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TITLE:
The Risk and Clinical and Molecular Characteristics of Breast Cancer in Women with Neurofibromatosis Type 1

PRINCIPAL INVESTIGATOR:

Xia Wang, M.D., Ph.D.

CONTRACTING ORGANIZATION:

Henry Ford Health System
Detroit, Michigan 48202

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Xia Wang, MD, PhD; Renee Tousignant, MS; Albert Levin, PhD; Bruce Korf, MD, PhD; Maria Acosta, MD; Jaishri Blakeley, MD; Michael Tainsky, PhD

E-Mail: xwang1@hfhs.org

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14. ABSTRACT: The purpose of this project is to characterize the breast cancer in women affected with Neurofibromatosis type 1 (NF1) in a multi-institutional setting. The first aim is to assess the incidence of breast cancer in this cohort and the clinical features of NF1 associated with breast cancer. The second aim is to investigate any characteristic NF1 gene germline mutations in women with breast cancer and to carry out whole exome sequencing to search for other germline mutations. For the third aim, at tumor tissue level, we plan to characterize the selected signaling pathway on archived breast cancer tissue from women with NF1 utilizing immunohistochemistry (IHC) methods. At the somatic level, we plan to study loss of heterozygosity (LOH) of the NF1 gene using ion semiconductor sequencing and MLPA copy number analysis. We also plan to sequence 30 other breast cancer genes on the breast cancer specimens. By the end of May, 2013, a total of 242 cases of NF1 women have been reviewed. Eleven women have had a diagnosis of breast cancer. Statistical analysis was conducted. The presence of plexiform neurofibroma has a trend to be inversely related to the occurrence of breast cancer, however, it has not reached statistical significance ($p=0.083$). A family history of cancer is associated with a personal history of breast cancer ($p=0.000119$). No other clinical features or family histories were found to be associated with the occurrence of breast cancer. To date, germline NF1 mutations has been investigated in 10 women with breast cancer. No significant pattern of mutation has been discovered. Nine breast cancer specimens have been collected and are ready to be analyzed. We are expected to review up to 450 cases of women with NF1 and collect another 5 archived breast cancer specimens. The analyses listed in the above aims are to be completed once the final cases have been reviewed and the specimens have been collected. The fourth aim is to study the senescence effects in response to hyper-activated Ras on human mammary epithelial cells (HMEC). The work in year one did not reach any conclusion and HMEC needs a longer period of culture to be tested again. The continuation of this work has just started.

15. SUBJECT TERMS
NF1; breast cancer; signaling pathway; germline mutation; somatic mutation; FFPE; IHC; ion semiconductor sequencing; whole exome sequencing, NF1 knockdown cells; senescence, Ras

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INTRODUCTION

Several reports from England and United States have described increased breast cancer occurrence in women affected with Neurofibromatosis type 1 (NF1). This study aims at identifying an accurate incidence of breast cancer in this group of women in a multi-center collaborative environment. There are 4 specific aims. Aim 1 is to confirm the increased breast cancer risk in women with NF1. All the participating centers, Henry Ford Health System (HFHS), University of Alabama at Birmingham (UAB), Children's National Medical Center in D.C. (CNMC), and Johns Hopkins University (JHU), will review the medical records of women affected with NF1. Clinical data will be analyzed to determine whether there are clinical features associated with the increased risk for breast cancer. At the same time, women with a history of breast cancer will be recruited to donate blood and their archived tumor specimen. Aim 2 is to analyze the germline *NF1* gene and whole exome sequencing in the subjects with history of breast cancer. The NF1 mutations identified will be analyzed for genotype-breast cancer correlation. Other germline gene changes identified may reveal germline breast cancer predisposition in addition to the NF1 gene. Aim 3 is to determine if NF1 associated breast cancers have unique signaling pathways and molecular tumorigenesis characteristics. Immunohistochemistry study of the signaling pathway will be performed on archived tumor blocks. NF1 gene copy number variation will be analyzed on these tumor specimens. Next generation sequencing of a 31 gene panel known to be associated with breast cancer will be performed as well. The NF1 gene is included in the panel. Aim 4 is to study the phenotype of NF1 knockdown in primary mammary epithelial cells. This study will provide information to help in determining when and how to screen for breast cancer in this group of women. It will also shed light on the molecular mechanisms of breast cancer in *NF1* deficient human subjects.

BODY

Aim 1:
To confirm the increased breast cancer risk in women with NF1. To identify any clinical features associated with the risk for breast cancer.

Clinical medical history data collection, analysis, patient contact and specimen retrieval

Methods:

Multicenter chart review was performed on 242 women with NF1. Information on common clinical features of NF1, tumor diagnosis, as well as family history of NF1 and cancer diagnosis was collected.

Statistical analysis was performed to detect any association between each clinical feature of NF1 and incidence of cancer. The analyzed features and cancers are illustrated as below.

Clinical features of NF1: Pigmentary changes on skin and eyes (café au lait spots and Lisch nodules), bony dysplasia, scoliosis, short stature, macrocephaly, learning disability, vasculopathy, cutaneous/dermal neurofibroma, plexiform neurofibroma, optic glioma, and MPNST.

Cancers: Malignant solid tumors, hematological malignancies and central nervous system tumors were classified as "cancer" in this study. Plexiform neurofibroma, cutaneous neurofibroma and schwannoma were not included in the "cancer" category in this report.

Data:

Table 1. Among 242 reviewed cases, there are 11 cases (4.54%) with a diagnosis of breast cancer.

Total cases of breast cancer	Family History of NF1		Family History of Cancer		Family history of Breast Cancer		
	Yes	Unknown	Yes	No or Unknown	Yes	No	Unknown
11	5	6	7	4	5	4	2

Table 2. Incidence of cancer and plexiform neurofibroma in 242 cases.

History of Cancer		History of Plexiform Neurofibroma (PN)	
Number of cases	Cohort Incidence (%)	Number of cases	Cohort Incidence (%)
41	16.9 %	72	29.7 %

Table 3. For certain number of cases, clinical information is unavailable during chart review for some of the NF1 features.

Traits	Information not available in the chart review	
	Number of cases	Percentage %
Café au lait spots	31	13
Dermal Neurofibromas	17	7
Lisch nodules	106	44
Plexiform neurofibroma (PN)	57	23.5
Learning disability (LD)	86	35.5
Vasculopathy	97	40
Scoliosis	72	29.8
Bone dysphasia	46	19
Short stature	74	30.6
Macrocephaly	70	28.9
Family history of NF1	48	19.8
Family history of breast cancer	80	33

Table 4. Number of different types of cancer in 242 cases review.

Cancer Type	Number of Cases	Cohort Incidence (%)
Breast	11	4.5
Optic glioma	20	8.2
MPNST	10	4.1
CNS tumor	12	5.0
GISTs	3	1.2
Hematological cancer	2	0.8
Adenocarcinoma of GI	1	0.4
Ovarian	2	0.8

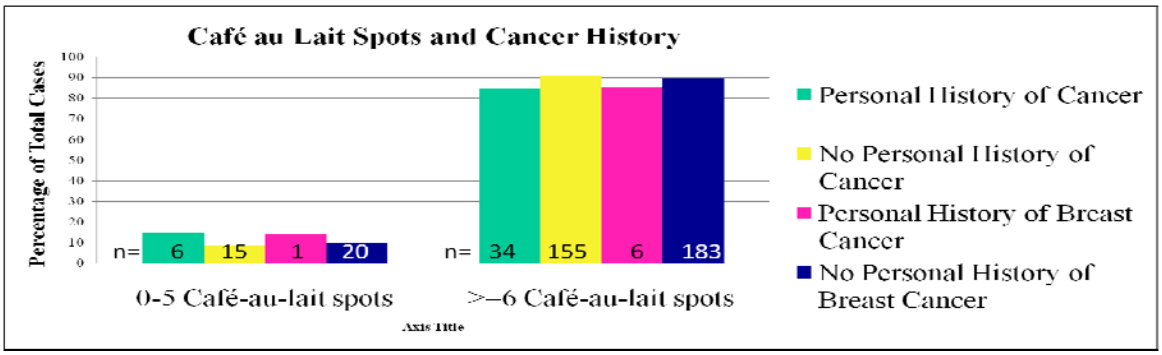

Figure 1. The number of café-au-lait spots is not a predictor for cancer risk.

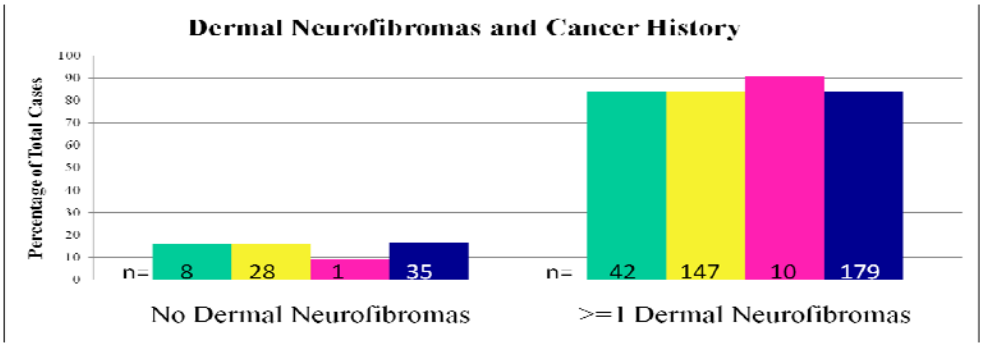

Figure 2. The presence or absence of dermal neurofibromas is not a predictor for cancer risk.

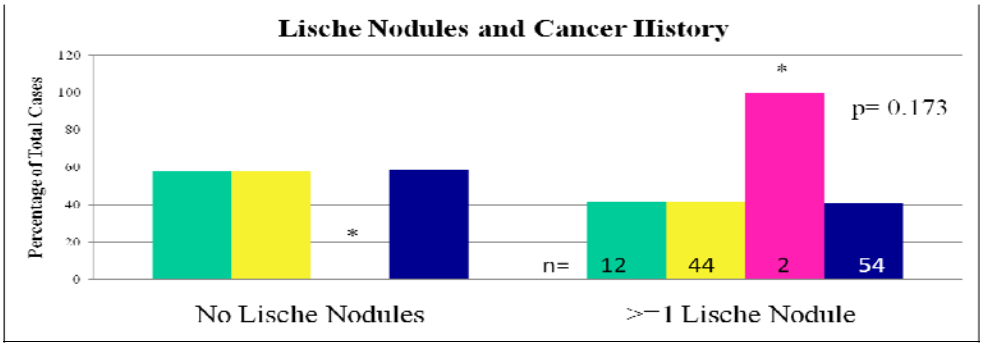

Figure 3. The presence or absence of Lisch nodules is not a predictor for cancer risk.

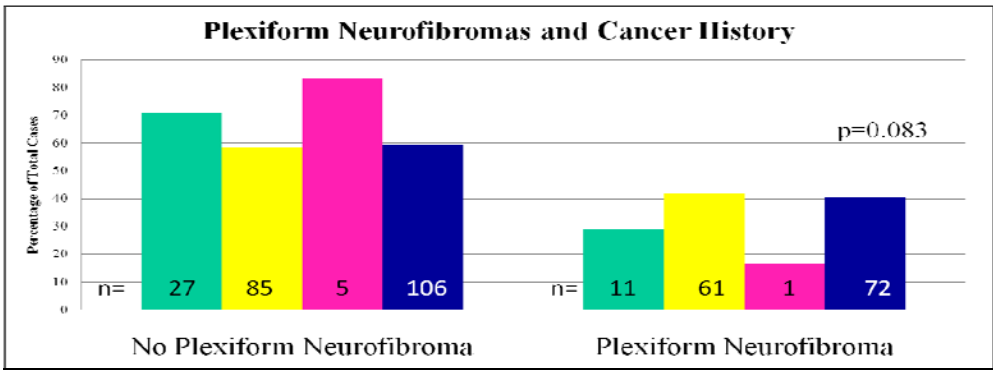

Figure 4. We observed a trend towards an inverse relationship between the presence of plexiform neurofibromas and a personal history of breast cancer, but it has not reached statistical significance.

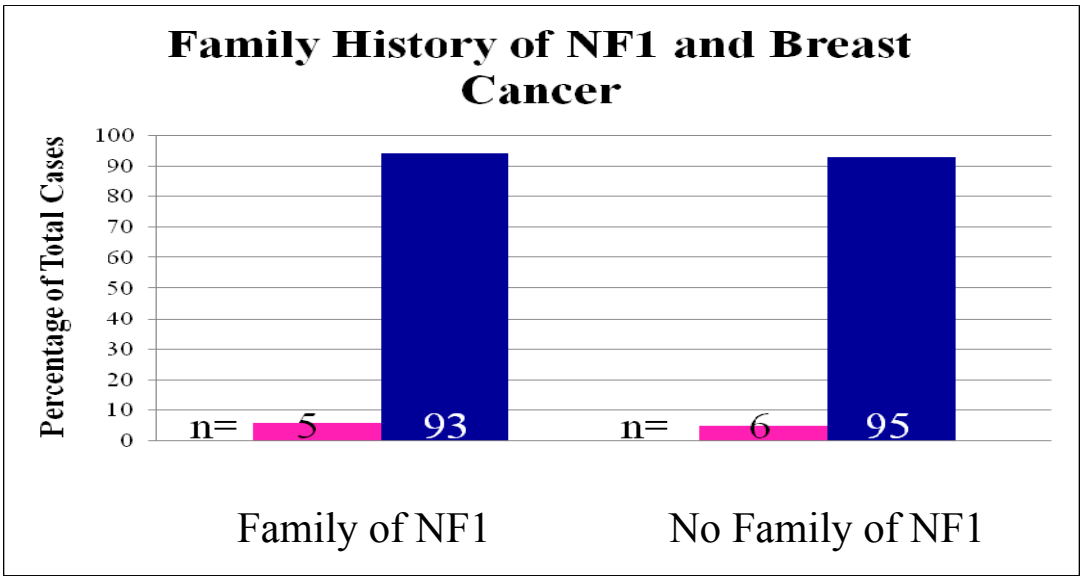

Figure 5. Breast cancer is not associated with a family history of NF1. Cases with history of breast cancer are in pink; cases without breast cancer are in blue.

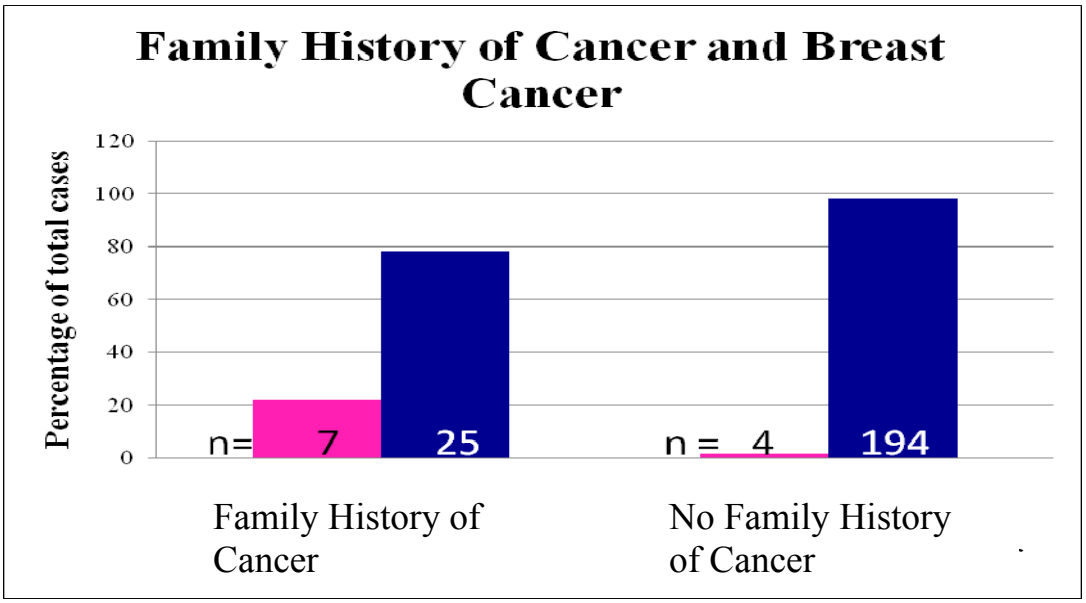

Figure 6. Breast cancer is seen more often in women with a family history of cancer (p=0.000119). Cases with history of breast cancer are in pink; cases without history of breast cancer are in blue.

Aim 2:
To analyze germline NF1 gene on the subjects with history of breast cancer. The mutations identified will be analyzed for genotype-phenotype correlation.

NF1 gene mutation testing and mutation data analysis.

A total of 10 participants who has had a clinical diagnosis of NF1 and breast cancer underwent germline NF1 gene analysis:

Table 5. Types of NF1 mutation found in 10 women who developed breast cancer.

Type of mutation	Number of cases
Nonsense mutation causing truncation	0
Missense mutation	3
Splicing mutation	2
Nucleotides deletion causing frame shift	1
Exon deletion	1
Nucleotides insertion causing truncation and splicing error	1
Missense variant of unknown significance	1
No mutation detected	1

Aim 3:
To determine if NF1 associated breast cancers have unique signaling pathway and molecular tumorigenesis characteristics.

A total of 9 tumor blocks have been collected. Since we are expecting more tumors to be collected, analysis has not been started.

Tumor specimen molecular analysis by LOH and methylation assay for NF1, p53, BRCA1, BRCA2, PTEN, and ATM genes and IHC assay for proteins, pMEK, ERK, pERK, AKT, mTOR, p53, PTEN, Her2, Ki67 proteins .(Up to 50 specimens and 100 controls)
To be completed

Full gene sequencing on formalin-fixed paraffin-embedded (FFPE) tissue: NF1, BRCA1, BRCA2, TP53, PTEN, and ATM genes, and additional breast cancer gene targets including; CDH1, RB1, MLL3, MAP3K1, CDKN1B, PIK3CA, AKT1, GATA3 TBX3, RUNX1, CBFB, AFF2, PIK3R1, PTPN22, PTPRD, 3F3B1, and CCND.
To be completed

Aim 4:
Phenotypic Analysis of NF1 knockdown in normal mammary epithelial cells.
Mammary cell line (MCF10A) and Human mammary epithelial cells (HMEC) siRNA NF1 lentivirus knockdown construction and phenotype testing – initially was only budgeted for 1 year (month 1-12). The continuation of the project has just started.
To be completed

KEY RESEARCH ACCOMPLISHMENTS

1. A total of 242 cases of NF1 women are reviewed. There are 11 cases (4.54%) with a diagnosis of breast cancer.
2. Statistical analysis was performed on the clinical features in women affected with breast cancer.
3. Nine women were recruited for germline NF1 analysis. One woman underwent NF1 analysis before participate this study. In all, germline NF1 gene mutation has been obtained for 10 women.
4. Nine breast cancer specimens have been collected.

REPORTABLE OUTCOMES

Not available at the present time.

CONCLUSIONS

1. No association has been found of statistical significance between cancer incidence and NF1 clinical features other than the ones discussed below (Figure 1, 2, and 3).

2. Plexiform neurofibroma (PN) was seen less often in women with a personal history of breast cancer (Figure 4). However, it has not reached statistical significance. This may suggest a fundamental difference between the tumorigenesis of PN and breast cancer.

3. Women with a family history of NF1 were no more likely to have a personal history of breast cancer than those without (Figure 5). However, women with a family history of cancer were more likely to have a personal history of breast cancer, $p= 0.000119$ (Figure 6). This may indicate that breast cancer in women with NF1 may be a result of familial germline cancer risk. This germline cancer risk is independent of NF1 and cannot be specified at this time. An alternative explanation is that this familial cancer risk is due to environmental exposures that are familial or geographically specific.

4. The weakness of this report is that the total number of breast cancer cases is relatively small. Clinical information is incomplete in a significant number of cases.

5. We expect to review more cases. Statistical analysis will be conducted again once we discontinue the case review.

REFERENCES

None

APPENDICES

None