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TITLE: Genetic Abnormalities in Breast Cancer Tumors and Relationships to Environmental and Genetic Risk Factors Using Twins

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CONTRACTING ORGANIZATION: University of California
Los Angeles, California 90033

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ANNUAL REPORT

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A. INTRODUCTION

Abnormalities relating to the p53 gene are one of the most commonly found genetic aberration in breast cancer tumors, and include overexpression of p53 protein, loss of heterozygosity at the p53 locus, and specific mutations in the p53 gene. However, it is unknown why some tumors have these changes and others do not. Further, little is known about what factors are involved in the interaction of oncogenes such as HER-2/neu with p53.

While investigators in previous studies have attempted to link p53 abnormalities to tumor histology, survival time, estrogen and progesterone receptor status, Her-2/neu, and, in some cases, risk factors for breast cancer, none has studied all of these factors within a large population of twins. These subjects offer great potential for distinguishing the role of predisposing genetic factors from environmental exposures. Specifically we will address the following issues in this study: 1) Are genetically similar tumors more likely to occur among identical twins than among fraternal twins? 2) Do environmental factors predispose to concordance or discordance of genetic abnormalities? 3) Do fraternal twins, concordant for environmental exposures, tend to be discordant for genetic abnormalities, suggesting that other predisposing genetic factors that can be identified? 4) Among identical twins discordant for disease, are specific environmental factors more related to tumors with a genetic abnormality than those without?

Three methods have been commonly used to detect p53 abnormalities: immunohistochemical methods of detecting overexpression of the mutant p53 protein, polymerase chain reaction (PCR) techniques for the detection and sequencing of specific p53 mutations, and Southern blots to detect loss of heterozygosity (LOH) at the p53 gene locus. Studies have indicated that 50-60% of breast tumors may have LOH in the 17p region; there may be overexpression of the p53 mutant protein in 27-54% of all breast tumors (3). Specific mutations in the p53 gene usually occur in the highly conserved exons 5-8 (4,5). Twenty-five percent have been shown to occur in codons 245, 248, 273, and 282 (6). From collaborative efforts of specific p53 mutations in more than 30 types of cancer it has been shown that different types of cancer evince different patterns of DNA base substitutions (7).

Rarely have all types of abnormalities been investigated within the same tumor tissue, but a few studies provide information on the correlations between them. Overexpression of the mutant p53 protein product has been seen in association with mutation of the p53 gene (8) but not invariably (9). LOH and overexpression of the p53 protein have been found to occur independently (9,10,11). The mechanism by which dysfunction in the p53 gene leads to malignant transformation is therefore unclear.

Under one hypothesis it would be necessary for both copies of the p53 gene to be inactivated by loss or mutation to prevent the transcription of the normal or 'wild-type' protein and hence prevent normal function of the gene. The failure by some investigators to demonstrate damage to or loss of both copies of the p53 gene suggests that additional steps or other mechanisms must precede malignant transformation. For example, under a hypothesis of co-dominance, a stable
mutant protein might bind to and inactivate any wild-type protein produced (12). Strong
immunohistochemical staining for p53 in normal cells has been found in a mother and daughter
with a family history of breast cancer (13). However, no p53 overexpression was found in
fibroblasts from individuals from families with the Li-Fraumeni syndrome who had germline DNA
mutations of the p53 gene (14). Thus another event (apart from damage to p53) sometimes may
be necessary for expression of mutant protein, or only certain mutations in p53 may be related to
overexpression of the mutant protein and subsequent malignant transformation.

Another mechanism by which the normal function of p53 gene may be interrupted is by nuclear
exclusion (15). When p53 protein is found in the nucleus of cells, mutations in the gene are
usually found, whereas when the protein is found in the cytoplasm, mutations are generally not
found. If the protein is sequestered in the cytoplasm (by binding with heat shock proteins) then it
may be unable to regulate nuclear division. Some studies have shown p53 protein to occur in the
cytoplasm of lobular breast cancers (16).

When p53 mutations in germline tissue were found in members of Li-Fraumeni families (17),
efforts to detect germline mutations in other high-risk families were intensified, largely without
success (18, 19, 20). While these studies were based on small numbers of families: 5 (18) and 25
(19), or cases: 19 individuals with bilateral disease (20). This failure has led to the presumption
that environmental factors or other genes may also determine the abnormalities in the p53 gene
that lead to breast cancer (21). In any event, the inactivation or disabling of the p53 gene appears
to be an important step in a large proportion of breast cancer cases, and studies have shown it to
be an early step, present in situ tumors and maintained throughout all stages of tumor progression
(8).

Since the etiology of breast cancer appears to be complex and heterogenous, other genes,
especially oncogenes, may sometimes interact with p53 in the development and progression of
breast cancer. HER-2/neu (or also referred to as c-erbB-2), located on the long arm of
chromosome 17 (17q12-21.32) has been shown to occur in 20% of invasive breast cancer tumors
and in 50% of all ductal carcinoma in situ (22). Studies that have examined the association of p53
with HER-2/neu have produced mixed results; at least four have found the two to be correlated
(23, 24, 25, 26), while others have not (27, 28). Barbareschi et al. (26) suggest that p53 and
HER-2/neu alterations may occur independently and at an early stage of tumor progression.
Escape from hormonal control may be associated with HER-2/neu overexpression (which has
been related to estrogen receptor negative tumors); while alterations in p53 may induce a high
proliferation rate, leading to tumor progression and further opportunities for genetic damage.

The association of p53 abnormalities and HER-2/neu overexpression with estrogen and
progesterone receptor status, histology, progression, and patient survival may provide insights
into the mechanisms of tumor development and progression. While some studies have linked p53
overexpression to tumors with a more aggressive phenotype (28), it may be that LOH is more
critical to tumor progression than any specific mutation (11). Nuclear p53 expression has been
associated with tumors of aggressive (ductal) as well as less aggressive (medullary) histology
(16); however neither LOH nor specific mutation sequences were assessed. HER-2/neu is
generally found in association with a poorer prognosis (29).

The relationship of p53 and HER-2/neu overexpression to environmental and other genetic risk factors has not been extensively studied. A higher proportion of tumors with p53 protein expression in familial than in sporadic cases has been reported (30). p53 has been associated with low levels of estrogen receptors (23, 26, 28) and late age at first full term pregnancy has been linked to the prevalence of estrogen receptors (McTiernan et al., 1986). An effect of breast-feeding on risk has been found to be dependent on expression of HER-2/neu (32).

To assess the interrelationships of tumor suppressor genes, oncogenes, specific mutations, loss of heterozygosity, and protein overexpression, it is essential that all factors be examined in the same material. This study presents the opportunity to study the several characteristics of breast cancer tumors in a large group of familial cases--concordant twin pairs--and relate these findings to genetic identity and to environmental risk factors. Secondly, a large number of disease discordant identical twin pairs offers the opportunity to further study association of environmental factors with specific genetic changes in breast cancer tumors.

**B. BODY**

Work done during the fourth year of the project has included the following:

1) Data management

2) Acquisition of Archived Tissue Blocks
   a) Ongoing contact with female twins (concordant MZ and DZ pairs, and discordant MZ pairs) from the International Twin Registry to obtain consent and release forms for acquisition of tissue blocks.
   b) Ongoing correspondence with hospitals to borrow tissue blocks and return them after slides have been made.

3) Laboratory procedures
   a) Processing: Logging in of received blocks and slides in database and processing of tissue blocks to cut and store slides.
   b) p53, HER-2/neu, and estrogen/progesterone receptor immunohistochemistry.
   c) DNA sequencing of the p53 gene from concordant pairs.
   d) FISH

4) Epidemiologic Analyses

5) Results

1). Data Management
A data management system using SAS was set up in the first year of the study and has been used to monitor the correspondence and follow-up efforts with the twins and their hospitals. The
laboratory information and the epidemiologic questionnaire information obtained from the twins when they first became part of the Registry have been linked using SAS datasets.

2). Acquisition of Archived Tumor Blocks
a. Contact with Twins
Three groups of twins have been contacted and results are shown in Table 1. There are a total of 1,220 cases for whom we are seeking archived tissue blocks. Our procedures for contacting the twins are the same for each group. Beginning with those who were diagnosed after 1975 and for whom we had already obtained pathology reports, we sent a letter explaining the study, the informed consent, and a release form to each twin for her signature. If we determined that a twin was deceased, these forms were sent to her next of kin. If we did not receive a response from a twin after 4 weeks, we have called the twin to be sure they received the forms and to answer any questions. Additional follow-up has been performed as required. For those with diagnosis dates before 1975, we called the hospitals first to determine if the tissue blocks were still available, before initiating the correspondence with the twin. Of the 85 hospitals called, blocks were available for approximately 30%.

b. Correspondence with Hospitals
Once the signed informed consent and release forms were obtained from a twin, a letter was sent to the hospital along with the release form requesting the tissue blocks, including one that was most representative of the tumor and one that contained normal tissue, such as a lymph node. If the hospital's policies prohibited sending the blocks, we requested that 20 unstained slides be cut from each of the blocks specified, and sent to us. For hospitals not responding follow-up efforts were initiated.

3). Laboratory Procedures
a. Processing: Once the blocks (or slides) are received, they are transferred to Dr. Press's Laboratory in padded envelopes which have the Twin ID number, name of submitting hospital, and number of blocks and/or slides provided. This information is logged into a master data file. Variables in this file include information the characteristics of the tissue, number of blocks, number of nodes sampled, and patient information. One H&E slide is cut from each block submitted. Since numerous blocks are sent with some specimens, this enables us to pick a block that is most representative of the tumor and one that is most representative of normal tissue. The 20 unstained slides are then cut from the chosen blocks and are then coated with paraffin so that antigenicity is not lost during storage. After this process has been completed, the blocks are sent back to the hospitals.

b. Immunohistochemistry: p53, HER-2/neu, ER and PR
When a specimen is selected to be stained, two slides per analysis are taken. One is for the antibody of interest and the other is used as a negative control. A positive control is used for every antibody on each day's run. The antibodies are scored on the basis of intensity of staining. HER-2/neu, being a membrane protein, is scored as low (+), over-expressed (+++), or highly over-expressed (+++). A tumor was considered to be positive if the staining was either over-expressed
or highly overexpressed. P53, ER, and PR, which are nuclear proteins, are scored both by staining intensity and by percentage of cells with that particular intensity, i.e. (27%, ++), (33%, ++), (10%, +). For the initial analyses, we considered a tumor to be positive if more than 10% of the cells were positive.

c. DNA sequencing of the p53 gene: Our original plan was to do SSCP and then sequence only the portion of the gene with a mutation indicated by SSCP. However, in order to avoid the problems with lack of sensitivity inherent in the SSCP process, and since the technology for sequencing the gene has advanced since the grant was written we are now sequencing the entire gene directly, and eliminating the SSCP process. DNA yield is lower in the paraffinized tissue than in frozen tissue and the sequencing gives weaker peaks. This has required us to request more material for some cases.

d. Fluorescence In Situ Hybridization (FISH). FISH is a method using DNA probes to localize genes in cytogenetic chromosomal spreads, in cytologic preparations of whole cells, or in histologic sections of tissue. Hybridization of the probes to their complementary genetic elements in cells is recognized by visualizing a fluorescence signal in cell nuclei with a fluorescence microscope. Initially, in our preliminary studies we used a series of 10 established breast cancer cell lines to confirm that each cell line, known to be amplified by Southern hybridization data, was amplified by FISH. The probe for HER-2/neu hybridized with both metaphase and interphase DNA to yield signals that are proportional with the known gene amplification level of the cell lines.

4). Epidemiologic Analyses
The laboratory findings will be linked to environmental factors obtained from, a detailed questionnaire that was sent to all of the breast cancer twins and co-twins, which covers reproductive, developmental, and putative environmental breast cancer risk factors. Because the questionnaire included many questions about the co-twin, pairs in which only one twin responded can be used in the analysis. We will address the following objectives:

a) Within the concordant pairs in each zygosity group, determine if discordance in genetic abnormalities in tumor tissue is associated with discordance in environmental risk factors.

b) Within the MZ discordant pairs, determine if discordance in breast cancer is associated with discordance in environmental risk factors.

c) Within the MZ discordant pairs, determine if the relationship between environmental risk factors and breast cancer is the same for tumors with and without somatic abnormalities.

5). Results

a. Status of block accrual (Table 1)

We have resolved 723 (an increase of 153 from a year ago) cases at this time with tissue blocks obtained from 330 (an increase of 54 from a year ago). The largest group pending is with the effort to obtain the consent and release form from the patient or next of kin. We have been making extensive follow-up efforts on this group and for those who cannot be located, and for those who are known to be deceased with no next of kin identified, we will be sending a request
for the tissue blocks directly to the hospital. Currently we have 48 requests pending with hospitals and have another 56 completed consent forms which we will be sending to the identified hospitals. We are also in the process of returning blocks to the hospitals after we have completed our laboratory studies with them.

1) **MZ concordant twins:** 206 pairs of identical female twins, concordant for breast cancer, were initially selected to obtain archived tissue blocks. From these 412 cases we have obtained tissue blocks from 167 or 40.5%. We have continued to follow-up our contacts with these twins and their next-of-kin to obtain consent and release forms.

2) **DZ concordant twins:** We initiated efforts to obtain consent and release forms from 130 DZ concordant pairs, by sending letters first to twins who were diagnosed after 1975 and known to be alive at last contact. We have also recently sent letters to the next of kin to those who were known to deceased. Currently we have obtained blocks from 39 or 15.0% of these 260 cases.

3) **MZ discordant twins:** We also selected 548 MZ discordant pairs who met the following criteria: a) they were diagnosed after 1975 and we had obtained their pathology report, and b) they had completed the epidemiologic questionnaire that was sent to all females pairs of twins with at least one member with breast cancer who participated in the International Twin Study Registry. We have received blocks from 124 (22.6%) of these cases.

Among concordant pairs for whom we have received blocks, we have 57 pairs with blocks received from both twins (50 MZ and 7 DZ) and 92 pairs with blocks received from one twin (67 MZ and 25 DZ). Thus, in total we have received blocks from at least one twin for 149 concordant pairs (117 MZ and 32 DZ). In addition we have received blocks from 124 of the 548 discordant MZ pairs. For some of these twins, the only blocks received were the ones without tumor tissue (we asked for both tumor and normal tissue). Thus of the 57 pairs with blocks from both, tumor tissue is available from both members of 45 of these pairs.

b. Immunohistochemistry for p53, HER-2/neu, ER, and PR.
The percent of tumors that stained positive for each of the biomarkers is shown in Table 2. For all tumor tissues studied (including 6 cases with both right and left tumors) the percent positive ranged from a high of 67.5 for ER and a low of 10.4 for HER-2/neu, based on high expression of HER-2/neu. These results are consistent with other reports in the literature. Some differences by pair type are evident as ER, p53, and HER-2/neu were more likely to be positive higher among concordant pairs than discordant pairs.
Table 1: Status of twin participation and acquisition of blocks/slides by category of pair (as of 9/28/98)

<table>
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<th>Status</th>
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<th>DZ Concordant</th>
<th>MZ Discordant</th>
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<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
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<tr>
<td>Total pairs</td>
<td>884</td>
<td>206</td>
<td>130</td>
<td>548</td>
</tr>
<tr>
<td>Total individuals</td>
<td>1220</td>
<td>412</td>
<td>260</td>
<td>548</td>
</tr>
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<td>(cases)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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Resolved cases:
- Blocks/slides rec.: 330 (27.0) 167 (40.5) 39 (15.0) 124 (22.6)
- Blocks/slides n/a*: 347 (28.4) 155 (37.6) 76 (29.2) 116 (21.2)
- Twin refused: 46 (3.8) 17 (4.1) 8 (3.1) 21 (3.8)

Total resolved: 723 (59.3) 339 (82.3) 123 (47.3) 261 (47.6)

In process:
- Hosp. Pending: 48 (3.9) 12 (2.9) 11 (4.2) 25 (4.6)
- Consent Rec.: 56 (4.6) 7 (1.7) 5 (1.9) 44 (8.0)
- Patient Pending: 393 (32.2) 54 (13.1) 121 (46.6) 218 (39.8)

Total in process: 497 (40.7) 73 (17.7) 137 (52.7) 287 (52.4)

*largely consists of cases who were diagnosed before 1975

Table 2: Immunohistochemistry Results for Individual Cases by Pair type

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<th>Percent Positive* for</th>
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<td></td>
<td>(N)</td>
<td>(261)</td>
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<tr>
<td>ER</td>
<td>67.5</td>
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<td></td>
</tr>
<tr>
<td>PR</td>
<td>63.1</td>
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</tr>
<tr>
<td>P53</td>
<td>26.1</td>
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<tr>
<td>HER-2/neu (M+H)</td>
<td>30.8</td>
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<td>HER-2/neu (H)</td>
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*Positivity for ER, PR, and p53 defined as expression in more than 10% of cells; positivity for HER-2/neu defined as both medium or high expression (M+H) and high expression alone (H).
The correlation of positivity for different biomarkers within tumors by pair type is shown in Table 3A, 3B and 3C. As expected, ER and PR were highly correlated with each other in each subgroup ($r=0.58$, $p<.05$ among tumors from concordant pairs and $r=0.53$, $p<.05$ among tumors from discordant pairs). P53 was negatively correlated with ER, especially within tumors from concordant pairs. ER and PR were both negatively correlated with HER-2/neu when the definition of positivity for HER-2/neu was based solely on those expressing high levels. There was a slight non-significant positive correlation between p53 and HER-2/neu.

Table 3A: Correlation coefficients between tumor biomarkers within same tumor: All tumor tissues (N=260)

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<th></th>
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<th>PR</th>
<th>p53</th>
<th>HER-2(M+H)</th>
<th>HER-2(H)</th>
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<td>ER</td>
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<td>PR</td>
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<td>p53</td>
<td>-0.16*</td>
<td>-0.06</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER-2/neu (M+H)</td>
<td>-0.003</td>
<td>-0.02</td>
<td>0.10</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu(H)</td>
<td>-0.25*</td>
<td>-0.26*</td>
<td>0.06</td>
<td>0.51*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*p<.05

Table 3B: Correlation coefficients between tumor biomarkers within same tumor: All tumor tissues from concordant pairs (N=180)

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>PR</th>
<th>p53</th>
<th>HER-2(M+H)</th>
<th>HER-2(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>0.58*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>-0.21*</td>
<td>-0.006</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER-2/neu (M+H)</td>
<td>0.04</td>
<td>0.08</td>
<td>0.09</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu(H)</td>
<td>-0.35*</td>
<td>-0.24*</td>
<td>-0.001</td>
<td>0.53*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*p<.05

Table 3C: Correlation coefficients between tumor biomarkers within same tumor: All tumor tissues from discordant pairs (N=81)

<table>
<thead>
<tr>
<th></th>
<th>ER</th>
<th>PR</th>
<th>p53</th>
<th>HER-2(M+H)</th>
<th>HER-2(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>0.53*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>-0.09</td>
<td>-0.18</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER-2/neu (M+H)</td>
<td>-0.12</td>
<td>-0.27*</td>
<td>0.12</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu(H)</td>
<td>-0.05</td>
<td>-0.30*</td>
<td>0.21</td>
<td>0.46*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*p<.05
Concordancy of Biomarkers in Within Concordant Pairs

From the 180 twins from the concordant pairs with immunohistochemistry completed, there were 41 pairs (36 MZ and 5 DZ) included with results available from both members of the pair. We next looked to see if these markers were identical between members of the same pair with the supposition being that, if they were, it might indicate that predisposing genetic factors were largely in control of the development of these markers. Table 4 shows the correlation for each of these biomarkers between members from these concordant pairs. The highest level of correlation was for ER (r=0.42, p<.05). PR and p53 had similar levels of correlation (r=.33 and .30, respectively). No correlation between members of a pair was found for HER-2/neu. Kappa (Table 5), which takes into account chance agreement, was also the highest for ER (0.42, p<.05), and lowest for HER-2/neu (0.03).

Table 4: Correlation coefficients between tumor biomarkers of individuals from the same twin pair: All Concordant pairs (N=41 pairs) and MZ pairs (N=36)

<table>
<thead>
<tr>
<th>Twin 1</th>
<th>Twin 2</th>
<th>ER</th>
<th>PR</th>
<th>p53</th>
<th>HER-2(M+H)</th>
<th>HER-2(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>MZ</td>
<td>All</td>
<td>MZ</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>MZ</td>
<td>All</td>
<td>MZ</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>MZ</td>
<td>All</td>
<td>MZ</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>MZ</td>
<td>All</td>
<td>MZ</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>ER</td>
<td>0.42*</td>
<td>0.35*</td>
<td>0.33*</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.30</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td></td>
<td></td>
<td>0.03</td>
<td>-0.03</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>HER-2/neu (M+H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HER-2/neu(H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*p&lt;.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 5: Measures of Agreement Between Biomarkers from Members of Concordant Pairs with Tissue Analyzed from Both Twins

<table>
<thead>
<tr>
<th>41 pairs</th>
<th>Percent Agree</th>
<th>Percent Expected</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>73.7</td>
<td>54.8</td>
<td>0.42*</td>
</tr>
<tr>
<td>PR</td>
<td>63.4</td>
<td>47.8</td>
<td>0.30*</td>
</tr>
<tr>
<td>P53</td>
<td>70.7</td>
<td>58.5</td>
<td>0.30</td>
</tr>
<tr>
<td>HER-2/neu (M+H)</td>
<td>58.5</td>
<td>57.4</td>
<td>0.03</td>
</tr>
<tr>
<td>HER-2/neu (H)</td>
<td>73.2</td>
<td>73.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*p<.05

c. DNA Sequencing of the p53 gene
The work of sequencing the entire p53 gene is well underway. The complete gene has been sequenced for 50 cases and mutations were detected in 25. We are re-requesting some tissue blocks to obtain additional tissue for this work. Work in this area will be completed during the next year.
d. FISH
We have completed FISH analyses to quantify HER-2/neu gene amplification for approximately 100 cases at this time. This work is ongoing.

e. Epidemiological Analyses: Association with Risk Factors
We will complete these analyses in our final report. Preliminary analyses were presented in last year’s report.

C. CONCLUSIONS
We have been granted a 1 year no cost extension to complete the work on this project. We will continue to obtain tissue blocks through Dec. 31, 1998. The additional year extension will include the completion of immunohistochemistry on all blocks received by Dec. 31, 1998, the completion p53 sequencing on all breast cancer concordant pairs with tumor blocks obtained from both twins (regardless of p53 immunohistochemistry results), and FISH to detect amplification of the HER-2/neu gene on the above mentioned concordant pairs. Analyses of the data will address concordance of these tumor markers within the same tumor tissue and within twin pairs, and look at associations of these markers with environmental exposures. In addition, among the breast cancer discordant pairs, we will conduct stratified analyses based on tumor characteristics to determine if specific risk factors are associated with the development of breast cancer that is positive or negative for the tumor characteristics under study, i.e. p53, HER-2/neu, and ER and PR receptors.

D. REFERENCES


51) Buckley J. *Epilog*, 1990, Pasadena