)	(0.73–1.56)
17/16	70	(8.0)	44	(7.2)	0.87	(0.58–1.30)	22	(6.9)	0.90	(0.54–1.50)	26	(5.6)	0.65	(0.40–1.05)	(0.40–1.05)
17/13	82	(9.3)	59	(9.6)	0.99	(0.69–1.42)	30	(9.4)	0.98	(0.62–1.54)	45	(9.6)	1.12	(0.75–1.67)	(0.75–1.67)
17/14	16	(1.8)	22	(3.6)	1.90	(0.98–3.66)	15	(4.7)	2.63	(1.27–5.44)	20	(4.3)	2.25	(1.12–4.51)	(1.12–4.51)
All others	113	(12.9)	70	(11.4)	0.86	(0.62–1.19)	39	(12.2)	0.98	(0.65–1.46)	58	(12.4)	0.98	(0.68–1.40)	(0.68–1.40)