The Sister Study

www.SisterStudy.niehs.nih.gov

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Overview

The Sister Study is a prospective cohort study of environmental and genetic risk factors for breast cancer and other diseases among 50,884 sisters of women who have had breast cancer. Such sisters have about twice the risk of developing breast cancer as other women, thus about 300 new cases of breast cancer are expected to be diagnosed each year. Study enrollment opened nationally in October 2004 and closed in July 2009. Eligible women were 35 to 74 years of age, lived in the United States, including Puerto Rico, and had a sister diagnosed with breast cancer but did not have breast cancer themselves. Multiple recruitment strategies were used to enroll a diverse cohort of women with a variety of different life experiences and exposures. Baseline data on potential risk factors and current health status were collected in telephone interviews and mailed questionnaires. Blood, urine, and environmental samples were collected during a baseline home visit and banked for future use in nested case-cohort or case-control studies of breast cancer or other diseases. Stored samples include whole blood, cryopreserved whole blood or lymphocytes (12% random sample), plasma, serum, urine, toenail clippings, and household dust collected with alcohol wipes. The cohort is being followed prospectively. Contact information and major changes to health are updated annually. Comprehensive triennial questionnaires update medical history and changes in exposures. Medical records, pathology reports, and tumor tissue blocks are sought for women who develop breast and (recently) ovarian cancer. For other cancers, pathology reports are requested. Other self-reported health outcomes are validated for special studies. Analyses assess the effects of environmental and lifestyle exposures and genetic factors on breast cancer risk and risk for other diseases (e.g. heart disease, osteoporosis, other hormonal cancers, and autoimmune diseases). Future studies of environmental and genetic influences on breast cancer prognosis are made possible by continuing to follow women in the cohort who develop breast cancer.

Background and Rationale

Breast cancer is the leading (non-skin) cancer among women with approximately 282,000 diagnoses of breast cancer and 44,000 deaths per year in the United States. Known risk factors explain less than 50% of variation in breast cancer risk and known breast cancer genes account for fewer than 10% of cases. The Sister Study was designed to study environmental and genetic

risk factors for breast cancer and other women's health conditions. The study creates a framework for addressing current and future hypotheses as science advances over the follow up period, including studies related to biological mechanisms. Studying sisters of women diagnosed with breast cancer is advantageous because it allows for a smaller cohort size and shorter follow-up than needed to study women in general. These sisters have about twice the risk of breast cancer as other women and the frequency of relevant genes and shared risk factors will also be higher, increasing the statistical power of the study. This enhances the ability to assess the interplay of genes and environment in breast cancer risk and to identify potential modifiable risk factors. In addition, sisters are often highly motivated to participate in long-term breast cancer research because their family member has experienced the disease so the response rates and compliance are high. The prospective design allows the assessment of exposures before the onset of disease thereby avoiding biases common to retrospective studies.

Recruitment and Enrollment

After a four-city vanguard phase in 2003, nationwide enrollment took place October 2004 through March 2009. Eligible women were 35 to 74 years of age, lived in the United States, including Puerto Rico, and had a sister diagnosed with breast cancer but did not have breast cancer themselves. Efforts were made to maximize the inclusion of women who are often under-represented in research, such as minoritized racial and ethnic groups, those with low education, and those aged 65 years and older, and to target women with possible relevant exposures because of their place of residence or occupation. Recruitment activities included outreach to volunteers and breast cancer organizations, networking with communities, direct mailings to specific lists, national media campaigns and the endorsement of the Sister Study by high profile celebrity supporters. Study materials were made available in Spanish in 2005. Additional details can be found in Sandler, et al. 2017 (PMID: 29373861)

Study Population

A total of 50,884 women completed required baseline activities and were fully enrolled in the study, including 8,311 women (16%) who self-identified as Hispanic/Latina or non-White, 8,874 women aged 65 years and older (17%), and 7,805 women with a high school education or less (15%). A smaller group of women who completed some but not all study requirements (n=3,066) is being followed passively through record linkage (vital statistics and possibly cancer registries) to assess differences in outcomes for those who did and did not fully enroll. This latter group includes a larger percentage of minority women (36%) and women with fewer years of schooling (18%), women who were the focus of intense recruitment efforts towards the end of the recruitment period. Reflecting the volunteer nature of the cohort and the recruitment of sisters of women with breast cancer, Sister Study participants have higher education levels and have higher prevalence of known breast cancer risk factors, including enhanced family history (Table 1). Additional information about the cohort can be found on the study's website – www.SisterStudy.niehs.nih.gov.

Baseline characteristics of women in the Sister Study

Characteristic	Number	Percent
Total	50,884	
Race/ethnicity		
Non-Hispanic White	42,558	83.6
Non-Hispanic Black/African American	4,600	9.0
Hispanic/Latina	2,377	4.7

Other[a]	1,334	2.6
Age		
35-44	6,578	12.9
45-54	17,520	34.4
55-64	17,912	35.2
65+	8,874	17.4
Education		
High school or less	7,805	15.3
Some college	9,957	19.6
Associates or technical degree	7,224	14.2
Bachelor's degree	13,714	27.0
Master's or doctoral degree	12,172	23.9
Smoking		
Nonsmoker	28,552	56.1
Past	18,141	35.7
Current	4,175	8.2
Alcohol consumption		
Never	1,949	3.8
Former	7,730	15.2
Current, < 1 drink/day	34,256	67.4
Current, ≥ 1 drink/day	6,862	13.5
Body Mass Index		
Normal/underweight	19,438	38.2
Overweight	16,151	31.7
Obese	15,278	30.0
Number of sisters (full or half) with breast cancer		
1	45,706	89.8
2	4,548	8.9
3+	629	1.2
Mother diagnosed with breast cancer	9,135	18.0

[a]Includes non-Hispanic Asian/Pacific Islanders, non-Hispanic American Indians, and non-Hispanic Other; women who self-identified as Black/African American and another race were included as Black/African American

Data Collection

Baseline

Computer-assisted telephone interviews (CATI): Telephone interviews were scheduled in two one-hour sessions to collect information on a broad range of exposures and lifestyle characteristics. Supporting materials, including a list of relevant medications and a chronological life calendar, were provided to help women prepare for the interviews. Topics included demographic and socioeconomic factors, lifestyle and environmental exposures, residential history, medical and medication-use history, reproductive history and hormone use, breast conditions and surgeries, occupational history, and physical activity. Questionnaires focused specifically on early life (before puberty) and reproductive years as well as the time of enrollment.

Self-administered questionnaires: Participants filled out three self-administered questionnaires: use of personal care products; prenatal (*in utero*) exposures and family medical history; and current diet (Block 98 food frequency questionnaire), with supplementary questions on

complementary and alternative medicines, childhood diet, special diets, and eating patterns.

Home Visit: Trained female examiners from a national in-home phlebotomy service (EMSI) visited participants' homes (or a mutually agreed upon alternate site such as a doctor's office) to draw blood, measure blood pressure, height, weight, hips, and waist and to retrieve consent forms, self-administered questionnaires and self-collected toenails, dust, and urine. Participants filled out a brief questionnaire on the day of the visit to report information on their diet, medication use, and activities over the past 24 hours. Examiners packed and shipped study samples and forms to the Sister Study Laboratory by FedEx Priority Overnight on the same day as the visit.

Biological Specimen Collection: Nearly all participants provided biological samples. Details of collection and processing can be found on the Sister Study website (https://sisterstudy.niehs.nih.gov/english/images/SIS_SpecimenSummary_20120323_webversion.pdf)

Toenails: Participants collected toenail clippings from each toe unless they had a medical or physical condition (e.g. diabetes) that would prohibit collection. Samples are stored at room temperature.

Dust: Participants collected dust samples from three rooms of their home using pre-packaged alcohol wipes. Wipes are stored in -20° C freezers.

Urine: Participants collected clean-catch midstream first morning urine specimens on the day of the home visit and kept them refrigerated until pick up by the examiner.

Blood: Participants were instructed to fast for at least eight hours prior to their blood draw. Examiners collected approximately 45 ml of blood using six BD Vacutainer® (Becton, Dickinson and Company) tubes, including two EDTA tubes (BD#s 367855 and 366643), two serum tubes (BD# 367820) and two ACD-B tubes (BD# 364816). In the rare event that a blood sample could not be collected due to an unsuccessful phlebotomy, participants were asked to provide a saliva sample using an Oragene™ DNA self-collection saliva kit (DNA Genotek, Ottawa Canada).

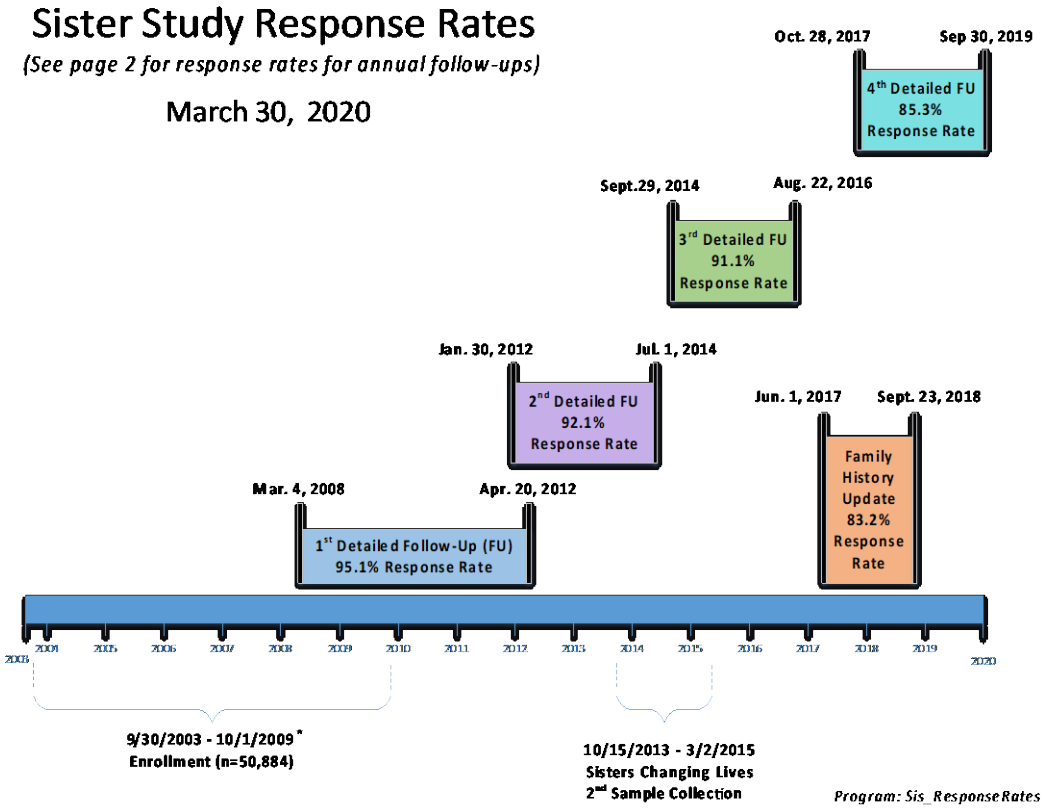
DNA: DNA was extracted from whole blood (90%), clot, or saliva. We initially extracted DNA for ~2,400 breast cancer cases, 140 ovarian cancer cases, a random sample of the cohort (n=2,350), and additional premenopausal women to maximize studies related to breast cancer in premenopausal women. These cases and non-cases have been included in large scale GWAS and methylation studies. Since then we have added more recently diagnosed breast and ovarian cancer cases, women diagnosed with other cancers, additional premenopausal women, and another random sample of the cohort. The other cancers selected were those likely to be hormonally related, to have reasonable sample size to support candidate-SNP analyses or pooling efforts, and to either have high rates of medical record confirmation or be those for which self-reports are likely to be valid. DNA is also available for cases and non-cases included in early pilot efforts. In all, DNA is available for 19,000 women in the cohort.

Follow-up

The Sister Study cohort is followed prospectively to identify incident breast cancer and other health outcomes. Participants can report a diagnosis of breast cancer or other conditions at annual updates (selected outcomes) or follow-up questionnaires, or they can contact a study helpdesk by telephone, mail, or email. Annual update forms and biennial/triennial follow-up questionnaires are available in English and Spanish. Starting in 2010, all study materials have been available on the web and women have the option of completing follow-up questionnaires on-line, by mail, or over the phone. Annual updates and questionnaires are administered in

"waves" representing groupings by enrollment date. Over time, waves have been combined to condense the time it takes to complete a single follow-up activity from 5 years to just over 2 years (see schematic below).

Women reporting a diagnosis of LCIS, DCIS, invasive breast cancer or ovarian cancer are asked to provide information on their diagnosis and treatment and provide authorization for medical record and tumor tissue sample retrieval. Women with other cancers are asked for pathology reports or permission to retrieve them from medical providers. Protocols for validating other incident conditions reported during follow up are developed as needed.



Annual updates: Women are contacted annually for a brief update or a scheduled detailed follow-up questionnaire. The annual update form collects changes in contact information and allows participants to report major changes in health, including breast cancer. Response rates for the annual updates have been 91% or higher throughout follow-up (range 91%-96%). Through the end of July 2021, 2,967 (5.8%) of Sister Study participants were known to be deceased.

Detailed questionnaires: More in-depth questionnaires collect information on medical diagnoses and symptoms, changes in environmental exposures and lifestyle, and special topics of interest. The first detailed (biennial) follow-up, completed in July 2012, consisted of three questionnaires: *Health and Medical History*, *Lifestyle*, and the special topic, *Stress and Coping*. Responses were obtained from 48,090 women for an overall response rate of 95%. For the next round of detailed follow-up, the study shifted to triennial administration to reduce participant burden and simplify workflow. The special topic for the 2nd detailed follow-up (completed April 2014) was *Quality of Life and other related topics.* The 3rd detailed follow-up introduced a streamlined reproductive section for participants >60 years of age; this follow-up was completed in August 2016. With this

questionnaire, we introducted an advocacy program, which provides more personal attention to those at higher risk for dropping out of the study. The result was a preservation of high response rates (91%), with an approximately 2% *increase* in response rate among minority women. A 4th follow-up was completed in 2019 (response rate 85%) and a 5th is currently in the field (starting fall 2020, response rate of 67% through July 2021, on track with prior surveys). Additionally, a detailed family history questionnaire was distributed in 2017-2018 to collect data on the history of cancer in first and second degree relatives (response rate of 83%), and a special COVID-19 survey was distributed in the fall of 2020 (response rate of 74% through July 2021).

Breast and Ovarian Cancer Follow-up

The breast cancer follow-up protocol has been streamlined over time to reduce participant burden and maximize response rates. We are now also getting more detailed follow-up information from ovarian cancer cases. Women are now contacted 6 months after diagnosis, closer to the end of their treatment. They are mailed a packet that includes instructions, breast cancer definitions, a self-administered questionnaire and authorization forms for requesting medical records and tumor tissue samples. The questionnaire was streamlined to focus on information only the woman can provide herself, such as how the tumor was detected, her health insurance status, and quality of life after diagnosis. It also covers basic information on tumor pathology and treatment in case the medical record is not obtained. We ask women to send us a copy of their pathology report if they have one. Medical providers are asked to complete a form about the breast cancer diagnosis and treatment and/or provide relevant pages from the medical record. They are also asked to send pathology reports, blocks of breast (ovarian) carcinoma and normal breast tissue, and diagnostic H & E slides.

As of the most recent Sister Study Data Release (9.0), 3,999 women had reported a diagnosis of incident DCIS, or invasive breast cancer. Out of the total incident breast cancer events at that time, medical records or pathology reports were obtained to confirm 3,269 (81.7%) incident breast cancer events, and 2,487 tissue samples have been retrieved. Among women for whom we obtain pathology reports or medical records, the positive predictive value (PPV) of a self-reported breast cancer is 99.4%.

Breast Tissue Microarrays (TMAs) and Tissue Cores: Tumor and normal tissue blocs are being used to create TMAs for immunohistochemical staining and to obtain tissue core biopsies. TMAs are prepared at the UNC Translational Pathology Laboratory. Mark Sherman, formerly at NCI and now at Mayo Clinic, Jacksonville Florida, serves as study pathologist. He oversees the review of slides, documentation of tumor features, and selection of tissue for sampling for TMAs and cores, which are extracted and placed into individual tubes. When available, TMAs and cores include invasive cancer tissue, co-occurring DCIS, and adjacent normal tissue. Initial immunohistochemical staining will include ER, PR, HER2, Ki67, EGFR, and CK6/6 to allow for comprehensive classification of breast cancer subtypes.

Other incident conditions

In 2010 we began validating other (non-breast) cancers, with prioritization of types based on relevance to hormonal-related hypotheses, frequency of diagnosis, and opportunities for consortia collaboration. Women are asked to mail a copy of the pathology report to us if available and to sign an authorization form allowing us to request it from their medical provider.

Second Specimen Collection

In 2014/2015 we carried out the Sisters Changing Lives initiative in which we invited 3,800 women to complete a second home visit for sample collection. Procedures were identical to those at the

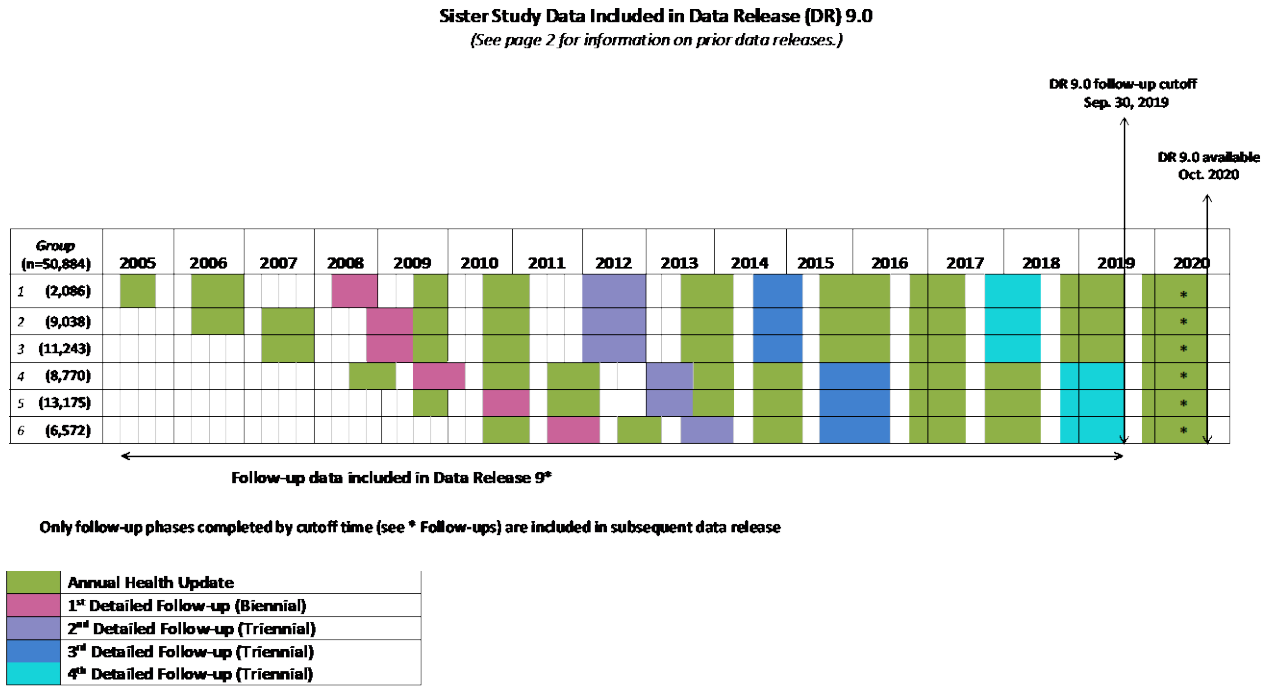
enrollment visit except an RNA Tempest tube was substituted for one of the baseline whole blood tubes. Women diagnosed with breast cancer by the time of the initiative and a random sample of the cohort were targeted. Samples were obtained for 61% of invited breast cancer cases and 65% of non-cases. A total of 2,434 women participated, of whom 1,227 were cases. This resource allows for studies of changes in biomarkers over time and of changes in biomarkers due to a breast cancer diagnosis.

Special COVID Survey

In response to the 2020-2021 COVID-19 pandemic, we designed a special questionnaire to collect data on coronavirus infections, testing, and COVID-related health behaviors. We also included questions about screening or treatment delays, mental health (including stress, anxiety, and sleep health), and many other factors. The survey was sent to all active participants in November 2020, with a 74% response rate through July 2021. Several COVID-related questions (e.g. ever infected, vaccine status) were also added to the annual follow-up questionnaire and 5th detailed follow-up questionnaire. Additionally, we joined a large collaborative group collecting COVID data from large cohorts in the US or UK via the Zoe app (https://covid.joinzoe.com/).

Data Management and Processing

Over the course of the study, data files were released for analysis for the first 10,000 (2006), 20,000 (2007), 30,000 (2008) and the final baseline cohort of 50,884 (2011) participants. To create continuity across analyses and papers, we are now using data releases. The first data release was created in January 2013. Data releases are issued approximately once per year to incorporate new follow-up data, including updated mortality data from the National Death Index, and any changes due to data cleaning. The most recent data release (DR 9.1) was in June 2021. Data Release 10.0 is expected in early 2022.



Data Sharing and Collaboration

In the interest of promoting scientific research on the environmental and genetic risk factors for breast cancer and other diseases, the Sister Study welcomes proposals for collaborative studies from within NIEHS and the wider scientific community. Proposals are reviewed to ensure scientific merit and to protect the integrity of the study and the confidentiality of participants. Acceptable study topics will take advantage of the unique characteristics of the Sister Study cohort and may involve the analysis of routinely collected data or specimens or involve new data collection. Information on available data, instructions on how to submit a research topic and proposal, and guidelines regarding the use of study data and specimens can be found on the Sister Study data portal at ***www.sisterstudystars.org***. This portal tracks study proposals, data requests, specimen use and manuscripts.

Consortia and data pooling

The Sister Study participates in the National Cancer Institute's Cohort Consortium, a group that facilitates the pooling of data from individual cohort studies to create high-quality databases large enough to investigate risk factors for rare cancers or to study low-penetrance genetic variants and other factors with small effects in relation to breast and other common cancers. More than 20 international cohorts are included.

Sister Study investigators (Dr. Sandler and former fellow Hazel Nichols, now at the University of North Carolina) are leading a Cohort Consortium project on premenopausal breast cancer in collaboration with investigators from the Institute of Cancer Research, London. Initial efforts aim to understand pregnancy-related breast cancer risk factors and other exposures that may differentially affect premenopausal versus postmenopausal breast cancer, such as obesity, physical activity, and hormone therapy use. Drs. O'Brien and Sandler have also led projects in the Ovarian Cancer Cohort Consortium (OC3), including studies of genital powder use and an upcoming project on hormone therapy.

The Sister Study also has contributed data to Cohort Consortium studies on head and neck, gallbladder, and thyroid cancers as well as large GWAS and sequencing-based studies of breast and ovarian cancer. This allows us to contribute to research on cancers for which we lack sufficient power on our own or to contribute to the large efforts needed for gene identification and risk prediction. These include:

- The Confluence Project, NCI
- Biomarkers and Breast Cancer Risk Prediction in Younger Women, NCI
- Diet and Cancer Consortium, NCI
- Breast Cancer Association Consortium (BCAC)
- Cancer Risk Estimates Related to Susceptibility Genes (CARRIERS) Consortium, Mayo Clinic
- Breast CAncer STratification (B-CAST) Consortium, Netherlands Cancer Institute
- Ovarian Cancer Association Consortium (OCAC)
- Biliary Tract Cancers Pooling Project, Epidemiology and Genomics Research, NCI
- Reproductive and Hormonal Factors and Thyroid Cancers Risk Pooling Project, NCI
- AMH and Breast Cancer Pooling Project (NYU and the NCI Cohort Consortium)
- Collaborative Study to Find Genetic Variants Associated with Variation in Anti-Müllerian Hormone Levels, Exeter University (UK)

The Sister Study also contributes to other meta-analyses and data pooling efforts outside the Cohort Consortium:

- Trauma and ovarian cancer, Moffitt Cancer Center
- Circulating hormones in premenopausal women and subsequent breast cancer risk, NCI
- Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BODICEA), Cambridge UK

Through an agreement with the PIs, we will be receiving a near complete copy of their study data (enrollment and follow-up questionnaires and selected cancer outcomes) for the **Breakthrough Generations Cohort** that will allow for pooling data from the two studies to evaluate new hypotheses that require a larger sample or to validate findings published from either cohort alone. https://www.cancerresearchuk.org/about-cancer/breast-cancer/research-clinical-trials/generations-study

Finally, The Sister study participates in research led by extramural collaborators, funded by NIH or other grants. For example, the Sister Study is one of several prospective cohorts included in a study (NIH RO1, Joel Kaufman, PI) of ambient air pollution and incident cardiovascular. As part of that effort, self-reported cases of cardiovascular disease in the Sister study were validated with medical records and probability-based algorithms were created to classify those with no available records. A spin-off study will include some of the same cohorts in a pooled study of air pollution and breast cancer.

Geocoding studies

Sister Study enrollment, longest-lived and childhood residences have been geocoded allowing linkage to air pollution, census, and other data. Follow-up addresses were recently geocoded as part of the cardiovascular disease collaboration with Dr. Kaufman. A new effort will attempt to identify Sister Study participant's interim addresses (post-1980) using the LexisNexis database.

Ancillary Studies

Two Sister Study

The Two Sister Study, which completed enrollment in December 2010, is a family-based study of genetic and environmental risk factors for young onset (before age 50) breast cancer. The study recruited the affected sister of Sister Study participants (the sister with breast cancer who was not in the Sister Study) if her diagnosis was before age 50 and within four years of screening for eligibility. Case-sisters completed the same computer-assisted telephone interviews as their sisters did when joining the Sister Study, provided saliva, dust, and toenail samples, provided detailed information about their breast cancer diagnosis and treatment, and were asked to authorize retrieval of medical records and tumor samples. In addition, participants invited their parents to provide a saliva sample as a source of DNA for genetic analyses. Over 1,400 young-onset sisters enrolled in the study by completing questionnaires and/or providing saliva samples for DNA. These index cases are the sisters of ~1,700 women in the Sister Study (who also provided questionnaires and samples for DNA). Of their parents, 1,438 provided a saliva sample. About 1,300 of the sisters with young-onset breast cancer completed all study requirements (all questionnaires and saliva sample) and are now being followed prospectively along with Sister Study participants who developed breast cancer after joining the study.

CDC Special Survey and Survivorship Survey

In response to a CDC mandate under the Young Women's Breast Health Awareness and Support of Young Women Diagnosed with Breast Cancer bill (the 2010 EARLY Act), the Sister Study teamed with researchers from the Epidemiology and Applied Research Branch in the Division of Cancer Prevention and Control at the Centers for Disease Control and Prevention (CDC) to 1)

survey breast cancer free Sister Study participants about breast cancer screening practices, family communication about cancer, and the effect of having a sister with breast cancer on participants and their families. About 18,000 women participated in 2012; 2) survey women diagnosed with breast cancer about topics of interest to younger women such as body image, work-life balance, relationships and intimacy, and fertility, as well as impact of cancer on the lives of breast cancer survivors and their families, survivors' quality of life, physical and emotional health, changes in lifestyle and environment, and coordination of cancer treatment and follow-up care. This survey was completed (2012-2013) by 2,537 women with breast cancer in the Sister Study and the Two Sister Study.

Validation of Early Life Factors

Data collection for a validation study of self-reported early life factors was completed in 2012. The aim was to evaluate how accurately women reported the information on early life collected at baseline, including information on their mother's pregnancy. A total of 1,802 of the participants' mothers completed a questionnaire after receiving an invitation from their daughters.

Mammography Initiative

In collaboration with investigators from Columbia University, the Sister study attempted to retrieve digital and film mammography images from a case-control sample of participants age ≤55, with a goal of studying factors related to breast density changes over time. Approximately 60% of women contacted for the study provided medical release forms, allowing for the collection of 10,000+ mammograms from more than 1,500 women.

Olfactory Impairment

Dr. Honglei Chen of Michigan State University received grant funding (Department of Defense and Parkinson's Foundation) to study airborne pollutants, the olfactory system, and Parkinson's disease. To support this research, a sample of 3,406 participants aged 50-79 completed a special survey about olfaction status and a Brief Smell Identification Test.

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Sister Study Publications 2017- August 2021

More than 220 Sister Study papers (primary papers and as part of large consortia) were published **since 2007**. A complete list of papers can be found on the Sister Study Website (https://sisterstudy.niehs.nih.gov/).

2017

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