# Breast Cancer and Family History:

## Levels of Lipid-Associated Sialic Acid in Plasma and Absent Family History of Breast Cancer in Women Who Have Breast Tumors

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### Abstract

**Background:** Breast cancer has a strong genetic component, and at least two breast cancer genes exist. But these genes probably play little role in most breast cancers. Other factors, such as environmental estrogens and diet, may cause the genetic changes involved in the genesis of sporadic breast cancer. A method of observing genetic changes indirectly might be to measure tumor markers known to be associated with breast cancer.

**Methods:** We measured, by biochemical extraction and partition, lipid-associated sialic acid in plasma (LASA-P), a circulating tumor marker, in a group of 239 women with benign or malignant breast tumors.

**Results:** The concentration of LASA-P was elevated in women with both benign and malignant tumors and no family history of breast cancer ($p = 0.046$, one-way ANOVA). Because LASA-P levels rise with age and number of pregnancies, we analyzed our data using multiple linear regression. Benign versus malignant character of the tumor, family history of breast cancer, number of pregnancies, and age were the independent variables. Family history of breast cancer had a significant effect on LASA-P levels ($p = 0.0146$) independent of the effects of age ($p = 0.011$), number of pregnancies (0.012), and whether the tumor was benign or malignant ($p = 0.31$).

**Conclusions:** We hypothesize that elevated LASA-P in women with breast tumors and no family history of breast cancer is a result of the genetic changes occurring in nonfamilial breast cancer. These genetic changes, possibly related to environmental estrogens or other environmental factors, are distinct from the changes due to mutations of BRCA1 or other familial breast cancer genes. Moreover, the elevation of LASA-P suggests that the surface membranes of breast cancer may differ in composition. Further study may lead to exact characterization of the genetic and cell membrane changes associated with familial and nonfamilial breast tumors, and perhaps to better methods of breast cancer prevention and treatment.


BREAST CANCER has a strong genetic component; there are at least two breast cancer genes, BRCA1 and BRCA2 (1, 2). But these two genes probably play little role in most breast cancers (2). Indeed, 90%–95% of "sporadic" breast cancers are unlikely to be due to inherited susceptibility (2). Thus, other factors, such as environmental estrogens and diet, may cause the genetic changes involved in the genesis of sporadic breast cancer (3, 4).

A method of observing genetic changes indirectly might be to measure tumor markers known to be associated with breast cancer. For example, levels of one circulating tumor marker, lipid-associated sialic acid in plasma (LASA-P), are ab-

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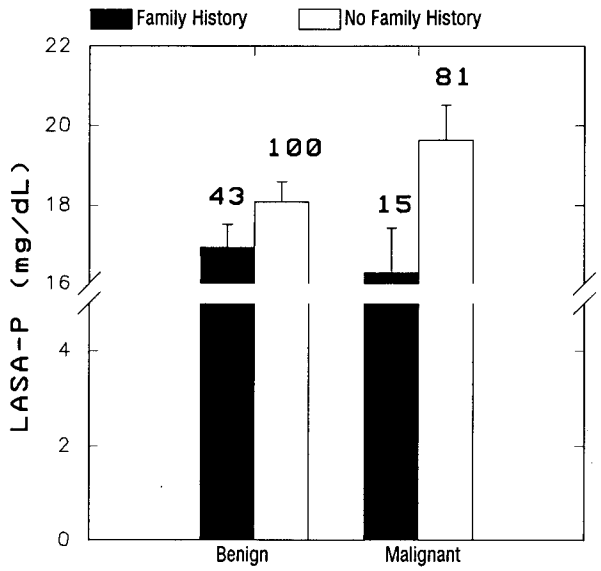


**Fig.** Family history of breast cancer and LASA-P levels (mean ± SEM) in 239 women with benign and malignant breast tumors. Number of women in each group indicated above corresponding error bar.

normally high in the plasma and serum of patients with gynecologic malignancies, including breast cancer (5–7).

We have measured LASA-P in a series of women with breast tumors. We now report that LASA-P is elevated in women with benign and malignant breast tumors who have no family history of breast cancer.

## Methods

We studied 239 women with benign or malignant breast tumors surgically treated in The Mount Sinai Medical Center between 1991 and 1994. Cases were selected for study if the family history was known and LASA-P had been measured. Family history was assessed by questioning the patient. No attempt was made to contact affected relatives.

Plasma specimens to be assayed for LASA-P were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and frozen until tested. LASA-P was determined by the procedure of Katopodis and Stock (8). Total lipid was extracted from 45 μL of plasma with 3 mL of chloroform/methanol (2/1 by volume) at 4–5°C. The extract was partitioned with 0.5 mL of cold distilled water, and the aqueous phase containing the sialolipid fraction was precipitated with 50 μL of a 1 g/mL suspension of phosphotungstic acid. After centrifugation (5 min, room temperature, 750 × g), the supernatant fluid was aspirated, the precipitate was determined by the resorcinol method (9, 10). The extracted blue color was assessed spectrophotometrically at 580 nm, and the sialic acid was calculated by use of a standard curve constructed from data on *N*-acetylneuraminic acid (purity 98%, Sigma Chemical Co., St. Louis, MO) in amounts ranging from 5 to 20 μg per tube (6). Dianon Systems, Stratford, CT, performed all assays. Statistical analysis was done with the SPSS System (11).

## Results

The average age of the 239 women studied was 48.4 years. The youngest woman was 16, the oldest 84. Women with malignant tumors were, on average, ten years older than women with benign tumors. Of the 15 breast cancer patients with a family history of breast cancer, six had a mother with breast cancer, and one had a sister with breast cancer. The others had more distant relatives with breast cancer (grandmothers, aunts, a cousin, or a niece). Of the 43 women with benign breast tumors and a family history of breast cancer, 16 had mothers with breast cancer, two had sisters, and the others had more distant relatives with breast cancer (grandmothers, aunts, cousins, or a niece).

Of the women with malignant tumors and a family history of breast cancer, 60% had ductal carcinoma, 33% had lobular carcinoma, and 7% had mixed ductal and lobular carcinoma. Of the women with malignant tumors and no family history of breast cancer, 83% had ductal carcinoma, 8% had lobular carcinoma, and 9% had mixed ductal and lobular carcinoma.

The concentration of LASA-P was elevated in women with both benign and malignant breast tumors and no family history of breast cancer ($p = 0.046$, one-way ANOVA, **Fig.**). Because LASA-P levels rise with age and number of preg-

**TABLE**

*Multiple Linear Regression Analysis of Effect of Family History of Breast Cancer on Levels of LASA-P in 239 Women with Breast Tumors*

| Variable | b | SE | *t* Test | *p* |
|---|---|---|---|---|
| Family history* | 2.27 | 0.92 | 2.46 | 0.0146 |
| Histologic character† | 0.90 | 0.89 | 1.01 | 0.31 |
| Pregnancies‡ | 1.24 | 0.49 | 2.54 | 0.01 |
| Age | 0.079 | 0.031 | 2.56 | 0.011 |

b, regression coefficient.
* Absent or present.
† Benign vs malignant.
‡ Zero, one, two or more.

nancies (12), we analyzed our data using multiple linear regression. Benign versus malignant character of the tumor, family history of breast cancer, number of pregnancies, and age were the independent variables (**Table**). Family history of breast cancer had a significant effect on LASA-P levels ($p = 0.0146$) that was independent of the effects of age ($p = 0.011$), number of pregnancies ($p = 0.012$), and whether the tumor was benign or malignant ($p = 0.31$). There was no significant correlation of LASA-P levels with estrogen receptor (ER) ($r = 0.13$) or progesterone receptor (PR) ($r = 0.06$) in the malignant tumors. Because ER and PR were measured only in malignant tumors, ER and PR were not included in the multiple regression analysis.

## Discussion

LASA-P is a biomarker useful in a wide range of malignancies. It reflects alteration in the surface membrane of tumor cells. The LASA-P assay measures total gangliosides and glycoproteins (6). Elevated LASA-P levels in breast cyst fluid have been associated with increased risk of breast cancer (13). Also, LASA-P levels are higher in women with benign or malignant breast tumors than in controls. Patel et al. (14) found LASA-P levels (mean ± SEM, in mg/gl) of 17.07 ± 0.607 for controls, 23.7 ± 1.66 for benign tumors, and 28.03 ± 1.9 for malignant tumors.

Some conditions other than cancer affect LASA-P. Among them are myocardial infarction (15), infections, rheumatoid arthritis, and collagen degeneration.

We hypothesize that the elevated LASA-P in women with breast tumors and no family history of breast cancer is a result of the genetic changes occurring in nonfamilial breast cancer. These genetic changes, possibly related to environmental estrogens or other environmental factors, are distinct from the changes due to mutations of BRCA1 or other familial breast cancer genes. Moreover, the elevation of LASA-P suggests that the surface membranes of breast tumor cells from women without and women with a family history of breast cancer may differ in composition. Further study may lead to exact characterization of the genetic and cell membrane changes associated with familial and nonfamilial breast tumors, and perhaps to better methods of breast cancer prevention and treatment.

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