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## Genes, Hormones, and Pathways to Breast Cancer

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Much current epidemiologic research aims to find the factors that trigger the development of breast cancer in women who are genetically predisposed to it. In this issue of the *Journal*, Hamilton and Mack<sup>1</sup> add to that rapidly growing literature some intriguing observations from a study of female twins. They find evidence that hormonal exposures at puberty may play an exaggerated part in some women who are likely to be at high genetic risk for breast cancer, even though the responsible genes are unknown. On the basis of a multifaceted examination of different risk factors in various groups of twins, they also infer that hormonal exposures after adolescence, such as pregnancy and menopause, exert very little influence on breast-cancer development in these high-risk women. They conclude that at least two distinct pathways to breast cancer must exist.

It would be premature to accept these conclusions until they are replicated in other studies, partly for the reasons that apply in general to research. In addition, research into molecular pathways by examination of gene-environment and gene-gene interactions can easily generate false positive findings because of the three-way and four-way comparisons intrinsic to the search. Whether the research focuses on finding genes that modify powerful environmental carcinogens, such as tobacco, or on finding the environmental or behavioral factors that modify powerful genes, such as *BRCA1*, false impressions arising from one or even two good studies abound.

Hamilton and Mack base their conclusions on a novel and complex analysis. They categorized participating pairs of twins into groups presumed to

have a high, low, or intermediate genetic predisposition to breast cancer, examined several of the established hormonal risk factors within each group, and thus created an overall mosaic of odds ratios. They combined a typical assessment of pairs in which breast cancer developed in just one twin (pairs discordant for disease) with a more unusual assessment of pairs in which breast cancer eventually developed in both members of the pair (concordant pairs). They did this by counting the first twin affected as the one with disease. The authors used this surrogate measure because they were keenly interested in the disease-concordant pairs of twins, especially those who are genetically identical to one another. They reasoned convincingly that most of the women in these pairs are especially genetically sensitive.

Hamilton and Mack compared the effects of surrogate measures of hormone exposure at different ages within these high-risk pairs of concordant twins. Earlier puberty in one twin correlated with an earlier age at diagnosis — weakly so in the dizygotic pairs and strongly so in the monozygotic pairs. Nulliparous women had a higher risk than women who had given birth, but the difference was not statistically significant. Earlier menopause (natural or by oophorectomy) in one twin also correlated with a higher risk, in the opposite of the usual pattern.

The investigators then contrasted that risk profile with the pattern of effects in the disease-discordant pairs, who were further divided into those who had bilateral cancer or an affected (nontwin) family member and those who had neither. In the

resulting large matrix of comparisons, the investigators traced one pathway for the presumptively highest-risk women, for whom earlier puberty was a critical trigger for an earlier diagnosis. They found at least one other pathway, in which the timing of the first full-term pregnancy, of menopause, and of other reproductive milestones mattered more. In their view, this was the pathway to breast cancer in most of the women who were not strongly predisposed to it by their genes.

Several issues complicate the examination of interactions between genetic and hormonal risk factors for breast cancer. First, the same genes may directly control both the hormonal risk factor and breast cancer — for example, through the production or metabolism of estrogens, other hormones, or growth factors. In principle, among the disease-concordant twins studied by Hamilton and Mack, earlier puberty in one twin than in the other may be a marker, rather than a cause, of the elevated risk of breast cancer. On the other hand, there may be an even stronger interaction between puberty and genes than the analysis of concordant twins can reveal, because these twins share a puberty-controlling genotype. The contrast between monozygotic and dizygotic twins helps but does not solve the problem.

Second, the hormonal risk factors associated with breast cancer are well established, but few account for the large variation in risk found in typical modern populations. A majority of women fall within a narrow range of variability in age at menarche, total number of full-term pregnancies, and age at first pregnancy. It is hard to detect any interactions with genetic background that may be present if there is little diversity in exposure.

Third, although one might not expect consistency among studies of other genetically susceptible women, it is notable that the pattern reported by Hamilton and Mack in high-risk twins has not been generally found in carriers of BRCA1 or BRCA2 mutations or in women who have family members with breast cancer; the latter women face roughly twice the usual risk,<sup>2-4</sup> with higher risks depending on the number of relatives in whom cancer developed and at what ages. Long before any breast-cancer genes were identified, investigators began comparing the effects of the established risk factors in women with a family history and those without one, precisely to see whether two pathways appeared. It is sobering to see that the question has not been fully answered. A collaborative reanalysis of 52 stud-

ies compared the effects of hormonal factors on the risk of cancer in 1500 women with breast cancer in the family to the effects in about 10 times as many without such a family history.<sup>4</sup> Later age at first full-term pregnancy, lower parity, and later age at menopause all increased the risk of breast cancer in women with a family history, with relative risks very similar to those seen in other women. Earlier menarche had a weak effect overall, one that was similar in women with a family history and those without one. A randomized trial has suggested that the antiestrogen tamoxifen reduces risk by half, regardless of whether women have three, two, one, or no relatives with breast cancer.<sup>5</sup>

Various studies have investigated the highly penetrant breast-cancer genes BRCA1 and BRCA2 to see how typical risk factors modify their effects.<sup>6</sup> It appears that oophorectomy reduces risk in carriers of BRCA1 mutations and probably in carriers of BRCA2 mutations and that tamoxifen reduces the risk in carriers of BRCA2 mutations<sup>7</sup> and probably in carriers of BRCA1 mutations,<sup>8</sup> despite the high frequency of estrogen-receptor-negative tumors in those who carry BRCA1 mutations. Earlier first pregnancy may not be protective in women with the mutations,<sup>6,9,10</sup> but further study is needed. A recent study looking for gene-hormone pathways in carriers of BRCA1 or BRCA2 mutations<sup>11</sup> reported that the risk was slightly higher in women with an earlier menarche, especially in women with long repeat sequences in AIB1, a gene involved in endocrine signaling. In that study, nulliparous women were at increased risk. In sum, many of the risk factors in other women appear to operate similarly in carriers of the mutations; pregnancy may not, but the issue has not been settled. To date, a strong effect of age at puberty, along the lines of the fivefold risk reported by Hamilton and Mack in disease-concordant pairs of twins, has not been noted in association with the presence of a BRCA1 or BRCA2 mutation.

BRCA1 and BRCA2 are probably exceptional among the genes that alter susceptibility to breast cancer. Common polymorphisms of lower penetrance are thought to explain many cases of breast cancer, but it has proved difficult to find the responsible genes. Hamilton and Mack's study of concordant twins as sentinels offers a critical insight, one with broad implications for the larger search for susceptibility genes. Breast cancer in identical twins probably does signal a background of high genetic risk. The authors appropriately urge further work on genotyping such twins. In the current study, participants

were not genotyped for mutations in BRCA1 or BRCA2. It will be important to confirm the authors' expectations that few of the monozygotic concordant twins carried BRCA1 or BRCA2 mutations. If, as the authors suspect, the twins had potent combinations of common genetic variants that, individually, would be less influential, study of these unusual twins may provide the key to finding polymorphisms important in many other women. In large studies of women in the general population, the relevant polymorphisms will probably be found to carry measurable risk, more limited alone than in combination, and some women will have the same potent combinations of polymorphisms as the high-risk twins. Continuing thoughtful exploration of data from twins will enhance the understanding we can gain from consortiums of clinics for women at high genetic risk, surveys of unique populations, and case-control and cohort studies in the population. As studies succeed in finding gene-hormone interactions, we can expect to illuminate the pathways to breast cancer and to reduce the chances that it will develop.

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