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GRANT NUMBER: DAMD17-94-J-4290

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

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REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1995	Annual 1 Oct 94 - 30 Sep 95

4. TITLE AND SUBTITLE
Genetic Abnormalities in Breast Cancer Tumors and Relationships to Environmental and Genetic Risk Factors Using Twins

5. FUNDING NUMBERS
DAMD17-94-J-4290

6. AUTHOR(S)
Thomas M. Mack, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Southern California
Los Angeles, California 90033

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Little is known about factors that produce genetic abnormalities in breast tumors; germline mutations explain only a few cases. Environmental determinants as well as unrecognized genetic mechanisms may be involved. Using identical and fraternal twin pairs concordant for breast cancer, patterns of somatic abnormalities will be compared to other tumor characteristics and to breast cancer risk factors. Breast cancer risk factors will also be compared in breast cancer-discordant identical twin pairs having proband tumors with and without specific somatic abnormalities. In this first year of the study, procedures have been developed for 1) contacting twins to explain the study and obtain an informed consent, 2) obtaining blocks and slides from hospitals, 3) cataloging and storing them in the laboratory, 4) processing the blocks and returning them to the hospitals, 5) performing immunohistochemistry for p53 and HER-2/neu, and 6) data base management. At present we have obtained 83 blocks/slides from MZ concordant twins and have performed immunohistochemistry on 33 specimens.

14. SUBJECT TERMS
Twins, genetics, p53, HER-2/neu, immunohistochemistry, DNA sequencing, epidemiology, breast cancer

15. NUMBER OF PAGES
23

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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A. INTRODUCTION

Abnormalities relating to the p53 gene are 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 do 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 that 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 first year of the project has included the following:

1) Selection of concordant MZ twin pairs from the Twin Registry.
2) Correspondence with twins and next of kin to obtain informed consent and release form to obtain tissue blocks.
3) Correspondence with hospitals to borrow tissue blocks.
4) Slide acquisition and storage.
5) p53 and HER-2/neu immunohistochemistry.

Selection of pairs

207 pairs of identical female twins, concordant for breast cancer, were initially selected to obtain archived tissue blocks. 66 of these pairs had at least one additional first degree relative with breast or ovarian cancer. This information on other family members was determined from questionnaire information that the twins had previously completed.

Correspondence with twins (Copies of Forms are included in Appendix)

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%.

Correspondence with Hospitals (Copies of Forms are included in Appendix)

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.

Slide Acquisition and Storage

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.

Immunohistochemistry: p53 and HER-2/neu

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 (+++). p53, a nuclear protein, is scored both by staining intensity and by percentage of cells with that particular intensity, i.e. (27%, +++), (33%, ++), (10%, +).

Results

a. Status of twin participation and acquisition of blocks/slides
(207 MZ concordant pairs, 414 individuals)

Status	Number	Percent
Resolved:		
Blocks/slides received	83	20.1
Blocks/slides not available*	84	20.3
Twin refused	20	4.8
Lost	6	1.4
In process:		
Need to send to twin	12	2.9
Need to send to next of kin	9	2.2
Pending with twin/next of kin	141	34.1
Need to send to hospital	15	3.6
Pending with hospital	44	10.6
TOTAL	414	100.0

*largely consists of cases who were diagnosed before 1975

Among pairs for whom we have received blocks, we have 16 pairs with blocks received from both twins and 51 pairs with blocks received from one twin.

b. Immunohistochemistry

33 specimens have been analyzed immunohistochemically for p53 and HER-2/neu overexpression. HER-2/neu was overexpressed in 6 and highly overexpressed in 2. Thus HER-2/neu overexpression of any degree was found in a total of 8 samples (24.2% of the 33 tested). Analysis of p53 overexpression in the same samples showed overexpression in 7 (21.2%), ranging from one case with only 16% of the cells stained with low intensity to a case where 90% of the cells stained and the staining intensity was evenly divided between low, moderate, and high. The remaining specimens are in the process of being analyzed.

C. CONCLUSIONS

Our process of obtaining the blocks and slides has been developed and we have streamlined procedures. Other than the hospitals who no longer have the blocks, we have had excellent cooperation from most of the hospitals contacted. We have had some delays in identifying next-of-kin for deceased cases; however, once located, nearly all are willing to participate. The laboratory procedures for processing the blocks are in place and immunohistochemistry procedures have been implemented.

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Appendix

1. Letter to twin describing study
2. Letter to next of kin describing study
3. Informed consent--twin
4. Informed consent--next of kin
5. Release form--twin
6. Release form--next of kin
7. Letter to hospital requesting blocks

1. Letter to twin describing study

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Parkview Medical Building B-105
(800) 421-9631 (213) 342-1642
(213) 342-1237 Fax

January 3, 1995

MS. ^F1^
^F2^
^F3^

Dear MS. ^F4^:

In the past, you have participated in studies of breast cancer carried out by the International Twin Study. I am writing to ask for your participation in an important extension of these studies. We propose to search for and study certain abnormalities of the tumor cells which appear after the tumor has already started. In certain cases, we will also search for inherited genetic abnormalities.

To do this study, we need your permission to borrow the specimen of the tumor from your hospital or clinic and take a small slice of it for study. The tumor specimen will be returned without delay. We will use the slice that is removed only to characterize certain elements of the DNA in the cells.

Your participation would involve 3 items:

1) Sign the enclosed release form (Page 2) giving us permission to obtain the tissue blocks from your hospital.

2) Read and sign the enclosed "Informed Consent" form for this study (Pages 3 and 4).

> This form outlines all of the parts of the study and provides you with other information about your rights as a participant. This form also requires that a second person sign as a witness. This can be a family member or any other adult who is willing to attest to the fact that it was you who signed the form. A second copy of this form is enclosed for you to keep.
>
> Your participation is voluntary. Parts of the consent form are included for reasons that are not obvious. Support for this breast cancer research project comes from the funds Congress appropriated to the Department of Defense, specifically to be administered by the U.S. Army (USAMRDAL). Even though participation in the International Twin Study was and is voluntary, Army regulations require that we include the sentences that are put in quotation marks, whether or not they are important for the subjects of our particular study. For example, the section on "Physical Injury" was drafted with research on different forms of medical treatment in mind, but it must be used in all studies, even when no physical danger is involved.

3) Review the enclosed "Follow-Up" form and provide us with any changes since we last communicated with you.

If you are willing to participate in the study, **please return the signed release form, the signed copy of the informed consent, and the Follow-Up form in the enclosed postage paid envelope.**

Thank you very much for your continued participation in our studies.

Sincerely,

Thomas M. Mack, M.D.
Professor

2. Letter to next of kin describing study

INTERNATIONAL TWIN STUDY
Department of Preventive Medicine
Parkview Medical Building B-105
(800) 421-9631 (213) 342-1642
(213) 342-1237 Fax

October 17, 1995

^F1^

Dear ^F2^:

In the past, your ^F3^ participated in studies of breast cancer carried out by the International Twin Study. I am writing to you as her next of kin for your assistance in an important extension of these studies. We propose to search for and study certain abnormalities of the tumor cells which appear after the tumor has already started. In certain cases, we will also search for inherited genetic abnormalities.

To do this study, we need your permission to borrow the specimen of the tumor from the hospital or clinic and take a small slice of it for study. The tumor specimen will be returned without delay to the health care provider. We will use the slice that is removed only to characterize certain elements of the DNA in the cells.

Participation is entirely voluntary. If you agree to participate, the only thing you need to do is to read, sign and return the enclosed forms. The first one (page 2) is the release form that will be sent to the hospital to enable us to obtain the tissue blocks. The second, longer, form (page 3-4) is a statement indicating that you understand and consent to the study. This form also requires that a second person sign as a witness. This can be a family member or any other adult who is willing to attest to the fact that it was you who signed the sheet. A second copy of this form is enclosed for you to keep.

Parts of the consent form are included for reasons that are not obvious. Support for this breast cancer research project comes from the funds Congress appropriated to the Department of Defense, specifically to be administered by the U.S. Army (USAMRDAL). Even though participation in the International Twin Study was and is voluntary, Army regulations require that we include the sentences that are put in quotation marks, whether or not they are important for the subjects of our particular study. For example, the section on "Physical Injury" was drafted with research on different forms of medical treatment in mind, but it must be used in all studies, even when no physical danger is involved.

After the necessary signatures have been added, please mail one copy of the informed consent form and the release form back to us in the enclosed postage-paid envelope.

Thank you very much for your assistance in our effort to unlock the secrets of this terrible disease.

Sincerely,

Thomas M. Mack, M.D.
Professor

INFORMED CONSENT

GENETIC ABNORMALITIES IN BREAST CANCERS OF TWINS

PURPOSE OF THE STUDY: The purpose of this study is to identify the genetic characteristics of breast tumor cells, especially those which only appear after the tumor has started. In certain instances, additional investigation will be made to determine if they may have been inherited and/or induced by life experiences.

FUNDING SOURCE: The study is funded by the U.S. Army, Department of Defense

PROCEDURES: By agreeing to participate in this study you give permission to release a small slice of the stored tumor specimen to the study for analysis. You must therefore voluntarily and freely donate this piece of tumor tissue to the investigators, who are acting on behalf of the U.S. Army, and you must therefore relinquish all right, title, and interest to it. It is always possible that it may be found useful for other scientific purposes in the future, and it is always possible that some unforeseen future use might result in a commercial application (no such uses have been proposed).

PARTICIPATION IS VOLUNTARY: Your participation in this study is entirely voluntary and would have no bearing on any medical care you may receive. You may withdraw participation at any time.

RISKS TO BE EXPECTED FROM THE STUDY: The only risks from participation in a study such as this are from the potential loss of privacy. You have already released the information about your diagnosis to us. As before, all results of this study will be kept entirely confidential and will not be associated with any person's name. The information which we obtain will only be released for publication in statistical form, such that no individual can be recognized. In the very unlikely event that any of the results from the study are of particular importance to your family, that information will be released, but only to you. No one other than study employees will have access to information about you, with one exception, as follows. "Representatives from the U.S. Army Medical Research, Development, Acquisition and Logistics Command are eligible to inspect the records of this research as part of their responsibilities to protect human subjects in research."

BENEFITS TO BE EXPECTED FROM THE STUDY: There are no direct benefits to you personally to be gained by participation. It will not affect any future treatment you may receive. Of course, you will have the satisfaction of knowing that you have helped in a very significant way in efforts to learn the causes of breast cancer.

The results are also unlikely to help anyone in your family determine a specific level of risk for breast cancer. You already know whether or not your female blood relatives are at a relatively high risk from your family history of breast cancer, and results from this study are not likely to give a clearer idea of the actual level of risk. While it is true that one or more locations on the chromosome have been identified that are highly related to breast cancer risk (recently one such location identified is called BRCA1), a family member's individual risk is dependent upon knowing which specific gene has been inherited at that location. At this time, even if we are successful in attempts to characterize the genes, we are not able to accurately translate the result into a specific level of risk.

PHYSICAL INJURY: "You are authorized all necessary medical care for injury or disease which is the proximate result of your participation in this research. Other than medical care that may be provided, you will not receive any compensation for your participation in this research study; however, you understand that this is not a waiver or release of your legal rights."

QUESTIONS: If you need more information about the study you may call the Principal Investigator (Thomas Mack, M.D., 213-342-1638) at any time, or write to him at USC School of Medicine, Department of Preventive Medicine, 1420 San Pablo St, PMB-B105, Los Angeles, CA 90033. If you have a question about the rights of human subjects generally, and you wish to speak with someone not involved in this particular study, you may call Dr. John Nicoloff at the USC Research Committee Office (213-223-2340).

AGREEMENT: I agree to participate in this study of the causes of breast cancer by granting the release of a piece of my tumor specimen to the investigators. I have read this form and understand it. I will keep one copy for myself.

Name of Subject or Guardian	Signature of Subject*	Date

Name of Witness (printed)	Signature of Witness*	Date

*Please also initial the first page of this informed consent. Thank you.

INFORMED CONSENT

GENETIC ABNORMALITIES IN BREAST CANCERS OF TWINS

PURPOSE OF THE STUDY: The purpose of this study is to identify the genetic characteristics of breast tumor cells, especially those which only appear after the tumor has started. In certain instances, additional investigation will be made to determine if they may have been inherited and/or induced by life experiences.

FUNDING SOURCE: The study is funded by the U.S. Army, Department of Defense

PROCEDURES: By agreeing to participate in this study you give permission to release a small slice of the stored tumor specimen to the study for analysis. You must therefore voluntarily and freely donate this piece of tumor tissue to the investigators, who are acting on behalf of the U.S. Army, and you must therefore relinquish all right, title, and interest to it. It is always possible that it may be found useful for other scientific purposes in the future, and it is always possible that some unforeseen future use might result in a commercial application (no such uses have been proposed).

PARTICIPATION IS VOLUNTARY: Your participation in this study is entirely voluntary. You may withdraw participation at any time.

RISKS TO BE EXPECTED FROM THE STUDY: The only risks from participation in a study such as this are from the potential loss of privacy. From our previous studies we have already been informed about her diagnosis. As before, all results of this study will be kept entirely confidential and will not be associated with any person's name. The information which we obtain will only be released for publication in statistical form, such that no individual can be recognized. In the very unlikely event that any of the results from the study are of particular importance to your family, that information will be released, but only to you. No one other than study employees will have access to information about you, with one exception, as follows. "Representatives from the U.S. Army Medical Research, Development, Acquisition and Logistics Command are eligible to inspect the records of this research as part of their responsibilities to protect human subjects in research."

BENEFITS TO BE EXPECTED FROM THE STUDY: There are no direct benefits to you personally to be gained by participation. Of course, you will have the satisfaction of knowing that you have helped in a very significant way in efforts to learn the causes of breast cancer.

The results are also unlikely to help anyone in your family determine a specific level of risk for breast cancer. You already know whether or not her female blood relatives are at a relatively high risk from her family history of breast cancer, and results from this study are not likely to give a clearer idea of the actual level of risk. While it is true that one or more locations on the chromosome have been identified that are highly related to breast cancer risk (recently one such location identified is called BRCA1), a family member's individual risk is dependent upon knowing which specific gene has been inherited at that location. At this time, even if we are successful in attempts to characterize the genes, we are not able to accurately translate the result into a specific level of risk.

PHYSICAL INJURY: "You are authorized all necessary medical care for injury or disease which is the proximate result of your participation in this research. Other than medical care that may be provided, you will not receive any compensation for your participation in this research study; however, you understand that this is not a waiver or release of your legal rights."

QUESTIONS: If you need more information about the study you may call the Principal Investigator (Thomas Mack, M.D., 213-342-1638) at any time, or write to him at USC School of Medicine, Department of Preventive Medicine, 1420 San Pablo St, PMB-B105, Los Angeles, CA 90033. If you have a question about the rights of human subjects generally, and you wish to speak with someone not involved in this particular study, you may call Dr. John Nicoloff at the USC Research Committee Office (213-223-2340).

AGREEMENT: I agree to participate in this study of the causes of breast cancer by granting the release of a piece of the tumor specimen to the investigators. I have read this form and understand it. I will keep one copy for myself.

Name of Next of Kin	Signature of Next of Kin*	Date

Name of Witness (printed)	Signature of Witness*	Date

*Please also initial the first page of this informed consent. Thank you.

INTERNATIONAL TWIN STUDY
Department of Preventive Medicine
Parkview Medical Building B-105
(800) 421-9631 (213) 342-1642
(213) 342-1237 Fax

REQUEST AND AUTHORIZATION FOR RELEASE OF HISTOPATHOLOGICAL TISSUE

I, hereby give my permission to release the tissue specimens from my breast cancer to:

Thomas M. Mack, M.D.
International Twin Study
University of Southern California
School of Medicine
1420 San Pablo Street, PMB B-105
Los Angeles, CA 90033

I understand that the specimens are to be used for research purposes only, and that they will be returned to their present location after review.

_____ _____

Signature Date

Patient Information

Name: ^F1^ **D.O.B.:** ^F2^

A copy of this authorization is valid as the original, and is valid for the length of the Study.

^F3^

INTERNATIONAL TWIN STUDY
Department of Preventive Medicine
Parkview Medical Building B-105
(800) 421-9631 (213) 342-1642
(213) 342-1237 Fax

REQUEST AND AUTHORIZATION FOR RELEASE OF HISTOPATHOLOGICAL TISSUE

I, as next of kin, hereby give my permission to release the tissue specimens from my ^F4^ breast cancer to:

Thomas M. Mack, M.D.
International Twin Study
University of Southern California
School of Medicine
1420 San Pablo Street, PMB B-105
Los Angeles, CA 90033

I understand that the specimens are to be used for research purposes only, and that they will be returned to their present location after review.

_____ _____

Signature of next of kin Date

Patient Information

Name: ^F1^ **D.O.B.: ^F2^**

A copy of this authorization is valid as the original, and is valid for the length of the Study.

^F3^

7. Letter to hospital requesting blocks

INTERNATIONAL TWIN STUDY
Department of Preventive Medicine
Parkview Medical Building B-105
(800) 421-9631 (213) 342-1642
(213) 342-1237 Fax

October 23, 1995

Attention: Pathology Dept.

^F1^
^F2^
^F3^

RE: ^F4^ **Twin Study No.:** ^F5^

Birth Date: ^F6^ **Date of DX:** ^F7^

PATH No.:

Dear Doctor:

The International Twin Study based at the University of Southern California School of Medicine is conducting epidemiologic research on genetic changes in breast cancer tumors.

To do this research we are requesting selected histopathological tissue blocks from the patient listed on the enclosed authorization form. A copy of the pathology report, which we have previously obtained from your hospital, is also enclosed. Please send us:

1) Tissue blocks that are most representative of the tumor, and
2) Tissue blocks that include normal tissue, preferably from a lymph node.

We will make approximately 20 unstained slides from each block and return the original blocks to you within a month's time or less. (If you prefer to make the slides and send them to us, this would be acceptable).

Please send the blocks to: International Twin Study
USC School of Medicine
1420 San Pablo Street, B-105
Los Angeles, CA 90033

Atten: Ruby Sidhu

Thank you for your cooperation.

Sincerely,

Thomas M. Mack, M.D.
Professor