# The epidemiology of Her-2/*neu* and P53 in breast cancer

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## Abstract

Breast cancer is an etiologically heterogeneous disease with marked geographical variations. Joint consideration of the relationship between specific molecular alterations and known or suspected epidemiologic risk factors for this disease should help distinguish subgroups of women that are at elevated risk of developing breast cancer. In this article, we present a comprehensive literature review of the etiologic and prognostic roles of Her-2/*neu* and P53 among women. In addition, we discuss the advantages and limitations of using biomarkers in epidemiological studies. We conclude that more research is needed to understand the complex relationships between genetic alterations and etiologic risk factors for breast cancer.

Key words: breast neoplasms; genes; risk factors; biological markers; racial stocks

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## Resumen

El cáncer mamario es una enfermedad con gran variabilidad geográfica y cuya etiología es heterogénea. La evaluación conjunta de los factores de riesgo que se conocen por estudios epidemiológicos y de las alteraciones específicas a nivel molecular, podría ser útil para identificar subgrupos de mujeres con alto riesgo de padecer dicho tumor maligno. En este artículo presentamos una revisión de la literatura acerca del papel que el Her-2/*neu* y el P53 tienen en la etiología y el pronóstico del cáncer mamario en mujeres. Además, discutimos las ventajas y limitaciones de utilizar biomarcadores en los estudios epidemiológicos. Concluimos que se requieren nuevas investigaciones orientadas a dilucidar las complejas relaciones que existen entre las alteraciones genéticas y los factores de riesgo para el cáncer mamario.

Palabras clave: neoplasmas de la mama; genes; factores de riesgo; marcadores biológicos; razas

Worldwide breast cancer incidence and mortality rates vary widely by country. The highest mortality rates (25/100,000 women) are found in nations such as Great Britain, New Zealand, the Netherlands and Uruguay, while the lowest rates (10/100,000 women) are found in most East Asian and Latin American countries.[1] In the United States breast cancer is the most commonly diagnosed cancer among women, accounting for 32% of all incident cancers in women while mortality from breast cancer is the second leading cause of cancer death, lung cancer being the first.[2]

Breast cancer is an etiologically heterogeneous disease and epidemiologic studies have pointed to particular subgroups of the population who are at increased

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risk of developing the disease. Molecular changes, such as overexpression of oncogenes (e.g., Her-2/*neu*) and tumor suppressor genes (e.g., P53), have been detected in breast tumors. However, they do not occur in 100% of the cases. The heterogeneity of molecular alterations may be indicative of distinct etiologic subgroups of breast cancer. Joint consideration of the relationship between specific molecular alterations and known or suspected epidemiologic risk factors associated with breast cancer and its treatment should help distinguish subgroups of women at high risk of breast cancer and resolve otherwise weak or inconsistent results.

Despite many epidemiologic studies identifying risk factors for breast cancer and numerous studies documenting the presence of molecular markers in breast cancer tissue, the relationship of molecular alterations with epidemiologic risk factors is practically unexplored. Since the study of molecular epidemiology is relatively new, most of the research has been conducted using hospital cases or small case series of patients, where risk factor information is either nonexistent or extremely limited. Thus the type of associations that can be made have been quite restricted and the focus has been on prognostic utility rather than on etiology. Below we present a comprehensive review of the literature on risk factors for breast cancer among women and, to the extent possible, the etiologic and prognostic roles of Her-2/*neu* and P53.

## Risk factors for breast cancer among Caucasian women

The many epidemiologic studies conducted on breast cancer have primarily focused on caucasian women. An increased risk of breast cancer among has been shown to be related to: older age; reproductive factors; higher socioeconomic status; urban residence; family history of breast cancer, fibrocystic breast disease;[3-6] obesity;[7-9] and ionizing radiation.[10-12] The odds in relation to several of these factors (such as obesity and family history of breast cancer) have been shown to vary with age at diagnosis or menopausal status.

Among these same women, oral contraceptive (OC) use in general has not been found to increase the risk of breast cancer[13] while results regarding long-term use are less consistent. Other factors that have also been identified in some studies to affect breast cancer include: diet;[14,15] alcohol;[16-20] smoking;[21-24] lactation;[15, 25,26] parity;[15,27] abortions;[11,28] and physical activity.[11,29,30]

In general, the longer the reproductive lifespan, the greater the risk of developing breast cancer. That is, early age at menarche,[31-34] late age at menopause,[33,35-37] and late age at first birth,[38-40] are all known to increase a women's risk. These consistent associations found in epidemiologic studies, suggest an important role of cyclic hormones (time between menarche and menopause) in breast cancer etiology.[9,41] Cumulative exposure to estrogens may provide a selective environment for the clonal outgrowth of cells which contain somatic mutations.[9] Although consistent, the relative risks associated with reproductive factors and breast cancer risk are weak, generally ranging below 2.0.

Several epidemiologic studies have identified subgroups of women with a family history of breast cancer who are at an especially elevated risk of developing breast cancer themselves: women with a first-degree relative who was diagnosed with breast cancer at an early age; women with more than one affected relative; women with a family history of ovarian cancer; and women with a first degree relative who had bilateral breast cancer.[42-47] Sattin *et al.* (1985) found that the relative risk for women with at least one affected first degree relative was 2.3, for women with at least one affected second degree relative the risk was 1.5, and for women with both an affected mother and sister the risk increased to 14.[48] In addition, a number of studies have examined the effect of age at onset on the relationship between a woman's risk of breast cancer and the presence of bilateral breast cancer in a first degree relative and found that relatives of younger breast cancer cases with bilateral disease are at an increased risk.[43,49,50] Formal genetic analyses have provided strong evidence for one or more rare autosomal dominant inherited gene(s) associated with increased susceptibility to breast cancer.[50-54] The elevated risk to carriers versus non carriers increases with decreasing age at onset, indicating that younger breast cancer cases are more likely to represent gene carriers than are older patients.

The actual extent to which the familial aggregation of most breast cancer is attributable to hereditary or environmental factors is not fully known. Some researchers suggest that the heritable component of breast cancer should only be considered in concert with environmental risk factors, such as diet, sun exposure, and smoking behaviors.[55] For example, perhaps mediated through genetics, there may be factors which predispose some women to an abnormal differentiation of cells in response to environmental risk factors.[56] It may be that genetic factors affect the probability of neoplastic transformation and/or expression of the

neoplastically transformed cells through control of hormonal or other stimuli and the cellular response to these stimuli.[57]

## Breast cancer risk factors among women of other ethnical backgrounds

*Breast Cancer in Hispanic Women.* Breast cancer incidence and mortality rates are consistently lower among Hispanic women than among white and black women.[58] In Central America for example, the annual incidence breast cancer rate in 1996, was 17 per 100,000 compared with 146.6 per 100,00 breast cancer cases in the U.S.A.[59] After cervical cancer, however, breast cancer is the second leading cause of female cancer mortality in most Latin American countries.[58]

Information on breast cancer risk factors among Hispanic women is scarce. From two case-control studies performed in Mexico[60,61] and one case-control study carried out in Colombia,[62] it has been confirmed that nulliparity and late age at first pregnancy double the risk of breast cancer while the familial history of breast cancer increases this risk two to six times. In addition to finding similar results for nulliparity, age at first pregnancy and family history, another case-control study of Hispanic women in the U.S. found an increased risk for women previously diagnosed with benign breast disease.[63] One study, although based on small numbers, found that Hispanics had the lowest rate of familial breast cancer compared to whites and blacks.[64] To date no information has been published regarding the importance of a positive history of breast cancer in a relative (i.e. sister, mother etc.) among Hispanic women. A protective effect, i.e. a risk reduction of 55% to 75% has been reported for Mexican[60,61] and Colombian women[62] who breastfeed their first child for more than 12 months, as compared to those who had children but did not breastfeed them. Also, a significant correlation has been reported from an ecological study between a decreasing trend of fertility and an increasing trend of breast cancer mortality in Mexico.[65]

It has been hypothesized that diet may play an important role in reducing breast cancer risk among Hispanics.[66] Such diet is generally rich in dietary fiber,[67] just as the Asiatic diet and both ethnic groups have low breast cancer rates. Experimental results showed that dietary fiber can reduce the levels of circulating estrogens[66,67] but additional work is needed to confirm whether a reduction in breast cancer risk could truly be achieved.

Exposure to xenoestrogens and the risk of breast cancer was evaluated by a study in Mexico City. The results indicated that the serum levels of Dichlorodiphenyl-trichloro-ethane (DDT), beta-hexachloro-cyclohexane and hexachloro-benzene were not associated with an increased risk for breast cancer.[68,69] In contrast, exposure to polychlorinated biphenyls (PCBs) might be doubling the risk for that disease.[69] Currently, in Mexico, an estimated 8,000 tons of PCBs are still in the electrical power sector, mainly in operational equipment and as residues.[69]

In the United States, breast cancer incidence and mortality rates for immigrant Hispanic women are also much lower than those reported for white or black women. However, those rates vary across geographic regions in the US with much higher incidence rates found among Hispanic women living in Illinois than those for to women who live in Texas.[70] Results from other studies in the US suggest that Hispanic women are also more likely to be diagnosed at a younger age ($p<.0001$) and have an increased likelihood of getting an advanced cancer when compared to Caucasians.[71] However, in a more recent study where access to healthcare was not an issue because all participants had access to a single source of care, Hispanic women were still significantly younger at the time of diagnosis, but no longer at an increased risk for suffering advanced forms of breast cancer.[72]

*Breast Cancer in African-American Women*. Among these female population, breast cancer is the most common cancer. The average annual age-adjusted incidence for breast cancer is lower among blacks (92.8 per 100,000) as compared with whites (112.2 per 100,000).[73] Age-specific incidence rates, however, vary by age and race. Among younger women blacks have a higher rate than whites, and among older women whites have higher rates than blacks, with the cross-over of rates occurring between the ages of 40 and 50 years.[74-76] Reasons for this extreme effect modification by age and race on breast cancer risk are unknown.

Risk factors for breast cancer among African-American women are still poorly understood. Only four major epidemiologic studies focusing on blacks have been published to date: one population-based[77] and three hospital-based case control studies.[29,78,79] All four, found that breast cancer among black women was positively associated with nulliparity or low parity. Other risk factors for breast cancer among whites were associated with higher risk among blacks in only one or two of the three studies (higher education, late age at first birth, larger body size among postmenopausal women, OC use, older age at menopause, history of benign breast disease, and family history of breast cancer). Meanwhile, other factors such as early age at

menarche were not associated with risk among blacks in any of the studies. In a more recent case-control study looking specifically at OC use and breast cancer risk among African American women, only moderate to long term OC use below the age 45 was found to increase the risk for breast cancer.[80] The only study which examined the effects of alcohol and smoking on breast cancer among black women found no association.[77] A fifth study, a segregation analysis using data from the Cancer and Steroid Hormone Study, found that a history of breast cancer among first-degree relatives is equally predictive of BC risk among black or white women.[81]

## Molecular Alterations

It is clear that human cancers are partly caused by alterations of some genes (oncogenes, tumor suppressor genes) involved in growth regulation. In most cases, tumorigenesis probably requires alteration of several of these genes. Inheritance of a mutated gene may predispose an individual to cancer, but, in general, somatic alteration of other genes over the course of an individual's life is also required for development of cancer. Several oncogenes and tumor suppressor genes have been shown to be altered in breast cancer. Genetic alterations which have frequently been reported include overexpression of the Her-2/*neu* oncogenes and the tumor suppressor gene P53.

The Her-2/*neu* proto-oncogene encodes a growth factor receptor-like molecule that is inserted into the cell membrane[82], and ligands that bind to Her-2/*neu* were identified recently.[83] Holmes et al. (1992) described the isolation, sequencing, and characterization of a family of Her-2/*neu* ligands and demonstrated their interaction with Her-2/*neu* protein.[84] They also showed biologic responses to treatment with the ligand in cell lines. A point mutation in the membrane spanning region in the Her-2/*neu* gene has been shown to cause oncogenic activation of the gene. Structurally normal Her-2/*neu* protein is overexpressed (2-40 fold) in a significant fraction (20-30%) of human breast cancers.[85,86] Overexpression usually is due to gene amplification, but in some cases occurs despite the presence of a normal gene copy number. One possible explanation for Her-2/*neu* overexpression in the absence of amplification (increase in net gene copy number) would be transcriptional up-regulation or post-translational modification. In most studies, overexpression of Her-2/*neu* in breast cancer has been associated with aggressive biological behavior and poor survival.[86-88] Several studies have shown the association between overexpression of the Her-2/*neu* oncogene and early stages of breast carcinogenesis in distinct histologic types of breast cancer,[86,89] perhaps suggesting a more important role in initiation than in progression.

The P53 gene encodes a nuclear protein that is found at low levels in virtually all cells.[90,91] It is thought that the P53 gene product normally acts to restrain inappropriate cellular proliferation. Although the precise mechanism by which P53 acts as a growth inhibitor is unknown, it binds to specific regions of DNA where it may regulate expression of other genes.[92,93] Loss of P53 due to deletion of this gene from the short arm of chromosome 17 has been associated with a malignant phenotype in vitro. In addition to its role as a tumor suppressor, it has been shown that P53 genes that have undergone mutations can act as dominant transforming genes to elicit malignant transformation, similar to proto-oncogenes.[94] This is thought to occur, in part, due to mutant P53 protein complexing with and inactivating the normal wild type P53 present in the cell.[95]

The frequency of P53 overexpression detected by immunohistochemistry in breast tumors varies from approximately 15% to 60%,[93,96,97] whereas frequency of mutations varies from 15% to 35% in breast cancers analyzed for P53 in only the conserved regions of the gene (exon 5-8, sometimes 4 or 9 too).[98] The broad range of P53 overexpression might be due to: a) the varying ways overexpression is defined;[99-102] b) differences in fixation of tumor tissue,[102] and c) the diversity of antibodies and immunohistochemical procedures used.[101,103,104] Why P53 overexpression is observed in breast cancers which apparently lack P53 mutations is a question that remains to be solved. It is possible that some of these cases have mutations in exons which were not analyzed or that overexpression is caused by stabilization of the P53-protein by other cell constituents .[105] On the contrary, there is also a proportion of cases (10 to 20%) with mutations which lack P53 immunostaining. Deletions, nonsense- and frame-shift mutations are the aberrations found in most of these cases.[98, 99,103,106,107] However, the most frequently observed type of P53 gene alteration in breast tumors is a single-base missense substitution which is expected to alter a single amino acid in the P53 protein and stabilize it.[96,97,108,109] The correlations found between overexpression of P53 protein and mutation are around 0.8 in the majority of studies.[99,100,106,109-111]

## Risk Factors and Molecular Alterations

Her-2/*neu* is one of the most commonly involved oncogenes in breast cancer etiology and P53 is one of the

most commonly involved tumor suppressor gene. Two studies compared risk factor patterns of breast tumors categorizing by Her-2/*neu* status.[112,113] The results showed thst Her-2/*neu* amplification was more frequently found among early oral contraceptive users,[112] and overexpression of the Her-2/*neu* protein (as a result of amplification) was associated with not having breast fed and also with a later age at first full-term pregnancy.[113] The prognostic value of Her-2/*neu* is generally accepted, since the larger studies support an association between Her-2/*neu* and poor prognosis.[114]

Studies comparing P53 mutational patterns of breast tumors between different parts of the United States and Europe,[107] and between the United States, Europe, and Japan,[115] suggest that the various patterns found may indicate variations and differences in exposures to specific risk factors. Biggs *et al* (1993) also suggested that exogenous factors may contribute to the P53 mutational patterns in breast tumors, by comparing mutational spectra of P53 in colorectal, lung, and breast cancer.[116] Several studies have implicated P53 protein expression as an independent prognostic factor in carcinomas of the breast and other cancer sites as well.[117] Studies have also shown that P53 mutations are associated with significantly reduced disease-free periods and/or overall survival.[97] Since P53 mutations correlate with other indicators of poor prognosis,[97] careful analyses are needed to assure that P53 status adds information to established prognostic markers.

## Survival and Overexpression of Her-2/*neu* and P53

As stated above, previous studies suggest that an overexpression of Her-2/*neu* is associated with poor prognosis. In a study performed by Slamon *et al*., Her-2/*neu* amplification was found to be independent of other prognostic factors predicting overall survival among breast cancer patients.[85] This finding was confirmed by Press *et al*. in a multivariate analysis where they showed Her-2/*neu* overexpression to be a marker of poor prognosis independent of histopathologic grade, tumor size, and involvement of regional lymph nodes.[118] However, a similar study published by Thor *et al*. did not confirm this finding.[119] Univariate analyses in the latter study suggested that there was an association between Her-2/*neu* overexpression and overall survival in certain patient subpopulations.[119] More recently, Thor *et al*., using immunohistochemical techniques demonstrated a significant association, independent of other prognostic factors, between P53 protein accumulation and overall survival in 199 breast cancer patients.[120]

The frequency of alterations in both Her-2/*neu* and P53 positively correlates with clinical stage at diagnosis of breast cancer. One of the largest studies to date, found correlations between Her-2/*neu* and various prognostic factors for breast cancer, including: premenopausal status, estrogen receptor status, and young age at diagnosis.[89] Importantly, these relationships were modified by stage at diagnosis. Press and coworkers found Her-2/*neu* amplification/overexpression level to be correlated with risk of developing recurrent disease among women with node-negative breast cancer.[118] They found the risk of developing recurrent disease in node negative women with any level of Her-2/*neu* overexpression to be 3 times greater than among women whose breast cancer lacked overexpression while the group of patients with high overexpression had a risk of recurrence 9.5 times greater than those whose breast cancers had normal expression ($p$=0.0001). This effect seemed to be significantly elevated across menopausal status.

## Molecular Expression among Non-White Women

Differences in molecular expression among various racial groups can also contribute to our understanding of breast cancer etiology and risk, however, published research among non-white women is very limited.

Among Hispanic women, very few studies have been conducted looking at expression of molecular indices and breast cancer. The majority of studies have focused on estrogen and progestrone receptor status with prognosis.[71,121] One study of 253 white, Hispanic and black women undergoing breast biopsies, looked at several prognostic factors (including Her-2/*neu* expression) in relation to ethnicity but found no significant difference in Her-2/*neu* expression levels. For each ethnic group, overexpression of Her-2/*neu* ranged from 10-15%.[122] A larger study of 4885 white, 1016 black and 777 Hispanic women by Elledge et. al also found no significant difference in Her-2/*neu* or P53 expression between the three ethnic groups.[123]

A 1995 study by Shiao *et al*. comparing 45 black with 47 white breast cancer patients found that black patients with P53 gene alterations had a significant 4-5 fold excess risk of death from breast cancer when compared to black subjects without P53 alterations.[124] In addition, they observed significantly poorer survival associated with P53 alterations for blacks

**SUMMARY OF RISK FACTORS FINDINGS PRESENTED IN TEXT FOR BREAST CANCER ACROSS ETHNICITY**

| *Risk factor* | *Whites* | *African American* | *Hispanics* |
|---|---|---|---|
| Age | Increased risk with increasing | Greater breast cancer rates at younger age compared to white women | -More likely to be diagnosed at younger age.<br>-Increased risk with increasing age results are consistent to those found in white women) |
| Family history | Increased risk for women with family history of breast or ovarian cancer | Increased risk for women with family history | Increased risk for women with family history (RR= 2-6) |
| 1st degree relative (mother, sister) with breast cancer | Great risk if 1st degree relative is diagnosed at an early age and/or had bilateral breast cancer | Greater risk if 1st degree relative had breast cancer, magnitude of risk similar to those found in white women | |
| # of affected relatives | Greater risk if more than one affected relative | | |
| Reproductive factors | Increased risk with longer exposure to endogenous estrogens/progesterones | | |
| Parity | Increased risk among nulliparous and low parous | Similar results as those found in white women | Similar results as those found in white women (RR= 2) |
| Age at first birth | Increased risk with later age at first full term pregnancy | Similar results as those found in white women | Similar results as those found in white women (RR= 2) |
| Age at menarche | Increased risk with earlier age at menarche | No association found to date | No association found to date |
| Age at menopause | Increased risk with older age at menopause | Similar results as those found in white women | No association found to date |
| Socioeconomic status/level of education | Increased risk with higher socioeconomic status and years of education | Increased risk with higher levels of education | No association found to date however the study used low, middle low, middle class categories which may not have enough variation |
| History of benign breast disease | Increased risk with history of benign breast disease (e.g., Fibrocystic breast cancer) | Increased risk with history of benign breast disease | |

than for whites ($p$=0.012). In another study, the types and frequency of P53 gene mutations found in an American black cohort of 45 breast cancer patients differed from previously studied white American and European population samples.[125] Specifically there was an excess of A:T to G:C transitions in this population of black women from Michigan, who have the highest breast cancer mortality rates in the US, compared to US white women from the midwest. These studies suggest that specific mutational patterns might exist in each ethnic group and may reflect ethnic variations in breast cancer etiology.

## Some Epidemiologic Considerations for the Use of Molecular Markers

Although analyses concerning etiologic issues in the incidence of first primary breast cancer have fewer immediate clinical implications than those concerning survival and second primaries, their ultimate implications for the prevention of breast cancer could be considerable. However, it must be acknowledged that the results of such investigations may not always permit the distinction between the role of the molecular alterations as: a) the mechanism through

which a particular risk factor (e.g., use of oral contraceptives) has its possible influence on the occurrence of breast cancer *versus* b) its roles as a co-factor that interacts with the risk factor in producing cancer.

A major challenge in using molecular markers for epidemiological research comes in choosing which molecular alterations will be most effective and practical. Understanding the role of a molecular marker, such as Her2/*neu* or P53, in the development of cancer and establishing its association to other breast cancer risk factors or to breast cancer itself helps to determine its most appropriate use in a study design. For example, for the purpose of prevention or altering the course of disease development, a tumor marker and its relationship with etiologic factors should be examined; for clinical applications, a marker and its relationship with tumor characteristics and treatment should be evaluated. Although difficult in practice, additional understanding of alternative pathways that may lead to the same cellular change or about the way in which multiple exposures may lead to the same biological event allows an investigator to make adjustments in study design or analysis to account for these alternative pathways.

Other important considerations involve assessing the sensitivity and feasibility of molecular markers for large scale screening or testing. The type of assay used will also lead to practical decisions about the types and quantity of biological samples that must be collected (e.g., blood, urine, hair, tumor tissue) and how samples should be handled. Finally the ethical and legal issues surrounding privacy and protection of the study subjects especially in the areas of genetic testing, gene therapy, eugenics, and insurance/employment are becoming significant considerations in epidemiologic studies using molecular markers.

## Wrap-up

Epidemiologic studies have identified many risk factors for breast cancer, including older age, family history of breast cancer, reproductive factors, and history of fibrocystic breast disease or prior breast cancer. Although consistent, the etiologic associations observed between these factors and breast cancer incidence are generally weak, with increases in risk mostly below 2.0. However, specific subgroups of women have been described in whom the incidence of breast cancer in relation to classic risk factors is substantially elevated. Variations in overexpression of oncogenes and tumor suppressor genes have here forth been virtually unexplored in relation to breast cancer risk. Now is the time to take advantage of recent insights into the molecular biology of breast cancer to classify breast cancer cases into more homogeneous subsets and to explore the interaction of nongenetic and genetic factors in its etiology. To a limited extent, this approach has already proven successful in relating Her-2/*neu* overexpression with increasing age at first pregnancy, ever having breastfed, and OC use at an early age.

## References


1. Levi F, Lucchini F, La Vecchia C. Worldwide patterns of cancer mortality, 1985-1989. Eur J Cancer Prev 1994;3(2):109-143.
2. Parker SL, Tong T, Bolden S, Wingo PA. Cancer Statistics 1997. CA Cancer J for Clincians 1997;47(1): 5-27.
3. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative disease. N Engl J Med 1985;312:146-51.
4. Dupont WD, Page DL. Breast cancer risk associated with proliferative disease, age at first birth, and a family history of breast cancer. Am J Epidemiol 1987;125:769-79.
5. Carter CL, Corle DK, Micozzi MS, Schatzkin A, Taylor PR. A prospective study of the development of breast cancer in 16 692 women with benign breast disease. Am J Epidemiol 1988;128:467-77.
6. Krieger N, Hiatt RA. Risk of breast cancer after benign breast disease: Variation by histologic type, degree of atypia, age at biopsy, and length of follow-up. Am J Epidemiol 1992;135:619-31.
7. Swanson CA, Jones YD, Schatzkin A, Brinton LA, Ziegler RG. Breast cancer risk assessed by anthropometry in the NHANES I epidemiologic follow-up. Cancer Res 1988;48:5363-7.
8. Morabia A, Wynder EL. Epidemiology and natural history of breast cancer. Surg Clin North Am 1990;70:739-52.
9. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progesterones, normal breast cell proliferation and breast cancer risk. Epidemiol Rev 1993;15:17-35.
10. Kelsey JL, Gammon MD. Epidemiology of breast cancer. Epidemiologic Rev 1991;12:228-40.
11. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993;15:36-47.
12. John EM, Kelsey JL. Radiation and other environmental exposures and breast cancer. Epidemiol Rev 1993;15:157-162.
13. Prentice RL, Thomas DB. On the epidemiology of oral contraceptives and disease. Adv Cancer Res 1987;49:285-301.
14. Howe GR, Hirohata T, Hislop TG *et al*. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 1990;82:561-569.
15. Kelsey JL, Horn PL. Breast cancer: Magnitude of the problem and descriptive epidemiology. Epidemiol Rev 199315:7-16.
16. Willet WC, Stampfer MJ, Colditz GA, et al. Moderate alcohol consumption and risk of breast cancer. N Engl J Med 1987;316:1174-80.
17. Longnecker MP, Berlin JA, Orza MJ *et al*. A meta-analysis of alcohol consumption in relation to risk of breast cancer. J Am Med Assoc 1988; 260:652-6.
18. Chu SY, Lee NC, Wingo PA *et al*. Alcohol consumption and the risk of breast cancer. Am J Epidemiol 1989;130:867-77.
19. Howe GR, Roharli A *et al*. The association between alcohol and breast cancer risk: evidence from the combined analysis of six dietary case-control studies. Int J Cancer 1991;47:707-10.
20. Rosenberg L, Palmer JR, Miller DR *et al*. A case-control study of alcoholic beverage consumption and breast cancer. Am J Epidemiol 1990; 131:6-14.

21. Brinton LA, Schairer C, Stanford JL *et al*. Cigarette smoking and breast cancer. Am J Epidemiol 1986;123:614-22.
22. Adami HO, Lund E, Bergstrom R *et al*. Cigarette smoking, alcohol consumption and risk of breast cancer in young women. Br J Cancer 1987; 58:823-37.
23. London SJ, Colditz GA, Stampfer MJ *et al*. Prospective study of smoking and the risk of breast cancer. J Natl Cancer Inst 1989;81:1625-31.
24. Chu SY, Stroup NE, Wingo PA *et al*. Cigarette smoking and the risk of breast cancer. Am J Epidemiol 1990;131:244-53.
25. Byers T, Graham S, Rzepka T *et al*. Lactation and breast cancer: Evidence for a negative association in premenopausal women. Am J Epidemiol 1985;121:664-74.
26. London SJ, Colditz GA, Stampfer MJ *et al*. Lactation and risk of breast cancer in a cohort of US women. Am J Epidemiol 1990;132:17-26.
27. Carter CL, Jones DY, Schatzkin A *et al*. A prospective study of reproductive, familial, and socioeconomic risk factors for breast cancer using NHANES I data. Public Health Rep 1989;104:45-50.
28. Parazzini F, La Vecchia C, Negri E. Spontaneous and induced abortions and risk of breast cancer. Int J Cancer 1991;48(6):816-820.
29. Schatzkin A, Palmer JR, Rosenberg L, Helmrich SP, Miller DR, Kaufman DW *et al*. Risk factors for breast cancer in black women. J Natl Cancer Inst 1987;78:213-7.
30. Bernstein L, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK. Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 1994;86:1403-8.
31. La Vecchia C, Decarli A, Di Pietro S. Menstrual cycle patterns and the risk of breast disease. Eur J Cancer 1985;21:417-22.
32. Brinton LA, Schairer C, Hoover RN, Fraumeni JF Jr. Menstrual factors and risk of breast cancer. Cancer Inves 19886:245-54.
33. Hsieh C-C, Trichopoulos D, Katsouyanni K, Yuasa S. Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: Associations and interactions in an international case-control study. Int J Cancer 199046:796-800.
34. Wang Q-S, Ross RK, Yu MC, Ning JP, Henderson BE, Kimm HT. A case-control study of breast cancer in Tianjin, China. Cancer Epidemiol Biomark Prev 1992;1:435-9.
35. Pike MC, Henderson BE, Casagrande JT. The epidemiology of breast cancer as it relates to menarche, pregnancy, and menopause. In: Pike MC, Siiteri PK, Welsch CW, eds. Hormones and breast cancer. (Banbury Report no. 8). Cold Spring Harbor, NY: Cold Spring Laboratory, 1981;3-18.
36. Ewitz M, Duffy SW. Risk of breast cancer in relation to reproductive factors in Denmark. Br J Cancer 1988;58:99-104.
37. Kvale G, Heuch I. Menstrual factors and breast cancer risk. Cancer 1988;62:1625-31.
38. Paffenberger RS, Jr, Kampert JB, Chang H-G. Characteristics that predict risk of breast cancer before and after menopause. Am J Epidemiol 1980;112:258-68.
39. Bruzzi P, Negri E, La Vecchia C, Decarli A, Palli D, Parazzini F *et al*. Short term increase in risk of breast cancer after full term pregnancy. BMJ 1988;297:1096-8.
40. Yuan J-M, Yu MC, Ross RK, Gao YT, Henderson BE. Risk factors for breast cancer in Chinese women in Shanghai. Cancer Res 1988;48: 1949-53.
41. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG. Hormonal risk factors, breast tissue age and the age-incidence of breast cancer. Nature 1983;303:767-70.
42. Go RCP, King MC, Bailey-Wilson J, Elston RC, Lynch HT. Genetic epidemiology of breast cancer and associated cancers in high risk families. I. Segregation analysis. J Natl Cancer Inst 198371:455-61.
43. Ottman R, Pike MC, King MC, Casagrande JT, Henderson BE. Familial breast cancer in a population-based series. Am J Epidemiol 1986;123: 15-21.
44. Goldstein AM, Haile RWC, Marazita ML, Paganini-Hill A. A genetic epidemiologic investigation of breast cancer in families with bilateral breast cancer. I. Segregation analysis. J Natl Cancer Inst 1987;78:911-8.
45. Claus EB, Risch N, Thompson WD. Using age of onset to distinguish between subforms of breast cancer. Ann Hum Genet 1990;54:169-77.
46. Claus EB, Risch N, Thompson WD. Age of onset as an indicator of familial risk of breast cancer. Am J Epidemiol 1990;131:961-72.
47. Lynch HT, Fitzgibbons RJ, Lynch JF. Heterogeneity and natural history of hereditary breast cancer, surgical implications. Surg Clin of North Am 1990;70:753-74.
48. Sattin RW, Rubin GL, Webster LA, et al. Family history and the risk of breast cancer. JAMA 1985;253:1908-1913.
49. Ottman R, Pike MC, King MC, Henderson BE. Practical guide for estimating risk for familial breast cancer. Lancet 1983;2:556-558.
50. Claus EB. Genetic epidemiology of breast cancer in young women. Monographs, J Natl Cancer Inst 1994;16:49-53.
51. Bishop DT, Cannon-Albright L, McLellan T, Gardner EJ, Skolnick MH. Segregation and linkage analysis of nine Utah breast cancer pedigrees. Genet Epidemiol 1988;5:151-169.
52. Newman B, Austin M, Lee M, King M. Inheritance of human breast cancer: Evidence for autosomal dominant transmission in high-risk families. Proc Natl Acad Sci 1988;3044-3048.
53. Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. Ann J Hum Genet 1991;48:232-42.
54. Iselius L, Slack J, Littler M, Morton NE. Genetic epidemiology of breast cancers in Britain. Ann Hum Genet 1991;55:151-9.
55. Goldgar DE, Easton DF, Cannon-Albright LA *et al*. Systematic population-based assessment of cancer risk in first degree relatives of cancer probands. J Natl Cancer Inst 1994;86:1600-8.
56. Petrakis NL, Ernster VL, King EB, et al. Epithelial dysplasia in nipple aspirates of breast fluid: Association with family history and other breast cancer risk factors. J Natl Cancer Inst 1982;68:9-13.
57. Petrakis NL. Genetic factors in the etiology of breast cancer. Cancer 1977;39:2709-15.
58. International Agency for Research on Cancer. Trends in cancer incidence and Mortality. Lyon, France: World Health Organization, 1993.
59. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the Prevetion of Cancer: A global perspective. Washington, DC, 1997.
60. Lopez-Carrillo L, Bravo-Alvarado J, Poblano-Verastegui O, Ortega-Altamirano D. Reproductive determinants of breast cancer in Mexican women. Ann NY Acad Sci 1997;837:537-550.
61. Romieu I, Hernandez-Avila M, Lazcano E, Lopez-Carrillo L, Romero-Jaime R. Breast cancer and lactation history in Mexican women. Am J Epidemiol 1996;143:543-552.
62. Olaya P. Cáncer de mama en mujeres del altiplano Cundoboyasence Colombiano (tesis). Cuernavaca, Morelos: Escuela de Salud Pública de México, 1996.
63. Mayberry RM, Branch PT. Breast cancer risk factors among Hispanic women. Ethn Dis 1994;4(1):41-46.
64. Bondy MI, Spitz Mr, Halabi S, Fueger JJ, Jogel VG. Low incidence of familial breast cancer among Hispanic women. Cancer Causes Control 1992;3(4):377-382.
65. López-Ríos O, Lazcano-Ponce CE, Tovar-Guzmán V, Hernández-Ávila M. La epidemia de cáncer de mama en México. Consecuencia de la transición demográfica? Salud Publica Mex 1997;39:259-265.
66. Lovell AJ, González R, Pillow PC, Gomez-Garza S, Foreman CJ, Chilton JA *et al*. Dietary fiber, Hispanics, and breast cancer risk? Annals of the New York Academy of Sciences 1997;837:524-536.
67. Martínez ME, Tortolero-Luna G. Fibra y cáncer de mama. Dieta y Salud 1997;7(1).

68. López-Carrillo L, Blair A, López-Cervantes M, Cebrian M, Rueda C, Reyes R *et al*. Dichlorodiphenyltrichloroethane serum levels and breast cancer risk: A case-control study from Mexico. Cancer Research 1997; 57:3728-3732.
69. López-Carrillo L, López-Cervantes M, Blair A, Torres-Sánchez L, Rueda C, Cebrian ME *et al*. Serum levels of beta-hexachlorocyclohexane, hexachlorobenzene and polychlorinated biphenyls, in relation to breast cancer risk among Mexican women. In press, 1998.
70. Jones LA, González R, Pillow PC, Gómez-Garza SA, Foreman CJ, Chilton JA *et al.* Dietary fiber, Hispanics and breast cancer risk. Ann NY Acad Sci 1997;837: 524-536.
71. Daly MB, Clark GM, McGuire WL. Breast cancer prognosis in a mixed Caucasian-Hispanic population. J Natl Cancer Inst 1985;74(4):753-757.
72. Zaloznik AJ. Breast cancer stage at diagnosis: Caucasians versus Hispanics. Breast Cancer Res Treat 1997;42(2):121-124.
73. Gloeckler-Reis LA, Hankey BF, Edwards BK (Eds). Cancer Statistics Review 1973 - 87. Washington, D.C.: U.S. Department of Health and Human Services, NIH 1990;90:2789..
74. Gray GE, Henderson BE, Pike MC. Changing ratio of breast cancer incidence rates with age of black females compared with white females in the United States. J Natl Cancer Inst 1980;64:461-463.
75. Janerich DT, Hoff MB. Evidence for a crossover in breast cancer risk factors. Am J Epidemiol 1982;116:737.
76. Polednak AP. Breast cancer in black and white women in New York State. Cancer 1986;58:807-815.
77. Mayberry RM, Stoddard-Wright C. Breast cancer risk factors among black women and white women: Similarities and differences. Am J Epidemiol 1992;136:1145-56.
78. Wynder EI, Bross IJ, Hirayama T. Study of the epidemiology of cancer of the breast. Cancer 1960;13:559-601.
79. Austin H, Cole P, Wynder E. Breast cancer in black American women. Int J Cancer 1979;24:541.
80. Palmer JR, Rosenberg L, Sowmya R, Strom BL, Warshauer ME, Harlap S *et al*. Oral contraceptive use and breast cancer risk among African-American women. Cancer Causes and Control 1995;6:321-331.
81. Amos CI, Goldstein Am, Harris EL. Familiality of breast cancer and socioeconomic status in blacks. Cancer Res 1991;51:1793-7.
82. Bargmann CI, Hung MC, Weinberg RA. The *neu* oncogene encodes an epidermal growth factor receptor-related protein. Nature 1986;319: 226-30.
83. Lupu R, Colomer R, Zugmaier G, Sarup J, Shepard M, Slamon D *et al*. Direct interaction of a ligand for the erbB2 oncogene product with the EGF receptor and p185erbB2. Science 1990;249:1552-5.
84. Holmes WE, Sliwkowski MX, Akita RW, et al. Identification of Heregulin, a specific activator of p185erb2. Science 1992;256:1205-10.
85. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE *et al*. Studies of the Her-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 1989;244:707-712.
86. Slamon DJ, Press MF, Godolphin W, Ramos L, Horan P, Shek L *et al*. Studies of the Her-2/*neu* proto-oncogene in human breast cancer. Cancer Cells 1989;7:371-380.
87. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL. Her-2/*neu* oncogene protein and prognosis in breast cancer. J Clin Onco 1989; I7:1120-8.
88. Berchuck A, Kamel A, Whitaker R, Kerns B, Oh G, Kinney R *et al*. Overexpression of Her-2/*neu* is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res 1990;50:4087-91.
89. Allred DC, Clark GM, Molina R, Tandon AK, Schmitt SJ, Gilchrist KW *et al*. Overexpression of Her-2/*neu* and its relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancer. Hum Pathol 1992;23: 974-9.
90. Finlay CA, Hinds PW, Levine AJ. The P53 proto-oncogene can act as a suppressor of transformation. Cell 1989;57:1083-93.
91. Levine AJ, Momand J, Finlay CA. The P53 tumor suppressor gene. Nature 1991;351:453-5.
92. Kem SE, Kinzler KW, Bruskin A *et al*. Identification of P53 as a sequence-specific DNA binding protein. Science 1991;252:1708-11.
93. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations of the P53 tumor suppressor gene: Clues to cancer etiology and molecular pathogenesis. Cancer Res 1994;54:4855-78.
94. Jenkins JR, Rudge K, Chumakov P *et al*. The cellular oncogene P53 can be activated by mutagenesis. Nature 1985;317:816-8.
95. Kraiss S, Quaiser A, Oren M *et al*. Oligomerization of oncoprotein P53. J virol 1986;62:4737-44.
96. Harris A. P53 expression in human breast cancer. Adv Cancer Res 1992;59:69-88.
97. Allred DC, Elledge R, Clark GM, Fuqua SA. The P53 tumor suppressor gene in human breast cancer, page 63-77. In: *Mammary Tumorigenesis and Malignant Progression*. Eds. R. Dickson and M. Lippman. Kluwer Academic Publisher, 1994.
98. Smith HS. Tumor-suppressor genes in breast cancer progression. page 79-96. In: *Mammary Tumorigenesis and Malignant Progression*. Eds. R. Dickson and M. Lippman. Kluwer Academic Publisher, 1994.
99. Anderson TI, Holm R, Nesland JM, Heimdal KR, Ottestad L, Borresen AL. Prognostic significance of TP53 alterations in breast carcinoma. Br J Cancer 1993;68:540-8.
100. Moll UM, Riou G, Levine AJ. Two distinct mechanisms alter P53 in breast cancer: Mutation and nuclear exclusion. Proc Natl Acad Sci 1992; 89:7262-6.
101. Jacquemier J, Moles JP, Penault-Llorca F, Adelaide J, Torrente M, Viens P *et al*. P53 immunohistochemical analysis in breast cancer with four monoclonal antibodies: Comparison of staining and PCR-SSCP results. Br J Cancer 1994;69:846-52.
102. Fisher CJ, Gillet CE, Vojtesek B, Barnes DM, Millis RR. Problems with P53 immuno-histochemical staining: The effect of fixation and variation in the methods of evaluation. Br J Cancer 1994;69:26-31.
103. Hall PA, Lane DP. P53 in tumor pathology: Can we trust immunohistochemistry?-revisited! J Path 1994;172:1-4.
104. Battifora H. P53 immunohistochemistry; a word of caution (editorial). Human Pathol 1994;25:435-37.
105. Pietenpol JA, Vogelstein B. No room at the P53 inn. Nature 1993; 365:17-18.
106. Marchetti A, Buttitta F, Pellegrini S, Campani D, Diella F, Cecchetti D *et al*. P53 mutations and histological type of invasive breast carcinoma. Cancer Res 1993;53:4665-9.
107. Sommer SS, Cunningham J, McGovern RM, Saitoh S, Schroeder JJ, Wold LE *et al*. Pattern of P53 gene mutations in breast cancers of women of the Midwestern United States. J Natl Cancer Inst 1992;84:246-52.
108. Coles C, Condie A, Chetty U *et al*. P53 Mutations in breast cancer. Cancer Res 1992;52:5291-8.
109. Harris CC, Hollstein M. Clinical implications of the P53 tumor-suppressor gene. N Engl J Med 1993;329:1318-27.
110. Davidoff AM, Humphrey PA, Inglehart JD, Marks JR. Genetic basis for P53 overexpression in human breast cancer. Proc Natl Acad Sci 1991; 88:5006-10.
111. Varley JM, Brammer WJ, Lane DP, Swallow JE, Dolan C, Walker RA. Loss of chromosome 17p13 sequences and mutation of P53 in human breast carcinomas. Oncogene 1991;6:413-21.
112. Olsson H, Borg A, Ferno M, Ranstam J, Sigurdsson H. Her-2/*neu* and INT2 proto-oncogene amplification in malignant breast tumors in relation to reproductive factors and exposures to exogenous hormones. J Natl Cancer Inst 1991;83:1483-7.
113. Treuniet HF, Rookus MA, Peterse HL, Hart AA, van Leeuwen FE. Differences in breast cancer risk factors to *neu* (c-erbB-2) protein overexpression of the breast tumor. Cancer Res 1992;52:2344-5.

114. Stancovski I, Sela M, Yarden Y. Molecular and clinical aspects of the *neu*/ErbB-2 receptor tyrosine kinase. page 161-191: In Mammary tumorigenesis and malignant progression. Eds. R. Dickson and M. Lippman. Kluwer Academic Publisher, 1994.

115. Sasa M, Kondo K, Komaki K, Uyama T, Morimoto T, Monden Y. Frequency of spontaneous P53 mutations (CpG site) in breast cancer in Japan. Breast Cancer Res Treat 1993;27:247-52.

116. Biggs PJ, Warren W, Venitt S, Stratton MR. Does a genotoxic carcinogen contribute to human breast cancer? The value of mutational spectra in unravelling the aetiology of cancer. Mutagenesis 1993;8:275-83.

117. Dowell SP, Hall PA. The clinical relevance of the P53 tumor suppressor gene. Cytopathol 1994;5:133-45.

118. Press MF, Pike MC, Chazin VR, Hung G, Udove JA, Markowicz M *et al*. Her-2/*neu* expression in node-negative breast cancers: Direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Cancer Res 1993;53:4960-70.

119. Thor AD, Schwartz LH, Koerner FC, Edgerton SM, Skates SJ, Yin S *et al*. Analysis of c-erbB-2 expression in breast carcinomas with clinical follow-up. Cancer Res 1989;49:7147-52.

120. Thor AD, Moore DH, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT *et al*. Accumulation of P53 tumor suppressor gene protein: an independent marker of prognosis in breast cancer. J Natl Cancer Inst 1992; 84:845-55.

121. Gasptur SM, Dupuis J, Gann P, Collila S, Winchester DP. Hormone receptor status of breast tumors in black, Hispanic, and non-Hispanic white women. An analysis of 13,239 cases. Cancer 1996;77(8):1465-1471.

122. Weiss SE, Tartter PI, Ahmed S, Brower ST, Brusco C, Bossolt K *et al*. Ethnic difference in risk and prognostic factors for breast cancer. Cancer 1995;76:268-74.

123. Elledge RM, Clark GM, Chamness GC, Osborne CK. Tumor biological factors and breast cancer prognosis among white, Hispanic, and Black Women in the United States. J Natl Cancer Inst 1994;86:705-712.

124. Shiao YH, Chen VW, Scheer WD, Wu CX, Correa P. Racial disparity in the association of P53 gene alterations with breast cancer survival. Cancer Res 1995;55:1485-90.

125. Blaszyk H, Vaughn CB, Hartmann A, McGovern RM, Schroeder JJ, Cunningham J *et al*. Novel pattern of P53 gene mutations in an American black cohort with high mortality from breast cancer. Lancet 1994;343:1195-7.