

# Breast Cancer and Family History:

## A Multivariate Analysis of Levels of Tumor HER2 Protein and Family History of Cancer in Women Who Have Breast Cancer

STEVEN LEHRER, M.D., PATRICIA LEE, PAUL TARTTER, M.D., BRENDA SHANK, M.D., PH.D., AND STEVEN T. BROWER, M.D.

### Abstract

**Background:** The HER2 gene, located on the long arm of chromosome 17, codes for a protein with the characteristics of a growth factor receptor. In a preliminary study, we reported that high levels of tumor HER2 (erbB-2/neu) protein are associated with a family history of breast cancer (that is, one or more female blood relatives with breast cancer).

**Methods:** We have now collected a larger number of subjects (94) and performed a multivariate analysis of the independent variables family history of breast cancer, tumor estrogen receptor, age, and tumor DNA index. Family history of breast cancer was assessed by questioning the patient, in many cases by telephone.

**Results:** HER2 levels were significantly higher in women with a family history of breast cancer ( $p = 0.015$ , two-tailed  $t$ -test). The 27 women with family history were predominantly postmenopausal, mean age  $61 \pm 2.3$  (mean  $\pm$  SEM), versus a mean age of  $56 \pm 1.7$  for the 67 women with no family history. Of the 27 women with a family history of breast cancer, 13 had a first-degree relative (mother or sister) with the disease. The remaining 14 women had other relatives (grandmothers, aunts, cousins, or a niece) with breast cancer. The results of multiple linear regression analysis, with HER2 as the dependent variable, showed that family history of breast cancer was significantly associated with elevated HER2 levels in the tumors ( $p = 0.0038$ ), after controlling for the effects of age, tumor estrogen receptor, and DNA index.

**Conclusions:** The association of family history of breast cancer and elevated tumor HER2 protein suggests that postmenopausal familial breast cancer may be associated with altered HER2 expression.

---

THE HER2 GENE, located on the long arm of chromosome 17, codes for a protein with the characteristics of a growth factor receptor (1, 2). Its ligand, a molecule which binds to the receptor and activates it, may belong to a family of proteins called heregulins (3). HER2 protein overexpression in breast cancers may be associated with poor prognosis (4), although this connection is controversial (5, 6).

HER2 overexpression is also associated with higher tumor mitotic activity (7,8) and young age (6). HER2 overexpression is negatively correlated with tumor estrogen receptor and progesterone receptor content (9). HER2 overexpression is found in over 90% of ductal carcinomas *in situ* of the comedo type, whereas other subtypes, such as the cribriform, solid, or papillary, rarely overexpress HER2 (6).

In a preliminary study (10), we reported that high tumor HER2 protein levels are also associated with a family history of breast cancer. We have now collected a larger number of subjects and performed a multivariate analysis to control for the effects of other factors which affect HER2 expression.

---

From the Departments of Radiation Oncology (SL, PL, BS) and Surgery (PT, STB), The Mount Sinai Medical Center, New York, NY. Address correspondence and reprint requests to Dr. Steven Lehrer, Box 1236, Radiation Oncology, The Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

### Methods

We measured HER2 protein levels in 94 women who had breast cancer excised in Mount Sinai Medical Center in 1991, 1992, and 1993. We included in the study only cases in which there was sufficient tumor tissue to determine HER2 protein level, estrogen receptor protein level, and DNA index.

Of the breast cancer cases, 57 (60.6%) were stage T1, 33 (35.1%) were stage T2, and 4 (4.3%) were stage T3. The smallest tumor was 0.6 cm; the largest was 10 cm. In 50 cases (53.2%) there was no node involvement; in 44 cases (46.8%) there was node involvement. A total of 73 cases (77%) were classified as ductal carcinoma, 11 cases (11.7%) as lobular carcinoma, 5 cases (5.3%) as mixed ductal and lobular carcinoma, and 4 cases as of other histologic structure (malignant phyllodes tumor, comedocarcinoma, colloid carcinoma). Ten cases were classified as *in situ*, and 84 cases as infiltrating.

HER2 protein was extracted from frozen breast tumor tissue and assessed by a well-behaved competitive enzyme immunoassay (EIA). Homogenized tumor tissue is trypsinized, centrifuged, and incubated with tris lysis buffer (10 mM tris-HCl, pH 7.6, 1.5 mM EDTA, 10% glycerol, 0.1% sodium azide). The proteins are then detergent extracted (11). After centrifugation, supernatants are collected and protein values determined by BCA assay (Pierce Chemical Company).

The enzyme immunoassay employs an affinity-purified sheep polyclonal antibody which was generated against a synthetic peptide composed of 19 amino acids derived from the cytoplasmic domain of the human c-erbB-2 (HER2) oncoprotein. This peptide has two amino acid substitutions which differ from the corresponding aligned sequence in rat neu. The synthetic peptide has approximately 50% alignment with the epidermal growth factor-receptor (EGFR) sequence. The immunogenic synthetic HER2 peptide is used as the calibrator for the assay. The antibody detects bands at 170 kDa and 185 kDa on immunoblots of SkBr-3 cell membrane preparations which produce high levels of HER2 protein. HER2 is the membrane-associated 185 kDa oncoprotein detected by this antibody; the 170 kDa band represents a degradation product of the 185 kDa HER2 oncoprotein. These bands can be blocked by preincubation of the antibody with the immunogenic synthetic peptide. The antibody does not recognize any bands on immunoblots prepared from A431 cells which produce high levels of EGFR. Furthermore, specificity of the antibody has been tested by Western blot; there is

no cross-reactivity with EGFR produced by breast cancer cell lines, or any other significant cross-reactivity (data on file, Dianon Systems, Stratford, CT). The antibody is specially produced for Dianon Systems and is not commercially available.

Two quality control specimens are run in each assay to ensure day-to-day precision. The negative control consists of a pool of 1,000 human breast biopsy specimens, which possesses a HER2 concentration of less than 2,000 fmol/mL. The positive control consists of SKBR-3 breast cancer cells (12), which produce HER2 at a level of greater than 2,000 fmol/mL, the cutoff point for overexpression.

The cutoff point was established in two ways: (a) 14 specimens of pathologically proven benign tissue, removed during reduction mammoplasty and fresh frozen, were assayed for HER2. Only one of these specimens had HER2 concentration greater than 2,000 fmol/mg; (b) more than 800 breast cancer biopsies were assayed for HER2. Thirty percent of the specimens were found to have HER2 levels of greater than 2,000 fmol/mg.

The results of the HER2 EIA are normalized by the amount of total protein extracted from the tissue specimen. The total protein in the extracted tissue is measured by a commercial dye assay method (Biorad Laboratories). Results of the HER2 assay correlate well, relative to percent positive cases, with those obtained by immunohistochemistry (data on file, Dianon Systems, Stratford, CT).

Specimens for flow cytometric analysis were prepared using a modification of the Thornthwaite procedure (13). The samples were analyzed with the Coulter Epics-XL Flow Cytometer (Coulter Corporation, Hialeah, FL). Analysis of ploidy was performed using Verity Software (Verity Software House, Topsham, ME) with a minimum sample size of 10,000 cells. DNA indices of 1.00 were considered diploid; those above 1.00 were considered aneuploid. Calibration for true diploidy was done using normal lymphocytes.

Tumor estrogen receptor was determined by enzyme immunoassay (EIA) (Abbott, North Chicago, IL). The cutpoint of the assay, 15 fmol/mg, was determined by the manufacturer. All assays were performed by Dianon Systems, Stratford, CT.

Family history of breast cancer was assessed by questioning the patient, in many cases by telephone. In other cases, the patient was interviewed in person. Family history was defined as any female blood relative with breast cancer. No attempt was made to verify family history by contacting other family members or their physicians.

To do multivariate analysis, we logarithmically transformed HER2 and estrogen receptor values to normalize variances. Statistical analyses were done with the SPSS System (14).

### Results

HER2 levels were significantly higher in women who reported a family history of breast cancer ( $p = 0.015$ , two-tailed  $t$ -test, Fig.). In 67 women without a family history, log of the mean tumor HER2 level was  $2.99 \pm 0.034$  (mean  $\pm$  SE), minimum value was 2.39, maximum value was 3.66. In 27 women with a family history, log of the mean tumor HER2 level was  $3.15 \pm 0.057$ , minimum value was 2.57, maximum value was 3.91. The log of the mean difference was 0.1587 (0.031–0.286, 95% CI).

The 27 women reporting a family history were predominantly postmenopausal, mean age  $61 \pm 2.3$  (mean  $\pm$  SEM), versus a mean age of  $56 \pm 1.7$  for the 67 women reporting no family history; there is no significant age difference in these two groups ( $p = 0.19$ , two-tailed  $t$ -test).

Of the 27 women with a family history of breast cancer, 13 (13.8% of all cases) had a first-degree relative (mother or sister) with the disease. This is comparable to the 12% of cases with an affected first-degree relative reported by Slattery and Kerber (15). The remaining 14 women had other relatives (grandmothers, aunts, cousins, or a niece) with breast cancer. There was no significant difference in HER2 levels between women who had a first-degree relative with breast cancer and women who had relatives of other degrees with breast cancer. There were too few patients to compare HER2 levels in women with affected first-degree relatives to women without family history.

The results of multiple linear regression analysis (Table), in which log HER2 was the dependent variable, were significant overall ( $F = 3.4$ ,  $p = 0.01$ ). DNA index, age, and tumor estrogen receptor concentrations showed the same effects on HER2 levels that have been previously demonstrated. However, of these, only the effect of DNA index ( $p = 0.02$ ) was significant. Moreover, family history of breast cancer was significantly associated with elevated HER2 levels in the tumors ( $p = 0.0038$ ).

There was no significant difference in HER2 levels between *in situ* and infiltrating tumors, nor was there a significant association between tumor size, node involvement, or stage and tumor HER2.

### Discussion

Recently, a familial breast-ovarian cancer gene, BRCA1, has been localized to the long arm

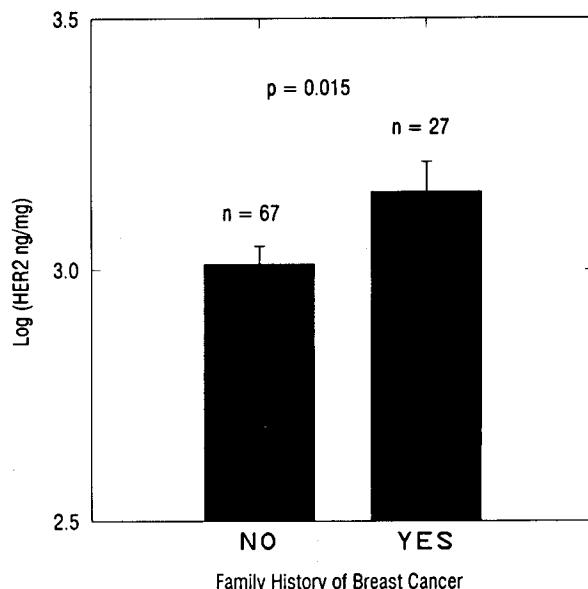


Fig. Log HER2 protein (mean  $\pm$  SD) in tumors of women with and without a family history of breast cancer. Number of cases in each group is indicated above the corresponding error bar.

of chromosome 17 (16, 17). This gene, associated with breast cancer in women under age 45, is not the HER2 gene (2, 18), which is also located on the long arm of chromosome 17. In addition, there is evidence implicating at least two genes on the short arm of chromosome 17 in breast cancer carcinogenesis (19, 20). Finally, although some studies suggest that the HER2 gene is not involved in the genesis of breast cancer (21), at least three new HER2 mutations in breast tumors have recently been identified (22–24).

Moreover, there is evidence that HER2 is involved in breast cancer initiation. For example, HER2 alterations are present in all clinical stages of the disease, are maintained during metastatic spread, are homogenously present throughout tumor sections, and are present in *in situ* as well as infiltrating tumors (25). Our own data, as mentioned, also showed no significant difference in HER2 levels between *in situ* and infiltrating tumors. Therefore, HER2 alter-

TABLE  
Multiple Linear Regression Showing Effects of Family History of Breast Cancer, DNA Index, Log Estrogen Receptor (ER), and Age on Log HER2 in the Tumors of 94 Women

Variable	Regression coefficient	Standard error	T	P
Family history	0.19	0.06	3.0	0.0038
DNA index	0.14	0.06	2.3	0.02
Log ER	-0.005	0.03	-0.16	0.87
Age	-0.002	0.002	-1.0	0.3

ations appear early and may be a causal factor (26).

HER2 is also implicated in animal studies of mammary tumor development, for example in mammary cancer in transgenic mice (27). And early mutations of HER2 are implicated in the development of other tumors, for example nitrosourea-induced neural malignancies in rat Schwann cells (28).

Based on these studies and on our finding of elevated HER2 protein in breast tumors associated with family history of breast cancer, we hypothesize that postmenopausal familial breast cancer may be associated with altered HER2 expression.

#### References

- Hoffman M. New clue found to oncogene's role in breast cancer. *Science* 1992; 256:1129.
- Roberts L. Zeroing in on a breast cancer susceptibility gene. *Science* 1993; 259:622-625.
- Holmes WE, Sliwkowski M, Akita RW, et al. Identification of heregulin, a specific activator of p185-erbB2. *Science* 1992; 256:1205-1210.
- Paik S, Burkhart E, Lippman ME. Clinical significance of erbB2 protein overexpression. In: Dickson RB, Lippman ME, eds. *Genes, oncogenes, and hormones: advances in cellular and molecular biology of breast cancer*. Boston: Kluwer Academic Publishers, 1992:181-191.
- Van de Vijver MJ, Peterse JL, Mooi WJ, Wisman P, Lomas J, Dalesio O, Nusse R. Neu-protein overexpression in breast cancer: association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med* 1988; 319:1239-1245.
- Lofts FJ, Gullick WJ. c-erbB2 amplification and overexpression in human tumors. In: Dickson RB, Lippman ME, eds. *Genes, oncogenes, and hormones: advances in cellular and molecular biology of breast cancer*. Boston: Kluwer Academic Publishers, 1992:161-179.
- Schimmelpenning H, Eriksson ET, Falkmer UG, Rutqvist LE, Johansson H, Fallenius A, Auer GU. Prognostic significance of immunohistochemical c-erbB-2 proto-oncogene expression and nuclear DNA content in human breast cancer. *Eur J Surg Oncol* 1992; 18:530-537.
- Gasparini G, Gullick WJ, Bevilacqua P, et al. Human breast cancer: prognostic significance of the c-erbB-2 oncprotein compared with epidermal growth factor receptor, DNA ploidy, and conventional pathologic features. *J Clin Oncol* 1992; 10:686-695.
- O'Reilly SM, Barnes DM, Campjohn RS, Bartkova J, Gregory WM, Richards MA. The relationship between c-erbB-2 expression, S-phase fraction and prognosis in breast cancer. *Br J Cancer* 1991; 63:444-446.
- Lehrer S, Lee P, Tarter P, Brower S. Tumour HER2 protein in breast cancer and family history. *Lancet* 1993; 341:1420-1421.
- Carney WP, Hamer PJ, Petit D, et al. Detection and quantitation of the human neu oncprotein. *J Tumor Marker Oncol* 1991; 6(2):53-72.
- Goldman R, Levy RB, Peles E, Yarden Y. Heterodimerization of the erbB-1 and erbB-2 receptors in human breast carcinoma cells: a mechanism for receptor transregulation. *Biochemistry* 1990; 29:11024-11028.
- Thornthwaite JT, Sugarbaker EV, Temple WJ. Preparation of tissues for DNA flow cytometric analysis. *Cytometry* 1980; 1(3):229-237.
- Norusis MJ. SPSS for Windows Advanced Statistics Release 5. Chicago: SPSS Inc., 1992.
- Slattery ML, Kerber RA. A comprehensive evaluation of family history and breast cancer risk: the Utah population database. *JAMA* 1993; 270(13):1563-1568.
- Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 1990; 250: 1684-1689.
- Narod S, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, Lenoir GM. Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 1991; 338:82-83.
- Feunteun J, Narod S, Lynch HT, et al. A breast-ovarian cancer susceptibility gene maps to chromosome 17q21. *Am J Hum Genet* 1993; 52:736-742.
- Coles C, Thompson AM, Elder PA, et al. Evidence implicating at least two genes on chromosome 17p in breast carcinogenesis. *Lancet* 1990; 336:761-763.
- Radford DM, Fair K, Thompson AAM, et al. Allelic loss on chromosome 17 in ductal carcinoma in situ of the breast. *Cancer Res* 1993; 53:2947-2950.
- King MC. Breast cancer genes: how many, where, and who are they? *Nature Genetics* 1992; 2:89-90.
- Ehsani A, Low J, Wallace RB, Wu AM. Characterization of a new allele of the human ERBB2 gene by allele-specific competition hybridization. *Genome* 1993; 15: 426-429.
- Hall JM, King MC. PCR detection of an MboI polymorphism in the ERBB2 (HER2/NEU) gene on chromosome 17q11.2-q12. *Nucleic Acids Res* 1991; 19:2515.
- Briscoe WT, Ray DB, Airhart JL, et al. A new high frequency polymorphism in the HER2/neu oncogene in normal tissue and breast tumors. *Breast Cancer Res Treat* 1993; 28:45-49.
- Liu E, Thor A, He M, Barcos M, Ljung BM, Benz C. The HER2 (c-erbB-2) oncogene is frequently amplified in in situ carcinomas of the breast. *Oncogene* 1992; 7:1027-1032.
- Iglehart JD, Kraus MH, Langton BC, Huper G, Kerns BJ, Marks JR. Increased erbB-2 gene copies and expression in multiple stages of breast cancer. *Cancer Res* 1990; 50:6701-6707.
- Bieche I, Champeme MH, Lidereau R. [Cancer of the breast. Genetic alterations and prognostic factors]. *Pathol Biol Paris* 1990; 38:727-734.
- Nikitin AY, Ballering LA, Lyons J, Rajewsky MF. Early mutation of the neu (erbB-2) gene during ethynitrosourea-induced oncogenesis in the rat Schwann cell lineage. *Proc Natl Acad Sci USA* 1991; 88:9939-9943.

*Submitted for publication August 1994.  
Final revision submitted June 1995.*