Review

Future possibilities in the prevention of breast cancer
Role of genetic variation in breast cancer prevention

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Abstract

Risk factors for breast cancer are related to endogenous hormones and reproductive events. As such, traditional cancer prevention strategies are not easily applicable. Tamoxifen and other selective estrogen receptor modulators (SERMs) offer a new preventive strategy for some high-risk women, but have not yet been shown to be efficacious for all women. New tools to identify high-risk women are needed. One such tool is the development of a multigenic model of breast cancer susceptibility that can be used to screen women in order to identify those who carry a combination of alleles that puts them at significantly increased risk.

Keywords: breast cancer, epidemiology, polymorphism, prevention, risk factors

Introduction

The traditional view of public health prevention strategies considers three broad categories. Primary prevention involves activities that are aimed at reducing and removing agents that increase risk. Secondary prevention includes early detection activities that are designed to reduce mortality. Finally, tertiary prevention aims to minimize or reduce long-term disability and suffering. Cancer prevention usually implies activities involved in primary or secondary prevention. For many cancer sites, we have made significant contributions in prevention. For example, the Pap smear has had a great impact on cervical cancer, and refrigeration and other safe food preservation practices have markedly reduced the incidence of stomach cancer. Largely because of the underlying hormonal etiology, however, primary prevention strategies for breast cancer have been limited.

Breast cancer risk factors

The biggest determinants of breast cancer risk are related to endogenous hormone levels and major reproductive events, and thus do not lend themselves to traditional prevention strategies. Table 1 lists the established breast cancer risk factors. Those with the greatest impact on risk are listed first, and unfortunately are the most difficult to modify by traditional public health measures. For example, surgical removal of the ovaries can hasten the onset of menopause, but is not considered a reasonable approach to reduce the risk of breast cancer. Age at menarche and the establishment of regular menstrual cycles may be delayed by vigorous physical activity and possibly diet, but rarely is breast cancer prevention a concern before menarche occurs.

Limiting the use of alcohol, hormone replacement therapy (HRT), and oral contraceptives may reduce the risk of

HRT = hormone replacement therapy; SERM = selective estrogen receptor modulator.

Table 1

Breast cancer risk factors

	Modifiable using traditional intervention strategies	
	Yes	No
Factors that confer highest risk		
Age		✓
Inherited susceptibility		✓
Atypical hyperplasia		✓
Endogenous hormone levels		✓
Early age at menarche	✓	
Late age at menopause		✓
Late age at first pregnancy		✓
Factors with small effects on risk		
Physical activity	✓	
Lactation	✓	
Weight/body mass	✓	
HRT	✓	
Alcohol consumption	✓	
Possible risk factors that confer small risk		
Oral contraceptive use	✓	
High dietary fat	✓	
Low dietary fiber	✓	

breast cancer, but the impact of these factors on breast cancer risk are modest. Furthermore, these agents have significant beneficial effects on the risks of other chronic diseases, and these risk–benefit ratios must be carefully weighed. Prolonged lactation may offer a small reduction in breast cancer risk, but is not likely to be readily accepted as a risk reduction strategy. The benefit of physical activity after menarche for the purpose of reducing breast cancer risk is unclear. Because physical activity can curb weight gain, it can be seen as an important preventive measure because postmenopausal obesity increases breast cancer risk. Although the literature is mixed on whether dietary fat and fiber are important in breast cancer etiology, these factors are readily modifiable. However, the effect of moderate dietary changes on breast cancer risk are likely to be small, at best.

Selective estrogen receptor modulators for breast cancer prevention

The newest hope in the area of breast cancer prevention came from the findings of a highly publicized tamoxifen trial reported in 1998 [1]. These data provided the first information from a randomized clinical trial to support the hypothesis that breast cancer can be prevented among high-risk women. Tamoxifen reduced the risk of invasive breast cancer by 49% ($P<0.00001$) and noninvasive breast cancer by 50% ($P<0.002$). The effect was limited to estrogen receptor-positive tumors. Two smaller trials [2,3] failed to find a benefit with tamoxifen; however, these seemingly different results may be due largely to differences in the age and family history of the populations studied, and (at least in one study) poor compliance with the treatment regimen [4].

At present, we do not know whether the Breast Cancer Prevention Trial (BCPT, NSABP-P1) [1] tamoxifen findings are generalizable to all groups of women, such as those with *BRCA* mutations or women from ethnic minority groups. We do not know whether the decreased incidence observed among tamoxifen users was due to a delay in the development of occult tumors that would ultimately be diagnosed after cessation of tamoxifen use. If so, how long can tamoxifen be safely administered. Finally, none of the studies to date have provided reliable data on mortality. The risks of tamoxifen chemoprevention must be weighed carefully against its benefits. Although tamoxifen reduces the risk of certain breast cancers and bone fractures, it increases the risk of endometrial cancer. Whether tamoxifen can reduce the incidence of heart disease is unknown, and data from the BCPT [1] suggest that the drug may increase the risk of stroke and cataracts. (See Gail *et al* [5] for a complete discussion of the risks and benefits of tamoxifen chemoprevention.)

Raloxifene is another SERM that has recently been suggested to reduce the risk of estrogen receptor-positive breast cancer, but without the subsequent increased risk of endometrial cancer [6]. However, that study also needs further confirmation and careful follow up.

Multigenic model of breast cancer susceptibility

What more can be done to reduce the risk of breast cancer? A long-standing strategy of risk reduction involves targeting intervention efforts toward high-risk groups, but who is at high risk for breast cancer? One in eight women in the USA will develop breast cancer, and we must find more effective ways to identify these women early. One possibility is to develop a multigenic model of breast cancer susceptibility, and then screen women to determine who carries a combination of alleles that puts them at significantly increased risk. Given the large and compelling body of epidemiologic and experimental evidence that implicates estrogens in the etiology of human breast cancer, we proposed a multigenic model of breast cancer predisposition that includes genes that are involved in estrogen biosynthesis and intracellular binding [7•]. We hypothesized that functionally relevant sequence variants in such genes would act together, and also interact with well-known hormonally related risk factors, to define a high-risk profile for breast cancer. The term 'functionally relevant sequence variants' refers to those mutations or polymorphisms that can be shown through laboratory experiments to alter encoded protein structure or function, interaction with other proteins, or half-life and stability within the cell.

We assumed that variation in genes that encode critically important enzymes in estradiol biosynthesis would, individually, result in only modest differences in the rate of biosynthesis. Presumably, there would be limited evolutionary tolerance for major variation in hormone synthesis, which could disrupt reproductive ability. However, a combination of genes, each with minor variation in expressed activity, could provide a degree of separation of risk that would be clinically useful. These small variations could result in a large cumulative effect after several decades. For example, the model of breast tissue age by Pike *et al* [8••] demonstrates that a 20% difference in levels of circulating estrogen can result in a more than twofold increase in lifetime breast cancer risk.

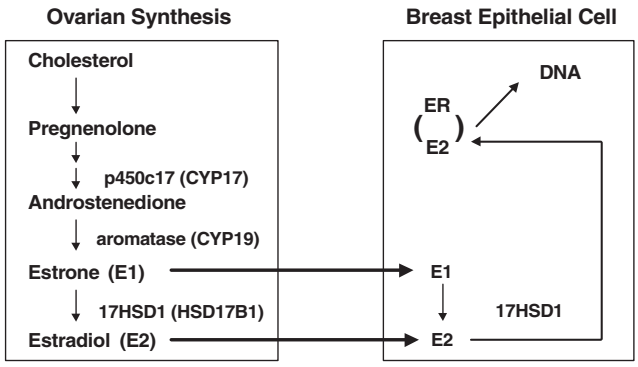
As shown in Figure 1, our original presentation of this model included three genes of interest: the 17β-hydroxysteroid dehydrogenase 1 gene (*HSD17B1*), the cytochrome p459c17α gene (*CYP17*), and the estrogen receptor alpha gene (*ESR1*). We later added the aromatase gene (*CYP19*) to the model, although a functional polymorphism was not known. Huang *et al* [9•] recently reported their findings of a similar model with the estrogen metabolizing genes *CYP17*; *CYP1A1*, which participates in estrogen hydroxylation; and the catechol-*O*-methyltransferase gene (*COMT*), which encodes the enzyme that is responsible for *O*-methylation leading to inactivation of catechol estrogen. The numerous studies of other polymorphisms are reviewed elsewhere [10].

At present, data to support a role for *CYP17* are most compelling. As summarized below, this gene has been shown to be associated with the risk of breast cancer, serum hormone levels, age at menarche, and use of HRT.

CYP17 encodes the cytochrome p450c17α enzyme, which functions at key branch points in human steroidogenesis [11]. The 5′-untranslated region of *CYP17* contains a single base-pair polymorphism that is 34 bp upstream from the initiation of translation, and 27 bp downstream from the transcription start site [12]. This base-pair change creates a recognition site for the MspAI restriction enzyme, and has been used to designate two alleles: A1 (the published sequence) and A2.

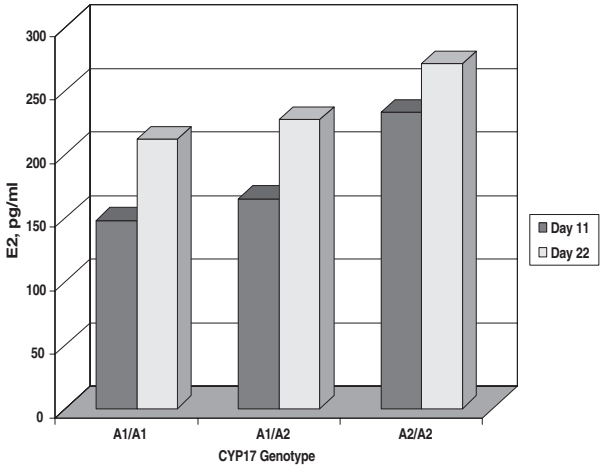
An association between risk for breast cancer and this *CYP17* polymorphism was first shown in 1997 [13]. In a case–control study of incident breast cancer among Asian, African-American and Latina women [13], we found a 2.5-fold increased risk of advanced breast cancer associated with the *CYP17* A2 allele. That study also presented preliminary evidence suggesting that the *CYP17* gene may be associated with age at menarche. The reduced risk of breast cancer associated with a later age at menarche was largely limited to A1/A1 women (odds ratio 0.47, 95% confidence 0.22–0.98 for breast cancer and later age at menarche) compared with women who carried the A2 allele (odds ratio 0.80, 95% confidence interval 0.51–1.27).

Figure 1



Estrogen metabolism in the ovaries and breast epithelium and four candidate genes that may play a role in breast cancer etiology. The genes of interest are the cytochrome P450c17α gene (*CYP17*), the aromatase cytochrome P450 gene (*CYP19*), the 17β-hydroxysteroid dehydrogenase 1 gene (*HSD17B1*), and the estrogen receptor alpha gene (*ESR1*).

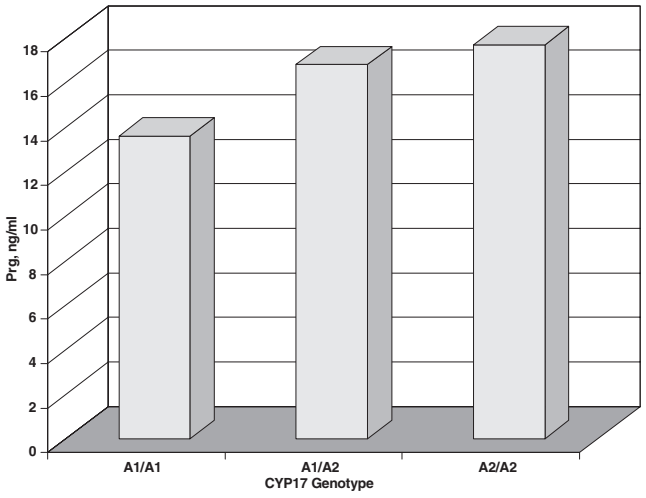
Figure 2



Geometric mean serum estradiol (E2) concentrations among young nulliparous women on days 11 and 22 of the menstrual cycle by *CYP17* genotype. Day 11 E2, *P* for trend 0.04; day 22 E2, *P* for trend 0.06.

These results suggested that serum hormone levels may differ by *CYP17* genotype. We pursued this finding in a separate study and found that the *CYP17* genotype was associated with serum estradiol and progesterone levels among young nulliparous women [14]. As shown in Figure 2, serum estradiol measured around day 11 of the menstrual cycle was 11 and 57% higher ($P=0.04$), respectively, among women heterozygous and homo-

Figure 3



Geometric mean serum progesterone concentrations among young nulliparous women on day 22 of the menstrual cycle by *CYP17* genotype (*P* for trend 0.04).

zygous for the *CYP17* A2 allele compared with A1/A1 women. Similarly, around cycle day 22, serum estradiol was 7 and 28% higher ($P = 0.06$) and progesterone (Fig. 3) was 24 and 30% higher ($P=0.04$). These data provide direct evidence of genetic control of serum hormone levels, but the sample size was small and the results need confirmation.

Finally, we recently reported that *CYP17* is associated with use of HRT [15•]. Among 749 postmenopausal women aged 44–75 years at baseline randomly selected from a larger multiethnic cohort, women who carry the *CYP17* A2/A2 genotype were about half as likely as women with the A1/A1 genotype to be current HRT users (odds ratio 0.52, 95% confidence interval 0.31–0.86). This association was present in all four of the racial/ethnic groups that were included in the study (white, African-American, Latino, and Japanese), and for women above and below the median weight of 150 pounds. Presumably, women with the A2/A2 genotype have fewer indications for HRT use due to their higher level of endogenous hormones.

Since this original study of *CYP17* was published [13], at least six other studies have reported on *CYP17* and breast cancer (Table 2) [9•,16–18,19•,20]. The results of these subsequent studies were largely negative and suggest heterogeneity by ethnicity. However, there may be several reasons for the discrepant results.

The study by Dunning *et al* [16] is the largest to date but had few advanced cases, and it did not examine the possible confounding effects of HRT. Four other smaller studies [9•,17,18,20] found a modest elevation in breast cancer risk with the *CYP17* A2 allele in some subgroups, but those findings did not reach statistical significance. Kristensen *et al* [20] suggested that the effect of *CYP17* might be limited to older patients (ie those aged over 55 years at diagnosis), which also may explain some of the inconsistent results.

Table 2

Summary of published studies of *CYP17* and breast cancer

							CYP17 (A2/A2 versus A1/A1) and breast cancer				
							All cases		Advanced cases		
Ref	Study design	Race/ ethnicity	Age of patients (years)	Age of control individuals (years)	Cases (*n*)	Advanced cases (*n*)	OR	95% CI	OR	95% CI	HRT use considered?
[7•]	Nested case–control	Afro-American, Asian, Latina	63.0 ± 8.4	61.4 ± 8.3	174*	40	1.32†	0.87–2.0	2.5†	1.07–5.94	No
[16]	Case–control	White	<55	45–74	835	24	1.17	0.92–1.49	0.88†	0.38–2.01	No
[17]	Nested case–control	White	60.4 ± 11.7	60.2 ± 11.5	115	30	0.89	0.41–1.95	1.39	0.26–7.28	Yes
[18]	Case–control	Afro-American Latina White	Not given	Not given	76 20 27	21 7 10	1.40† 1.93† 0.80†	0.44–4.38 0.75–5.01 0.45–1.43	0.6† 0.2† 1.7†	0.1–4.0 0.0–1.3 0.6–5.1	No
[19•]	Nested case–control	White	58.3 ± 7.1‡		464	107	0.91	0.61–1.34	0.84†	0.54–1.32	Yes
[9•]	Case–control	Taiwanese	Not given	Not given	150	Not given	1.28	0.73–2.27			Yes
[20]	Case–control	White	59 (27–91)	20–44	510	93§	0.94	0.54–1.65	1.38§	0.62–3.06	No

Ages are expressed as mean ± standard error, mean (range), or range. *Total cases, not specified by ethnicity; †odds ratio (OR) reflects A1/A2 + A2/A2 combined; ‡age not specified by case–control status; §advanced cases over age 55 years.

Only one study, that by Haiman *et al* [19•], was both significantly larger than our original study and gave adequate consideration to potential confounding. Those investigators did not find an association between *CYP17* and breast cancer. However, their results are compatible with the potential modification of breast cancer risk due to late age of menarche. As in our study (and one other [18]) the protective effect of later onset of menarche was limited to women with the A1/A1 genotype (odds ratio 0.57, 95% confidence interval 0.36–0.90 for breast cancer and later age at menarche). Furthermore, among 297 postmenopausal women, those with the A2/A2 genotype had statistically significantly elevated levels of estrone (+14.2%, $P=0.01$) and dehydroepiandrosterone (+14.4%, $P=0.02$), and modest nonsignificant elevations in estradiol (+18.8%, $P = 0.08$), testosterone (+8.6%, $P=0.34$), androstenedione (+17.1%, $P=0.06$), and dehydroepiandrosterone sulfate (+7.2%, $P=0.26$) compared with women with the A1/A1 genotype. That study did not show evidence of an association between *CYP17* and HRT use. However, the patterns of HRT use among a cohort of predominately white nurses may be substantially different from patterns of use among women in our multiethnic cohort [15]. Additional studies are needed, but they must be of sufficient size and quality to evaluate adequately the role of *CYP17* in advanced breast cancer while examining the influence of HRT use and other potentially important confounders and effect modifiers.

An important piece of information about *CYP17* is still missing. What is the functional relevance of this polymorphism, or what is it marking? It was recently shown [20] that this T27C polymorphism in *CYP17* converting the sequence CACT into CACC does not influence Sp-1 binding, as had been suggested based on its similarity to other known Sp-1 binding sequences. Gene function studies are needed to determine whether the A2 allele confers specifically a higher expression level of *CYP17* [20]. Such studies will need to be carefully designed and evaluated because we would not expect these polymorphisms to result in a large difference in circulating hormone levels. Standard assays may not detect the relatively small differences in activity we would predict from the epidemiologic data.

Conclusion

The primary risk factors for breast cancer are not easily modifiable because they stem from prolonged endogenous hormonal exposures. The genetic basis of hormone levels as an important risk factor for breast cancer has only recently been recognized. It is now increasingly obvious that genetic susceptibility, acting through germline polymorphisms in metabolic genes, plays a critical role. Certainly the 'complete' multigenic model of breast cancer susceptibility would include several important genes. Further study is necessary to determine which genes consistently predict known breast cancer risk factors, serum hormone levels, and breast cancer.

The prevention and control of breast cancer is multidimensional. First, we must identify the key genetic components and use our knowledge of underlying genetic susceptibility to identify those women who are at highest risk. Second, we must identify effective lifestyle modifications, if any, that demonstrate a measurable and sustainable reduction in risk. Third, we must develop earlier screening tools using genetic markers of risk. Finally, we must develop suitable chemopreventive agents, such as tamoxifen, that can prevent tumor development or progression.

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- •• of outstanding interest

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