Project Abstracts & Specific Aims (1996 – 2008)
Breast Cancer Family Registry
National Cancer Institute

Application ID: B-AU-0597-02-C03-A3 **Status:** Approved, Ongoing

Principal Investigator: Chenevix-Trench, Georgia **Year:** 2008

Institution: Queensland Institute for Medical Research

Title: *Association Studies to Identify Low-Risk Breast Cancer Genes*

Abstract:

We intend to use material already collected by the Australian site of the Breast Cancer Family Registry (BCFR) to identify single nucleotide polymorphisms (SNPs) that appear to be associated with breast cancer risk. The scale of this study allows us to test both recessive and dominant models with reasonable power to detect moderately small effects. Having determined which SNPs, genes or pathways appear to be of most relevance we will then apply to the BCFR for collaborative studies to test these associations in material from the other population-based sites, and for additional funds to support the work.

Aim 1: to genotype selective SNPs in women affected with breast cancer, and controls frequency-matched for age, and thereby identify SNPs in predisposition genes acting in pathways that could be associated with a low risk of female breast cancer - Phase 1

Aim 2: to genotype relatives in the nuclear families for any SNPs found to show an association with breast cancer risk in Phase 1 in an attempt to replicate the finding by family-based analyses that will provide an essentially independent validation free from stratification bias – Phase 2

Aim 3: having identified SNPs associated with breast cancer risk, to genotype additional SNPs in the gene(s) to determine which SNPs or haplotypes are most strongly associated with risk and may be functional.

Selected SNPs will include those in:

a) genes whose products interact with BRCA1 and BRCA2
b) genes involved in de-toxification and hormone metabolism pathways
c) genes located under the highest linkage peaks from a genome scan of multiple-case families

In addition, under our original approval (B-AU-0597-02) we have a very valuable collaboration with Drs Doug Easton in which we have been providing rapid testing of positive associations found in the Anglian Breast Cancer Study in a variety of genes. We would like to be able to continue this collaboration and be able to respond rapidly to requests from them, and others, for analysis of SNPs found to be associated with breast cancer in any relevant gene.

Application ID: B-EX-1206-01-A2 **Status:** Approved, Ongoing

Principal Investigator: Easton, Douglas **Year:** 2008

Institution: Strangeways Research Laboratories

Title: *The Breast Cancer Association Consortium (BCAC) Collaborative Studies*

Abstract:

Many groups have attempted to identify low penetrance breast cancer susceptibility variants through case-control association studies. These studies have largely concentrated on variants in “candidate” genes thought to be functionally relevant to the disease. Some may be true associations, but these have been impossible to identify because most studies are too small to provide enough power to detect the modest associations that are likely to be present. Recently we have conducted a genome-wide association study of breast cancer, which offers much better prospects for identifying true associations than candidate gene studies. However, genome-wide association studies will also generate many false positive associations, and replication in larger case-control series are required to sort the true positives from the false positives. The general aim of this proposal is to evaluate the breast cancer risk and survival associated with germline genetic variation, through large-scale collaborative association studies. Specifically, this proposal requests that the Northern California, Ontario and Australian population-based sites of the Breast CFR participate in the Breast Cancer Association Consortium (BCAC) studies. These analyses will provide much more reliable estimates of the risks associated with specific variants than is possible through individual studies. Identification of such variants will provide more reliable and rational risk assessment, with important implications for counseling and prevention.

Ongoing analysis will involve the possibility of breast cancer risk-associated genotypes being associated with definable breast cancer subtypes and other lifestyle factors.

Specific Aim(s):

Hypothesis

Whole genome studies offer much better prospects for identifying true associations than candidate gene studies. However, genome-wide association studies will also generate many false positive associations, and replication in large case-control studies are required to sort the true positives from the false positives.

Application ID: B-UT-1206-01-A2 **Status:** Approved, Ongoing

Principal Investigator: Goldgar, David **Year:** 2008

Institution: University of Utah

Title: *Analysis of Candidate Genetic Modifiers of BRCA1/2 Risks*

Abstract:

There is increasing evidence that cancer risks conferred by germline mutations in BRCA1 and BRCA2 can be modified by both lifestyle and other genetic factors. Identification of these factors could lead to more precise risk estimates tailored to individual carriers. However, because the effects of genetic risk modifiers are likely to be quite small, large samples sizes are required to have sufficient power to detect these effects. To this end, the CIMBA (Consortium of Investigators of Modifiers of BRCA1/2) consortium was organized in 2005. Currently there are ~30 participating groups with >10,000 mutation carriers, with additional centers in the process of joining. A preliminary analysis of RAD51 SNPs has been completed. The goal of this proposal is to provide a mechanism to include the ~1300 Breast CFR carrier samples in ongoing CIMBA collaborative efforts efficiently in order to maximize the utility of this material. Initially it is proposed to genotype six SNPs that have been proposed by the CIMBA consortium for analysis in April 2007. These include 5 SNPs identified from a genome-wide association study of sporadic breast cancer and validated in a large case-control analysis, as well as a SNP in the MTHFR gene. In addition to the combined analyses of SNPs proposed by the CIMBA consortium, the Breast CFR is in a unique position to do more detailed analyses of gene-environment interaction, for those SNPs shown unambiguously to be associated with breast or ovarian cancer risk in BRCA carriers.

Specific Aim(s):

Our principle hypothesis is that genetic variation at other loci can modify cancer risks conferred by BRCA1 and BRCA2 and that these effects can be detected by association studies in large series of BRCA carriers. To address this question, our objective is to develop a procedure whereby the Breast CFR can participate in the large CIMBA consortium that is applying a rigorous strategy to identify modifiers of BRCA1 and BRCA2 risk. The Breast CFR has identified over 1300 BRCA1 and BRCA2 carriers to date and will continue to identify others. This is one of the largest collections of biospecimens from carriers with corresponding uniform pedigree and epidemiological data. As such, it is an extremely valuable resource that should be widely available to the scientific community for collaborative studies on genetic and environmental modifiers.

Application ID: B-UT-0408-02 **Status:** Approved, Ongoing

Principal Investigator: Goldgar, David **Year:** 2008

Institution: University of Utah

Title: *Retrospective and Prospective Investigations into the Polygenic Basis of Familial Breast Cancer*

Abstract:

It has been known for a long time that having a first degree relative with breast cancer is associated with a two-fold increased risk of developing the disease. Although BRCA1 and BRCA2 confer high risks of breast cancer, studies have shown that they account for only about 20% of this familial risk. It is likely that a substantial fraction of the remaining familial effect is due more common genetic variation conferring only modest increased risks of breast cancer (RR<1.5). Through both candidate SNP studies and 3 genome-wide association studies (GWAS) there are already on the order of 7-10 validated common modest risk variants identified; more will no doubt be arising in the next year through additional GWAS studies Thus it is likely that there will be a panel of perhaps 20-30 such SNPs that are known to be associated with breast cancer. We will genotype this panel of SNPs in BRCA1/2 negative families with blood/DNA samples of at least two affected women diagnosed under age 60 and at least one unaffected female relative over age 30 in order to estimate the proportion of familial risk explained by these loci and evaluate their utility as a predictive tool in the context of familial breast cancer. We will also identify a group of women in the BCFR resource who were unaffected at time of enrollment/blood draw, define their entry into a cohort in order to examine the predictive power of such a SNP panel in a prospective manner. In order to achieve maximum power for these studies (particularly the prospective followup study) we will work collaboratively with a number of other groups with similar resources. In particular, Dr. Gillian Mitchell and Prof. John Hopper in Australia are leading a parallel effort in the kConFab familial breast cancer resource in Australia, and the INHERIT project led by Jacques Simard in Quebec City in which we are also collaborating.

Specific Aim(s):

Hypothesis/Objectives:

The hypotheses that we wish to test in this proposal are: 1) that the known (including those that will become known during the course of this proposal) common breast cancer susceptibility SNPs explain a significant fraction of the familial clustering not due to BRCA1 or BRCA2; 2) This SNP panel together with traditional lifestyle/reproductive breast cancer risk factors panel will provide a clinically useful risk assessment in women with a strong family history of the disease. Our objectives in this proposal are to 1) genotype the probands and family members for the selected panel of validated SNPs; 2) explore their contribution to family history of the disease using a variety of statistical approaches; 3) use both retrospective and prospective cohort designs to examine the utility of the SNP panel and selected lifestyle risk factors for prediction of risk; 4) develop statistical methods necessary to accomplish objectives 2 and 3. In addition to the stated goals above, we believe that this study will greatly enhance the utility of the registry in providing the genotype at a large number of breast cancer susceptibility loci in a large number of study participants as part of the resource for many other studies by other investigators.

Application ID: B-UT-0408-01 **Status:** Approved, Ongoing

Principal Investigator: Goldgar, David **Year:** 2008

Institution: University of Utah

Title: *Participation in the IMPACT Study of Male BRCA Carriers*

Abstract:

Although female carriers of BRCA1 and BRCA2 mutations have been extensively studied, male carriers have not been the subject of much research. However, genetic epidemiological studies have shown that male carriers are at increased risk of developing several cancers, most notably prostate cancer. The IMPACT project, led by Dr. Eeles, is a multicenter screening study of male BRCA1/2 carriers and control men who are non-carriers in BRCA1/2 families.

The goals of the project are to:

1. Establish an international targeted prostate cancer screening study in BRCA1 and BRCA2 mutation carriers

2. Determine the incidence of elevated PSA and abnormal biopsy as a result of screening in this group

3. Determine if the incidence of elevated PSA and pathology is different from screen-detected disease in controls

4. Determine sensitivity and specificity of PSA screening for prostate cancer in BRCA1/2 carriers

5. Evaluate new markers of early prostate cancer (eg HK2, new markers from proteomics from prostate cancer cases) in BRCA1/2 carriers.

The study is a prospective cohort study with 5 years recruitment, and a further 5 years for followup. The target is 850 BRCA1/2 mutation carriers and 850 controls (men from BRCA families who have tested negative for the mutation). Subjects will undergo annual PSA, serum/plasma, urine for other markers, (e.g., testosterone) and free: total PSA in the pilot cohort.

The Utah and Fox Chase Cancer Center sites have identified a substantial number of male carriers and thus are ideally suited to contribute to this study. Since we have no clinical research studies for these men, the IMPACT study will be of interest to a large fraction of our male carriers. It is important to note that we will not be using any existing BCFR biospecimens in this study but will be collecting new biospecimens (plasma and urine) from males who consent to participate.

Specific Aim(s):

Hypothesis/Objectives:

With respect to the BCFR, the objective of this proposal is to allow collaboration of the BCFR (specifically the FCCC and Utah sites) in the international multi-center IMPACT study of male BRCA carriers.

The goals of the IMPACT study are:

* To establish an international targeted prostate cancer screening study in male BRCA1/2 carriers as well as men who are members of families with a known BRCA1/2 who did not have the mutation (controls) where biological samples can be taken and assessed in this cohort.

* To determine the incidence of elevated PSA and abnormal biopsy as a result of PSA screening in this group and determine if the incidence of elevated PSA and pathology is different from screen-detected disease in controls which comprise:

(i) a group of men who are age matched (+/- 5 years) and who have a negative predictive genetic test

(ii) two population based screening studies

* To determine the sensitivity and specificity of PSA screening for prostate cancer in male BRCA1/2 gene mutation carriers and controls.

* To prospectively collect serial serum and urine samples to evaluate new markers of early prostate cancer in BRCA1/2 carriers and controls.

* To gain a better understanding of the pathogenesis of prostate cancer in men with BRCA1 or BRCA2 mutations. This will be done through further investigation by genomics and postgenomic technologies (including micro-arrays, biochemistry, biological functional assays, proteomics and metabonomics).

Application ID: B-EX-0408-03 **Status:** Approved, Ongoing

Principal Investigator: Haiman, Christopher **Year:** 2008

Institution: University of Southern California

Title: *A Genome-Wide Breast Cancer Scan in African Americans*

Abstract:

Genome-wide association studies of breast cancer have been completed or are in progress among populations of European ancestry, and several regions have been identified that appear to contribute susceptibility to this cancer. Recent data suggests that not all risk alleles for common cancers will be revealed however by studies limited to Whites of European ancestry, and that similar efforts in other racial and ethnic populations will be needed to identify the full spectrum of common risk alleles that contribute to disease risk in the population. In this application, we propose to identify genetic risk alleles for breast cancer risk among African American women by performing a well-powered whole-genome association scan using a two-staged design. This study

is specifically designed to identify genetic variants that contribute modest to high risk of breast cancer in this understudied minority population. In stage 1 we will genotype 1,000,000 single nucleotide polymorphisms (SNPs) in 1,500 invasive breast cancer cases and 1,500 controls. In stage 2 we will perform follow-up of ≥7,000 SNPs in 1,500 cases and 1,500 controls. The top hits from a combined analysis of stages 1 and 2 will be evaluated in a third stage among ~1,200 cases and ~1,200 controls. Using the combined stage 1 + 2 +3 dataset, we will assess main effects and examine interaction between these SNPs and risk factors (age and BMI) and disease severity (e.g. estrogen receptor status, grade). For this project we have established a collaborative network on investigators whose careers have been dedicated to studying breast cancer in minority populations. We expect this work to significantly advance knowledge of the etiology of breast cancer among African Americans, guiding the development of future preventive, early detection, prognostic and even therapeutic measures.

Specific Aim(s):

Hypothesis:

We propose the following hypothesis: (a) that common inherited DNA variation influences risk of breast cancer, and that an unbiased, comprehensive search for association between common germline variants and breast cancer risk in African Americans is more likely to reveal the full range of causal variants in the population, and genes and pathways contributing to breast cancer risk that will not be found in studies limited to Whites.

Application ID: B-EX-0408-02 **Status:** Approved, Ongoing

Principal Investigator: Huo, Dezheng **Year:** 2008

Institution: University of Chicago

Title: *Unconjugated Bilirubin and the Risk of Breast Cancer*

Abstract:

Breast cancer is heterogeneous in both disease outcome and etiology. Risk factors for ER– tumor, or more specifically for “triple negative” and/or “basal-like” breast tumors, remain unknown and understudied. Bilirubin, a degradation product of heme, is toxic to many cells types if its molar concentration in blood exceeds albumin. However, it is also a strong antioxidant so higher level in normal range or mild hyperbilirubinaemia may be protective and lower level of bilirubin is associated with cardiovascular diseases. As oxidative stress may promote carcinogenesis and in vitro experiment showed unconjugated bilirubin induced apoptosis, it is plausible that bilirubin is also protective against cancer development. One study showed lower total bilirubin level was associated with increased risk of colorectal cancer. Another study showed that total bilirubin was lower in breast cancer patients than controls. UGT1A1 is the primary enzyme that convert unconjugated bilirubin to water-soluble conjugated bilirubin in the liver. Number of TA repeats in the promoter of UGT1A1 gene is one of important determinants of the variation of unconjugated bilirubin: 7 and 8 TA repeats alleles are associated with higher level of bilirubin, whereas 5 and 6 alleles for lower bilirubin level. In our recent case-control study in Nigeria, found risk of breast cancer was reduced by 53% for women with low-activity TA repeat genotypes (contains 7 or 8 TA repeats) in premenopausal women. These data suggest that unconjugated bilirubin may be protective against breast cancer. As ER– breast tumors may be predominant in indigenous African, we further hypothesize that bilirubin play a role in the development of ER– breast cancer. We propose a case-control study utilizing resource of Breast CFR. Cases are patients with breast cancer and controls are women without breast cancer. We plan to measure both conjugated and unconjugated bilirubin in plasma and genotype TA repeats and other SNPs in the UGT1A1 gene.

Specific Aim(s):

The hypotheses being evaluated by this project are:

Hypothesis 1: Higher plasma bilirubin in normal range and mild hyperbilirubinaemia is protective against breast cancer.

Hypothesis 2: The protective effect of bilirubin is mainly against ER – breast cancer.

Application ID: B-EX-0806-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Longacre, Teri **Year:** 2008

Institution: Northern California Cancer Center

Title: *Characterization of DCIS in BRCA1/BRCA2*

Abstract:

Distinct histologic, immunohistologic and molecular phenotypes have been reported in association with invasive breast cancers in women with BRCA1 and BRCA2 germline mutations. When compared to sporadic cancers, BRCA1 invasive cancers are of higher grade (decreased tubule formation, increased mitotic index), have pushing margins with a lymphocytic infiltrate, are estrogen and progesterone receptor negative, rarely overexpress HER2/neu, more likely to overexpress p53 and exhibit a basal cytokeratin 5/14 phenotype (so-called basal phenotype).1-3 In contrast, when compared to sporadic cancers, BRCA2 associated invasive cancers exhibit a luminal phenotype (estrogen receptor positive, luminal cytokeratin 8/18 expression); these tumors are also more likely to be high grade (decreased tubule formation, nuclear pleomorphism, and increased mitotic index), with pushing margins, a moderate to marked lymphocytic infiltrate and rare overexpression of HER2/neu.3,4 Despite recent advances in our understanding of invasive cancer in the BRCA patient population, there is little information concerning the incidence and phenotype of in situ cancers.

Specific Aim(s):

Hypothesis

As invasive cancers in BRCA germline mutation carriers differ from sporadic carcinoma, it is likely that precursor lesions in this population also differ from those in sporadic cancer, both morphologically and in the expression of immunohistochemical markers. Preliminary data from our recent morphologic study (B-EX-0806-01: “Characterization of DCIS in BRCA1/BRCA2”) confirms the morphologic aspect of this hypothesis.5 Further investigation is needed to characterize immunohistochemical expression patterns in precursor lesions. Identification of these differences would enhance screening protocols for BRCA patients and provide additional insight into the development of BRCA-associated invasive carcinoma.

Application ID: B-PH-0408-01 **Status:** Approved, Ongoing

Principal Investigator: Offit, Kenneth **Year:** 2008

Institution: Memorial Sloan-Kettering Cancer Center

Title: *International Study of Modifiers of BRCA2 Penetrance*

Abstract:

We propose utilizing 2ug of native DNA samples (if unavailable, 1ug will suffice) from BRCA2 mutation carriers from the Breast Cancer Family Registry (B-CFR) for a multi-centered genetic epidemiology study to identify genes that modify breast cancer risk associated with BRCA2 mutations. In addition to the B-CFR samples, the study will assemble DNA specimens from over 5,000 individuals who carry BRCA2 mutations through the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). These samples have been collected for clinical research use according to IRB or other human subjects’ oversight board-approved studies at participating centers. DNA samples will be annotated with available demographic and epidemiologic data as well as tumor characteristics of the breast cancer cases, but will contain no personal identifiers. Samples will be amassed at the Broad Institute in Cambridge, Massachusetts and checked for quantity and quality of DNA. A whole genome scan of more than one million single nucleotide polymorphisms (SNPs) will be performed on older unaffected individuals (>40 years of age) and younger affected individuals (<40 years of age). Primary data analysis will be preformed by collaborators at the Broad Institute, MSKCC, and NCI. SNPs significantly associated with breast cancer risk after adjustment for multiple comparisons will be genotyped and evaluated in subsequent stages using additional BRCA1 carrier breast cancer cases and controls. Replicated SNPs will be evaluated in relation to interaction with known breast cancer risk factors as well as molecular subtypes of breast cancer. Additional mapping of identified genes may be necessary to identify the functional loci. It is planned that ascertainment and preliminary genotyping of samples will be carried out by January 1, 2009. The study is funded by peer-reviewed grants, a reviewed grant from the multi-institutional Starr consortium, and the Breast Cancer Research Foundation. Based on the success of previous whole genome scans within large consortia of sporadic breast cancer, the conclusion of our project will likely be the identification of new and confirmed genes that modify the penetrance of BRCA2-associated breast cancer and may also provide novel genes for the study of sporadic breast carcinogenesis.

Specific Aim(s):

Hypothesis

The objective of this study is to perform a whole genome association scan utilizing linkage disequilibrium to identify genetic variants modifying BRCA2 penetrance. Associations will be studied both in isolated populations (e.g., Ashkenazi Jews) as well as in outbred, diverse pools consisting of BRCA2 mutation carriers assembled as part of CIMBA, an international consortium of BRCA2 carrier studies. The success of this project will yield the elucidation of genetic modulators of BRCA2 penetrance and may inform the study of important genes of non-BRCA-associated tumorigenesis, transforming our understanding of pathways associated with both predisposition as well as resistance to these tumor types.

The central hypothesis of this proposal is that the predisposition to breast cancer conferred by a germline mutation in BRCA2 is subject to modulation by genetic cofactors. It is hypothesized that differences in modifying loci may account for a substantial proportion of variation in BRCA2 penetrance estimates derived from population-based and clinic-based ascertainments.

Specific Aims

To explore these hypotheses, we aim:

* To utilize linkage disequilibrium with whole genome scan to identify putative modifier loci associated with age-specific penetrance as well as cancer resistance at late age in carriers of the BRCA2 founder mutation,

* To confirm the association of particular alleles identified using a multi-staged approach,

* To evaluate interaction between confirmed SNPs, breast cancer risk factors, and tumor characteristics in the association with breast cancer among BRCA2 mutation carriers

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Application ID: B-EX-1202-01-A3 **Status:** Approved, Ongoing

Principal Investigator: Pasche, Boris **Year:** 2008

Institution: Northwestern University

Title: *Association between the TGFBR1*6A Allele and Breast Cancer*

Abstract:

TGF-β and cancer Unrestricted cell growth due to a lack of growth inhibitory activity appears to be the most important of the possible consequences of a defect in TGF-ß function. This hypothesis was confirmed by the discovery that Tgfb1 +/- and Tgfbr2 +/- haploinsufficient mice resulting in decreased TGF-ß signaling have an increased susceptibility to develop cancer than their wildtype counterparts. Specific targeting of the TGF-ß receptors in human cancer has been demonstrated by the identification of inactivating mutations in TGFBR2 in colon and head and neck (HN) cancers, homozygous deletion of TGFBR1 in pancreatic and biliary carcinomas and in lymphoma, and a TGFBR1 tumor-specific mutation in breast cancer. Additionally, restoration of functional receptor expression reverses the transformed phenotype of several human cancer cell lines that inherently lack functional TGF-ß receptors. We have recently shown that a combined assessment of two well-characterized functionally-relevant variants of TGFB1 (T29C) and TGFBR1 (TGFBR1*6A) may predict breast cancer risk in 30% of the general population.

We have shown that TGFBR1*6A acts as a dominant allele in MCF-7 breast cancer cells and we have preliminary data indicating that TGFBR1*6A modifies the expression level of TGFBR2 and enhances the migration and invasion of MCF-7 cells. We also have new data showing that TGFBR1*6A enhances the migration and invasion of breast cancer cells and may thus affect affect patient outcome. In this study, we propose an independent confirmation of this important hypothesis studying breast cancer families on whom DNA samples and detailed questionnaire data have already been collected. A family-based association study is proposed by examining the allelic distribution among breast cancer cases and their unaffected siblings/cousins participating in BCFR. By using a family-based association design, we avoid population stratification bias that may affect standard case-control association analysis. We also propose to study the outcome of patients with breast cancer.

If the level of TGF-β signaling is found to have an inverse correlation with breast cancer risk, the next logical step would consist in an interventional trial whereby at-risk patients may be offered medications susceptible to upregulate TGF-β signaling. At-risk individuals with a family history of breast cancer and low TGF-β signaling are assessed by TGFB1 and TGFBR1 genotyping.

Specific Aim(s):

The specific aims of the proposed family-based study are to:

1. examine whether the TGFBR1*6A polymorphic allele is associated with the risk and outcome of familial breast cancer; examine whether the TGFB1*CC genotype is negatively associated with the risk and outcome of breast cancer

2. estimate the absolute (penetrance) and relative risk of breast cancer as well as outcome for the TGFBR1*6A allele and TGFB1*CC genotype in these families;

3. explore whether the association between TGF-β signaling level as assessed by the various combination of TGFBR1 and TGFB1 polymorphisms and breast cancer risk and outcome is different in specific subgroups, as determined by BRCA1/2 mutation status, presence of male breast cancer in family, age, and ethnicity.

4. amplify the DNA samples by whole-genome amplification, perform haplotype analysis of 65 genes: BMP2, BMP4, GDF5, GDF6, GDF7, BMP10, GDF2, BMP5, BMP6, BMP7, BMP8A, BMP8B, GDF1, GDF3, BMP3, GDF10, MSTN, GDF11, GDF15, AMH, INHBA, INHBB, INHBC, INHBE, BMP15, GDF9, NODAL, A2M, BAMBI, CER1, DAND5, CHRD, TDGF1, NBL1, GREM1, FST, FSTL1, CHRDL1, NOG, GREM2, ENG, TGFBR3, ACVR1C, ACVR1B, BMPR1B, BMPR1A, ACVRL1, ACVR1, AMHR2, BMPR2, ACVR2A, ACVR2B, SMAD1, SMAD5, SMAD9, SMAD6, SMAD7, TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, SMAD2, SMAD3, SMAD4.

This will provide a comprehensive coverage of the TGF-beta pathway superfamily and determine the association of the haplotypes with breast cancer risk as well as outcome.

Application ID: B-EX-0408-01 **Status:** Approved, Ongoing

Principal Investigator: Shen, Jing **Year:** 2008

Institution: Columbia University

Title: *Telomere Length, SNPs in Telomere Pathway and Breast Cancer Risk*

Abstract:

The Breast Cancer Family Registry (BCFR) provides a unique resource to identify breast cancer susceptibility biomarkers involved in different biological pathways. The telomere pathway is one of the most important in breast tumorigenesis. Telomeres consist of a large number of tandem repeats of the sequence TTAGGG at the ends of linear chromosomes1 and a multi-protein complex, which are essential for maintaining the integrity and stability of the genome. There are at least three ways leading to telomere dysfunction: (1) progressive loss of telomere repeat sequences due to the end-replication problem; (2) activation of telomerase; and (3) disruption of higher-order telomere structures due to defects in various telomere-binding proteins. These reasons can lead to telomere shortening, genomic instability, and finally drive tumorigenesis.

The goal of current proposal is to expand telomere length measurement from the New York site to the other five BCFR sites. Our hypothesis is that short telomere length or single nucleotide polymorphisms (SNPs) in the telomere-related pathway will influence telomere function, and be associated with breast cancer susceptibility. A case-control analysis will be conducted using the BCFR resource. Breast cancer cases (affected sisters) and two types of controls (unaffected sister controls and unrelated population-based controls) will be selected from the six BCFR sites. An estimated 1,200 sister-pairs will be studied. Each sister-pair will include one affected sister with breast cancer and one unaffected sister. A similar number of population-based controls (1,200 subjects) will be randomly selected and frequency matched for age and race/ethnicity with cases (unmatched by site). Telomere length and SNPs in the telomere pathway will be measured in DNA extracted from white blood cells (WBC), instead of target breast tissues. Available epidemiologic and family-history questionnaires will provide detailed information for these subjects. Real Time Quantitative PCR (Q-PCR) and BioTrove OpenArray™ SNP Genotyping approaches have been set up in our laboratory to detect telomere length and SNPs in the telomere pathway. These results will provide solid evidence to clarify the role of telomere pathway in breast tumorigenesis.

Specific Aim(s):

Our hypothesis is that with telomere length shortening, an elevated breast cancer risk will be observed. SNPs in telomere pathway may influence telomere length, and modify breast cancer susceptibility. The specific aims are: (1) To identify whether telomere length measured in WBC DNA has statistically significant difference between breast cancer cases and controls (unaffected sister control and population control); (2) To distinguish multiple SNPs across 25 genes (TERT, TERC, TERF1, TERF2, PINX1, POT1, RAP1, RAD50, RFC1, TINF2, TNKS-1 etc.) encoding proteins in the telomere pathway, and to examine the associations of these SNPs with breast cancer risk; (3) To evaluate whether there are dynamic changes for telomere length by SNPs in the telomere pathway, cigarette smoking, body mass index (BMI) and physical activity.

Application ID: B-NY-0806-02-A1 **Status:** Approved, Ongoing

Principal Investigator: Terry, Mary Beth **Year:** 2008

Institution: Columbia University, School of Public Health

Title: *Epigenetics and Breast Cancer Along the Life Course*

Abstract:

Extensive epidemiologic evidence has linked many environmental factors to breast cancer risk, including reproductive history, exogenous hormone use, body size, and selected dietary factors (including alcohol intake). One plausible mechanism for the environment to alter breast cancer susceptibility is through epigenetic effects on somatic cells, leading to activation or silencing of key genes in critical pathways. Epigenetic processes include DNA methylation, genomic imprinting, and altered packaging with histones; aberrations of these processes play a role in causing cancer. There is increasing evidence that DNA methylation and histone packaging are altered not only within tumors, but in some cases in precursor lesions, or in the whole body. Human studies have led to an understanding that changes in methylation occur with age. However, it is not yet understood how environmental factors may influence these changes. Studies are only now underway to investigate whether smoking and other environmental factors influence epigenetic changes later in life. Using resources from the Breast Cancer Family Registry (BCFR), we propose to examine the association between DNA methylation patterns (including global hypomethylation and gene- specific hypermethylation of selected genes) and breast cancer risk. Specifically, we will measure DNA global hypomethylation and gene-specific hypermethlation in the lymphocytes of 3,828 unaffected sisters and 1,831 population-based controls and examine DNA methyation and epidemiologic risk factors among women unaffected with breast cancer. We will compare whether these associations between DNA methylation and risk factors differ among higher risk women (unaffected sisters) and average risk women (population-based controls). With separate funding, the unaffected sisters are currently being prospectively and will be used in a nested case control design. We currently have 225 cases identified whose blood was collected prior to cancer. We explect to ascertain at least 430 cases prospectively but we have conservatively powered the study at 350 cases. We will also assess DNA methylation in the plasma and tissue of these 350 cases and we will measure DNA methylation in the plasma of 350 controls matched by date of blood draw and age at blood draw. We will determine the association between DNA methylation patterns and epidemiologic risk factors including 1) reproductive history; 2) exogeneous hormone use; 3) cigarette smoking; 4) alcohol exposure; 5) radiation exposure; 6) body size; and 7) diet (including measures of folate and methionine). Our investigator team brings together scientists with expertise in DNA methylation, environmental science, genetics, biostatistics and breast cancer epidemiology. We have generated preliminary data suggesting strong associations between DNA methylation and breast cancer risk and various epidemiologic factors. Altered DNA methylation has already been found in breast cancer tissue. We seek to understand whether measurements in lymphocytes and plasma years before cancer diagnosis can be used as a biomarker of risk.

Application ID: B-EX-0805-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Ziv, Elad **Year:** 2008

Institution: University of California, San Francisco

Title: *Admixture Mapping of Breast Cancer in Latinas*

Abstract:

Breast cancer rates vary substially among women of different racial/ethnic groups in the US. Recent studies have identified several new genetic variants associated with the risk of breast cancer in studies that comprised women of mainly European descent. The degree to which these risk factors are also important in women of other ethnic groups, and the degree to which other genetic risk factors play a role in women of other ethnic groups is unknown.

Latina/Hispanic women are a population of complex genetic ancestry with a diverse environmental exposures. From a genetic standpoint, Latina women are of mixed ancestry with European, Indigenous or Native American ancestry and African ancestry. From an environmental standpoint, Latina women are often immigrants (predominantly from Mexico and Central American countries in California) or are childen of immigrants.

Latina women have substially lower incidence and mortality from breast cancer compared to Caucasian women in the U.S. The degree to which this is due to genetic vs. environmental factors is unknown. Migrant studies have suggested that the risk of Latina women who are born in the US is higher compared to Latina women who are first generation immigrants. Our own studies have also suggested that genetic ancestry may be a factor in breast cancer among Latinas. Specifically, we have found that European genetic ancestry was higher among our older cases (age>50) compared with age matched controls. In contrast, our younger cases tended to have more Native American ancestry than our older cases.
We propose to perform a genome wide association study to investigate genetic risk of breast cancer among Latina women cases and controls. Specifically, we proposel the following specific aims:

(1) To perform admixture mapping for breast cancer among Latinas. Specifically, we will use the subset of markers on the Affymetrix 6.0 array that are

ancestry informative to estimate locus specific ancestry for cases and controls and identify regions where cases share more ancestry (either European or Native American) than expected by chance.

(2) To perform genome wide association. Specifically, we will test for differences in allele frequencies and copy number variant frequencies between cases and controls using the Affymetrix 6.0 array. The study will include cases and controls from the CFR as well as cases and controls from Dr. John's case-control study in the Bay Area and for a case-control study in Mexico.

Application ID: B-TO-0402-01-A2 **Status:** Approved, Ongoing

Principal Investigator: Andrulis, Irene **Year:** 2007

Institution: Cancer Care Ontario

Title: *Use of CGH Arrays to Identify Genes Involved in Hereditary Breast Cancer*

Abstract:

The overall objective of this work is the identification and characterization of novel genes that are involved in breast cancer susceptibility and progression. We hypothesize that some of these genes will have undergone chromosomal losses and gains that can be detected by CGH microarrays of the tumor DNAs from women who are at "high-risk" of carrying a (non-BRCA1/2) cancer susceptibility gene mutation. CGH microarrays have been developed to provide a measure of DNA copy number variation across a whole genome and can be used to increase gene mapping resolution. In addition to genome wide scans that search the entire genome to find novel breast cancer susceptibility genes, we plan to take advantage of available tumor tissue as well as germline DNA and will use CGH microarraysas well as a SNP genotyping as a screen to select regions of potential importance.

It has recently been found that, in addition to single nucleotide polymorphisms (SNPs) and small insertion/deletion polymorphisms, human inter-individual genomic variation includes large-scale copy number variations (CNVs) that involve gains or losses of up to hundreds of kilobases of DNA differing from the NCBI reference sequence of the human genome. These large-scale CNVs have been detected in genomic DNA from 'normal' healthy individuals. We hypothesize that there are regions of normal genomic CNVs that may be associated with risk of breast cancer.

We propose to take advantage of two approved and funded BCFR studies to identify these genomic regions through comparisons of constitutional and tumor DNAs from individuals participating in the BCFR. In addition to array CGH studies for detection of gains and losses, we propose to address genomewide loss of heterozygosity (LOH) by comparing the tumor genomic data among a subset of affected sisters with the constitutional genomic data generated in the association and linkage scan.

1. To characterize chromosomal regions of gains and loss in breast tumours DNA from 100 unrelated women with early-onset disease. The cases will be selected for being at risk of carrying a (non-BRCA1/2 and non-CHEK2*1100delC) cancer susceptibility gene mutation because of their personal history (age at diagnosis < 35 years) and family history (having at least an affected sister) of breast cancer. We will also conduct BAC aCGH studies on 100 DNAs extracted from blood of the early-onset probands.

2. To identify and compare chromosomal regions of gains and losses using sib-pairs with breast cancer. We will perform aCGH on 100 tumours from siblings of the probands. We will identify alterations that are concordant or discordant within sib-pairs, and for selected regions will assess whether the agreement within a sib-pair is greater than that expected.

3. To identify genes that discriminate between early-onset breast cancer and hereditary breast cancer. We plan to compare the aCGH profiles of the tumours from early-onset breast cancer cases without BRCA1/2 mutations and CHEK2 variant to profiles of tumours from each of two other groups of women (50 BRCA1 mutation carriers and 50 BRCA2 mutation carriers).

4. To investigate whether the Illumina 550 arrays can be used to generate information on regions of loss and gain similar to that obtained from the aCGH studies.

5. If aim 4 is successful, we plan to use the Illumina 550 SNP array platform in addition to our aCGH studies to scan the entire genome to characterize LOH in tumor DNA as well as the extent and frequency of CNV, and its potential to cause or influence susceptibility to disease.

Application ID: B-TO-0802-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Boyd, Norman **Year:** 2007

Institution: Ontario Cancer Institute

Title: *Circulating IGF-1 and Risk of Breast Cancer*

Abstract:

Background and Objectives
In general, studies indicate that greater circulating IGF-I concentration is associated with increased breast cancer risk in premenopausal women. Both breast cancer risk and circulating IGF-I concentrations appear to be partly heritable. Therefore, IGF1 is a suitable candidate gene to examine in association with breast cancer risk.

Methods
DNA and risk factor data from 840 premenopausal probands with breast cancer and their first degree relatives were obtained from the Ontario Familial Breast Cancer Registry (OFBCR) and the Australian Breast Cancer Family Registry (ABCFR). The association between allelic variants of three CA repeat polymorphisms, including a previously investigated 5¢ repeat, with breast cancer risk in premenopausal women was examined.

Results
Some nominally significant associations (5¢ 20 allele, P=0.03; intron 2 212 allele, P=0.04; intron 216 allele, P=0.04) were observed in the combined ABCFR OFBCR sample, but adjustment for multiple comparisons indicated that these had a high probability of being false discoveries. In additional analyses, a stronger association between the intron 2 216 allele with risk (P=0.01) was observed under a recessive model and ad hoc grouping of 5¢ polymorphism alleles resulted in a significant positive association of with risk for alleles of length 18 to 20 (P=0.02) and a inverse association for alleles greater than 20 repeats in length (P=0.01). These same associations were observed in the OFBCR (intron 2 216 allele recessive, P=0.02; both 5¢ 18 to 20 allele grouping and >20 allele groupings, P=0.01) but were not strongly supported in the ABCFR (intron 2 216 recessive, P=0.14; 5¢ 18 to 20 grouping, P=0.25; 5¢ > 20 grouping P=0.20). Analysis of haplotypes resulted in few nominally significant associations that could have been due to chance.

Conclusions
Given the number of comparisons performed and the lack of strong consistency across samples, the results provide limited evidence for an association between genetic variants of IGF1 and breast cancer risk.

Project Report

2003

Our sample consists of pre-menopausal probands and first degree relatives from the OFBCR and the ABCFR. Three repeats along the IGF-I gene are being genotyped. Genotyping is complete for most of the Ontario sample (705 of 781). For 76 subjects there were no samples available for DNA extraction at the time of our initial request. We have requested an update on the status of these samples. The data from the 705 genotyped samples is now being reviewed and a sub-sample will be retested to determine the reliability of our genotyping results. To date, 720 of the 2014 Australian samples have been genotyped. Further genotyping for this sample and testing of sub-samples for reliability is in progress.

Specific Aim(s):

The purpose of obtaining this data is to compare characteristics (e.g. risk factors) of premenopausal breast cancer cases from the ABCFR and OFBCR that were not eligible for my study to those cases that were included in my study.

Application ID: B-EX-1205-01-A1 **Status:** Approved, Ongoing
Principal Investigator: Broome, Carolyn **Year:** 2007
Institution: Howard University
Title: *Genetics and Epigenetics of Breast Cancer in African Americans*

Abstract:

The molecular basis of the initiation of breast cancer is believed to be a consequence of cumulative damages leading to genetic and/or epigenetic alterations that result in activation of proto-oncogenes and inactivation of tumor suppressor genes. This proposal focuses on the detection of hereditary mutations in CHEK2 (including genomic rearrangements) and BRCA1 genomic rearrangements in African Americans with breast cancer, an area that has not been intensely studied. This investigation will provide information about the spectrum of mutations in African Americans for genetic testing, genetic counseling, risk assessment, and treatment. Using a highly sensitive method, quantitative multiplex methylation-specific PCR (QM-MSP), a methylation profile for African Americans that may differentiate among BRCA1, BRCA2, hereditary non-BRCA1/2 and sporadic tumors will be investigated. This profile could identify families for BRCA1/2 testing and provide clinical markers for early detection, prognosis, and treatment using very small samples, such as ductal lavage (Fackler et al, 2004).

Application ID: B-AU-0406-02-A1 **Status:** Approved, Ongoing
Principal Investigator: Byrnes, Graham **Year:** 2007
Institution: The University of Melbourne
Title: *Identifying Breast Cancer Subgroups Using Breast CFR Family, Epidemiological and Pathology Data*

Abstract:

Breast cancer is a heterogeneous disease, which complicates attempts to understand its etiology or to optimize its treatment. Mutations in the genes BRCA1 and BRCA2 are examples of distinct etiologies, however these account for only a small fraction of familial cases (Cui et al 2001). This heterogeneity greatly reduces the power of linkage and association analyses to discover new cancer-predisposing genes.

It is reasonable to expect different etiologies to be reflected in the pathology of the tumors they produce and the circumstances and characteristics of women who have these tumours. Hence we propose to identify homogeneous subgroups of breast cancer by analyzing data held by the Breast CFR on tumor pathology, individual epidemiological characteristics and family histories of breast cancer patients. Planned analyses include principal components for noise reduction and a variety of clustering techniques, detailed below. Carriers of known mutations in BRCA1 and BRCA2 will serve as training data to identify which features, or combinations of features, are likely to identify etiological subgroups. Family data will allow us to identify subgroups which segregate within families and are therefore suggestive of genetic or shared environmental causes.

Our preliminary analyses of Australian population-based data collected through local funds has shown that three of the first four principal components based on pathology data alone are predictive of BRCA1 or BRCA2 germline mutation status. They have also shown that there is a major (first) principal component generated from studying cases diagnosed before age 40 and this results in a continuously-distributed dimension of early-onset breast cancer that is strongly predictive of BRCA1 mutation status in the germline, and perhaps also mortality (unpublished data). These analyses have been the primary motivation for us now wanting to use this approach to include the NIH-funded Australian Breast CFR resources. If successful, we will then apply to the AC for approval to use data from the North American sites of the Breast CFR, which to date has been denied us, so as to hopefully provide an independent confirmation and further elaboration of this approach to later onset cases, cases identified through cancer family clinics, and cases in Ashkenazi Jewish women.

We anticipate that the analyses proposed here will lead to new ways to subdivide breast cancers, and new dimensions for differentiating breast cancers from one another, that may prove to be useful for many other existing and future studies. For example, existing case-control (Milne et al) and prognosis studies (Goodwin et al) can be revisited in terms of subgroups or new continuously-distributed dimensions. Gene discovery studies (eg Southey et al) will benefit from being able to identify subgroups and dimensions that may be more strongly associated with family history. Gene expression studies using micro arrays (eg Andrulis et al) will benefit from being able to incorporate pathology-review related subgroups into their attempts to define new molecularly-defined subgroups.

The analytic techniques developed in the proposed study would also facilitate future work on data types not currently available: comparative studies of treatment outcomes; comparative microarray expression studies and other tumour genetics assays such as comparative genomic hybridization (CGH) or fluorescent in-situ hybridization (FISH); case-case studies to identify germ-line genetic characteristics in candidate genes; genome wide or candidate gene case-control studies using cases selected from a selected subgroup. Where the subgroups are related to mutations in known genes (such as BRCA1 or BRCA2), subgroup identification based on pathology and epidemiological data will enable better targeted genetic testing of relatives of affected women, potentially preventing disease and reducing cost. That is, we anticipate (based on our preliminary analyses) that we will be able to produce algorithms for predicting mutation status with both high sensitivity and specificity, with areas under the ROC curve (at least for BRCA1 mutations) in excess of 0.9 (most family history based algorithms typically produce areas under the ROC curve of less than 0.8).

Specific Aim(s):

Hypothesis

That different etiological pathways to breast cancer manifest in the pathology and immunohistochemistry of the tumors, as well as the age of onset, reproductive history and family history of the affected women. Consequently, principal components analysis and un-supervised clustering methods of epidemiological and pathology data will identify subgroups of breast cancer related to known and unknown genetic risk factors.

Application ID: B-AU-0802-01-A2 **Status:** Approved, Ongoing

Principal Investigator: Chenevix-Trench, Georgia **Year:** 2007

Institution: Queensland Institute of Medical Research

Title: *The Role of the ATM Gene in Familial Breast Cancer*

Abstract:

The role of the ATM gene in breast cancer predisposition is controversial. Studies of carriers of ATM mutations have indicated that females have on average a 4-7 fold increased risk of breast cancer, but mutation analysis of the ATM gene in unselected breast cancer cases has failed to find a convincingly increased frequency compared with controls. However, we have shown that two specific pathogenic mutations of the ATM gene do occur in 4% of multiple-case breast cancer families, and confer high risks of breast cancer, equivalent to a risk to age 70 years of 60%. The broad aim of this proposal is to extend our findings by estimating the penetrance and frequency of breast cancer-causing ATM mutations by analysis of the gene in almost 3000 putative hereditary breast cancer families from North America and Australia.

Our specific aims are to:

1) Perform mutation screening of ATM in the youngest affected female from at least 900 breast cancer families.

2) Identify putative ATM mutations among the variants found by analyses of the behaviour of these variants in tumors and cell lines from women who carry them.

3) Screen 2000 additional breast cancer families for putative ATM mutations defined in Aim 2.

4) Genotype the affected and unaffected family members of those individuals found to carry putative ATM mutations, and estimate the penetrance of these mutations.

5) Estimate the frequency of ATM mutations in defined groups of unselected population-based breast cancer cases AND SCREEN FEMALE CONTROL

SAMPLES FOR THE SAME ATM MUTATIONS (NEW AIM) IN ORDER TO ESTIMATE THE RELATIVE RISKS ASSOCIATED WITH THESE MUTATIONS.

6) Use LCLs from ATM 7271 T>G carriers to validate the expression profile we have identified in carriers with this mutation from other sources.

Application ID: B-EX-1207-04 **Status:** Approved, Ongoing

Principal Investigator: Couch, Fergus **Year:** 2007

Institution: Mayo Clinic

Title: *Genome-Wide Association Studies in BRCA1 Carriers*

Abstract:

The penetrance of breast cancer in BRCA1 mutation carriers appears to vary considerably in populations and even in families with some reports suggesting that the lifetime risk of breast cancer for a BRCA1 mutation carrier can be anywhere from 55% to 85%. In addition, there is considerable variability in the age of diagnosis of breast cancer in BRCA1 carriers. As a result, we are still limited in our ability to predict whether a carrier of a BRCA1 mutation will develop breast cancer. Our goal in this study is to identify previously undefined genetic risk factors that modify breast cancer risk in BRCA1 carriers so that it will be possible to predict who will develop cancer. We propose to identify these genetic risk factors through a genome wide association study that will comprehensively evaluate common variants throughout the genome. We will rigorously evaluate common variants through a multi-stage approach using DNA samples from BRCA1mutation carriers that have been collected through an international consortium. In brief, we will genotype 1,500 BRCA1 carriers with young onset breast cancer and 1,500 older unaffected BRCA1 carriers on 550,000 common variants and identify variants associated with risk of breast cancer. In stage 2 we will evaluate the 13,180 variants most significantly associated with breast cancer risk in 2,000 affected BRCA1 carriers and 2,000 unaffected carriers and combine the data with stage 1 to increase statistical power. In stage 3 the 384 most significant variants will be further evaluated in 2,000 affected and 2,000 unaffected BRCA1 carriers and the data will be combined with data from stages 1 and 2. In stage 4 fine mapping of the genomic regions containing the most significantly associated variants will be conducted in an effort to identify the variants that likely account for the modification of breast cancer risk in BRCA1 carriers. The identification of these factors will be useful for understanding the etiology of breast cancer, developing novel therapeutic targets, developing improved cancer risk assessment models for mutation carriers and for identifying women at particularly high-risk for prevention strategies.

Specific Aim(s):

The main aim of this study is to identify common SNPs associated with breast cancer risk in BRCA1 carriers, by correlating disease phenotype with SNP genotypes.

Aim 1: To conduct a genome-wide association scan in 1,500 BRCA1 carriers with young onset breast cancer and 1,500 older unaffected BRCA1 carriers (Stage 1).

Aim 2: To further evaluate observed associations between breast cancer risk and SNPs implicated in Aim 1 by genotyping 2,000 breast cancer affected and 2,000 unaffected BRCA1 carriers (Stage 2).

Aim 3: To confirm observed associations between age of onset of breast cancer and SNPs selected in Aim 2 in an additional 2,000 affected and 2,000 unaffected BRCA1 carriers (Stage 3).

Aim 4: To identify the SNPs most strongly associated with age of onset of breast cancer in BRCA1 carriers in the chromosomal regions and genes containing the SNPs displaying the most significant associations in Aim 3 (Stage 4).

Hypothesis

The cumulative risk of breast cancer by age 70 for BRCA1 mutation carriers is estimated at 56-85% (1-4). While these risk estimates show that women carrying deleterious BRCA1 mutations are at extremely high risk for developing breast cancer they also indicate that not all carriers will be diagnosed with breast cancer. In addition to incomplete penetrance, there is also considerable variability in the age of diagnosis of the carriers who develop breast cancer. On the basis of these and other findings we hypothesize that there are genetic factors in the form of common genetic variants that modify the age specific risk of breast cancer for BRCA1 mutation carriers.

Our goal in this study is to identify previously undefined genetic risk factors involved in the modification of cancer risk in BRCA1 carriers in order to improve risk assessment, prevention and therapy for these and other breast cancer patients. We propose to identify these genetic risk factors through a genome wide association study of samples from BRCA1 mutation carriers collected through an international consortium. The Breast CFR has identified many BRCA1 carriers to date and will continue to identify others. This is one of the largest collections of biospecimens from carriers with corresponding uniform pedigree and epidemiological data. As such, it is an extremely valuable resource that is of great importance to the success of this project. Using BCFR and other BRCA1 carrier samples we will rigorously evaluate common variants through a multi-stage approach and select the most significantly associated with breast cancer risk as candidate genetic modifiers.

Application ID: B-EX-0207-02 **Status:** Approved, Ongoing

Principal Investigator: Gomez, Scarlett **Year:** 2007

Institution: Northern California Cancer Center

Title: *Improving Utility of Cancer Surveillance Data for Studying Cancer in Immigrant Populations*

Abstract:

At the Greater Bay Area Cancer Registry, the Surveillance Research group conducts studies seeking to improve the utility of population-based cancer registry data for identifying and understanding population disparities in cancer incidence and outcomes. We have previously demonstrated that although the cancer registry collects information (via hospital medical records and death certificates) on patients' place of birth, this information in the registry is missing for nearly half of the patients, and furthermore, patients missing the information were more likely to be US-born than those not missing the information in the registry data. Therefore, cancer registry data are currently limited for studies of immigrants and the impact of immigration on cancer incidence, stage, treatment, and outcomes. Through two funded SEER RRSSs (Surveillance, Epidemiology, and End Results Rapid Response Special Studies), we aim to improve the utility of cancer registry data for studying immigration effects on cancer patterns. The first study "Obtaining Information on Language Preference among Minority Cancer Patients" will examine the availability and accuracy of medical record information on patients' language preference with the intent of evaluating the feasibility of including this information as a routine cancer registry data item. The second study "Trends of Cancer Incidence among Asian and Hispanic Sub-Populations by Age, Period, and Immigration Cohort" will explore novel methods to develop an enhanced cancer registry data resource for examining age, period and cohort effects on cancer incidence among Asian and Hispanic immigrants. Epidemiologic and sociodemographic data collected from patients enrolled in the Northern California Family Registry for Breast Cancer (FRBC) were previously included in an analysis of patient interview data from 25 studies for the purposes of evaluating the accuracy of registry data on race, ethnicity, and birthplace (AC approved project B-SF-1203-01). We propose to now use these data (total N=23,900 patients, of whom 2262 are FRBC participants) for the following aims: 1) determine the accuracy of information on language preference abstracted from patient medical records; 2) examine how accuracy of data on patient language preference in the medical record varies by patient and hospital characteristics; 3) validate statistically imputed data on nativity and age/year of immigration; and 4) assess performance of an area-based acculturation measure derived from US Census data. Thus, the epidemiologic data collected from the FRBC will be invaluable towards improving the utility of SEER cancer registry data for studying patterns in incidence, diagnosis, treatment, and outcomes among the rapidly growing immigrant population.

Specific Aim(s):

Our aims are descriptive in nature in that we will use the self-reported interview data (collected from 25 interview studies, including the FRBC) to determine the accuracy and performance of certain data items with the intention of determining their utility for enhancing analyses of SEER cancer registry data for studying immigrant populations.

Currently, we have been funded through two SEER RRSSs to: 1) determine the feasibility of abstracting patient language preference information from medical records and including this information as a routine registry data item ("Obtaining Information on Language Preference among Minority Cancer Patients"); and 2) compile a data resource to examine age, period, cohort effects on cancer incidence among Asian and Hispanic immigrants ("Trends of Cancer Incidence among Asian and Hispanic Sub-Populations by Age, Period, and Immigration Cohort").

Thus, we are seeking approval to use patient epidemiologic interview data collected from the Northern California Family Registry for Breast Cancer (FRBC) to address the following aims:

1. Determine the accuracy of information on language preference abstracted from patient medical records.

2. Examine how accuracy of data on patient language preference in the medical record varies by patient and hospital characteristics.

3. Validate statistically imputed data on nativity and age/year of immigration.

4. Assess performance of area-based acculturation measure derived from US Census data.

Application ID: B-SF-0907-01 **Status:** Approved, Ongoing

Principal Investigator: John, Esther **Year:** 2007

Institution: Northern California Cancer Center

Title: *Collaborative Epidemiologic Analyses of Environmental Risk Factors for Breast Cancer Using Population-based Data Collected in Australia, Ontario and Northern California: The Role of Physical Activity*

Abstract:

This application is an extension of a previously approved project B-AU-1203-03 titled "Collaborative epidemiologic analyses of environmental risk factors for breast cancer using population-based data collected in Australia, Ontario and Northern California". This application was submitted by the Analytic Working Group and approved by the AC in 2003. Several publications have resulted from these collaborative analyses, including breast cancer risk in relation to oral contraceptive use (Milne et al 2005), ovarian cysts (Knight et al. 2006), and medical radiation (John et al. 2007). The approved project also covered case-control analyses to assess physical activity and breast cancer risk in white women. Analyses are underway by the applicant and show inverse associations in the expected direction. This new application requests approval to extend these on-going analyses on physical activity to non-white women. Relatively few studies have assessed the role of physical activity in non-white women. The large number of non-white families enrolled at the Northern California site will allow separate analyses in non-white women. The extended analyses will be based on white and non-white women (incident case probands, unaffected sisters, population controls) recruited during phase I (diagnoses 1995-1998) at the 3 population-based sites and non-white women (incident case probands, unaffected sisters) recruited during phase II (diagnoses 1999-2003) at the Northern California site.

Specific Aim(s):

This collaborative case-control analysis will test the hypotheses that:

1) high lifetime physical activity is associated with reduced breast cancer risk,

2) risk reductions are similar in white and non-white women,

3) risk may be modified by hormone receptor (ER/PR) status, family history of breast or ovarian cancer, age, menopausal status, HRT use, and body mass index (BMI).

Application ID: B-EX-1204-02-A1 **Status:** Approved, Ongoing

Principal Investigator: Katki, Hormuzd **Year:** 2007

Institution: National Institutes of Health

Title: *Extending BRCAPRO to Handle Errors in Family History*

Abstract:

People with familial history of breast and ovarian cancer often consult with genetic counselors about their chance of carrying mutations in BRCA1 and BRCA2. To aid such people, genetic counselors use Mendelian models (like BRCAPRO) that predict whether the patient carries a BRCA mutation based on the person's family history of the age of diagnosis of each disease or current age or age of death. Such models rely on accurate reporting of each member's diagnosis and age of diagnosis, but this information may be inaccurate. Commonly encountered errors in family history can significantly distort predictions, and thus can alter the clinical management of people undergoing counseling, screening, or genetic testing. Given population-based sensitivities and specificities of reported family history for true family history (such as those reported by Ziogas and Anton-Culver (2003)), we have developed two extensions to BRCAPRO that attempt to handle inaccurate family history. The first extension can be applied to any risk prediction model, but requires knowledge of the predictive values for each relative given the entire family. The second is restricted to Mendelian models like BRCAPRO, but only requires the sensitivity/specificity conditional on a single individual's history alone. We want to compare the performance of these two extensions against the current BRCAPRO using data from the CFR. Data from the population-based sites is useful since BRCAPRO can be used by scientists to plan studies in population-based settings. However, data from clinic-based sites is important as well since a major application of BRCAPRO is for genetic counseling at clinics.

Specific Aim(s):

My application is currently approved to receive data from the three population-based Breast CFR sites (Australia, Ontario, Northern California).

For this amendment, I would also like to include data from the three clinic-based Breast CFR sites (Utah, New York, Fox Chase). The reason is that a major application of BRCAPRO is for genetic counseling at clinics, so if my proposed methods are to be used in clinics, they must have good performance for clinic-based families.

Application ID: B-EX-0107-01 **Status:** Approved, Ongoing

Principal Investigator: Keegan, Theresa **Year:** 2007

Institution: Northern California Cancer Center

Title: *The Influence of the Built Environment on Outcomes After Breast Cancer*

Abstract:

Although high breast cancer (BC) survival rates in the United States have led to a growing population of BC survivors in the United States, survivors are at an increased risk for recurrence, second primary cancers, diabetes, cardiovascular disease, osteoporosis, and premature death. Therefore, it is critical that modifiable factors contributing to these adverse outcomes be identified. Previous studies have suggested that body size, dietary intake, and physical activity may be associated with BC outcomes. Other studies have found physical activity and overweight/obesity to be related to neighborhood characteristics such as the number of walkable destinations, the abundance of fast food restaurants and supermarkets, and the availability of recreation facilities, commonly referred to as the "built environment." As no studies have directly assessed the relationship between the built environment and BC outcomes, this study aims to explore the hypothesis that women who live in neighborhoods that facilitate walking and access to healthy food will be less likely to be obese and will be more likely to have better survival following BC. The proposed study will combine existing epidemiologic data from women with invasive breast cancer in two resources: the Multiethnic Case-Control Study of Breast Cancer (PI: Esther John) and the Northern California Family Registry for Breast Cancer (PI: Esther John). In addition to these two population-based studies, this study will utilize California Cancer Registry data on breast cancer diagnosis and follow-up, self-reported data on recurrence, and geodata from a previously developed built and social environment resource.

Specific Aim(s):

We hypothesize that women who live in neighborhoods that allow them to walk more to accomplish their daily activities, spend less time driving, get more physical activity, and have access to healthy food are less likely to be obese and more likely to have better survival following BC.

Application ID: B-EX-0307-01 **Status:** Approved, Ongoing

Principal Investigator: Kurian, Allison **Year:** 2007

Institution: Stanford University

Title: *Breast Cancer Risk in Women Who Test Negative for a Familial BRCA1/2 Mutation*

Abstract:

Background:

Compared to the general population, women who inherit a mutation in the BRCA1 or BRCA2 tumor suppressor genes have a four to six-fold higher risk of developing breast cancer [Antoniou 2003]. First-degree relatives (FDRs) of BRCA1/2 mutation carriers (BRCA+s) who test negative (BRCA-) for the familial mutation are usually considered to have equivalent breast cancer risk to that of the general population. A recent clinic-based study reported that BRCA- FDRs of BRCA+s have up to a five-fold elevation in breast cancer risk [Smith 2007], but this study was limited by its extrapolation of average breast cancer incidence rates to a highly screened referral clinic population. We propose to evaluate breast cancer incidence among BRCA- FDRs of BRCA+ probands in the Breast Cancer Family Registry (Breast CFR), using FDRs of BRCA- probands as a control group with similar anticipated levels of breast screening and breast cancer risk.

Our hypothesis is that BRCA- FDRs of BRCA+ women with breast cancer have breast cancer incidence similar to that of FDRs of BRCA- women with breast cancer.

Our specific aim is to compare breast cancer incidence in BRCA- FDRs of BRCA+ probands to that of FDRs of BRCA- probands, all accrued by the population-based sites of the Breast CFR.

Methods and Analysis Plan:

We will identify all female BRCA- FDRs of BRCA+ probands in the three population-based sites of the Breast CFR: Ontario, Northern California, and Australia. We will match each female BRCA- FDR of a BRCA+ proband to three female FDRs of BRCA- probands, matching on study site, date of birth, and race (using racial categories of Caucasian, African-American, Asian-American, and other). We will perform a Cox proportional hazards analysis of age at breast cancer incidence in FDRs versus the BRCA1/2 mutation status of the proband.

Potential Significance:

Currently, BRCA- FDRs of BRCA+s are advised that they have no significant increase in breast cancer risk, and are not referred for high-risk breast cancer screening or preventive measures. A finding that these women have substantially elevated breast cancer risk would warrant changes in their clinical care, and re-consideration of the determinants of breast cancer risk in BRCA1/2 mutation-carrying families.

Application ID: B-EX-0402-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Olopade, Olufunmilayo I. **Year:** 2007

Institution: University of Chicago

Title: *Genetics of Breast Cancer in Blacks*

Abstract:

African Americans and Africans experience a disproportionate burden of pre-menopausal breast cancer for reasons that remain unknown. The identification of persons carrying breast cancer susceptibility genes is a promising approach to understanding the etiology of the disease and developing more effective early detection and prevention strategies. With the cloning of BRCA1 and BRCA2 genes, there is an urgent need to identify mutations among different ethnic populations in order to study mutation spectrum, age-specific penetrance, risks of other cancers, epidemiological risk factors, and effects of modifier genes for mutation carriers. Our proposal aims to narrow the knowledge gap by examining a large cohort of African American and African breast cancer cases. We propose to ascertain a total of 1000 Nigerian women diagnosed with breast cancer at, or before, age 65, and 1000 controls. All women under the age of 45 and women with a family history of breast or ovarian cancer, or bilateral breast cancer will be screened for BRCA1 and BRCA2 mutations. The incidence and spectrum of mutations identified in these cases will be compared to that previously reported in Caucasian women and to that obtained in 360 African American women from Northern California who are participating in the Cooperative Family Registry for Breast Cancer Studies (CFRBCS). Detailed family cancer history and exposure information will be collected on each participant to determine whether differences exist in exposure history and clustering of breast and other cancers in the families of women with breast cancer, in Nigeria and the United States. Penetrance of BRCA mutations will be estimated for African American and Nigerian kindreds that are segregating a deleterious mutation. UGT1A1 enzyme is one of the major UGT involved in estradiol glucunonidation and also constitutes a major detoxification pathway for toxic or carcinogenic compounds. We have found that populations of African origin harbor four different alleles of UGT1A1 while non African populations appear to have only two alleles. In addition, alleles associated with lower gene expression levels reach the highest frequency in populations of sub-Saharan African. Using the resources of the CFRBCS and those collected in this study, the association between single nucleotide polymorphisms and in a number of the genes in the UGT1A and UGT2B gene cluster and breast cancer will be assessed in a Nigerian case-control study and an African-American case-control study. In addition, UGTIA1 and UGT2B polymorphisms will be examined as a potential modifies of BRCA1 or BRCA2 cancer risk.

Specific Aim(s):

1. UGT1A1 Polymorphisms, Bilirubin, and Breast Cancer in Blacks

Recently, we reported that there is a significant difference in UGT1A1 single nucleotide polymorphisms (SNPs) between Caucasian (n=181) and African American females (n=335) enrolled at the Northern California site of the Breast CFR and University of Chicago (Hong et al, 2007). In Caucasians, three common haplotypes accounted for 71.8% of chromosomes while five common haplotypes accounted for only 46.6% of chromosomes in African Americans. In a subset of 125 women, total serum bilirubin levels were available. We showed that the number of TA repeats in UGT1A1 promoter is associated with total bilirubin: 7 and 8 TA repeats alleles are associated with higher level of bilirubin, whereas 5 and 6 alleles are associated with lower bilirubin level. In addition, we found TA repeats accounts for 29% of variations in total bilirubin among Caucasians (n=89) and 17% among African Americans (n=36). It is conceivable that the observed differences between the two groups is due to the higher diversity in the African Americans and the relatively small sample of African Americans patients with total bilirubin levels available for study. In African Americans, a study with larger sample size is desired to elucidate the function of other variants and haplotypes in determining bilirubin levels.

In the case-control study conducted in Nigeria, we examined the relationship between the risk of breast cancer and UGT1A1 gene polymorphisms, especially the number of TA repeats in the promoter of the gene (Aim 2 of the protocol). We found that the risk of breast cancer was reduced by 53% for women with low-activity TA repeat genotypes and 36% for women with moderate-activity genotypes, compared with women with high-activity genotypes in premenopausal women (p=0.009) (Huo et al 2007). Taken together, we hypothesize that polymorphisms in UGT1A1 gene may affect breast cancer development through the bilirubin pathway and women with lower bilirubin levels have increased risk of breast cancer.

In order to better estimate the impact of TA repeat polymorphism in UGT1A1 gene on bilirubin levels in African Americans and elucidate the function of other variants and haplotypes in determining bilirubin levels, we propose to extend the pilot study by analyzing plasma bilirubin in African American women who had genotyped for UGT1A1 previously. We plan to measure both indirect (unconjugated) and direct (conjugated) bilirubin levels in the plasm. The plasm analysis will be completed by the Collaborative Laboratory Services, a NHANEs collaborator for biochemical analysyis. They have provided their standard protocol for which they run this assay.

Application ID: B-EX-1207-02 **Status:** Approved, Ongoing

Principal Investigator: Palmer, Lyle **Year:** 2007

Institution: WA Institute of Medical Research, University Western Australia

Title: *Genetic-Based Prediction of Breast Cancer Radiation Therapy Outcomes (GenePBreast)*

Abstract:

Breast cancer is the most common cancer in females in developed countries. The increasing incidence of breast cancer in the ageing population is producing a larger absolute number of cases and is increasing the already considerable healthcare burden. We propose to identify genes predisposing to poor response to radiotherapy therapy for the treatment of breast cancer through a systematic, comprehensive analysis of 550,000 single nucleotide polymorphisms (SNPs) in a minimum of 1500 individuals from the Breast Cancer Family Registry (Breast CFR) who received breast irradiation. We hypothesize that genetic factors are responsible for inter-individual variation in response to radiotherapy. Development of DNA-based biomarker assays to evaluate individual breast cancer treatment response will have exciting implications for the care of individual patients and for the cost effectiveness of health systems.

Many genetic variations are implicated in clinical radiosensitivity, although the expression may differ across tissue type. Ionizing radiation produces cytotoxic effects through the generation of highly reactive DNA radicals that evolve into stable/long lived DNA lesions, such as double strand breaks, or through interactions with the cell membrane, leading to cell death. Studies have shown that deficient DNA repair capacity is associated with increased risk of breast cancer and selected DNA repair polymorphisms may contribute differentially on familial and sporadic breast cancer susceptibility. The use of family-based sampling in the Breast CFR, would ensure an enriched sample of familial breast cancer individuals, homogenous with respect to treatment region, tissue irradiated and adverse reaction experienced.

A Cox regression model will be used to investigate the association of genome-wide SNPs with time to locoregional recurrence, adjusting for appropriate covariates. Radiation complications will be analysed similarly using logistic regression, for those sites that collected radiation adverse events. An exhaustive search for pairwise gene-gene, gene-environment and gene-treatment interactions will also be performed. Significant treatment, genetic and environment factors will be included in a multi-locus model to predict radiotherapy outcome for the treatment of breast cancer.

Specific Aim(s):

Hypothesis

We propose to identify genes predisposing to poor response to radiotherapy for the treatment of breast cancer through a systematic, comprehensive analysis of 550,000 single nucleotide polymorphisms (SNPs) in a minimum of 1500 individuals from the Breast Cancer Family Registry who received breast irradiation. We hypothesize that genetic factors are responsible for inter-individual variation in response to radiotherapy.

2.1 To investigate the predictive effect of genetic variants (SNPs) on 5 year locoregional recurrence and radiation toxicity following radiation therapy for breast cancer.

2.2 To investigate potential gene-gene, gene-environment and gene-treatment interactions between genetic variants (SNPs), radiation exposure and environmental factors.

Application ID: B-EX-1107-01 **Status:** Approved, Ongoing

Principal Investigator: Phillips, Kelly-Anne **Year:** 2007

Institution: Peter MacCallum Cancer Centre

Title: *Tamoxifen and Contralateral Breast Cancer in BRCA1 and BRCA2 Carriers*

Abstract:

Whether tamoxifen reduces risk of breast cancer (BC) in BRCA1 and BRCA2 mutation carriers is uncertain from current data, but the question is a critical one. Here we propose a collaborative study of women in the Breast CFR, kConFab and the International BRCA1/2 Carrier Cohort Study to address this question. Subjects will be women in these cohorts with prevalent or incident breast cancer but without a history of other invasive cancer or tamoxifen use prior to their initial breast cancer diagnosis. Cox proportional hazards models will be used, adjusting for age and calendar year at diagnosis of the initial BC, and with and without adjusting for chemotherapy, oophorectomy, ER status of the initial BC, axillary nodal status and tumor size (if data are available). The main exposure variable will be tamoxifen after initial BC (yes/no) and the primary outcome will be development of contralateral BC. Results from this study will have direct and immediate relevance for the management of women with BRCA1 and BRCA2 mutations.

Specific Aim(s):

Hypotheses

1) That tamoxifen taken following an initial BC reduces risk of contralateral BC in:

a) BRCA1 mutation carriers,

b) BRCA2 mutation carriers and

c) women not known to carry mutations in either gene.

Hypothesis, (c) has been clearly proven in several large randomised controlled trials, but the analysis will be undertaken in this setting to confirm the internal validity of the data. The size of the effect in this unique cohort of women who are, in general, at increased familial/genetic risk may be informative.

2) That the effect of Tamoxifen on BC risk is independent of the ER status of the primary tumor

3) That the effect of Tamoxifen on BC risk is independent of oophorectomy status

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Application ID: B-NY-0804-01-A4 **Status:** Approved, Ongoing

Principal Investigator: Santella, Regina **Year:** 2007

Institution: Columbia University

Title: *Gene Methylation in Tumor DNA in Blood*

Abstract:

It is now well established that CpG island promoter methylation aswell as global hypomethylation are common events in carcinogenesis including that in the breast. In addition,it is also now well established that tumor DNA can be found in blood in a subset of cancer cases and that this DNA frequently contains the same genetic alterations (gene mutations and promoter methylation) found in the tumor. For this reason, it has been suggested that detection of tumor DNA in blood may serve as an early marker of disease. In this application, we propose to test the hypothesis that global hypomethyaltion and gene specific promoter methylation will be detectable in BRCA1, ER, PR, p16, HIC1, RASSF1A and APC in DNA isolated from plasma of blood collected (1) from women without cancer who later went on to develop breast cancer and (2) in women who gave blood before a recurrence. We will also analyze blood samples from control women who did not develop cancer, matched to cases on age of blood donation. When tumor tissue is also available, we will analyze DNA for the presence of methylation in the same genes. Promoter methylation and global hypomethylation will be determined using the MethyLight assay in a 384 well-based format. The frequency of promoter methylation for the different genes will be compared between tumor and blood, between cases and controls and to the time between blood collection and diagnosis. For global hypomethylation, differences between cases an controls will be determined. As follow up data are collected from CFR participants, a longer term goal will be to determine if there is a relationship between methylation and prognosis.

Specific Aim(s):

We would like to add analysis of global methylation to our proposal that currently encompasses gene specific methylation. We specifically propose to use the MethyLight assay to determine levels of methylation in the repetitive elements Alu, Line-1 and Sat2. Methylation levels in these elements have been found to correlate with global methylation levels as determined by HPLC analysis of 5 meC content. No additional biospecimens will be required since we have recently switched to 384 well-based assays that allow smaller amounts of DNA to be used.

Application ID: B-NY-0499-01-C05-A2 **Status:** Approved, Ongoing

Principal Investigator: Santella, Regina **Year:** 2007

Institution: Columbia University

Title: *DNA Repair Capacity as a Susceptibility Factor in Breast Cancer*

Abstract:

Our ongoing project (B-NY-0499-01) on DNA repair capacity has used lymphoblastoid cell lines from sisters discordant for breast cancer to determine the role of nucleotide excision repair (NER) in breast cancer risk. We have found a significant association between increased risk and decreased repair capacity, using an in vitro phenotyping assay with cells treated with the carcinogenic metabolite of benzo(a)pyrene. We have also been genotyping for single nucleotide polymorphisms (SNPs) in genes involved in NER both to determine whether they differ in frequency between cases and controls and to determine if there is a relationship with phenotype. We now plan to extend these studies in several areas. We will continue to genotype new sister pairs for candidate SNPs in NER and expand to other SNPs. We will also extend our phenotype studies to the ability of lymphoblastoid cell lines to repair oxidative DNA damage using an in vitro assay measuring removal of 8-oxo-deoxyguanosine. We will also genotype subjects for SNPs in this pathway to determine case-control differences and relationship to phenotype. Finally, in a basic research project to determine the phenotype of the XRCC1 codon 399 SNP, we will carry out some descriptive studies. Cell lines of known genotype will be used in two in vitro assays to determine ability to repair etheno-adenine adducts and the mutation spectrum inducted in HPRT by this adduct.

A previous amendment encompased measurement of telomere length in mononuclear cell DNA of the sister pairs. This amendment will now look at a polymorphism in hTERT, a gene coding for a catalytic subunit of telomerase.

Specific Aim(s):

We would like to amend our approved proposal to use some of the remaining DNA aliquoted for our DNA repair genotyping studies to determine a functional genetic variation of the human telomerase reverse transcriptase (hTERT) gene in sister pairs.

Application ID: B-EX-0805-02-A1 **Status:** Approved, Ongoing

Principal Investigator: Schildkraut, Joellen M. **Year:** 2007

Institution: Duke University Medical Center

Title: *Genetic Modifiers of BRCA1 and BRCA2*

Abstract:

Women who carry germline BRCA1 or BRCA2 (BRCA1/2) mutations have an elevated lifetime risk of developing breast or ovarian cancer. There is substantial variability in penetrance estimates of breast and ovarian cancers, with estimates of 68% and 36% respectively among BRCA1 mutation carriers from a recent population-based study.1 The cancer risks of BRCA2 mutation carriers are somewhat lower. The Breast Cancer Linkage Consortium 2 reported differences in the histology of breast cancer in women with a BRCA1 or BRCA2 mutation as compared to sporadic cases, lending further support to the idea that there may be differences in etiologic factors that contribute to disease risk among mutation carriers. We hypothesize that additional genetic or environmental factors may modify the manifestation of cancer in BRCA1/2 mutation carriers. This hypothesis has been motivated by evidence that polymorphisms of certain DNA damage and repair genes modify risk of developing breast cancer among BRCA1/2 carriers. Furthermore, results generated in the context of the Cancer Genetics Network pilot project entitled 'Genetic and Environmental Modifiers of Penetrance in BRCA1 and BRCA2 Mutation Carriers' also support this hypothesis. The current proposal is motivated by preliminary results of this pilot study.

Our goal is to identify factors that influence the incidence of breast cancer in germline BRCA1/2 mutation carriers. The focus of the proposed study is on potential genetic modifiers involved in DNA damage and repair. This is supported by findings that BRCA1 and BRCA2 are associated with protein complexes involved in various aspects of genome surveillance and repair and two recent studies reporting an interaction between a RAD51 polymorphism and BRCA2. We hypothesize that variants in the genes that encode these proteins may interact with mutations in BRCA1 and BRCA2. A case-only design will be employed where cases are defined as women with breast cancer who have a known BRCA1/2 mutation status.

Application ID: B-AU-0807-01 **Status:** Approved, Ongoing

Principal Investigator: Southey, Melissa **Year:** 2007

Institution: The University of Melbourne

Title: *Estimate of the Average Age-Specific Cumulative Risk of Breast Cancer for Mutations in BRIP1 and PALB2*

Abstract:

OVERVIEW AND BACKGROUND

It has been known for more than a decade that there are “high risk” breast cancer genes, such as BRCA1, BRCA2 and Tp53; breast cancer predisposing mutations in these genes are very rare but they are associated with a large increase in risk. Over the last 6 months it has been unequivocally demonstrated that there are genes, such as CASP8 and the new genes identified by a genome-wide scan by the Breast Cancer Association Consortium (BCAC), which have common variants (not necessarily functional) that are associated with a very small increase in risk. (It is possible, however, that these common variants may be in linkage disequilibrium with rarer functional variants).

It has also become apparent that there are genes, such as ATM, CHEK2, BRIP1 and PALB2, for which mutations are rare but whose effect on risk is a matter of controversy. It would appear that they are associated with, on average, a moderate increase in risk. Moreover, three recent papers in Nature Genetics, and another in Nature, have suggested that this increased risk may depend on other familial or genetic factors. Therefore, women with a strong

family history may be at high lifetime risk, comparable to that for BRCA1 and BRCA2 mutation carriers, if they carry a mutation in these genes.

We will focus on BRIP1 and PALB2 and first try to replicate and refine the recently published findings on these two genes. We will then apply a large population-based case-control-family design that we have already shown is capable of clarifying issues about risk for two rare variants in ATM. Resolving the risks for mutations in BRIP1 and PALB2 and their relationship to family history will be critical for translating genetic testing for these genes to clinical genetics services.

Specific Aim(s):

Specific Aims

To characterise the breast cancer risk associated with genetic variation in the BRIP1 and PALB2 genes. We will do this by:

1. Trying to replicate the findings of the two published papers by genotyping the earliest onset case available from 2,000 familial cases from the Breast CFR and kConFab and 2,000 controls from the Breast CFR (including the ABCFS).

2. Testing the prediction of the published model that the carrier frequency in unselected cases is lower than in familial cases by genotyping 5,200 population-based cases from the Breast CFR (including the ABCFS) and comparing with data on familial cases generated by aim 1.

3. Quantifying the extent to which carrier frequency in cases might depend on family history and age at diagnosis by pooling data on all 7,200 cases by aims 1 and 2 above.

4. Trying to resolve if the relatives of carrier cases (population-based and clinic-based) are also carriers, by genotyping all relatives of carriers for whom a DNA sample is available.

5. Fitting statistical models to estimate breast cancer risk for carriers under models that assume the effect of being a carrier: (a) is a constant independent of family history of non-carriers, (b) multiplies underlying unmeasured polygenic risk, and (c) multiplies measured risk associated with family history, using all the genotyping data generated above.

6. Comparing the tumour characteristics of identified of roughly an expected 130 carriers with those of four times as many age-matched non-mutation carriers.

Hypothesis

A study of this breadth and magnitude can resolve the breast cancer risk associated with genetic variation in the BRIP1 and PALB2 genes and that this information will have immediate clinical utility in the field of breast cancer clinical genetics.

Application ID: B-EX-1207-05 **Status:** Approved, Ongoing

Principal Investigator: Vahdat, Linda **Year:** 2007

Institution: Weill Cornell Medical College

Title: *Chemoprevention Agents in Women At Increased Risk for Breast Cancer*

Abstract:

Note: only data-usage has been approved for this CFR project to date.

Breast cancer is the most commonly diagnosed cancer among women. In the past decade great effort has been devoted to the identification of chemoprevention agents. Tamoxifen and raloxifene are selective estrogen receptor modulators (SERMs) that are approved agents for breast cancer prevention in higher than average risk populations. These drugs are estimated to reduce the risk of hormone receptor positive invasive breast cancer by approximately 40%. The therapeutic index of SERMs, which are associated with several adverse side effects, is maximized with increasing breast cancer risk. Women who harbor mutations in either the BRCA 1 or BRCA2 genes are at greatly elevated risk of breast cancer and represent ideal candidates for chemoprevention agents. The specific efficacy of either tamoxifen or raloxifene in mutation carriers has not been established. Other agents including aspirin, non steroidal antiinflammatory drugs (NSAIDs), and inhibitors of peroxisome proliferator-activated receptor gamma (PPAR-gamma) are also believed to have activity as chemoprevention agents. Again, specific efficacy among mutation carriers is unknown. Using the unique resources of the Breast Cancer Family Registry (Breast CFR) we propose to assess the impact of chemoprevention agents on breast cancer incidence among BRCA1/2 mutation carriers using. Data will help guide clinical practice as well as provide greater understanding of distinct molecular pathways involved in BRCA1/2-associated breast cancer.

Specific Aim(s):

Aim 1: To assess the impact of therapy with SERMs (tamoxifen or raloxifene) on invasive and/or noninvasive breast cancer incidence among BRCA1/2 mutation carriers.

Aim 1a: To assess whether the association between SERM therapy and breast cancer risk among BRCA 1/2 mutation carriers is modified by duration of SERM use.

Aim 1b: To assess whether the association between SERM therapy and breast cancer risk among BRCA 1/2 mutation carriers is modified by CYP2D6 genotype.

Aim2: To assess the impact of aspirin and NSAID therapy on invasive and/or noninvasive breast cancer incidence among BRCA1/2 mutation carriers.

Application ID: B-EX-0807-02 **Status:** Approved, Ongoing

Principal Investigator: Weitzel, Jeffrey **Year:** 2007

Institution: City of Hope Cancer Center

Title: *Hereditary Breast Cancer and Novel BRCA Mutations in Hispanics*

Abstract:

There is a critical void of research on BRCA mutations in Hispanics. Lack of Hispanic-specific research has hindered the development of prevention and treatment efforts and limited the scope of comparative studies of genetic factors that influence breast cancer (BC) risk. We recently reported on the prevalence of BRCA mutations in an urban clinic-based Hispanic cohort (CEBP 2005;14:1-6), with deleterious BRCA mutations detected by conventional analysis in 31% of 110 Hispanic families. The BRCA1185delAG mutation was prevalent (3.6%) in this cohort of predominantly Mexican descent, and shared the Ashkenazi Jewish founder haplotype. We analyzed DNA from 45 of the 76 patients without an identifiable mutation for the presence of large rearrangements and identified a deletion of four exons in BRCA1 in 3/45 (6.7%) families. All three families reported Mexican ancestry and were unrelated through at least a 4-generation pedigree, suggesting founder effect. A manuscript describing the characterization of the break point and development of a simple targeted assay is in press in CEBP.

Specific Aim(s):

Our objective is to obtain data on the prevalence and nature of BRCA mutations among Hispanics.

Our hypotheses are:

1) The Jewish founder mutation, BRCA1 185delAG, is prevalent in Hispanic populations;

2) The BRCA1 rearrangement, the result of an Alu repeat-mediated recombination event, is deleterious and accounts for a substantial proportion of high-risk Hispanic families; and

3) A panel assay for recurrent BRCA mutations will account for a substantial proportion of BRCA mutations when applied prospectively to a high risk clinic cohort and will be prevalent in additional Hispanic cohorts. Both population-based and high risk clinic cohorts from the B-CFR will be used to characterize the prevalence and mutational age of the recurrent large rearrangement mutation, BRCA1 del (ex 9-12).

Application ID: B-EX-0807-05 **Status:** Approved, Ongoing

Principal Investigator: Zhang, Fang Fang **Year:** 2007

Institution: Texas Health Science Center

Title: *Energy Balance and Breast Cancer Risk: the Inflammation Pathway*

Abstract:

Energy restriction has been consistently associated with a reduction in mammary tumor development in animal models, whereas epidemiologic studies fail to demonstrate a clear link between energy intake and breast cancer risk. The inconsistency may be due to the difficulty to examine the independent effect of energy intake since both physical activity and body size are important determinants of energy expenditure. Few epidemiologic studies, however, have investigated the integrated effect of energy intake, body size and physical activity on breast cancer development. Few studies have also explored potential pathways relating energy balance to breast cancer risk. Recent findings reporting a reduced breast cancer risk in association with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) point to the important role inflammation may play in breast carcinogenesis. Using data collected from breast cancer cases and controls in Breast Cancer Family Registry (Breast CFR), we will examine the integrated effect of energy intake, body size and physical activity on breast cancer risk. We will further investigate whether polymorphisms in genes involved in the inflammation pathway affect cancer susceptibility, and whether these genetic polymorphisms modify the association between energy balance and breast cancer risk. Although exploratory, we will examine these associations by menopausal status, hormonal receptor status, and BRCA1/2 carrier status. In addition to use conventional analytical approaches to study the effect of multiple inflammation-regulating genes, we will apply a hierarchical modeling approach that incorporates prior knowledge on biological pathways to improve the estimation on genetic associations. Because of the unique design of Breast CFR, we will be able to construct both population- and family- based analyses to address our research questions. These analyses will advance our understanding on the role of energy balance and inflammation in the development of breast cancer. Such knowledge is of public health and clinical importance because both energy intake and expenditure are modifiable. If inflammation is etiologically important, anti-inflammatory agents could be implemented to reduce the risk of breast cancer.

Specific Aim(s):

Aim 1: To determine whether an unfavorable energy balance profile characterized by high energy intake, large body size and low physical activity is associated with an increased risk of breast cancer. Specifically, we will:

Aim 1a: examine whether energy intake, body size and physical activity individually and jointly affect the risk of breast cancer;

Aim 1b: investigate whether the effect of energy balance on breast cancer risk differs by menopausal status (pre- and post-menopause), hormonal receptor status (positive vs. negative), and BRCA1/2 carrier status (carrier vs. noncarrier).

Aim 2: To evaluate whether polymorphisms involved in the inflammation pathway including IL-1α, IL-1β, IL-6, IL-1RN, TNF-α, CRP, MCP-1, RANTES, COX-2, MnSOD, MPO, CAT and GSTP1 affect the susceptibility of breast cancer. Specifically, we will:

Aim 2a: examine whether genetic polymorphisms associated with pro-inflammatory status increase the risk of breast cancer, using both conventional analytical approaches and hierarchical modeling approach;

Aim 2b: investigate whether genetic associations between inflammation and breast cancer risk differ by menopausal status, hormonal receptor status, and BRCA1/2 carrier status.

Aim 3: To assess whether the effect of energy balance on breast cancer risk is modified by genes regulating the inflammation pathway. Specially, we will:

Aim 3a: examine whether the associations between energy balance and breast cancer risk are appreciably different in women with genetic polymorphisms related to pro-inflammatory status and in women without these polymorphisms;

Aim 3b: as a secondary aim, explore whether the interaction between energy balance and inflammation-regulating genes in association with breast cancer risk differs by menopausal status, hormonal receptor status and carrier status.

Application ID: B-EX-1207-03 **Status:** Approved, Ongoing

Principal Investigator: Zhao, Hua **Year:** 2007

Institution: Roswell Park Cancer Institute

Title: *MicroRNAs: Novel Breast Cancer Predisposition Factors*

Abstract:

MicroRNAs (miRNAs) are endogenous non-coding ~22 nucleotide (nt) RNAs, which suppress gene expression in a sequence-specific manner. Although it is known that there is widespread misexpression of miRNAs in many cancer tissues, including breast cancer, little is known regarding how inherited variability in miRNA genes and their responsive elements in target genes may predispose to cancer. Inherited variability in miRNAs may be extremely relevant for breast cancer, as family history has consistently been regarded as a major risk factor for breast cancer. This proposal intends to fill this gap in breast cancer. We propose to screen DNA from 152 non-related women with breast cancer identified from the Breast Cancer Family Registry (BCFR) for selected human miRNA genes and their responsive elements in target genes (Aim 1). Then, we will assess the genetic susceptibility of the identified genetic variants in the development of hereditary and sporadic breast cancers in two case-control analyses (Aim 2 and 3). We hypothesize that genetic variations in miRNA genes and their responsive elements in targets genes alter various biological processes by influencing the biological functions of miRNAs, and thereby modify genetic predisposition to breast cancer. This innovative and important area has not been investigated yet. The results from this study will help us to elucidate the biological significance of miRNA in breast cancer, explore the functional significance of these inherited variations, and establish a solid foundation for understanding the role of miRNA in breast cancer predisposition.

Specific Aim(s):

Hypothesis

The discovery of miRNAs in the last decade, and the realization of their growing importance in carcinogenesis and cancer prognosis through regulation of transcription of oncogenes and tumor suppressor genes (TSGs), has led to an era of excitement and discovery regarding these small molecules. Although it is known that there is widespread misexpression of miRNAs in many cancer tissues, including breast cancer, little is known regarding how inherited variability in miRNA genes and their responsive elements in target genes may predispose to cancer. As a consequence of the particular way in which miRNAs function – by targeting a number of functionally important protein-coding genes, such as oncogenes and TSGs – genetic variations in miRNA genes and their responsive elements in target genes could be important in cancer predisposition. Inherited variability in miRNAs may be extremely relevant for breast cancer, as family history has consistently been regarded as a major risk factor for breast cancer. Mutations in the currently known high-risk breast cancer genes (such as BRCA1/2, etc) are common in familial breast cancer, but they can explain at best 20–25% of the overall excess familial risk, suggesting the presence of other unidentified predisposition genes, which confer susceptibility to breast cancer. Considerable efforts have made to discover breast susceptibility genes, however so far few have been identified. The dilemma might be due to the fact that susceptibility alleles reside in protein non-encoding genes, such as miRNAs, or miRNA responsive elements at 3'UTR of the target genes, which are traditionally overlooked in genetic screening. We propose to screen DNA from women with breast cancer identified from the BCFR for selected human miRNA genes and their responsive elements in target genes. We hypothesize that genetic variations in miRNA genes and their responsive elements in target genes alter various biological processes by influencing the biological functions of miRNAs, and thereby modify genetic predisposition to breast cancer. This innovative and important area has not been investigated yet.

Specific Aim 1: To identify genetic variants in selected human miRNA genes and their responsive elements in target genes in women with hereditary breast cancer.

One hundred and forty-six miRNAs, which are predicted to regulate key TSGs or oncogenes in breast carcinogenesis, and 221 responsive elements in these key TSGs and oncogenes will be selected for screening. Sequence analysis will be performed in 152 non-related women who were diagnosed with breast cancer younger than 55 years old, have at least two first or second degree relatives with breast cancer, and are non-carriers of BRCA1/2 mutations.

Specific Aim 2: To evaluate whether genetic variants in miRNA genes and their responsive elements in target genes are associated with hereditary breast cancer.

To achieve this goal, we will estimate the frequencies of novel genetic variants (identified from Aim 1) in 268 family sets (287 breast cancer cases and 350 sister controls) from families with at least two sisters discordant for breast cancer.

Specific Aim 3: To evaluate whether genetic variants in miRNA genes and their responsive elements in target genes are associated with sporadic breast cancer.

To achieve this goal, we will estimate the frequencies of novel genetic variants (identified from Aim 1) in a sample of 1500 breast cancer patients who have no family history of cancer and 1500 population-based healthy controls.

Application ID: B-EX-0406-01 **Status:** Approved, Ongoing

Principal Investigator: Agurs-Collins, Tanya **Year:** 2006

Institution: National Cancer Institute, NIH

Title: *Glutathione S-Transferase Polymorphism, Behavioral Lifestyle Factors, and Premenopausal Breast Cancer Risk*

Abstract:

Glutathione S-transferase (GST) may play an important role in preventing breast cancer risk. GSTs have been shown to be protective through their defense against free radicals, detoxification of toxic compounds, DNA synthesis, cell differentiation, gene expression and apoptosis. However, persons with a deletion in GSTMI and GSTT1 polymorphisms are at increased risk for developing cancer. In particular, genetic polymorphisms resulting in deletions of GSTM1 and GSTT1 have been associated with increase breast cancer risk in pre-and postmenopausal women. Studies suggest that the interaction of certain healthy lifestyle factors with GST deletion polymorphisms may be protective of breast cancer. In particular, interactions of cruciferous vegetable consumption with GSTA1 and GSTP1 deletions showed statistically significant interactions in colorectal adenoma risk. Also, consumption of cruciferous vegetables was inversely associated with breast cancer risk in premenopausal women. GST genotype is also believed to have an interaction with levels of physical activity. Inverse correlations have been documented between serum levels of reduced glutathione peroxidase and plasma level of glutathione reductase with physical activity. However the interactions between GST genotype and levels of physical activity have not been examined. We propose to examine the associations and interactions of GST polymorphisms with lifetime physical activity and cruciferous vegetable intake on risk for early onset breast cancer. The study design is a population-base case-control study (2541 cases and 1447 controls) of premenopausal women participating in the Breast Cancer Family Registry (Breast CFR). The study aim is to determine whether GST candidate genes and tag SNPs modulate the association between diet and physical activity and risk of premenopausal breast cancer. The results from this study may provide evidence that healthy lifestyle behaviors are protective of premenopausal breast cancer risk in women with at risk GST genotypes.

Specific Aim(s):

Hypotheses

(1) Genetic polymorphisms of phase II enzymes GSTA1, GSTT1, GSTM1, and GSTP1 are associated with risk of premenopausal breast cancer.

(2) Genetic polymorphisms of phase II enzymes GSTA1, GSTT1, GSTM1, and GSTP1 will modulate the relationship between level of physical activity and risk of premenopausal breast cancer.

(3) Genetic polymorphisms of phase II enzymes GSTA1, GSTT1, GSTM1, and GSTP1 will modulate the relationship between cruciferous vegetable intake and risk of premenopausal breast cancer.

(4) Women with a combination of at-risk GST genotypes, low physical activity, and low cruciferous vegetable intake will have an increased risk of premenopausal breast cancer.

Application ID: B-AU-0406-01 **Status:** Approved, Ongoing

Principal Investigator: Apicella, Carmel **Year:** 2006

Institution: The University of Melbourne

Title: *Predictors of Breast Cancer Screening Practices in Australian Breast Cancer Survivors*

Abstract:

Breast cancer survivors are at increased risk of developing a second primary breast cancer, in either the same or the contra-lateral breast. Early-detection of breast cancer (screening) is important because it has been shown to be an effective means of reducing mortality from the disease. There are several screening modalities available for detecting breast cancer, such as mammography, physical breast examination, and ultrasound. However, screening practices in this group of women at increased risk are not well understood. That is, while screening using mammography and other modalities is usually determined by the woman's doctor in the first 5 years after diagnosis, it is not known what breast cancer survivors are doing in the long term. To address this, we plan to use screening behavior data collected using the B CFR pilot follow-up questionnaire, administered as part of the Australian site's follow-up, conducted in 2005, of 1143 probands recruited between 1996 and 2000 through the Breast CFR. Further, we will combine this with data collected using the same questionnaire, as part of follow-up conducted in 2004, of 248 probands recruited at the Australian site between 1992 and 1995. These data are held locally on a database at the Australian site, and would not require provision of data by RTI. The screening behaviours of interest in this study are: age at commencement, time since most recent, frequency, and timing, of: mammography, breast ultrasound, physical breast examination by a health professional, and breast self-examination.

Specific Aim(s):

The specific hypotheses we wish to address are that, in women with breast cancer:

(i) screening intensity increases with family history. That is, with increasing family history, women with breast cancer have a greater frequency and more recent use of: mammography, breast ultrasound, physical breast examination by a health professional, and breast self-examination.

(ii) screening behaviors vary according to: age; education; socio-economic status; state of residence; number of relatives with breast cancer; age at diagnosis of relatives with breast cancer; relatedness of women with breast cancer; side of the family of breast cancer cases; long-term survival of relatives with breast cancer; stage of disease at diagnosis; treating physician, and perceptions about population risk of breast cancer.

Application ID: B-EX-0406-03 **Status:** Approved, Ongoing

Principal Investigator: Brown, Melissa **Year:** 2006

Institution: The University of Queensland

Title: *Identification of New Mutations Important in Breast Cancer*

Abstract:

Disruption of both transcriptional and post-transcriptional control of many oncogenes and tumour suppressor genes contributes to human cancer. The number of detectable mutations in the breast cancer susceptibility gene BRCA1 falls well below expectations in both sporadic (based on expression data) and early-onset breast cancers (based on linkage data and histopathology data). BRCA1 is regulated transcriptionally by several regulatory elements and post-transcriptionally by several mechanisms including control of mRNA stability. The possibility that the cis-elements and trans-factors controlling the regulation of BRCA1 are abnormal in human breast cancer has not been fully investigated.

The overall aim of this research is to assess the status of these regulatory processes in breast cancer cases.

The specific aims of this research are:

1. Mutation analysis of novel BRCA1 cis-elements in the non-coding regions of BRCA1 (introns and UTRs) and the genes encoding trans-acting proteins that regulate the function of the BRCA1 transcript (e. g. HuR) in clinical material. DHPLC and DNA sequencing of DNA from early-onset breast cancer cases with a BRCA1-associated tumour pathology but no BRCA1 or BRCA2 coding region mutation.

2. Expression analysis of BRCA1 and trans-acting proteins (e. g. HuR) in clinical material. Allele-specific expression analyses of clinical material from early-onset breast cancer cases.

The outcome of this research will be a better understanding of the molecular pathways underlying the regulation of this important breast cancer susceptibility gene and the identification of new mutation targets that are critical to early-onset breast cancer tumorigenesis. The identification of additional regions of the BRCA1 gene susceptible to germline mutation that predispose to the development of breast cancer will be of immediate use to the clinical management of breast cancer families that have not had their cancer predisposition explained by current methods. Demonstration that trans-acting regulators of BRCA1 expression can rescue some effects of loss of BRCA1 expression may have therapeutic implications for the treatment of some breast cancers.

Application ID: B-NY-0806-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Chung, Wendy **Year:** 2006

Institution: Columbia University

Title: *Identification of Genetic Modifiers of BRCA1 or BRCA2 in Ashkenazi Mutation Carriers*

Abstract:

The risk of breast cancer is between 46 to 85% and 26 to 74% by age 70, respectively, for carriers of BRCA1 and BRCA2. However, it remains unclear why some women develop breast cancer, others ovarian cancer, some both, and others neither. Three common founder mutations in the breast cancer susceptibility genes BRCA1 (185delAG, 5382insC) and BRCA2 (6174delT) account for over 98% of mutations in BRCA1 and BRCA2 in the Ashkenazi population and have previously been genotyped in all the Ashkenazi subjects in the BCFR. The Ashkenazi subjects represent a genetically homogeneous population that should be more amenable to studying genetic modifiers of BRCA1 and BRCA2 for cancer susceptibility due to the homogeneity of mutations in BRCA1 and BRCA2 as well as the potential for genetic homogeneity of other interacting genetic factors. We propose to study known Ashkenazi specific genetic variations in the candidate genes CHEK2, MSH2, P53, RAD51, MTHFR, APC, and BRIP1 involved in DNA damage and repair in BRCA1/BRCA2 carriers for association of these variants with breast cancer and age of diagnosis. Although BRCA1 and BRCA2 modifying genes will be initially studied in the Ashkenazi population, the information provided by these studies will be widely applicable to patients carrying other BRCA1 and BRCA2 mutations and potentially other breast cancer susceptibility genes identified in the future. These studies may ultimately improve our ability to stratify risk for BRCA1 and BRCA2 mutation carriers and individualize plans for prevention and treatment of breast cancer.

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Application ID: B-EX-0406-04 **Status:** Approved, Ongoing

Principal Investigator: Dobrovic, Alexander **Year:** 2006

Institution: Peter MacCallum Cancer Centre

Title: *Do BRCA1 Epimutations Underlie Some Early Onset Breast Cancer?*

Abstract:

A small proportion of breast cancer is caused by mutations in BRCA1 or BRCA2 but the majority of breast cancers have no identifiable genetic predisposition. Epigenetic silencing of BRCA1 occurs in breast cancer, but the significance of this, either as a predisposing event, or as a step in carcinogenesis is unknown. In this study, we propose that BRCA1 can be methylated as a germline or somatic epimutation and this epimutation can predispose to BRCA1-like cancers. Recent evidence from the MLH1 gene in HNPCC suggests that epimutations may occur in early onset patients without a strong family history.

Specific Aim(s):

Hypotheses

1) Patients with somatic methylation of BRCA1 are likely to develop tumours that show BRCA1 mutation-associated morphology

2) Individuals with early-onset breast cancer without a family history or somatic mutation, particularly those with multifocal disease, and BRCA1-like tumour morphology are candidates for harboring germline “epimutations” (epigenetic silencing not involving sequence change) of BRCA1.

Application ID: B-SF-1203-01 **Status:** Approved, Ongoing

Principal Investigator: Gomez, Scarlett L. **Year:** 2006

Institution: Northern California Cancer Center

Title: *Immigration Effects on Racial/Ethnic Cancer Patterns*

Abstract:

Cancer surveillance data have been instrumental in documenting differences in racial/ethnic patterns. More recently, investigators have included measures of area-level socioeconomic status in their analyses of registry data and these studies have furthered our understanding of the contribution of socioeconomic status on racial/ethnic variations in cancer patterns. We propose to evaluate methods to enhance registry data with information on patients’ neighborhood social and physical (built) environment and birthplace/timing of immigration, as a means to improve the utility of the data for population-based studies of race/ethnicity vis-à-vis cancer burden. The aspect of the study that pertains to the Northern California Breast CFR data is the assessment the validity of methods to add measures of immigration to the cancer registry. Ultimately, we will apply these methods to California Cancer Registry data to examine the independent and joint contributions of area-level socioeconomic status, neighborhood social and built environment, and immigration on racial/ethnic differences in cancer incidence, diagnosis, treatment, and survival.

Specific Aim(s):

This study, which is being funded by the SEER Rapid Response Special Studies mechanism, bears two parts: 1) methodological research on the development and feasibility of methods in appraising immigration as well as social and built environments; and 2) application of these methods with California Cancer Registry (CCR) data to examine the extent to which SES, immigration, and social and built environments affect racial/ethnic patterns in cancer and whether their influences vary by race/ethnicity. Listed here are the aims that will incorporate the epidemiologic data collected from the approximately 2,265 probands enrolled in the Northern California Family Registry for Breast Cancer:

1. To compare self-reported data on year of immigration as a reference standard to that estimated via social security numbers (SSN) obtained from the Social Security Administration (SSA).

2. To statistically impute birthplace data for patients in the registry lacking this information and validate conclusions based on self-reported birthplace.

This study will not involve contact with patients.

Application ID: B-AU-0806-02 **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2006

Institution: The University of Melbourne

Title: *Hormones, Hormone Metabolism and Familial Breast Cancer*

Abstract:

Family history is an established risk factor for breast cancer. A consequence of this is that women vary widely in their underlying 'familial' breast cancer risk, and this is likely to be due to the effects of multiple genes and/or environmental factors. About 9 in every 10 breast cancers occur in women who are at above average familial/genetic risk. Virtually nothing is known definitively about the environmental or genetic factors that influence risk in women at known or implied familial/genetic risk.

The general aims are to use the core-funded follow-up to conduct follow-up of all participants, and thereby estimate prospectively the risk of breast cancer in women at familial/genetic risk, including those known to carry a germline mutation in BRCA1 or BRCA2, associated with: use of exogenous hormones, oral contraceptive (OC) or hormone therapy (HT); menstrual and reproductive variables; variants and haplotypes in a set of 25 known genes involved in the metabolism of sex hormones; and gene-gene and gene-environment interactions.

Specific aim 1: To estimate prospectively the risk of breast cancer, in women at familial/genetic risk including those known to carry a germline mutation in BRCA1 or BRCA2, associated with use of exogenous hormones, oral contraceptive (OC) or hormone therapy (HT).

Specific aim 2: To estimate prospectively the risk of breast cancer, in women at familial/genetic risk including those known to carry a germline mutation in BRCA1 or BRCA2, associated with variations in menstrual and reproductive variables, specifically age at menarche, parity, and the number and timing of child births, and age at menopause.

Specific aim 3: To estimate prospectively the risk of breast cancer, in women at familial/genetic risk including those known to carry a germline mutation in BRCA1 or BRCA2, associated with variants and haplotypes in a set of known genes involved in the metabolism of sex hormones.

Specific aim 4: To determine whether the risk estimates in Aims 1, 2 and 3 are not independent, due to gene-gene or gene-environment interactions.

The novelty and strength is that we will study women enrolled in the Breast Cancer Family Registry (Breast CFR) who are, by design, known to be at increased risk due to their personal or family history of the disease or because they have been found to be a mutation carrier, and who as a group are at 2-3 times population risk.

We will identify incident cases of breast cancer through the prospective follow-up of all 18,711 registrants enrolled in the Breast CFR between 1996 and 1999 (i.e. a 10 year follow-up), including 1,006 mutation carriers who were interviewed between 1996 and 2004. We will systematically update vital status, personal and family cancer histories, and ask more detailed questions about OC and HT use.

The design of the questionnaire and protocols has been almost completed, and pilot activities have shown that at least one member of virtually every family can be found, and that repeat interviews can be conducted with more than 90% of living case probands and more than 80% of relatives. We will use the Los Angeles and Hawaii Multiethnic Cohort Study and the international collaborative efforts of the Breast and Prostate Cohort Consortium to identify putative disease-predisposing variants and haplotypes in the 25 candidate genes. We estimate that we will identify about 1,000 cases of breast cancer diagnosed in the cohort since baseline, and 250 in mutation carriers. Statistical aalysis will be by standard methods for analysing cohort studies, using robust estimators to adjust for the fact that there is clustering due to the famiy structure of the cohort. Due to the size of this study there will be good power to detect modest and even small effects of the major exposures and genotypes.

The collection of data on outcomes for this study has either been collected as part of current core funding for the Breast CFR up until June 30, 2006, and/or as a consequence of core funding for the renewal grant.

Application ID: B-AU-0806-01 **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2006

Institution: The University of Melbourne

Title: *Statistical Analyses of Breast Cancer Risks for Australian BRCA1 and BRCA2 Mutation Carriers*

Abstract:

Aims: We will analyse large sets of both retrospective and prospective data collected from Australian women and their families to determine, for the genes BRCA1 and BRCA2:

(i) the average age-specific cumulative risks of breast and/or ovarian cancer for women who carry a germline mutation found in the setting of a cancer family clinic, and in the setting of an unselected case from the general population; and

(ii) the probability of being a mutation carrier, as a function of family cancer history and, for affected women, of their age at diagnosis and pathology features of cancers. We will develop simple algorithms, such as we have developed and validated for Ashkenazi Jewish women, to predict an Australian woman s likelihood of being a carrier as a function of her personal and family s cancer histories, and of the pathology characteristics of cases, and compare its performance to other models.

Background: Since the discovery in the mid 1990s that women with germline mutations in the genes BRCA1 and BRCA2 are at high risk of breast and some other cancers, there has been a great interest especially from the cancer family clinics that provide genetic counseling to determine accurately and precisely the actual risks (penetrance) and how best to triage women as likely carriers. There has been a considerable debate about penetrance estimates, especially if they differ depending on how families are sampled, and we are in an excellent position to answer these questions.

Research Plan: We will use data from the Australian Breast Cancer Family Study (ABCFS), a population-based case-control-family study of 1,600 families of women with breast cancer sampled irrespective of their family history, and the Kathleen Cuningham Foundation Consortium for Research on Familial Breast Cancer (kConFab), which has recruited more than 1,000 multiple-case breast cancer families through Australian and NZ cancer family clinics. We will determine the extent of mutation testing conducted, and the sensitivity from the literature and other sources. For each test, we will

determine the outcomes, and classify variants. We will use data, based on the same questionnaire used by kConFab and ABCFS, for age at interview, sex, breast and ovarian cancer status, age at diagnosis, and surgery, as well as family history data.

For (i) we will estimate the age-specific hazard rations and cumulative risks using a modified segregation analysis and maximum likelihood methods we and others have used several times previously, and also using a new method. For (ii) we will use multiple linear logistic regression, as we have previously. We will also conduct methodological research around some of the statistical issues raised by this research.

Outcomes and Significance: We will provide accurate and precise information to assist genetic counselors inform women who consider mutation testing for BRCA1 and BRCA2, and those who come to know their mutation status, about their cancer risks. The information will be relevant to Australian women attending cancer family genetics services. We will also develop new simple and valid algorithms to help triage women according to their likelihood of being a mutation carrier.

This will be an important step in making breast cancer genetics cost effective. This work is the result of more than 15 years planning, and no other comparable study includes retrospective and prospective data from clinic-based and population-based families from the same country.

Specific Aim(s):

Hypothesis

This project is about estimating risks and developing algorithms to predict mutation carriers. We intend to test if the estimates of penetrance from multiple-case clinic-based families are different to those from population-based families, but we do not a have any preconceived position that it should be different (even though there is widespread a belief that the estimates from clinic-based families are higher); i.e. will conduct a two-tailed test of the null hypothesis that there is no difference. It is plausible that our findings will be relevant to other western countries, including the USA.

Application ID: B-EX-0406-05 **Status:** Approved, Ongoing

Principal Investigator: McLaughlin, John **Year:** 2006

Institution: Samuel Lunenfeld Research Institute, Mt. Sinai Hospital

Title: *Ontario Population Genomics Platform*

Abstract:

To provide a foundation for genomic epidemiology in Canada, investigators at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital (Toronto, Ontario, Canada) have linked with the Centre for Applied Genomics (TCAG) at the Hospital for Sick Children (HSC) that will help investigators overcome the above barriers, which are currently faced in applying genomics to study human health. The Ontario Population Genomics Platform aims to provide a resource for determining population frequencies of allelic variants, including polymorphisms.

In order to provide a unique platform for genomic studies, a database and repository is needed that contains information on a representative sample of the population, and which can enrich and complement existing research resources (e.g., familial cancer registries) in order to enhance the feasibility, validity and statistical power of studies that aim to identify disease-causing alleles.

Specific Aim(s):

Hypothesis

The Population Genomics Repository will be a comprehensive database referring to volunteers sampled from the population of Ontario that contains demographic information, information on phenotype including medical history, epidemiological risk factors, pedigree information, and banked biospecimens collected from blood samples (genomic DNA, serum, plasma, Guthrie card, and lymphocytic cell lines). The Repository will contain information on approximately 3500 adult men and women (ages 20-79) who reside in Ontario, and who are representative of the Ontario population. This series is large enough to provide adequate statistical power to detect associations for even very rare alleles (e.g., with a population prevalence of 1 per 1000) among Caucasians, who constitute the majority of the Ontario population (Statistics Canada; 1996 Census; www.statcan.ca). The series is also large enough to provide reliable estimates even for infrequent genetic variants (e.g., prevalences of 5% or less) within the other major ethno-racial subgroups of the population, thereby providing an important improvement on the rather limited resources that are often relied on in genetic studies.

Application ID: B-EX-0406-06 **Status:** Approved, Ongoing

Principal Investigator: Milne, Roger **Year:** 2006

Institution: Spanish National Cancer Centre

Title: *Methodological Issues in Assessing Modifiers of BreastCancer Risk for BRCA1 and BRCA2 Mutation Carriers Using Retrospective Data*

Abstract:

Women for whom a mutation in BRCA1 or BRCA2 has been detected have a very high lifetime risk of breast cancer, yet little information is available about what they can do to reduce their risk apart from prophylactic surgical interventions (mastectomy and oophorectomy). While there have been number of publications on this issue in the last few years, the effects on breast cancer risk, for mutation carriers, of factors clearly associated with risk in the general population remain unclear. Recruitment of subjects for current studies of mutation carriers is largely carried out through multiple-case families with known carrier cases (and virtually all unaffected carriers are relatives of cases) and potential risk factors are assessed retrospectively. These

conditions mean that standard analytical methods cannot necessarily be applied and it is not immediately obvious what underlying biases are inherent in those methods that are applied. However, until data are available from prospective studies of cohorts of unaffected carriers, we must endeavour to analyse what data are available and make appropriate inference in order to inform mutation carriers correctly. We propose to assess the effect of exposures (e.g. OC use, smoking, parity, weight, alcohol consumption, HRT use, breast feeding, etc.) collected by the Breast CFR Risk Factor Questionnaire for all BRCA1 and BRCA2 mutation carriers identified by the Breast CFR, kConFab (and hopefully also OCGN), using a range of designs and methods in order to elucidate how these issues might influence the results obtained. This is novel, because previous work using carriers from the Breast CFR, kConFab and OCGN has been restricted to one analytic approach (unmatched case-control analysis) applied to a restricted data set (carriers under the age of 50 when completing the questionnaire within 5 years of diagnosis) that included only one-third of all known carriers for whom epidemiological data has been collected (i.e. 803 out of nearly 3,000). Our analyses will assess the sensitivity of estimates to design and analytic method.

Specific Aim(s):

We aim to determine if the estimated relative risks from analyses for associations between exposures and breast cancer risk for BRCA1 and BRCA2 mutation carriers depend on the design or analytic method used, or the age group considered.

Application ID: B-TO-0506-01 **Status:** Approved, Ongoing

Principal Investigator: Mulhall, Cara **Year:** 2006

Institution: Cancer Care Ontario

Title: *Optical Spectroscopy of Breast Tissue, IGF1 and Growth Hormone in Youth with a Family History of Breast Cancer*

Abstract:

A full-scale cohort study of the female children (aged 5-17) of participants in the Breast Cancer Family Registry (BCFR) is being planned by Dr. I Andrulis and colleagues to investigate the hormonal, genetic, and environmental determinants of intermediate endpoints (IEs) that are associated with breast cancer risk in adulthood. This study is called LEGACY (Lessons in epidemiology and Genetics of Adult Cancers from Youth). Physical development and early-life events are important in determining breast cancer risk in adulthood. It is difficult to obtain accurate measures of early-life development and exposures retrospectively, thus, the prospective study of intermediate endpoints like breast tissue characteristics and development in youth, is necessary to improve current understanding of breast cancer risk and etiology. This knowledge may lead to more effective prevention strategies applicable earlier in life when they can have greatest impact on lowering disease incidence.

Currently, there is no ideal method to measure one of the intermediate endpoints of interest in LEGACY: breast tissue development. Optical spectroscopy (OS) is a safe, reproducible, and non-invasive method that can measure fat and glandular tissues in the breast quantitatively even on what appear to be non-existent breasts as seen in early stages of puberty. In adults, the predicted percent density from OS correlates strongly with percent mammographic density, which is a heritable trait and a strong risk factor for breast cancer. Thus, OS may provide an improved method to study breast development that will be superior to the current gold standard of Tanner Staging (TS) by trained observer to assess breast development and we intend to pilot OS for LEGACY.

We have obtained funding from the Susan G Komen Foundation to pilot this new tool among the daughters of BCFR participants at the Ontario site. A cross-sectional study of approximately 300 daughters aged 7-17 of participants in the OFBCR will be conducted. At a clinic visit, OS will be used to measure breast tissue development, epidemiologic and anthropometric data will be collected, and TS will be conducted by a registered nurse. Blood samples will be obtained for the measurement of hormones and genes of the sex hormone and GH-IGF1 pathways. No mutation analysis will be conducted on the DNA of these participants. The relationship between hormone levels, polymorphisms, TS, and OS will be evaluated using regression analysis.

OS is a new tool that may have broad research applications to the scientific study of pubertal development. The quantitative data obtained from OS may prove useful for the study of genetic and environmental determinants of breast tissue traits relevant to adult breast cancer risk. Furthermore, similar research has not been conducted among daughters at high risk of breast cancer based on family history of the disease, and this study will provide information on development in youth at higher risk of disease than the general population.

Specific Aim(s):

Specific Aims

First, to measure breast development using OS, TS by health profession, mother-reported and self-reported TS and to analyze the relationships between these measures in this population.

Second, to assess the participant burden and acceptability of each modality of measuring breast development: nurse assessment of TS, OS, self-reported TS and mother-reported TS.

To assess the impact of body image perception measured using the Body Rating Scale on self-reported TS.

Lastly, to measure hormones in the GH-IGF1 pathway, and sex hormone metabolizing genes including IGF1 and its binding proteins and their relationship to breast tissue development measured by OS, and to measure the relationship between genes in sex hormone and GH-IGF pathways for associations with OS.

Hypothesis

We hypothesize that optical spectroscopy (OS) of breast tissue will provide quantitative measures of breast tissue characteristics in puberty that may correlate with TS assessed by a health professional during a clinic visit. Furthermore, serum sex and growth hormones that were associated with measures of breast tissue, such as mammographic density, may be associated with breast tissue characteristics in young females measured using OS.

Specifically, we expect that serum levels of insulin-like growth factor 1 (IGF1) and its primary binding protein, IGFBP3, may correlate with breast tissue characteristics measured by OS. Furthermore, data obtained from this pilot will assist in designing the full-scale LEGACY. First, we will verify that the measures of breast development and characteristics gained from OS are useful for the large-scale cohort study that we are currently planning. Second, the data will be used to calculate power for the full-scale cohort study, and will help with design issues such as number of samples to obtain from subjects in the full-scale study.

Application ID: B-UT-1206-02 **Status:** Approved, Ongoing

Principal Investigator: Neuhausen, Susan L. **Year:** 2006

Institution: University of Utah

Title: *Genetic Variation in the IGF Pathway and Clinical Outcomes in Breast Cancer*

Abstract:

Breast cancer remains a significant cause of morbidity and mortality. There is an important need to identify parameters that accurately predict clinical outcome and lead to improved cancer control. The role of genetic factors in prognosis and treatment of disease is still largely unknown. Single nucleotide polymorphisms (SNPs) are the most frequent genetic changes in the human genome, and can be common on a population-level, a fact that coincides with the large inter-individual variation in disease progression and response to therapy that exists. There are several pathways involved in cancer progression and treatment response. Candidates include pathways involved in steroid hormone/carcinogen metabolism, angiogenesis, proteolysis, motility, apoptosis, and growth control. The importance of these pathways in clinical outcomes in breast cancer has been highlighted by several studies, but the role of specific genes and variation within those genes remains poorly understood. The objective of this study is to conduct a comprehensive investigation of genetic variants in genes involved in the IGF signaling pathway to understand their role as prognostic and predictive factors for breast cancer using data from the Prognostic Study of Dr. Pam Goodwin of the Ontario Family Breast Cancer Registry. The Prognostic Study is studying clinical, follow-up and outcome data prospectively on breast cancer cases in the three population-based registries. Clinical and treatment data were extracted from patient medical records and include: demographics, clinical staging, treatment, and status of breast and ovaries. Data including surgical procedure, tumor size, lymph node status, and hormone receptor status were extracted centrally from the pathology report issued at the time of diagnosis. For the majority of patients, pathology slides were also reviewed centrally in a standardized fashion. Follow up data were collected for recurrences of breast cancer (loco-regional, distant), new primary cancers, and death (date, cause). Candidate genes include IGF1; IGF2; the IGF binding proteins IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, IGFBP7, IGFALS; the IGFBP proteases MMP3, MMP7, KLK3, CTSD, PRSS11; IGFIR; the IGFIR docking proteins IRS1, IRS2, SHC1; INS; INSR; ESR1; ESR2; and SHBG. SNPs will be genotyped using standard high-throughput methods (e.g., TaqMan, SNPlex, Illumina). Our primary research objective is to determine the association between genetic variants in genes in IGF signaling and distant recurrence. The Cox proportional hazards model will be used to test our hypotheses. This application adds the IGF pathway to a currently approved application of Dr. Andrulis (B-TO-0805-01) using the same set of samples, outcomes, and statistical analyses as described in that proposal.

Specific Aim(s):

Hypothesis

Genetic variants in genes involved in IGF signaling are associated with distant breast cancer recurrence and prognosis.

The objectives are to determine whether genetic variants in the IGF signaling pathway are associated with distant recurrence and mortality. Secondary objectives are to investigate whether the effect of these genetic variants on distant recurrence is modified by BRCA-mutation status and whether they interact with treatment.

Application ID: B-AU-1006-01 **Status:** Approved, Ongoing

Principal Investigator: Southey, Melissa **Year:** 2006

Institution: The University of Melbourne

Title: *Molecular Pathology Features of Breast and Colon Cancers Occurring Together in Families*

Abstract:

Reports of breast and colorectal cancer occurring together in families date back to 1904. Although many subsequent examples have been dismissed as the simultaneous occurrence of common cancers, recent evidence has highlighted non-independence between the two. In addition to having both breast and colorectal cancer cases, several features which suggested a genetic causation to familial clustering of breast and colorectal cancer, have been described. These include apparent Mendelian inheritance, at least one case of early onset disease below age 50, individuals with both breast and colon tumours, and synchronous and metachronous tumours. In addition, the presence of breast cancer in HNPCC has been described in several reports. Individual cases have been presented where there has been clear evidence of mismatch repair deficiency in breast cancers occurring in mutation carriers. Further, some registry studies have shown an excess of breast cancers in colorectal cancer families.

The study will proceed in 2 steps

Step 1) First, we will examine the resources of both the Colon and Breast CFRs for families where the number and age of onset of cases of both breast and colon cancer suggest that there may be a common genetic causation for the two tumours. From this we will perform preliminary analyses and generate hypotheses.

Step 2) We will submit an RO1 in June 2007 to examine the molecular pathology phenotypes of the tumours in a subset of breast colon families. Families with multiple cases of breast and colon cancer present a clinical problem in terms of their management, risk assessment and predictive testing.

This particular proposal is a DATA ONLY application and will cover step 1). The expected outcome of this work will be to address in part the interesting question as to whether there are a set of characteristics including age of onset, ratio of breast to colon cancer, the presence of other cancers in the family and pathology features which may identify a breast colon cancer predisposition.

Specific Aim(s):

Specific Aims

The specific aims of the study in the longer term will be to describe the families including molecular pathology specifically…

1) To determine the pathology features of breast and colon cancers occurring together in families

2) To determine the molecular profiles of breast and colon cancers occurring together in families with respect to

a) IHC of Wnt pathway proteins (APC, beta-catenin, SFRPs), hormone receptors (ER, PR, her2), p53, PTEN, MGMT, MSI, and PIK3CA mutations

b) tumour LOH arrays and methylation arrays in a subset of cases

3) to analyse the prevalence of variables obtained in 1) and 2) against strength of family history, the spectrum of other cancers in a family and ages at onset

Hypothesis

This study will interrogate the resources of the colon and breast CFR for characteristics of families in which the phenotypic structure of the kindred suggests that there is a predisposition to develop both breast and colorectal cancers. From this we will develop specific hypotheses which may be tested in a more detailed study involving biospecimens forming the basis of an RO1 to be submitted in June 2007.

The primary aim of the longer term study is to examine whether a subset of multiple-case breast and colon families (including those where there are individuals with both breast and colon cancer) demonstrates molecular histopathology and/or immunohistochemistry features that may be associated with increasing family history and decreasing age of onset thereby suggesting a genetic etiology.

Application ID: B-AU-0406-03 **Status:** Approved, Ongoing

Principal Investigator: Southey, Melissa **Year:** 2006

Institution: University of Melbourne

Title: *MYH Mutations and Breast Cancer Risk*

Abstract:

Mutations in the base excision repair gene MYH have been have been known for some time to be associated with recessive colorectal cancer risk (Al-Tassan et al 2002, Sieber et al 2003). Several studies have observed a higher prevalence of monoallelic mutation carriers in colorectal cancer cases compared with controls but this had not reached statistical significance but when taken together had suggested that mutations in MYH may have an additional dominant role (Croitoru et al 2004 and others). Jenkins et al (in press) have now demonstrated, via a kin-cohort design study, using a modified segregation analysis, that the colorectal cancer risk estimate of mono- and bi-allelic carriers of MYH mutations is 3-fold and 50-fold respectively.

The possible role of MYH mutations in breast cancer predisposition is largely uninvestigated. However, breast cancers have been observed in some selected colorectal cancer families carrying MYH mutations. In a study of 40 Dutch patients with MYH associated polyposis coli, breast cancer occurred in 18% of female MAP patients, significantly more than expected (Nielson et al 2005). In addition, 14 of the 39 MYH mutation carrying probands studied in Jenkins et al (in press) had a total of 22 breast cancers reported in their family.

Specific Aim(s):

Aims

1) Screening population-based recruited probands and the youngest affected members of clinic-based families for the common MYH mutations Y165C and G382D.

This will be done via high resolution melt curve analysis and confirmation via sequencing.

2) Screening any monoallelic carriers of these common MYH mutations for other mutation in MYH using a gene mutation screening technique.

This will be done via high resolution melt curve analysis utilising the molecular primer design described in Jenkins et al (in press) and Croitoru et al, 2004, and confirmation via sequencing.

3) Screening the population-based case controls for the identified MYH mutations.

This will be done via high resolution melt curve analysis and confirmation via sequencing.

4) Screening the family of each mutation carrier for the family-specific MYH mutation/s.

This will be done via DNA sequencing.

Hypothesis

Germline mutations in the MYH gene predispose to breast cancer development.

Application ID: B-EX-0404-02-A2 **Status:** Approved, Ongoing

Principal Investigator: Tavtigian, Sean **Year:** 2006

Institution: International Agency for Research on Cancer

Title: *Large Scale Mutation Screening of Strong Candidate Breast Cancer Susceptibility Genes*

Abstract:

Every new publication of a "strong candidate susceptibility gene" can be viewed as a hypothesis generating report. Unfortunately, such studies rarely make accurate measures of attributable risks and have an appreciable false-positive rate. Thus there is considerable utility in creating projects that are poised to test the hypotheses resulting from such initial publications. The project described here is directed towards those candidate susceptibility genes for which the most appropriate test is a case-control comparison of the summed frequency of sequence variants that are likely to alter gene function. Examples of genes that would have fit well in this project at some point in their publication history include ATM, CHEK2, and BARD1. The project design combines two elements that have not often been used in the past: (i) extensive mutation screening in familial cases and population controls in order to enable a test of the summed frequencies, in cases and controls, of sequence variants that are likely to alter gene function; and (ii) systematic use of comparative genomics techniques, both to identify conserved sequence elements in the candidate genes and to distinguish sequence variants that are likely to alter gene function from those that are not. The format of this project will enable a direct test of the relative contributions of category_c and category_d sequence variants to familial breast cancer.

The project is comprised of 4 principal Aims. Within the study design, we plan to maximize our power for detecting risk-conferring sequence variants by using high-risk (early onset/ familial) breast cancer cases for our case series. The reason for this application to the CFR is the first Aim of the project: to assemble an extensive series of lymphoblastoid lines (LCLs) derived from high-risk breast cancer patients and ethnicity-matched population controls. During 2005 we expect to write an R01 proposal based on this project; the proposal will probably also be included as a workpackage in at least one EU grant proposal. Please also note that the project as described here will be paired with a prostate cancer genetics project of very similar study design.

Specific Aim(s):

Aim 1. Assemble an extensive series of LCLs derived from high-risk breast cancer patients and population controls.

Aim 2. Resequence the coding exons and 5' UTR in both cases and controls.

Aim 3. Examine cross-species conservation of coding sequence.

Aim 4. For either class of gene, perform a test of "summed frequency of interesting sequence variants" in cases vs. controls.

Application ID: B-SF-0606-01 **Status:** Approved, Ongoing

Principal Investigator: Whittemore, Alice **Year:** 2006

Institution: Stanford University

Title: *Cancer Risks in Carriers of Unclassified Variants of BRCA1: A Feasibility Study*

Abstract:

Protein inactivating mutations of the BRCA1 gene are associated with elevated breast and ovarian cancer risks. However there is considerable uncertainty about the effects of other BRCA1 variants, particularly the single base changes that alter amino acids of the protein, termed nonsynonymous unclassified variants (ns UCVs). Because these variants are rare, no single source of data is sufficiently informative to unambiguously classify them as either neutral or pathogenic. Our goal is to assess the feasibility of adding useful new information about the risks of ns UCVs of BRCA1 by assessing cancer incidence in first-degree (FD) relatives of a population-based multi-ethnic series of incident breast cancer cases with and without ns UCVs.

If we find that the results of this feasibility study are promising, we will seek separate funding to extend it to other UCVs of BRCA1 and BRCA2, using a larger series of population-based families from the Breast CFR and other population-based breast and ovarian cancer family registries.

Note the similarity of the following specific aims with the information in the "Specific Aim(s)" field.

Our specific aims are:

1) to compare the prevalence and types of nsUCVs detected among Hispanic, Asian-American, African-American and non-Hispanic white (NHW) breast cancer cases ascertained from the Northern California component of the Breast Cancer Family Registry (Breast CFR);

2) to estimate risk ratios and standardized residuals for BRCA1-related cancers among FD relatives of 66 cases who carry ns UCVs, compared to those

of 1729 cases who carry at most neutral polymorphisms;

3) to combine results of Aim 2 with available pathogenicity scores of the variants to classify them as benign or deleterious; and

4) to evaluate agreement between this classification and one obtained using a function-based statistical learning algorithm.

Our ultimate goal is to assess the potential of using cancer incidence in relatives of carriers to help classify other variants in disease-susceptibility genes. If we find that the results of this feasibility study are promising, we will seek separate funding to extend it to other UCVs of BRCA1 and BRCA2, using a larger series of population-based families from the Breast CFR and other population-based breast and ovarian cancer family registries.

Specific Aim(s):

We propose to assess the feasibility of evaluating nsUCV risks by assessing the incidence of breast and ovarian cancer in first-degree relatives of African-American, Asian-American, Hispanic and non-Hispanic white (NHW) breast cancer probands ascertained from the Northern California component of the Breast Cancer Family Registry (CFR).

Our specific aims are:

1) compare the prevalence and types of nsUCVs in population-based samples of African-American, Hispanic, Asian-American and NHW breast cancer probands ascertained in the Northern California component of the Breast CFR;

2) evaluate standardized incidence ratios (SIRs) comparing breast & ovarian cancer incidence in first-degree relatives of these probands to that in the general population using SEER rates specific for calendar year, age and race, and to that in relatives of age- and race-matched CFR probands who carry only neutral polymorphisms. To do so, we will fit Poisson regression models to the relatives' incidence data.

3) assess whether trends in the SIRs for specific subgroups of nsUCVs increase with increasing values of their mean pathogenicity scores on two sequence-homology-based programs, Sort Intolerant from Tolerant (SIFT; http://blocks.fhcrc.org/sift/SIFT.html/) and Polymorphism Phenotype (PolyPhen; http://bork.embl-heidelberg.de/PolyPhen/) (Johnson et al., 2005).

Application ID: B-SF-0406-01 **Status:** Approved, Ongoing

Principal Investigator: Whittemore, Alice **Year:** 2006

Institution: Stanford University

Title: *Breast Cancer Metastasis: A Heritable Trait?*

Abstract:

Cancer metastasis is thought to reflect the somatic mutational spectra that arise during the development of a primary cancer. Recently, a new genetic modifier of metastatic efficiency has been identified in mice. This discovery supports the hypothesis that metastatic potential is a heritable trait influenced by the host genetic background. If this hypothesis is verified in humans, it would have profound implications for the screening and treatment of breast cancer. The identification of individuals with a metastatic phenotype could allow for increased intensity of screening in those at greatest risk for lethal metastatic disease, and for tailored treatment of patients who do develop cancer.

The hypothesis needs testing in human data. We propose to use existing demographic, prognostic and time-to metastasis data for breast cancer patients from large multiple-case families (i.e., those containing at least two first- or second-degree relatives with invasive breast cancer) to test the hypothesis that times to breast cancer metastasis are correlated within families, and to see if the strength of pairwise correlations increase with the patients' genetic relatedness. Our objectives are:

1. Use analysis of variance (ANOVA) methods to evaluate within- and between-family components of variation in tumor prognostic features (tumor size, lymph node status, tumor grade, and hormone receptors).

2. Test the hypothesis that, after adjusting for within-family correlation in tumor prognostic features, family members' (possibly censored) times to metastasis are correlated.

3. Examine whether times to metastasis are more strongly correlated in close relatives (e.g. sisters) than in more distant ones (e.g. cousins).

We will assemble data for 329 breast cancer patients in 128 families enrolled in the Breast Cancer Family Registry (Breast CFR). These data include prognostic factors (tumor size, nodal status, grade, receptor status), date of diagnosis and date of cancer spread beyond the primary tumor. We will use established statistical methods to evaluate correlations in times to metastasis within families and to look for evidence of increasing correlation in patients with closer genetic relationships.

The proposed research is an important step in a critical path toward advancing our understanding of the mechanisms underlying metastasis. Previous steps include: 1) the theory that the host's genetic background may influence metastatic efficiency; 2) identifying the gene Sipa1 that influences metastatic potential in mice, and functional assays showing that concentrations of the Sipa1 protein affect the metastatic phenotype of human cells. The work proposed here would test the theory in breast cancer patients, independent of possible roles for any specific genes. Support for the theory would stimulate the search for genes affecting metastatic potential, and enhance the prospect of tailoring therapy aggressiveness to patients' genetic needs.

Specific Aim(s):

Hypothesis

The occurrence of cancer metastasis is thought to reflect the somatic mutational spectra that arise during the development of a primary cancer. Recently, however, a new genetic modifier of metastasic efficiency has been identified in mice. This discovery provides experimental support for the hypothesis that metastatic potential is a heritable trait influenced by the host genetic background. If this hypothesis is verified in humans, it would have profound implications for the early detection, screening, diagnosis and treatment of breast cancer. Indeed, the identification of patients with a metastatic phenotype could allow the concentration of scarce resources on those with greatest risk for lethal metastatic disease.

The hypothesis that metastatic potential is a heritable trait needs testing in human data. We propose to use previously collected demographic, prognostic and time-to metastasis data for 329 breast cancer patients from 128 large multiple-case families (i.e., those containing at least two first- or second-degree relatives with invasive breast cancer) to test the hypothesis that times to breast cancer metastasis are correlated within families, and to examine if the magnitudes of pairwise correlations increase with the patients' genetic relatedness.

Application ID: B-EX-1206-05 **Status:** Approved, Ongoing

Principal Investigator: Ziv, Elad **Year:** 2006

Institution: University of California, San Francisco

Title: *Admixture Mapping for Breast Cancer in African American Women*

Abstract:

Breast cancer incidence rates vary significantly among different racial and ethnic groups. Although African American women have a lower overall incidence compared to Caucasian women, they are diagnosed more frequently at a younger age. African American women are also diagnosed more frequently with tumors which are higher grade and negative for estrogen receptor (ER) expression. Although environmental, reproductive and socioeconomic differences may play a role in explaining some of these differences, genetic factors may also contribute.

African Americans are an admixed population of mixed African and European ancestry. Particular interest has been focused on genetic studies in admixed populations such as African Americans partially because these populations provide a unique opportunity for efficient mapping of susceptibility genes that account for ethnic differences in disease in these populations, by an approach called "admixture mapping." Admixture mapping allows for a relatively sparse set of genetic markers (~1000-2000) to be used to detect genetic loci.

We propose to use an admixture mapping approach to search for breast cancer genes in African Americans. In particular, we propose to type ~1500 markers informative for African vs. European ancestry among African Americans in the Breast CFR. We will use the data from these markers to estimate genetic ancestry for each individual woman and to determine if subtypes of breast cancer (defined by ER-status, tumor grade) are associated with genetic ancestry. We will also use these markers to estimate African vs. European ancestry at each locus for each woman. We will use these ancestry estimates at each locus in the genome to determine if there is a particular locus where ancestry is associated with breast cancer risk or with subtypes of breast cancer.

Specific Aim(s):

Specific Aims

(1) We will examine which clinical phenotypes of breast cancer (defined by hormone receptor status, tumor grade, histological type) are most strongly correlated with African ancestry vs. European ancestry among African Americans. Specifically, we will estimate genetic ancestry among African American women using a set of ancestry informative markers. We will determine which subtypes of breast cancer defined by clinical parameters are most strongly associated with either African or European ancestry. This will allow us to determine which subtypes of tumors are most promising for admixture mapping.

(2) We will perform whole genome admixture mapping to identify genomic regions which underlie differences in breast tumor subtypes among African Americans. These genomic regions should lead us to genomic regions which harbor susceptibility genes for breast cancer.

Hypothesis

We hypothesize that among African Americans, different subtypes of breast cancer are due to genetic variants (alleles) from different ancestral populations. More specifically, we hypothesize that certain alleles which predispose to certain types of tumors (ER-negative, high grade) may be more common in African ancestral populations. Conversely, other alleles which may predispose to other types of tumors (ER-positive, low grade) may be more common among European populations. Furthermore, we also hypothesize that these genes may be identifiable by admixture mapping in African Americans, a population of mixed African and European ancestry.

Application ID: B-NY-0805-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Ahsan, Habibul **Year:** 2005

Institution: Columbia University

Title: *Genome-Wide Association Scan of Early-Onset Breast Cancer*

Abstract:

We plan to conduct a genome-wide association study of young (<50 years) invasive female breast cancer to identify new genes responsible for young cases who are negative for BRCA1 and BRCA2 gene mutations. This collaborative study will exploit the availability of biological samples and epidemiological data from 2,700 population-based, individually matched case-control pairs ascertained by large breast cancer study resources in Australia, Canada, the US and Germany. We will focus on early-onset BRCA1- and BRCA2-negative invasive cases because this group has high public health importance and high likelihood of harboring unidentified breast cancer genes.

To enhance cost-efficiency and validity, the study will proceed in two phases. Phase I will genotype and analyze population-based samples of 1,700 non-Hispanic Caucasian matched case-control pairs. We will perform Phase I in two stages. In Stage 1, we will genotype and analyze 1000 case-control pairs, using the Illumina Human Hap 550k SNP array containing 550,000 tagging SNPs covering ~90% of human genome variations. In Stage 2, we will genotype the remaining 700 case-control pairs only for the SNPs identified as promising in Stage 1. We will then analyze these SNPs using all 1,500 case-control pairs, adjusting for established breast cancer risk factors.

Phase II will genotype and analyze an independent set of 1,000 population-based sister case-control pairs for all promising SNPs from Phase I and also the surrounding haplotype-tagging and functional SNPs. Phase II provides robustness against false positives due either to confounding by population structure or to multiple comparisons in Phase I analyses. This genome-wide association study offers several strengths, including the availability of large numbers of population-based, well-matched young cases and controls, the ability to control confounding by population structure using a robust sister-pair design, and the extended genomic coverage provided by the 550,000 tagging SNP panels.

In conclusion, this research aims to identify new genes for early-onset breast cancer, which is a major source of morbidity, mortality and loss of life expectancy throughout the world.

Specific Aim(s):

In stage-1 of Phase-1: The set of DNA samples, which will be used in Infinium-II assay, the minimum requirement is 750 ng (15 ul of 50 ng/uL concentration) of genomic DNA.

In stage-2 of Phase-1: We propose to genotype ~10,000 important SNPs, based on stage-1 of Phase-1 analyses. Therefore in this set of DNA samples, we will need to run each DNA sample in at least 4-7 Sentrix Array Matrix (SAM), each of which will presumably genotype 1000 - 1500 SNPs. The minimum requirement of DNA per SAM is 250 ng which means that the minimum requirement would be 2ug (8 x 250) of genomic DNA per sample.

In Phase-2, we would genotype different set of DNA and use the same GoldenGate assay.

Final confirmatory analyses: Once both Phase I and Phase II have been completed our plan is to run the surrounding functional and tagging SNPs of the final set of replicated SNPs across Phases among all cases and controls in the study to attempt to identify the causal alleles underlying the observed replicated associations. This final run of assay is what we estimated to involve one GoldenGate SAM requiring this additional at least 250 ng of genomic DNA. All the DNA samples would be tested for about 1000 - 1500 SNPs, which would include not only the significant SNPs found through Phase-1 and Phase-2 but also their surrounding probable non-synonymous &/or coding region SNPs for identifying disease causing SNPs. Therefore we would need to run the DNA sample on at least 1 SAM which would need minimum 250 ng of DNA.

Application ID: B-TO-0805-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Andrulis, Irene **Year:** 2005

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada

Title: *Studies on Genetic Variation Related to Clinical Outcomes in Breast Cancer*

Abstract:

Breast cancer remains a significant cause of morbidity and mortality. There is an important need to identify parameters that accurately predict clinical outcome and lead to improved cancer control. The role of genetic factors in prognosis and treatment of disease is still largely unknown. Single nucleotide polymorphisms are the most frequent genetic changes in the human genome, and can be common on a population-level, a fact that coincides with the large inter-individual variation in disease progression and response to therapy that exists. There are several genes involved in cancer progression and treatment response. Candidate genes include pathways involved in steroid hormone/carcinogen metabolism, angiogenesis, proteolysis, motility and growth control. The importance of these pathways in clinical outcomes in breast cancer has been highlighted by several studies, but the role of specific polymorphisms remains poorly understood. The objective of this study is to conduct a comprehensive investigation of multiple polymorphisms within these pathways to understand their role as prognostic and predictive factors for breast cancer using data from the Prognostic Study of the Ontario Family Breast Cancer Registry (OFBCR). The Prognostic Study has accrued clinical, follow-up and outcome data prospectively on a large proportion of participants in the OFBCR (one of six international sites of the NIH-funded Breast Cancer Family Registries) for nearly a decade. Eligible participants were females without a prior cancer diagnosis (except Breast CIS, Cervix CIS or non-melanoma skin cancer) who provided a blood sample and consent to access medical records. Clinical and treatment data were extracted from patient medical records at government-financed centers that provide nearly all cancer treatments in the province of Ontario, by registered nurses using validated data collection forms. Collected data include: demographics (age, race/ethnicity/religion, menopausal status, family history of cancer, BRCA1/2 testing status), clinical staging (T including inflammatory, N, M), surgical treatment, radiation treatment, chemotherapy/hormone therapy and status of breast and ovaries. Data including surgical procedure, tumor size, lymph node status, and hormone receptor status (estrogen and progesterone) were extracted centrally from the pathology report issued at the time of diagnosis. For the majority of patients, pathology slides were also reviewed centrally in a standardized fashion. From this review we have collected information on pathology tumor size, tumor grade, histologic subtype, presence of endothelial-lined space invasion, status of resection margins, presence of dermal lymphatic invasion, and nodal involvement. Follow up data was collected approximately annually for recurrences of breast cancer (loco-regional, distant), new primary cancers, and death (date, cause). Candidate polymorphisms in several genes involved in metabolism (including SULT1A1, MTHFR, TYMPS, RFC1, CYP2D6, CYP2C19, CYP2C9, CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1, UGT2B15) and tumor progression (including TGFB1, CD44, NGFB, MMP9, MMP10, AGER, TNFR2, ERBB2IP, MYC, ELF1, EP300, TRAP1, TGFBR3, CDKN1B) have been selected based on their potential biological importance determined using bioinformatic tools (SIFT, PolyPhen) and literature sources and genotyped using standard high-throughput methods (TaqMan). Our primary research objective is to determine the association between polymorphisms and distant recurrence. The Cox proportional hazards model was used to test our hypotheses.

Specific Aim(s):

This study proposes to investigate the role of polymorphisms in metabolism and tumor progression and metastasis as prognostic or predictive factors in breast cancer. We initially chose the following genes in metabolism: CYP2D6, CYP2C9, CYP2C19, CYP3A4, MTHFR, TYMS, SULT1A1, RFC1, GSTP1, and the transforming growth factor-beta (TGF-β) family: TGFB1, TGFB2, TGFB3, TGFBR1, TGFBR2, TGFBR3, SMAD2, SMAD3, SMAD4, SMAD6, SMAD7, SMAD8, SKI, SNON, CMYC as potential candidates.

Application ID: B-EX-1205-03 **Status:** Approved, Ongoing

Principal Investigator: Bajdik, Chris **Year:** 2005

Institution: British Columbia Cancer Research Centre

Title: *Predicted Outcomes for a Genetic Testing Service*

Abstract:

Genetic testing allows for targeted surveillance of mutation carriers and avoids the unnecessary surveillance of non-carriers. The cost of a genetic testing service is partly determined by the number of users. The effect of the service's eligibility criteria depends on the population's characteristics and the likelihood of a positive test result. Our team has developed a computer simulation model that predicts the sensitivity, specificity and post-test likelihoods of eligibility criteria for a cancer susceptibility genetic testing service. The model allows criteria to be based on family history of cancer, and further allows the estimate to be restricted based on the age, gender and family size. Combining weighted estimates allows a user to predict outcomes for a general population. For each institution in the Breast and Colon FCR, our hypothesis is that the positive post-test likelihood (PTL+), also known as the positive predicted value, is equal to that predicted by our computer model. Values of PTL+ will be predicted using the computer model and a comparison with the observed PTL+ values from the CFR will be performed.

Specific Aim(s):

Hypothesis

For each institution in the Breast and Colon FCR, our hypothesis is that the positive post-test likelihood (i.e., positive predicted value) is equal to that predicted by our computer model.

Application ID: B-EX-0802-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Bernstein, Jonine **Year:** 2005

Institution: Mount Sinai Medical School

Title: *ATM and CHK2 Mutations in Population-Based Families from the Breast CFR*

Abstract:

We have recently completed screening of the Breast CFR samples for the CHEK2*1100delC variant and identified 30 carriers. Our results from this study are in agreement with others and suggest that the 1100delC variant increases risk for breast cancer. We wish to follow up these genetic studies with functional studies of the radiation response in cell lines derived from specific CHEK2*1100delC carriers.

Although the CHEK2*1100delC variant is predicted to truncate CHEK2 and thus not produce any full-length product, there are no studies published that address the functional consequences of being a carrier for this variant. Thus, the mechanism whereby it increases risk for breast cancer is unknown. Our data suggesting that radiation exposure may play a role in this mechanism suggests that studies of the radiation response in cells from carriers might provide useful insights into the mechanism. Therefore, we wish to obtain cell lines from carriers identified in our Breast CFR screening study in order to measure radiation phenotypes in cell lines from a group of carriers and determine the inter-individual variation.

Both ATM and CHEK2 operate in the same cellular pathway responding to ionizing radiation damage, raising the likelihood that variants in either gene may predispose to breast cancer by a common mechanism. There are few variants in ATM that have been clearly demonstrated to increase risk for breast cancer. One of these is the variant 7271T>G and we have recently identified carriers for this variant by screening the Breast CFR collection. Therefore, we propose to carry out functional studies, characterizing the radiation phenotypes of cells from carriers of CHEK2*1100delC and ATM 7271T>G. In these studies, the ATM 7271T>G carriers will serve as controls, providing information as to the radiation phenotype of cells from breast cancer patients with known defects in the cellular pathway that includes CHEK2.

Application ID: B-AU-0803-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Campbell, Ian **Year:** 2005

Institution: Peter MacCallum Cancer Center

Title: *Polymorphic Variants in Genes Involved in DNA Methylation and Methyl Group Metabolism and Breast Cancer Risk*

Abstract:

Genetic factors are increasingly been recognised as a major contributor to cancer risk and it is thought that low penetrance cancer susceptibility alleles may be responsible for the majority of the heritable risk. The identification of low penetrance alleles associated with cancer will have a wide range of applications for developing diagnostic, therapeutic, and preventative strategies. The recognition of this has prompted numerous epidemiological studies searching for common polymorphisms in genes that may represent susceptibility alleles (1, 2). Among the most promising candidates are genes involved in maintenance of genomic integrity and DNA repair, which include genes such as BRCA1, BRCA2, ATM, and TP53. These and other functionally related genes are currently under intense scrutiny for common low penetrance cancer predisposing alleles. Another class of gene, which has not received much attention, are those responsible for epigenetic modification of DNA through CpG methylation. This process can be used to maintain patterns of gene expression and may also influence genomic stability. In recent times, it has become clear that disturbances in DNA methylation are critical in the development of cancer and may be among the earliest changes. A family of genes that include DNA methyl transferases, methyl binding proteins and genes involved in methyl-group biosynthesis and metabolism, controls these processes. Polymorphic variants in these genes may be associated with increased risk of breast cancer. For example, we showed that a polymorphic C>T substitution at nucleotide 677 in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene may be associated an increased risk of breast cancer (3). Few of the other genes involved in DNA methylation have been investigated and the studies which have been conducted have lacked statistical power. We will harness the strengths of the Cooperative Familial Registries of Breast Cancer Studies (CFRBCS) to investigate the role of common single nucleotide polymorphisms (SNPs) in genes involved in DNA methylation and methyl-group biosynthesis and metabolism on the risk of breast cancer using a case-control-family design. The Identification of novel breast cancer predisposing alleles will increase our understanding of the molecular events underlying the development of this disease and provide a means for the rational targeting of early intervention strategies.

Specific Aim(s):

Hypothesis and Aims

Silencing of genes via epi-genetic mechanisms may be of greater relevance in cancer development than the acquisition of genetic mutations. Genetic variants in genes involved in DNA methylation increase an individuals propensity to hypermethylate DNA which increases breast cancer risk through silencing of tumour suppressor genes. Maintenance of DNA methylation and de novo DNA methylation involve the interaction of many genes and there is likely to be interaction between functional SNPs in these genes. Individually each genetic
variant may confer only a small absolute increase in breast cancer risk, but since they are very common in the population their contribution to population risk of breast cancer may be high. To date there have been no substantive studies of this class of gene in relation to cancer susceptibility.

The aims of this study are:

1) To screen population-based cases and controls (frequency matched for age) for polymorphisms in candidate genes to assess their role as low-risk cancer predisposition genes. The polymorphisms investigated will include published variants and those identified as a part of our search for BRCAX genes among multiple-case breast cancer families identified by KConFab.

2) To study the interaction of genes with potentially functional SNPs from the same pathway.

3) To validate the positive associations of DNA methylation SNPs using a family-based approach.

Application ID: B-NY-0805-02 **Status:** Approved, Ongoing

Principal Investigator: Chung, Wendy **Year:** 2005

Institution: Columbia University

Title: *Identification of Novel Breast Cancer Susceptibility Genes in Ashkenazi Jewish Families Without BRCA1 or BRCA2 Mutations*

Abstract:

The lifetime risk of breast cancer is approximately 12% in Ashkenazi Jewish (AJ) women living in the United States, which is higher than in most other ethnic groups. A proportion of this increased frequency is due to common founder mutations in the breast cancer susceptibility genes BRCA1 (185delAG, 5382insC) and BRCA2 (6174delT). Mutations in these two genes have a combined allele frequency of 1/40 in Ashkenazi Jews, unselected for personal or family history of breast cancer. However, all mutations in BRCA1 and BRCA2 account for only approximately 30% of the cases of familial breast cancer in this population. Segregation analysis in the AJ population suggests the existence of at least one additional major breast cancer susceptibility gene with a penetrance of 85% by age 70 with 4% of the population carrying the high risk genotype. Our goal is to identify novel, highly penetrant breast cancer susceptibility genes with immediate clinical utility. Because of the tendency to intermarry as well as the population bottlenecks that have occurred throughout history in the AJ population, there is much less genetic diversity amongst Ashkenazi Jews that is highly advantageous in gene identification. The genetic isolation of this population increases the likelihood of a small number of founder mutations in a small number of genes for hereditary diseases. This has been repeatedly proven within the AJ population for genetic diseases such as Tay Sachs, familial dysautonomia, Niemann Pick, Fanconi Anemia, Bloom syndrome, and hereditary nonpolyposis colon cancer. Because founder mutations are associated with conserved haplotypes across a genomic interval, these founder mutations and their associated genes can be identified by determination of shared haplotypes or genomic intervals among affected individuals that differ from unaffected individuals. With the completion of the human genome project and ongoing work by the human single nucleotide polymorphism (SNP) consortium, highly informative polymorphic sites have now been identified approximately every 1000 base pairs throughout the human genome. With the availability of such a high density of genetic markers, it is now possible to get much higher resolution for gene mapping. Panels of 600,000 single nucleotide polymorphisms evenly spaced throughout the genome are now commercially available. We propose to perform a two staged genome wide association study on a total of 500 cases and 500 controls. Stage one will consist of 250 BRCA1/BRCA2 negative subjects with breast cancer or ductal carcinoma in situ who have documented family histories including 1) three or more cases of men or women on the same side of the family with breast cancer or DCIS with at least one of those cases occurring before age 50 or 2) four or more cases of breast cancer on the same side of the family. We will use as controls, 250 BRCA1/BRCA2 negative Ashkenazi Jewish women without a personal or family history of

breast cancer in any first or second degree relatives. By comparing the allele frequencies between the cases and controls for each of these 600,000 SNPs, we hope to identify one or more regions of adjacent SNPs with significantly different allele frequencies between cases and controls. The second stage will consist of targeted, higher resolution genotyping in regions of preliminary association in a second independent set of 250 cases and 250 controls using the same clinical criteria as stage one to test for independent replication and decrease the number of false positive results on the initial screen. We will go on to finer physical mapping and ultimately gene and mutation identification in AJ subjects in those regions that are independently replicated in the second stage. If a putative breast cancer susceptibility gene is identified in AJ subjects, non-AJ subjects with breast cancer and strong family histories will also be tested to provide additional evidence that the gene identified is responsible for the breast cancer susceptibility.

Specific Aim(s):

Hypothesis

We hypothesize that there is at least one additional highly penetrant breast cancer susceptibility gene beyond BRCA1 and BRCA2 with founder Ashkenazi Jewish mutations that can be identified through genome wide association studies in BRCA1 and BRCA2 negative subjects with early onset breast cancer or DCIS and a strong family history of breast cancer.

Application ID: B-PH-0405-01 **Status:** Approved, Ongoing

Principal Investigator: Daly, Mary **Year:** 2005

Institution: Fox Chase Cancer Center

Title: *Ethno-Cultural Differences in Cancer Beliefs and Attitudes*

Abstract:

Over the past eight years a familial resource of individuals at high risk for breast cancer, the Breast Cancer Cooperative Family Registry (CFR), has been developed through a collaborative endeavor of the National Cancer Institute (NCI) and six medical research institutions located in the United States, Canada and Australia. The combined resources available in the CFR have already begun to create unprecedented opportunities for a range of hypothesis-testing studies involving the discovery of new genes related to breast/ovarian cancer, the exploration of gene-gene and gene-environment interactions, and the histopathologic and clinical characteristics of inherited breast and ovarian cancer. Ultimately these studies will reveal new opportunities to offer preventive options to these and other high risk family members. Critical to the successful translation of the new information emerging from the field of molecular genetics to the area of cancer prevention and control, however, will be a clear understanding of the psychosocial and cultural issues inherent in the personal experience of familial risk for cancer, and in the choice of health-related behaviors in response to this risk. The culturally diverse representation within the CFR provides an opportunity to begin to explore these issues in a collaborative cross-cultural international setting which will yield insights critical for progress in human genetics. The proposed pilot study utilizes a qualitative approach to advance our understanding of the meaning of cancer and genetic risk across a range of different cultural settings, and to examine the dynamics of familial communication patterns as they relate to cancer risk and cancer treatment information.

The specific aim of the pilot is: to conduct a series of qualitative, in-depth interviews with a sample of affected and unaffected CFR participants who represent four cultural/ethnic groups: African American, Hispanic, Chinese and non-Hispanic Caucasian. These interviews will explore five dimensions:

1) the core beliefs within different cultural settings regarding the determinants of cancer causation, including the role of genetics;

2) components of personal cancer risk perception;

3) the dynamics of health information communication within the family;

4) the personal and familial response to perceived cancer risk; and

5) personal and familial response to a cancer diagnosis.

We hypothesize that the modern western model of disease attribution and reliance on the established health care system will be modified by sociocultural beliefs and experiences. Other modifying factors may include age, educational level, disease status (affected with cancer or not), genetic status (status of genetic testing for BRCA1/2) and exposure to cancer risk counseling.

Specific Aim(s):

Extended families with a high prevalence of cancer or cancer family syndromes provide a unique research resource for epidemiologic, molecular genetic, and prevention studies. Over the past eight years a familial resource of individuals at high risk for breast cancer, the Cooperative Family Registry for Breast Cancer Studies (CFR), has been developed through a collaborative endeavor of the National Cancer Institute (NCI) and six medical research institutions located in the United States, Canada and Australia. The six participating international sites have accumulated a large computerized data base with both genetic and environmental risk information from a racially, ethnically and culturally diverse set of families with a history of breast and/or ovarian cancer. Plasma and DNA is available for participants in the Registry, and tumor specimens have been obtained from those with a diagnosis of breast/ovarian cancer. The combined resources available in the CFRBCS have already begun to create unprecedented opportunities for a range of hypothesis-testing studies involving the discovery of new genes related to breast/ovarian cancer, the exploration of gene-gene and gene-environment interactions, and the histopathologic and clinical characteristics of inherited breast and ovarian cancer. Ultimately these studies will reveal new opportunities to offer preventive options to these and other high risk family members. Critical to the successful translation of the new information emerging from the field of molecular genetics to the area of cancer prevention and control, however, will be a clear understanding of the psychosocial and cultural issues inherent in the personal experience of familial risk for cancer, and in the choice of health-related behaviors in response to this risk. The fast pace of discovery in the area of basic science related to cancer genetics must also be accompanied by an attempt to understand the meaning of familial cancer within each cultural milieu, and to examine the impact of familial cancer risk information on individual and family health behavior in order to maximize the preventive potential of the new science. The culturally diverse representation within the CFR provides an opportunity to begin to explore these issues in a collaborative cross-cultural international setting which will yield insights critical for progress in human genetics. The

multicultural composition of the CFR allows us to compare and contrast sociocultural differences as they apply to the understanding of cancer risk, the response to perceived risk, the dynamic between family members and the health care system, and the role of the family in managing cancer risk.

The proposed pilot study utilizes a qualitative approach to advance our understanding of the meaning of cancer and genetic risk across a range of different cultural settings, and to examine the dynamics of familial communication patterns as they relate to cancer risk and cancer treatment information.

The specific aim of the pilot is: To conduct a series of qualitative, in-depth interviews with a sample of affected and unaffected CFR participants. Interviewees will be chosen to provide representation of four cultural/ethnic groups: African American, Hispanic, Chinese and non-Hispanic Caucasian. These interviews will explore five dimensions: 1) the core beliefs within different cultural settings regarding the determinants of cancer causation, including the role of genetics; 2) components of personal cancer risk perception; 3) the dynamics of health information communication within the family; 4) the personal and familial response to perceived cancer risk; and 5) personal and familial response to a cancer diagnosis.

We hypothesize that the modern western model of disease attribution and reliance on the established health care system will be modified by sociocultural beliefs and experiences. Other modifying factors may include age, educational level, disease status (affected with cancer or not), genetic status (status of genetic testing for BRCA1/2) and exposure to cancer risk counseling.

Application ID: B-AU-1205-01 **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2005

Institution: University of Melbourne

Title: *Gene Discovery for Early-Onset Breast Cancer*

Abstract:

Breast cancers in young women are a priority area of the Breast CFR, important in terms of morbidity, and have received relatively little attention. The proportion of early-onset breast cancer due to germline mutations in BRCA1 and BRCA2 is less than 10% (Dite et al., 2003). BRCA1 and BRCA2 mutations combined explained less than 20% of the increased risk associated with having a close relative with early-onset breast cancer (Dite et al., 2003). We have found that about one-quarter of early-onset case with two or more affected first- or second-degree relatives with breast or ovarian cancer carry a mutation in BRCA1 or BRCA2, and in total less than one-third of these cases carry a mutation in any of the above genes. That is, the majority of early-onset strongly familial breast cancer is caused by factors other than mutations in the currently known breast cancer susceptibility genes.

The Breast CFR has an extensive resource to progress gene discovery studies on early-onset breast cancer, having recruited the families of 1,113 women who have been diagnosed with breast cancer before the age of 40 years and who have at least two other first- or second-degree relatives affected with breast cancer, one-third of whom have at least one of these affected relatives also diagnosed before the age of 40. Extensive mutation testing for BRAC1 and BRCA2 has been conducted to exclude families segregating mutations in those genes.

Aim: to conduct classic linkage and affected pairs studies using members of multiple-case early-onset breast cancer families to identify genomic regions containing novel breast cancer susceptibility genes, and to conduct fine mapping studies so as to identify these genes.

Pathology review data from the Breast CFR will be analysed so as to identify particular features that characterise cases with early-onset that have multiple-case families and are therefore most likely to be due to a germline mutation. Attempts will be made to identify families in which particular pathology features are common to affected relatives.

Genotyping for the ABI microsatellite panel will be conducted at the International Agency for Research on Cancer (IARC) in Lyon, France under the supervision of Dr Melissa Southey. This group is currently completing a similar genome-wide scan for multiple-case breast cancer families (PI: Dr David Goldgar) that has used about 25 Breast CFR families. Other methods, such as genome-wide SNP chips, may be used. Should regions be identified that are suggestive of containing breast cancer susceptibility genes, we will conduct fine mapping studies. We may also use DNA chip technology to conduct genome-wide scans.

Standard linkage analyses will be conducted, under both rare dominant and rare recessive models of inheritance, using the software packages MENDEL and GeneHunter-Plus and ASM (see Kruglyak et al., 1996; Kong and Cox, 1997), as used by the International Consortium for Prostate Cancer Genetics (ICPCG).

In addition, affected sister pairs will be used for linkage study using the Affymetrix Xba SNP chip, which has approximately 58K SNPs.

Specific Aim(s):

Hypothesis

There are mutations in genes other than BRCA1 and BRCA2 that, when present in the germline, predispose women to a high risk of breast cancer at a young age, and these mutations are segregating in families with multiple-cases of early-onset breast cancers not found to carry germline mutations in BRCA1 and BRCA2.

Application ID: B-EX-1205-02 **Status:** Approved, Ongoing

Principal Investigator: Huntsman, David **Year:** 2005

Institution: British Columbia Cancer Agency

Title: *E-Cadherin, A Susceptibility Gene for Lobular Breast Cancer*

Abstract:

Mutations in BRCA1 and BRCA2 have clearly been linked to hereditary breast cancers. However, 60% of breast cancer families do not have mutations in either of these two genes and are genetically unexplained. The identification of new molecular markers of breast cancer could significantly aid in the identification of individuals who may be susceptible to breast cancer. We believe that the E-cadherin gene may be a marker for breast cancer susceptibility. Currently, this gene is associated with gastric cancer susceptibility. However, preliminary investigations have revealed that many families with diffuse gastric cancers have an increased incidence of lobular breast cancer. Further, genetic analysis of these lobular breast cancer patients demonstrated that many of them carried mutations in the E-cadherin gene. Furthermore, diffuse gastric cancers have remarkable pathological similarity to lobular breast carcinomas. In order to definitively identify E-cadherin as a susceptibility gene for breast cancer, it is necessary to study patients with lobular breast cancer who do not carry mutations in either the BRCA1 or BRCA2 genes. Up to this point, we have only been able to study a biased population of lobular breast cancer patients who have been identified after preliminary diagnosis of a family member with gastric cancer. With samples obtained from the Breast Cancer Family Register, we hope to determine the incidence of E-cadherin mutations in families with lobular breast carcinomas. This could lead to the identification of E-cadherin as a novel molecular marker for breast cancer and might eventually result in a new breast cancer screening strategies. This would certainly improve the early diagnosis of patients which might lead to better survival.

Specific Aim(s):

Hypothesis

Ascertainment bias has led to an underestimation of the role of germline E-cadherin (CDH1) mutations in lobular breast cancer development. Analysis of material from the Breast CFR will remove this bias and will reveal that CDH1 is a susceptibility gene for familial and early onset lobular breast cancer.

Application ID: B-EX-1203-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Mouchawar, Judy **Year:** 2005

Institution: Kaiser Permanente Colorado

Title: *Estimating the Contribution of TP53 Mutations to Breast Cancer*

Abstract:

Background

While BRCA1/2 likely account for the majority of dominantly-inherited forms of hereditary breast cancer, investigators are finding that mutations in the BRCA1/2 genes are not common among multiple-case pedigrees with very early onset breast cancer. We have recently utilized the Australian Breast Cancer Family Study (ABCFS), and found a 5% prevalence for TP53 germline mutations among 66 early onset breast cancer cases known to be negative for BRCA1/2. This work needs to be validated with larger numbers, but indicates that germline alterations in TP53 may play a larger role for early onset breast cancer risk than previously thought. In addition, gene alterations that attenuate TP53 (MDM2-SNP309) may have similar implications.

Objective

To determine the proportion of breast cancer, in particular in those with early onset and those with a strong family history of breast cancer, due to mutations in TP53 and MDM2-SNP309 that can be identified by direct sequencing.

Methods

The data for this case series will come from the NCI-funded Breast Cancer Family Registry (Breast-CFR). We have already utilized the Australian portion of the Breast-CFR, and now wish to extend testing to samples from the other Breast-CFR sites. Controls will not be used for this case series study. We will test for germline TP53 and MDM2-SNP309 mutations among cases meeting the following criteria: female with a personal history of invasive breast cancer diagnosed under age 40, two or more family members with breast or ovarian cancer, and no deleterious BRCA1/2 mutation detected within the pedigree. We aim to obtain samples on up to 300 cases meeting our criteria.

Results

Results will be shared with the Breast-CFR participating sites, and will be presented at a Breast-CFR meeting. Results will be submitted for peer-reviewed publication. We will use the results to determine if grant funding should be sought to allow for testing of cases with more broad cancer risks.

Application ID: B-EX-0805-02 **Status:** Approved, Ongoing

Principal Investigator: Schildkraut, Joellen M. **Year:** 2005

Institution: Duke University Medical Center

Title: *Genetic Modifiers of BRCA1 and BRCA2*

Abstract:

The goal of the proposed investigation is to identify factors that influence the incidence of breast cancer in germline BRCA1/2 mutation carriers. The focus of the proposed study is to examine whether genetic factors involved in DNA damage and repair pathways act as modifiers of BRCA1 and BRCA2. Our hypotheses are supported by findings that BRCA1 and BRCA2 are associated with protein complexes involved in various aspects of genome surveillance and repair and by two recent studies reporting an interaction between a RAD51 polymorphism and BRCA2. We hypothesize that variants in the genes that encode proteins involved in DNA repair may interact with mutations in BRCA1 and BRCA2.

A case-only design will be employed where cases are defined as women with breast cancer who have tested positive for a BRCA1 or BRCA2 mutation, or negative for both mutations. The specific aims of this study are 1) to enroll approximately 1000 female cases; 2) to assess gene-gene interactions between BRCA1/2 and polymorphisms in DNA damage and repair genes; and 3) to assess previously reported interactions between hormonally related genetic and environmental factors and BRCA1 and BRCA2 in a much larger dataset than in prior reports.

Epidemiologic data and DNA will be collected for the analyses using instruments and methods developed in the context of a Cancer Genetic Network feasibility study. Women will be identified from clinic populations from 9 collaborating institutions. The molecular analyses for each of the genetic modifiers will be conducted at the Laboratory for Molecular Epidemiology by Dr. Timothy Rebbeck at the University of Pennsylvania. The Molecular and Cell Technology Core, directed by Dr. Jeffrey Marks, will perform genotyping and SNP discovery for genes involved in DNA damage and repair for which either 1) the frequency of the polymorphism is not known or polymorphisms have yet to be identified that interact with BRCA1 and BRCA2, or 2) the interaction was not known at the start of the study. Innovative statistical methods will be employed to address the complex statistical issues related to examining modifiers of BRCA1 and BRCA2. The genetic epidemiology studies proposed here could potentially have profound ramifications on breast cancer management: genetic counseling, prophylactic surgery, chemoprevention, and cancer therapeutics.

Specific Aim(s):

Specific Aims

1. To enroll approximately 1000 female breast cancer cases (1/3 who have tested positive for a mutation in BRCA1 and/or BRCA2).

2. To assess gene-gene interactions between BRCA1/2 and polymorphisms in DNA damage and repair genes. Promising candidate loci, including BASC (BRCA1-associated genome surveillance complex) genes, SWI/SNF complex genes (chromatin remodeling enzymes), XPD, RAD51, BARD1, BACH1, and
GADD45 will be evaluated.

3. To assess previously reported interactions between hormonally related genetic and environmental factors and BRCA1 and BRCA2 in a much larger dataset than in prior reports. Specifically we will examine:

3a. Previously reported gene-gene interactions between BRCA1/BRCA2 and genes involved in hormonal pathways, including the AIB1 and AR genes.

3b. Interactions between BRCA1 and BRCA2 mutations and environmental exposures related to endogenous hormones (pregnancy, age at menarche, age at first birth, body mass index) and exogenous hormone use (oral contraceptives, hormone replacement therapy).

Hypothesis

Our goal is to identify factors that influence the incidence of breast cancer in germline BRCA1/2 mutation carriers. The focus of the proposed study is on potential genetic modifiers involved in DNA damage and repair. This is supported by findings that BRCA1 and BRCA2 are associated with protein complexes involved in various aspects of genome surveillance and repair and two recent studies reporting an interaction between a RAD51 polymorphism and BRCA2. We hypothesize that variants in the genes that encode these proteins may interact with mutations in BRCA1 and BRCA2. A case-only design will be employed where cases are defined as women with breast cancer who have tested positive for a BRCA1 or BRCA2 mutation, or negative for both mutations.

Application ID: B-TO-0404-01 **Status:** Approved, Ongoing

Principal Investigator: Briollais, Laurent **Year:** 2004

Institution: Mount Sinai Hospital- Samuel Lunenfeld Research Institute

Title: *Analysis of BRCA-Negative Early-Onset Breast Cancer Cases*

Abstract:

Mutations in genes other than BRCA1 and BRCA2 may be associated with a high-risk of breast cancer, especially in young women. We will investigate in this study genetic models that could best explain familial breast cancer not due to the BRCA1 and BRCA2 genes by segregation analyses in a multicentre population-based series of breast cancer (BC) patients diagnosed before age 40 years and their relatives. Complex segregation analyses will be performed using two formulations of the class D regressive approach (Bonney 1984,1986): the logistic hazard model (LHM) from Abel and Bonney (1990) and a liability formulation that we have recently developed (Briollais and Demenais, 2002). Both approaches are implemented in the program REGRESS. They both have interesting features to model the residual familial correlation. To analyze BRCA-negative early-onset breast cancer families, the following strategies will be used: 1) Take out families in which mutations in BRCA1 or BRCA2 segregate and search for the genetic model best fitting the data. 2) Include families with mutations in BRCA1 or BRCA2 and estimate the effect of residual familial correlation and/or a second major gene. To account for the segregation of the known mutation we will use an extension of the regressive model to perform combined-segregation linkage analysis. This analysis will complement another proposal submitted by Drs Cui and Hopper (B-AU-0803-02) since our models are based on different assumptions (especially for the specification of the age-dependent penetrance function and the residual familial correlation) and the program used to fit the models has also different features. We also intend to extend our segregation models to accommodate the two-sampling designs of the Ontario and California registries. Comparisons of the results obtained by these different approaches on the same data sets should give us a better insight into the methodological issues related to the detection of major genetic factors in complex diseases, especially when the data arise from complex study designs.

Specific Aim(s):

Hypothesis

1) There is significant residual familial correlation not due to BRCA1 and BRCA2 genes in early-onset breast cancer.

2) The pattern of residual familial correlation can be explained by the presence of one or two major genetic factors.

3) The segregation of the major gene (or one of the two major genes in a two-locus model) has a recessive mode of inheritance.

4) The magnitude of the residual familial correlation is different in families segregating BRCA1 or BRCA2 gene than in other families.

Application ID: B-AU-0404-01 **Status:** Approved, Ongoing

Principal Investigator: Chenevix-Trench, Georgia **Year:** 2004

Institution: Queensland Institute of Medical Research

Title: *Evaluation of BRCA1 and BRCA2 Unclassified Sequence Variants*

Abstract:

Background

Many of the BRCA1 and BRCA2 sequence variants found in multiple-case breast cancer families are truncating mutations that have obvious effects on protein structure and function. However, a significant number of them are rare missense variants of unknown pathogenicity. These unclassified variants create a dilemma with respect to genetic counselling within such families, and improved knowledge of the pathogenicity and functional significance of such variants would be of great benefit to patients. Very little work has been done to address this issue. However variants within the BRCA1 and BRCA2 genes can now be evaluated using a number of different assays, some of which are designed to test the proposed function of specific domains. We therefore propose to assess, using a variety of genetic and molecular approaches, the pathological and functional significance of unclassified sequence variants detected in multiple-case breast cancer families.

Specific Aims

Aim 1: To assess the pathogenic potential of BRCA1 and BRCA2 unclassified sequence variants in multiple-case breast cancer families using genetic analyses (including estimating the frequency in controls, estimating penetrance in multiple-case breast cancer families, and analysis of loss of heterozygosity).

Aim 2: To assess the functional significance of a subset of BRCA1 and BRCA2 unclassified sequence variants which show features consistent with a cancer predisposing mutation from experiments in Aim 1 using functional analyses (including in vitro transcription and translation assays, transactivation assays, in vitro and in vivo rescue assays, and in vitro transformation, radiosensitivity and differentiation assays for dominant negative mutations).

Study Design: We will identify families from the ABCFS and Utah sites with unclassified variants in BRCA1 or BRCA2. The analyses of these variants will be prioritized according to the nature and location of the variant initially, and then according to the linkage and penetrance estimates. Not all assays will be performed on all variants, either because they are inherently unsuitable, or because the results of early investigations suggest that further analysis is not necessary. A combination of genetic and functional data will ultimately be used to classify the variants, and this information will be relayed to the relevant families by genetic counselors.

Specific Aim(s):

Hypothesis

Certain 'unclassified' missense mutations in BRCA1 and BRCA2 are pathogenic and confer high risks of breast cancer, and can be identified by a combination of genetic and functional assays.

Application ID: B-EX-0404-01 **Status:** Approved, Ongoing

Principal Investigator: Couch, Fergus **Year:** 2004

Institution: Mayo Clinic

Title: *Comprehensive Evaluation of BRCA1 and BRCA2 Unclassified Variants (UCVs)*

Abstract:

Background

Interpretation of results from mutation screening of tumor suppressor genes, such as BRCA1 and BRCA2, is becoming an increasingly important part of clinical medical practice. In most cases, this is quite straightforward. However, classification of so called unclassified variants (UCVs) in the BRCA1 and BRCA2 genes presents a difficult problem because it is not known whether these mutations alter function sufficiently to predispose the cell to cancer

development and appropriate genetic studies that could be used to establish associations between UCVs and cancer predisposition have not been undertaken. Thus, counseling and risk assessment for individuals found to carry these UCVs is problematic. As unclassified variants account for approximately 45% of all known variants detected by clinical mutation screening in BRCA1 and BRCA2 and are present in 13% of all women tested, this has become a significant clinical genetics issue.

To address this issue we have initiated a systematic and comprehensive scheme for classifying these UCVs as likely deleterious/disease causing, moderate/low risk, unclassified, or likely neutral/benign. The scheme integrates information on 1) the nature and position of the amino-acid substitution, 2) the degree of conservation among species, 3) frequency of the variants in different family types compared with their frequency in the general population, 4) the extent of co-segregation of the variant with disease in affected families, 5) the extent of co-segregation of the variants with other known deleterious mutations, and 6) results of functional assays. By combining these various factors it will be possible to classify a number of UCVs leading to improved risk assessment and counseling for carriers of these mutations. In addition, it will be possible to validate a series of functional assays using each of the other approaches. The validated assays will then be used for classification of rare UCVs that cannot be evaluated using the standard genetic approaches.

Study Design

The most important components of this approach are genetic co-segregation and mutation co-occurrence, because it is possible to develop likelihood estimates of disease causality for the UCVs based on the cancer phenotype using these data. These likelihood estimates can then be used to classify UCVs and to validate functional assays. However, the success of this approach is heavily dependent on access to pedigrees with UCVs in which multiple family members have been genotyped for the relevant UCVs. In this application, we propose to identify families from the CFRBCS, excluding the Utah and ABCFS sites, that carry BRCA1 and BRCA2 UCVs. The UCVs will be prioritizied based on frequency, the nature of the alteration, and the location of the alteration in the BRCA1 and BRCA2 genes, and genotyping data from as many relatives as possible from the families carrying the highest priority UCVs will be collected. These data will be combined with data from other collaborating groups and likelihood estimates of disease causality will then be calculated based on co-segregation and mutation co-occurrence. The results will also be combined with those from the other classification methods in order to establish the relevance of the UCVs to cancer and to validate the functional assays. The outcome will be relayed to all collaborators and/or participants.

Specific Aim(s):

Hypothesis

A combination of genetic and functional analysis can be used to establish the cancer relevance of unclassified variants in BRCA1 and BRCA2.

Specific Aims

AIM 1: To assess the disease predisposition associated with BRCA1 and BRCA2 unclassified sequence variants in multiple-case breast cancer families using genetic analyses.

AIM 2: To assess the functional significance of BRCA1 and BRCA2 unclassified sequence variants.

Application ID: B-TO-0804-01 **Status:** Approved, Ongoing

Principal Investigator: Davey, Scott **Year:** 2004

Institution: Queen's University

Title: *Development of a Novel Screen of BRCA1/BRCA2 Heterozygotes in Lymphoblastoid Cell Lines*

Abstract:

Mutations in either BRCA1 or BRCA2 in humans result in a 50-80% risk of developing breast and/or ovarian cancer by age 70 1. Identification of individuals carrying such mutations is critical for optimization of clinical management and initiation of appropriate monitoring practices. Current strategies for identification of BRCA mutations are labour intensive, expensive, time-consuming and of unknown sensitivity. We propose the development of an alternative method for detection of BRCA mutations that exploits the normal role of these proteins in cellular response to DNA damage. The new test will be rapid, economical and informative.

Specific Aim(s):

Specifically, we propose to:

1) Optimize a protocol for obtaining an enriched population of proliferating cells that express BRCA1 and BRCA2 from lymphoblastoid cell lines following the protocols we have already developed.

2) Apply the optimized protocol to 20 samples from individuals with breast cancer who carry a mutation in the BRCA1 gene, 20 samples from individuals with breast cancer who carry a mutation in BRCA2 and 20 samples from individuals with breast cancer who do not carry a BRCA1/2 mutation. The purified cells will be used as a source of total RNA for microarray experimentation.

3) Compare gene expression profiles for all 60 samples relative to their unirradiated controls using long oligo microarrays representing 19,200 human sequences, and analyze the data to identify groups of genes with expression patterns that correlate with the presence or absence of a BRCA mutation.

Hypothesis

We propose that the loss of activity of a single copy of either BRCA1 or BRCA2 will result in a measurable effect on the expression of genes that play a role downstream of their normal role in DNA damage monitoring and repair processes. We further hypothesize that these changes in gene expression can be measured in lymphocytes from patients who carry mutations in either gene using microarray technology.

Application ID: B-EX-0804-01 **Status:** Approved, Ongoing

Principal Investigator: Gago-Dominguez, Manuela **Year:** 2004

Institution: USC

Title: *A Genetic Epidemiological Study of Lipid Peroxidation in Breast Cancer*

Abstract:

A consideration of the animal and in vitro literature suggests that the most powerful and consistent influence on breast cancer protection relates to the generation of lipid peroxidation products. The following lines of experimental evidence in rodents and cultured breast cancer cells suggest that increased cytotoxic lipid peroxidation products may play an important role in breast cancer protection: (1) Polyunsaturated fatty acids (PUFA) including marine n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), alpha-linolenic acid (ALA), conjugated linoleic acid (CLA) and gamma linolenic acid (GLA) have been shown to inhibit the growth of breast cancer in vivo and in vitro and this inhibition is correlated with the extent of lipid peroxidation generated in tumor tissues or cells. (2) This suppression in cancer growth is enhanced by drugs that increase lipid peroxidation. (3) The suppression in cancer growth is eliminated by antioxidants and this elimination is proportional to the inhibition of lipid peroxidation products by antioxidants. (4) In humans, a number of clinical studies have shown that patients treated with marine n-3 fatty acids have marked increases in lipid peroxidation products, and (5) Marine n-3 fatty acids enhance the cancer-killing effect of chemotherapic drugs, which is thought to be achieved through increased lipid peroxidation products.

In addition, several lines of evidence suggest that this mechanism may play an important role in human breast cancer. (1) Estrogens, known to increase breast cancer risk, inhibit lipid peroxidation in a dose-dependent manner. (2) There is a long-term protective effect of pregnancy in breast cancer and pregnant women exhibit significantly higher lipid peroxidation levels than non-pregnant women. (3) Breast cancer risk is significantly reduced by oophorectomy and menopause, conditions both associated with significant increases in lipid peroxidation. (4) Combined estrogen plus progestin replacement therapy increases breast cancer risk and significantly decreases lipid peroxidation in postmenopausal women. (5) Tamoxifen increases lipid peroxidation in breast cancer tumors and this elevation disappears when tumors become resistant to tamoxifen. (6) A recently confirmed finding that a history of pre-eclampsia/pregnancy induced hypertension is associated with a reduced risk of breast cancer and women suffering from this condition exhibit significantly higher lipid peroxidation levels than those associated with a normal pregnancy. (7) Physical activity is associated with a decreased risk of breast cancer and has been consistently shown to increase lipid peroxidation in human and animal studies. (8) Alcoholic beverages have been consistently shown to increase breast cancer risk, and are associated with decreased lipid peroxidation.

The present application addresses genotypes as well as biomarkers involved in the oxidative stress pathway. We have the following specific aims.

1. To conduct a family-based case-control association study to assess the roles of oxidative stress-related genes, including MPO, ecNOS, MnSOD, GPX1, CAT, GSTA1, GSTM1, GSTT1, GSTP1, NQO1, COMT, PON1, HO1, and PPARg, in breast cancer development.

1.1. To assess the potential modifying effects of the candidate genes on the inverse associations between protective factors for breast cancer, including early age at first full-term pregnancy, number of pregnancies, early oophorectomy, late age at menarche, early age at menopause, pre-eclampsia/pregnancy-induced hypertension, and physical exercise, and breast cancer risk.

1.2. To assess the potential modifying effect of the candidate genes on the positive associations between alcohol intake and hormone replacement therapy, and breast cancer risk.

2. To determine if certain protective (early age at first full-term pregnancy, number of pregnancies, early oophorectomy, late age at menarche, early age at menopause, pre-eclampsia/pregnancy-induced hypertension, and physical exercise) and risk-enhancing (alcohol intake) factors for breast cancer are associated with plasma lipid peroxidation products [malondialdehyde, (MDA) and lipid peroxides] among controls.

2.1. To determine if oxidative stress-related genes are associated with lipid peroxidation products (MDA and lipid peroxides) among control subjects.

2.2. To determine if the protective/risk factor-lipid peroxidation associations are modified by oxidative stress-related genes.

3. To conduct a case-case association study to determine if morphological markers of apoptosis in tumor and surrounding free tissue differ among cases with varying levels of reproductive factors (early age at first full-term pregnancy, number of pregnancies, early oophorectomy, late age at menarche, early age at menopause, hormone replacement therapy, pre-eclampsia/pregnancy-induced hypertension), physical exercise and alcohol intake.

In addition, there is available evidence implicating increased lipid peroxidation products in the anti-carcinogenic effect of other suspected protective factors for breast cancer, including soy7, 78-84, vitamin D85 and calcium86-89, folate90, isothiocyanates (ITC)91-94, and tea95-99.

The role of lipid peroxidation in conferring protection against breast cancer may be considered as an episodic phenomenon linked to stages in a woman's life where lipid peroxidation-induced growth arrest or apoptosis is required for maturation, development, differentiation and tissue turnover. The consideration of lipid peroxidation as a protective factor for the prevention of breast cancer does not contradict the conventional view that it may be a cytotoxic process that is generally undesirable. This may remain the case for general biochemical processes in so far as lipid peroxidation in excess disturbs normal cell processes. However, in the case of stages in development where no longer useful or functional cells require disposal, lipid peroxidation-induced apoptosis may provide an important component for cell death and turnover. In addition, the human population is heterogeneous regarding ROS levels and lipid peroxidation generation. Factors that increase lipid peroxidation could increase degenerative diseases in people with innate or acquired high levels of ROS. However, factors that increase lipid peroxidation can increase apoptosis of precancerous and cancerous cells and thus protect against cancer, particularly in people with a low innate baseline level of ROS.

In summary, we believe that the effect of anti-oxidants and lipid peroxidation may depend on the baseline level of ROS. In the net, anti-oxidants may protect people from selected cancers if their baseline level of ROS is higher, but, in the net, may not protect people from selected cancers if their baseline ROS levels are lower because this may place a greater importance on the suppression of lipid peroxidation and apoptosis, as explained by Salganik100. We will measure lipid peroxides and MDA as a measure of ROS, and plan to investigate whether, for example, levels of MDA modify the effects of

risk/protective factors for breast cancer.

If lipid peroxidation plays a role in breast cancer protection, it is likely that differences in the ability to protect cells from beneficial lipid peroxidation products will determine, to some degree, the protective effect of hormonal/non-hormonal factors on breast cancer. We have recently published the first set of prospective results linking intake of marine n-3 fatty acids to breast cancer protection101. In a subsequent study, we found that women with genetic polymorphisms encoding lower or no activity in detoxifying genes (GSTM1, GSTT1, GSTP1) experienced more protection from marine n-3 fatty acids than those with common alleles, putatively because more cytotoxic peroxidation agents could reach the pre-cancerous/cancerous cell and cause damage102.

In this proposal, we will examine the overall effect of lipid peroxidation on breast cancer development as well as determine if the protective/risk-enhancing effect of established protective/risk factors for breast cancer is mediated through increased/decreased lipid peroxidation products. We will also examine the overall effects of genes mediating the target cell's response to oxidative stress on breast cancer development, as well as examine these candidate genes' potential modifying effects on the respective exposure/cancer associations.

Application ID: B-TO-1204-01 **Status:** Approved, Ongoing

Principal Investigator: Glendon, Gordon **Year:** 2004

Institution: Cancer Care Ontario

Title: *Lessons in Epidemiology and Genetics of Adult Cancers from Youth (LEGACY): Collaborative Pilot Studies on Parental Interest in their Daughters' Enrollment in a Research Study*

Abstract:

Breast cancer risk is likely influenced by genetic and environmental factors that have effects before adulthood. We propose a pilot study to determine parents' receptiveness to enrolling their minor children in a prospective cohort investigating ealry events in breast cancer. This may be a period when preventive strategies show greatest benefit. We will conduct a series of semi-structured telephone interviews of Cancer Family Registry (CFR) participant parents to gauge their receptiveness to such a cohort study. We will utilize a semi-structured interview for fdata collection and qualitative methodology to analyse the telephone transcripts.

Specific Aim(s):

Hypothesis

Breast cancer risk is influenced by genetic and environmental factors that exert effects prior to adulthood. The Cancer Family Registry cohort consists of a large number of families who have a wide spectrum of cancer risk. Family history, epidemiological and clinical data and biological specimens (blood and tumour blocks) have been collected for each participating family. The expansion of this existing cohort to include family members under the age of 18 would permit investigation of important early events in breast cancer etiology.

The participation of minors in research, however, is associated with special issues that will first need to be addressed before recruitment proceeds. In the proposed study, we will conduct semi-structured, in-depth interviews with adult-parent participants of the CFR to gain detailed understandings of their interests and concerns regarding their offspring being asked to contribute data and biological specimens as part of a CFR youth cohort. This youth cohort is entitled LEGACY (Lessons in Epidemiology and Genetics of Adult Cancers from Youth). Qualitative analyses of these interviews will be undertaken to ascertain the full range of concerns, facilitating and barrier factors to participation as well as alternative approaches to LEGACY enrollment.

Application ID: B-TO-1204-02 **Status:** Approved, Ongoing

Principal Investigator: Goodwin, Pamela **Year:** 2004

Institution: Cancer Care Ontario

Title: *Proposal for Supplementary Survival Studies in the BCFR*

Abstract:

The Prognostic Working Group has previously received approval from the Steering Committee to examine effects of mutations in BRCA1 and BRCA2, as well as family history and pathologic characteristics on breast cancer outcomes (local recurrence, distant disease free survival, overall survival) in probands enrolled at three population-based sites of the BCFR (Ontario, California, Australia). Funds were obtained from national funding agencies in Canada and Australia to collect clinical data at baseline, and to prospectively follow probands for local recurrence, distant recurrence and overall survival. A recent R01 application submitted to the National Institutes of Health (June 2004) for similar data collection in California, to continue follow-up in Ontario and Australia, and to conduct combined analyses was scored 172 (9.7 percentile). If this proposal is funded, we will be ready to perform our planned analyses in 2006.

With the upcoming BCFR renewal, there is a desire to conduct some outcome analyses on an earlier timeline. For the most part, these analyses would use existing Registry data, focusing on data available on the Epidemiology questionnaire. This proposal outlines those supplemental analyses that can be conducted during the next 12 to 18 months. The population-based nature of the data at three sites (Ontario, Australia, Northern California), coupled with the large sample size and uniformly collected data provide a strong experimental foundation for our proposed research that will provide important information on the role of reproductive and lifestyle factors in breast cancer survival.

Using existing Registry data, supplemented by SEER data in California and extraction of some pathology data from pathology reports, we propose to evaluate the survival effects of several key variables that have been collected as part of the Family History Questionnaire and the Personal History Questionnaire. Items on the Personal History questionnaire that are potentially relevant include date of birth (age), alcohol and smoking history, reproductive factors (oophorectomy, oral contraceptives, pregnancy, hormone replacement therapy, menopause, infertility) and physical activity. Items on the Family History Questionnaire that are relevant would include family history in 1st, 2nd and 3rd degree relatives on both the maternal and paternal sides of the family. The outcome variable for all of these analyses will be overall survival as reported to the Registry during routine follow-up of probands.

Specific Aim(s):

Our key hypotheses are as follows:

1. Oral contraceptive use (ever use, use for > 12 months, duration of use, use at diagnosis, age at first use) prior to breast cancer diagnosis is not an independent prognostic factor in breast caner.

2. Recent childbirth prior to diagnosis is an adverse prognostic factor, and in particular, this effect may be stronger in women with a family history; see 9 below.

3. Breast feeding (history and duration) is not a prognostic factor.

4. Ever use of hormone replacement therapy is a favourable prognostic factor.

5. Cigarette smoking (ever) is an adverse prognostic factor.

6. Alcohol consumption (standard drinks per day as a continuous variable) is not a prognostic factor.

7. Young age (continuous factor, by decade, <40 years) at diagnosis is an adverse prognostic factor; this effect will be modified (reduced) when family history of breast cancer in first or second degree relatives is considered.

8. Obesity (body mass index treated as a continuous factor, modeled linearly and quadratically) is an adverse prognostic factor; this effect will be modified by systemic adjuvant therapy and tumor characteristics (hormone receptor status).

9. History of breast cancer in a first or second degree relative is an adverse prognostic factor; the effect will be modified by age at diagnosis of both the proband and the family member (greatest effect with younger age in both).

Application ID: B-EX-1204-01 **Status:** Approved, Ongoing

Principal Investigator: Haiman, Christopher **Year:** 2004

Institution: USC/Norris Comprehensive Cancer Center

Title: *Genetic Variation in DNA Pathway Genes in the Breast Cancer Family Registry*

Abstract:

Both twin and family-based studies have long suggested that a substantial fraction of breast cancer susceptibility is heritable. A number of genes have been identified (BRCA1, BRCA2, TP53) that are associated with familial cancer syndromes that confer very high risks of breast cancer. Rare, high penetrant mutations in BRCA1 and BRCA2 however, only explain a quarter of cases in high-risk families with multiple affecteds. Rare mutations of varying penetrance in ATM, CHEK2, PTEN and TP53 may only help explain, at most, an additional 5% of cases in these families. A greater challenge has been the identification of inherited differences that convey susceptibility to breast cancer in families where the genetic contribution may be more modest. The combination of environmental factors and low-to-moderate penetrance alleles at multiple candidate loci most likely contribute to this risk. Advancements in our understanding of variation in the human genome and the development of novel genetic and epidemiologic methods have provided the necessary tools to comprehensively examine genetic variation in candidate genes as risk factors for familial and sporadic breast cancer. In this application, we propose to conduct linkage disequilibrium (LD)-based analyses to study common variation in DNA repair pathway genes in relationship with breast cancer risk.

Specific Aim(s):

In this proposal, analyses centered on linkage disequilibrium mapping will be employed to identify novel breast cancer susceptibility alleles. Based on the unequivocal link between DNA repair pathway genes and breast cancer, we propose to analyze genetic variation in key genes involved in DNA repair (see Appendix, Table 1 for the list of genes).

The specific aims of this research project are:

1. To assess the independent effects of known missense SNPs, tagging SNPs that delineate underlying genetic variation (i.e. haplotype patterns) in candidate DNA repair genes in relation to breast cancer risk.

2. To evaluate the combined effects of missense and tagging SNPs in DNA repair pathway genes on breast cancer risk.

Application ID: B-AU-0804-01 **Status:** Approved, Ongoing

Principal Investigator: Proos, Anna **Year:** 2004

Institution: Royal North Shore Hospital, Sydney, Australia

Title: *The Jewish Founder Mutations in the Fanconi Gene and the Blooms Syndrome Gene and Risk of Breast Cancer*

Abstract:

Among the Ashkenazi populations, it is clear that the founder mutations in BRCA1 and BRCA2 do not explain all familial aggregation of breast cancer, nor the majority of breast cancer families. In this study we will assess if the Jewish founder mutations in the FANCC and BLM genes, both involved in DNA repair, are associated with an increased dominantly-inherited risk of beast cancer. These genes are involved with recessively-inherited risk of Fanconi's anemia and Bloom's syndrome, respectively. BRCA1 and BRCA2 are part of the pathway leading to DNA repair which is defective in Fanconi anemia patients. Patients with Fanconi anemia in the FANCD1 complementation group have been shown to have homozygous mutations in BRCA2. Patients with Bloom syndrome have a high incidence of early onset adenocarcinoma and breast cancer. We shall measure the frequencies of the two common Jewish founder mutations in these two genes in 2,742 women from the Breast CFR who are self-reported to have Jewish heritage and from whom there is a blood sample and completed epidemiology questionnaire. We will test for association with risk of breast cancer by comparing the frequencies in the 1,288 women with a prior diagnosis of breast cancer (cases) and the 1,454 women without a prior diagnosis (controls). Given the allele frequencies of these founder mutations are in the order of 1 in 100, we will have 80% statistical power (at the 0.05 level of statistical significance) to detect odds ratios of 2.5 or more for risk of breast cancer, or 50% power to detect odds ratios of 2.0 or more. We will also use within-family analyses of founder mutation-carrying families to estimate penetrance. We will also use this data to see if there is any evidence that these mutations modify breast cancer risk in women with a BRCA1 or BRCA2 mutation.

Specific Aim(s):

Hypothesis

That the Jewish founder mutations in the FANCC gene (IVS4+4A->T) and the BLM gene (2281del6ins7) are associated with an increased risk of breast cancer, and may modify risk in BRAC1 and BRCA2 mutation carriers.

Application ID: B-SF-0804-01 **Status:** Approved, Ongoing

Principal Investigator: Ziv, Elad **Year:** 2004

Institution: University of California San Francisco

Title: *Admixture and Breast Cancer Risk Among Latinas*

Abstract:

Breast cancer incidence rates vary widely among different racial and ethnic groups. Admixed populations, in which two or more ethnic groups have mixed, may provide insight into the etiology of these differences. Latinos Americans are largely an admixed group descended from European, Native American, and African ancestors. In California, the majority of Latinos are immigrants from Mexico and Central American countries, and are mainly of European and Native American descent. Latinas have breast cancer incidence rates that are substantially lower than Caucasians but higher than Native Americans, consistent with their mixed European and Native American descent. However, these differences may also be due to differences in hormonal and lifestyle factors or the interaction of such factors with genetic factors. Genetic ancestry may be estimated using genetic markers that are known to have high allele frequency differences between ancestral populations (ancestry informative markers).

We propose to type a series of ancestry informative markers in an established case-control study of Latinas in the Bay Area to address the following specific aims:

(1) To determine the proportion of Caucasian, Native American and African ancestry in a population-based sample of Latinas residing in the San Francisco Bay Area and compare the genetic ancestry of Latina immigrants from different regions of Mexico, Central America, and South America.

(2) To compare ancestry between Latina breast cancer cases and age-matched controls and determine whether genetic case-control studies of breast cancer in this population may be confounded by recent admixture. If ancestry is associated with breast cancer in Latinas, then the association will be adjusted for known hormonal and lifestyle factors associated with breast cancer risk.

(3) To test several newly developed statistical methods to adjust for the confounding that may arise due to differences in genetic ancestry of cases and controls.

Based on these results we will determine whether genetic association studies of breast cancer risk may be confounded by population stratification in the Latina population. In addition, this project will test the feasibility of using the increased linkage disequilibrium in admixed populations to identify genetic variations associated with breast cancer among Latinas.

Specific Aim(s):

Hypothesis

(1) There are differences in genetic background between Latina breast cancer cases and Latina controls. These differences may explain the association between geographical origin and breast cancer risk in this population. Particularly, we hypothesize that European ancestry among Latinas will be associated with a higher risk of breast cancer.

(2) If differences in genetic backgrounds between cases and controls are identified, the excess false positive associations that would result could be adjusted for using unlinked markers.

Application ID: B-TO-0597-02-C03 **Status:** Approved, Ongoing

Principal Investigator: Andrulis, Irene **Year:** 2003

Institution: Cancer Care Ontario

Title: *Studies of Hereditary Breast Cancer Genes and Their Association with Somatic Molecular Alterations and Histopathological Features in Cases from the Ontario Familial Breast Cancer Registry (OFBCR) - Continuation*

Abstract:

Since the cloning of BRCA1 and BRCA2 genes, there have been a number of studies published that report on detection of mutations in these genes. The mutations, which have been reported, are predominantly frameshift or nonsense mutations, which result in truncations of the BRCA1 and the BRCA2 gene products and loss of function of the proteins. Single base substitutions that result in amino acid changes in the BRCA1 and BRCA2 genes have also been reported in families, although the frequency of these mutations is much lower than the truncation mutations. However, the frequency of missense mutations may be underestimated due to difficulty in detecting these changes relative to truncation mutations and inconclusive evaluation of the missense changes detected in families because the functions of BRCA1 and BRCA2 are not known. The analysis of both BRCA1 and BRCA2 is also complicated by the fact that the mutations observed are variable in nature and their location appears to be distributed throughout the large coding sequence, making the complete analysis of these genes very difficult. To date, mutational studies have focused mainly on families with a very strong history of breast and/or breast/ovarian cancers. In addition to these high risk families, there are also low and medium risk families with a history of breast cancer. The incidence of decreased risk in such families may be explained by the presence of novel BRCA1 and BRCA2 mutations, mutations in other predisposition genes, or BRCA1 and BRCA2 mutations identical to those observed in the high risk group, whose phenotypic expression is influenced by modifier genes or environmental factors.

Specific Aim(s):

Aims

We propose to use the population-based Ontario Familial Breast Cancer Registry (OFBCR):

1) to determine the frequency and the types of BRCA1 and BRCA2 mutations in a population-based sample of breast cancer cases considered familial according to defined criteria and a sample of nonfamilial cases chosen from the same population;

2) to assess whether there are associations between family history of cancer and other characteristics and mutations in BRCA1 and BRCA2;

3) to estimate the penetrance of BRCA1 and BRCA2 mutations in this population-based sample;

4) to examine risk factors associated with being an affected carrier as opposed to an unaffected carrier and associated with age at onset in carriers;

5) to perform descriptive studies on the families in this population-based registry including the prevalence of gene mutations in these families.

Analysis will be done on the first 200 registered familial cases and, eventually, 100 registered nonfamilial cases in the OFBCR.

Hypothesis

The hypothesis of this pilot study is that different molecular alterations in breast cancer predisposition genes may be associated with different types of family history of cancer and penetrance. A second hypothesis is that epidemiologic risk factors play a role in the development of cancer in gene mutation carriers.

Application ID: B-TO-1203-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Chiarelli, Anna M. **Year:** 2003

Institution: Cancer Care Ontario

Title: *Screening Behaviours and Outcomes among Relatives of Women with Breast Cancer*

Abstract:

Although there is substantial evidence for the efficacy of screening mammography for women 50-69 years of age, the impact of screening on reducing mortality from breast cancer in women with a family history of breast cancer is unknown. This evidence is required for the development of definitive breast cancer screening guidelines for women with varying levels of family history risk of breast cancer. A retrospective cohort study is proposed. The study cohort will be identified from female relatives of cases of invasive breast cancer (probands) who were diagnosed in Ontario between 1996-1998 in the Ontario Familial Breast Cancer Registry. Breast and ovarian cancer screening behaviours among women with high, moderate and low risk family history of breast cancer will be compared. In addition, the determinants of these behaviours will be examined and the outcomes will be followed until December 31, 2007. Information on breast and ovarian cancer screening behaviours and knowledge, attitudes and beliefs about breast cancer risk and screening will be collected by a telephone-administered questionnaire. The cohort will be followed for two years by annual questionnaires to identify breast screening outcomes. Data on the spectrum of benign breast lesions and prognostic features of breast cancer will be obtained from tissue specimens and breast cancer treatment will be abstracted from medical records.

Specific Aim(s):

1) To compare breast and ovarian cancer screening behaviours among women with high, moderate and low risk family history of breast cancer.

2) To determine if knowledge, attitudes and beliefs on breast cancer risk and screening influence breast screening behaviours among women with high, moderate and low risk family history of breast cancer

3) To determine if breast screening outcomes (benign/premalignant breast lesions and prognostic features of breast cancers) differ by breast screening behaviours among women with high, moderate and low risk family history of breast cancer

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Application ID: B-AU-0403-03 **Status:** Approved, Ongoing

Principal Investigator: Dite, Gillian **Year:** 2003

Institution: University of Melbourne

Title: *Collaborative Population-Based Analyses of Familial Risks of Breast and Other Cancers Associated with Early-Onset Breast Cancer*

Abstract:

This study is a collaborative investigation of the familial risks of breast and other cancers in the Breast CFR. We propose to use family history data from population-based case and control families that were recruited by three of the Breast CFR centres (Australia, Northern California, and Ontario) in an analysis of the familial aggregation of breast and other cancers. This work will build on analyses from the Australian Breast CFR that investigated the risk of breast cancer in first-degree and second-degree relatives of women diagnosed with breast cancer under the age of sixty years (Dite et al., 2003). The study is an undertaking of the Breast CFR's Analytical Working Group and will involve active participation from investigators in Australia, Northern California, and Ontario.

The study will focus on the familial risks associated with early-onset breast cancer. We will employ the same eligibility criteria for the selection of case and control families that have been developed for the collaborative case-control analyses of the Breast CFR's population-based data from Northern California, Ontario, and Australia. Data from the Australian Breast Cancer Family Study (ABCFS), a population-based case-control-family study of breast cancer with data collected prior to the establishment of the Breast CFR, but with almost identical study procedures and protocols, will also be included in the analyses.

A cohort of first-degree relatives (Australia, Northern California, and Ontario) and second-degree relatives (Australia and Ontario) of case and control probands will be assembled from the Breast CFR and the ABCFS. We will calculate time to diagnosis of breast and other cancers in groups of relatives from case families compared to groups of relatives from control families and population data. In addition to a particular focus on breast cancer, we will also look at other cancers (for example, ovarian, colorectal, pancreatic, prostate, and melanoma). We will also investigate the incidence with familial risks of breast and other cancers that remains after the exclusion of relatives of case probands with known BRCA1 and BRCA2 mutations.

The cohort of relatives from control families will be compared to population data to assess the extent (if any) of under-reporting of breast and other cancers in these families.

Specific Aim(s):

Hypothesis

The risk of breast and other cancers in first- and second-degree relatives of probands with early-onset breast cancer:

- Is increased compared to the relatives of the control probands
- Is increased compared to the population risk
- Is greater the earlier the age at diagnosis of the proband
- Is higher for first-degree relatives than for second-degree relatives
- Remains elevated if relatives of known BRCA1 and BRCA2 mutation carriers are excluded
- Is higher for relatives with one or more affected first-degree relatives (other than the proband)

The risk of breast and other cancers in first- and second-degree relatives of control women:

- Is the same as the population risk
- Is the same for first-degree and second-degree relatives

Application ID: B-SF-0403-01 **Status:** Approved, Ongoing

Principal Investigator: Glaser, Sally **Year:** 2003

Institution: Northern California Cancer Center

Title: *Breast Cancer, Endometriosis and Pyloric Stenosis: A New Breast-Cancer Family Syndrome?*

Abstract:

This pilot family-association study will explore whether breast cancer, endometriosis, and pyloric stenosis in families represent a breast-cancer family syndrome. It will determine if endometriosis and pyloric stenosis occur in breast-cancer families beyond the level expected by chance, using the efficient strategy of testing the hypothesis in the large group of breast cancer families and breast cancer patients at high risk for genetic predisposition already enrolled in the Northern California Family Registry for Breast Cancer (FRBC) and contacted annually for follow-up, thus facilitating inquiry about the other two diseases. The study aims are to: (1) identify FRBC probands participating in an annual follow-up interview, who are at presumed high risk of genetic susceptibility to breast cancer (i.e., diagnosed at < age 35, or < age 50 with bilateral disease, or being from multi-case families with >= one female relative diagnosed with breast cancer < age 60) but low risk for BRCA1 or BRCA2 mutations (i.e., no personal or family history of ovarian cancer, no breast cancer in male relatives, or testing negative), (2) interview the 850 eligible probands (or next-of-kin) about prior endometriosis and infantile pyloric stenosis in themselves and their already enumerated 1st- and selected 2nd-degree relatives, (3) determine whether the number of families with both these conditions exceeds that expected by chance, based on population probabilities from the scientific literature and assuming that the two conditions are independent.

Specific Aim(s):

We propose to conduct a pilot family-association study, that is, to explore whether breast cancer, endometriosis, and pyloric stenosis in families represent a breast-cancer family syndrome.

Application ID: B-AU-1203-01 **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2003

Institution: University of Melbourne

Title: *Mutations in Estrogen Metabolism Genes and Breast Cancer*

Abstract:

Although epidemiological data strongly suggest that estrogen and other hormones play the major role in explaining breast cancer risk, much remains to be learnt about genetic susceptibility to breast cancer. Less than 25% of the increased risk associated with having an affected first-degree relative diagnosed before the age of 40 is explained by mutations in the susceptibility genes BRCA1 and BRCA2. Only one-third of population-based early-onset case families with two or more affected first- or second-degree relatives carry a mutation in a known susceptibility gene. Segregation analyses suggest that, in addition to BRAC1 and BRCA2, there are major genes associated with a high risk of early onset breast cancer that have yet to be discovered. Recently a number of genes have been identified that play a role in the metabolism of estrogen and other hormones. These “candidate” genes are being studied extensively by the NIH-funded Cohort Consortium to identify common variants and haplotypes, and the genetic data is being made freely available to other researchers. That work aims to identify if particular variants or haplotypes are associated with breast cancer risk. Association with a haplotype could be due to the combination of functions of one or more of the alleles that constitute the haplotype, or be due to linkage disequilibrium with as yet undetected variant(s) in or close to the measured variants. We aim to investigate the latter hypothesis by using the population-based case families of the Australian Breast Cancer Family Study. We shall conduct mutation screening, using the highly sensitive denaturing gradient gel electrophoresis (DGGE), followed by direct sequencing of identified novel mutations, and/or DNA sequencing, for the major candidate genes, including those for which there is published evidence that there may be an association in early-onset disease in particular, and those for which the Cohort Consortium finds evidence for an association with breast cancer. We will initially focus on the 50 cases diagnosed before age 40 who have two or more affected relatives, and for whom extensive mutation testing has failed to identify a pathogenic mutation in BRCA1, BRCA2, Tp53, ATM or CHK2. This mutation screening will be carried out in laboratories at the Garvan Institute in Sydney, the Genetic Epidemiology Laboratory at The University of Melbourne, and the Australian Genome Research Facility at the Walter & Eliza Hall Institute in Melbourne. Should one or more likely pathogenic mutations be detected for a particular gene, we will test all DNA samples from relatives for that mutation. We will also extend mutation screening to the untested early-onset cases, and if more carriers are identified and sufficient resources are available, to cases of later age at diagnosis. We will also test all cases and controls for each putative pathogenic mutation so as to exclude the possibility that the variant is as common in unaffected women as it is in affected women. Extensions to use the resources of other sites of the Breast CFR will be sought should this prove to be a successful way of identifying genes and mutations associated with an increased risk of breast cancer. Penetrance estimates will be calculated using a modified segregation analysis fitted under maximum likelihood theory with the package FISHER.

Application ID: B-AU-1203-03 **Status:** Approved, Ongoing

Principal Investigator: Milne, Roger **Year:** 2003

Institution: University of Melbourne

Title: *Collaborative Epidemiologic Analyses of Environmental Risk Factors for Breast Cancer Using Population-Based Data Collected in Australia, Ontario and Northern California*

Abstract:

This study proposes to utilise the resources of the Breast CFR for collaborative analyses of risk factors for breast cancer in non-Hispanic White women of all ages, using questionnaire data collected by the three registries that have population-based recruitment of families (the Australian CFR, the Northern California CFR and the Ontario CFR). It will further the work already done using similar analyses for women under age 40 years, for which Advisory Committee approval has been granted. This collaborative work is clearly in line with the stated purpose and aims of the Breast CFR.

The Australian, Ontario and Northern California sites of the Breast CFR have been working together, through a sub-committee (Case-Control Group) of

the Analytical Working Group, over the last 12 months. We now plan to extend analyses to include women of all ages.

Application ID: B-AU-0403-01 **Status:** Approved, Ongoing

Principal Investigator: Milne, Roger **Year:** 2003

Institution: University of Melbourne

Title: *Collaborative Epidemiologic Analyses of Early-Onset Breast Cancer Using Population-Based Data Collected in Australia, Ontario, and Northern California*

Abstract:

This study proposes to utilise the resources of the Breast CFRs for collaborative analyses of risk factors for breast cancer in non-Hispanic, White women under age 40, using questionnaire data collected by the three registries that have population-based recruitment of families (the Australian CFR, the Northern California CFR, and the Ontario CFR). It will be the first large scale Breast CFR study pooling epidemiological data and we hope it will prompt further collaborative work such as extending analysis to older women and studies of candidate polymorphisms. This is clearly in line with the stated purpose and aims of the Breast CFRs.

The Australian, Ontario, and Northern California sites of the Breast CFR are currently working together, through a sub-committee (Case-Control Group) of the Analytical Working Group, to develop and implement uniform data cleaning methods and variable definitions. We plan to perform collaborative analyses on a defined set of exposures as potential risk factors for breast cancer and to investigate how these modify risk in: (1) women known to carry a mutation in BRCA1 or BRCA2, and (2) women with a strong family history of breast cancer. The sub-committee has developed a list of analyses to be carried out and has assigned people and sites to take the lead role in each of these, as detailed below.

Specific Aim(s):

Hypotheses

(i) That for non-Hispanic white women under age 40 (both including and excluding those known to carry a mutation in BRCA1 or BRCA2), increased risk of breast cancer is associated with:

1. having a family history of breast cancer
2. having a first child
3. having fewer children subsequent to the first
4. later age at first birth
5. earlier age at menarche
6. never breast feeding
7. being taller
8. being lighter (in weight)
9. having lower body mass index
10. ever using contraceptives
11. using oral contraceptives for longer
12. high alcohol consumption
13. never having smoked
14. smoking less total cigarettes
15. ever being exposed to radiation
16. having a higher number of radiation treatments
17. low physical exercise

(ii) That the above increased risks are greater for women who are known to carry a mutation in either BRCA1 or BRCA2

(iii) That the increased risks in (i) are greater for women who have a strong family history of breast cancer, but who are not known to carry a mutation in BRCA1 or BRCA2

Application ID: B-UT-0403-01 **Status:** Approved, Ongoing

Principal Investigator: Neuhausen, Susan L. **Year:** 2003

Institution: University of California Irvine

Title: *The IGF Pathway and Breast Cancer Risk*

Abstract:

We propose a comprehensive analysis of genes in the insulin-like growth factor signaling pathway, a pathway integrally involved in cellular proliferation. We will use powerful new approaches that combine multiple linked variants in a single gene to form haplotypes and that allow evaluation of multiple genes in a single mechanistic pathway. We will study population-based female breast cancer cases with population-based controls matched for ethnicity, center, and birth year and confirm results using family-based cases and their unaffected sister controls. Our specific aims are: (1) to screen Single Nucleotide Polymorphisms (SNPs) in IGF1, IGF2, IGF1R, IGFBP1, IGFBP3, IGFBP5, IRS1, INS, GH and SHBG in 150 unrelated breast cancer cases (50 African Americans, 50 Asian Americans, and 50 Caucasians; our objective is to mark the common variation across the genes, while minimizing genotyping in the larger cohort, and Haplotype-tagging SNPs, including all those with known function, will be selected, (2) to genotype affected cases and unaffected controls for SNPs selected in Aim 1, (3) to evaluate the association of SNPs in genes in the IGF pathway with risk of breast cancer, age at diagnosis, and stage and grade; we will confirm significant associations in a family-based study, and (4) to assess the biological effects of genetic variants/haplotypes that were significantly associated with breast cancer risk. We aim to identify genetic risk factors for developing this disease in the hope that this information can be used to better understand the etiology of breast cancer and to target women for prevention or treatment strategies.

Specific Aim(s):

Our specific aims are:

1. To screen Single Nucleotide Polymorphisms (SNPs) in genes involved in the insulin-growth factor signaling pathway. We will identify SNPs in IGF1,IGF2, IGF1R, IGFBP1, IGFBP3, IGFBP5, IRS1, INS, GH, ER and SHBG in currently existing SNP databases and then genotype in 150 unrelated breast cancer cases. We will then identify haplotype blocks by linkage disequilibrium analysis and select haplotype-tagging SNPs, including those with known function, for genotyping in Aim 2.

2. To genotype affected (cases) and unaffected (matched controls) for SNPs selected in Aim 1. We estimate that we will genotype 70 SNPs in the cases and controls.

3. To evaluate the association of SNPs in genes in the IGF pathway with risk of breast cancer, age at diagnosis, and stage and grade. Using a case-control design, we will evaluate the association of the SNPs with breast cancer risk. Using a case-case design, we will evaluate the association of the SNPs with age at diagnosis and pathologic stage and grade of breast cancer. We will examine main effects and gene-gene and gene-environment interactions. Significant associations will be validated in a family-based design of breast cancer cases and their relatives.

4. To assess the biological effects of genetic variants/haplotypes that were significantly associated with breast cancer. We will examine plasma levels of proteins in controls with and without specific genetic variants. We will perform immunohistochemistry in tumor tissue to correlate genetic changes with expression.

Hypothesis

Variants in genes involved in the insulin-like growth factor (IGF) signaling pathway are associated with breast cancer risk. We will focus on this pathway as it plays a key role in the regulation of cell proliferation, which is central to the carcinogenic process.

Application ID: B-AU-1203-02 **Status:** Approved, Ongoing

Principal Investigator: Southey, Melissa C. **Year:** 2003

Institution: University of Melbourne

Title: *Large Genomic Alterations in BRCA1 in Young Women with Breast Cancer*

Abstract:

Population-based studies of the genetic epidemiology of female breast cancer have shown that only a small proportion of familial aggregation and familial clusters of the disease can be explained by what is currently known about its genetic and environmental causes.

We have applied a variety of mutation detection methods to DNA extracted from a blood sample provided by participants with early onset breast cancer in the Australian Breast Cancer Family Study (ABCFS) to try to identify BRCA1 and BRCA2 mutations. We have used sequencing and the protein truncation test (PTT) to identify coding and splice site region mutations and we have applied a specific PCR-based test to detect the protein truncating, BRCA1 exon 13 duplication. These methods are suitable for identifying frameshift, non-sense, missense and splice site mutations within these genes. However, only a small proportion, of the cancers arising in the case probands of the ABCFS can be explained by mutations (identifiable by current methods) in BRCA1 or BRCA2. Specifically, only 13% of case probands diagnosed before the age of 40 years with 2 or more affected first- or second-degree relatives have been found to carry a BRCA1 or BRCA2 mutation with current methods.

We have tested for putative disease-predisposed variants in several other genes (ATM, CHK2, p53) that might explain some of the familial aggregation of breast cancer but only a small proportion (15%) of the cancers arising in case probands with the strong family history above can be explained by non-familial causes or by other genetic variants known or likely to be associated with increased risk of breast cancer.

It has been known for sometime that breast cancers in individuals carrying a germline mutation in BRCA1 have certain distinguishing histological features. The Pathology Working Group of the BreastCFR has devised a standard pathology review form that has been applied to all the tumours collected from clinic-based cases with a strong family history of breast and ovarian cancer and the population-based case probands (Melbourne and Sydney, Australia; Ontario, Canada and Northern California, USA). This creates a resource of approximately 3,000 breast tumours that have all had a common review process.
Analysis of the data from the ABCFS has revealed that 30% of early-onset breast cancers with a strong family history have BRCA1-associated morphological features. Of these cancers, 30% of these cancers do not appear to have an identifiable mutation in BRCA1 despite extensive genetic testing (In-house testing and BRCAnalysis by Myriad Genetics). These tumours are referred to as “BRCA1-like”. We wish to investigate the molecular mechanisms responsible for the generation of this distinctive group of “BRCA1-like” early-onset breast cancers.

There are possibly several good explanations of the BRCA1-like phenotype. We believe that a proportion of BRCA1-like cases would be resolved by

identifying other mechanisms for BRCA1 silencing. These mechanisms could include large deletions, insertions and duplications in BRCA1 that our current methods are insensitive to detecting and/or disruptions to BRCA1 promoter methylation and loss of heterozygosity (LOH).

This application proposes to screen the germline DNA of:

Population-based case probands diagnosed with breast cancer under the age of 40 who have a strong family history of breast or ovarian cancer and

Case probands whose breast cancer morphology is consistent with carrying a BRCA1 mutation but for whom a BRCA1 mutation has not yet been identified.

The results of this study could have an immediate impact on the clinical management of young women with breast cancer and women with a strong family history of breast and ovarian cancer.

Application ID: B-NY-0803-01 **Status:** Approved, Ongoing

Principal Investigator: Terry, Mary Beth **Year:** 2003

Institution: Columbia University, School of Public Health

Title: *Epidemiology, Pathology, and Breast Cancer Risk*

Abstract:

Most breast cancer risk factors are modest (< 2-fold) in nature making it difficult to rule out bias and draw causal conclusions. One explanation for the modest associations is that despite the heterogeneity, most epidemiologic studies lump all types of breast cancer into a single case group. Laboratory and clinical data point to different phenotypes for breast cancer characterized by pathologic characteristics. These different phenotypes indicate that multiple pathways are involved in breast carcinogenesis. Thus, it is important to understand if known and suspected risk factors for breast cancer operate differently according to specific pathway. Some epidemiologic studies have already attempted to examine whether risk factors differ by pathologic characteristics, particularly by hormone receptor status. However, the data are inconsistent likely owing to the lack of uniform-pathologic review and insufficient sample size. Such uniform review is critical given the likely measurement error in some of the pathologic parameters which hampers the detection of differences. Using already collected data from the epidemiology and pathology instruments of the NCI's Breast Cooperative Family Registry (BCFR), we plan to examine whether breast cancer risk factors such as reproductive history, exogenous hormone use, body size, alcohol and tobacco use, physical activity, family history, age and race differ by (a) histologic subtype; (b) ER/PR status; and (c) a composite phenotype variable including grade and stage. The BCFR is an excellent resource with which to conduct these analyses for several reasons including its uniform review of cases, the overall size, its vast information on family history and carrier status, and its uniqueness in examining these issues both in a population-based way as well as by family-based methods.

Specific Aim(s):

Specific Aims

We plan to examine whether breast cancer risk factors such as reproductive history, exogeneous hormone use, body size, alcohol and tobacco use, physical activity, family history, age, and race differ by:

(a) histologic subtype (ductal, lobular, medullary, tubular, mucinous);

(b) ER/PR status (ER+PR+, ER-PR+, ER+PR-, ER-PR-); and

(c) phenotype (combination of grade and stage and other pathologic criteria)

The specific aims will be accomplished first by using data from the three population-based sites (Ontario, Northern California, and Australia (Melbourne and Sydney) and using a case-control design which will allow multiple case groups to be compared to the control group (and accordingly case/case differences estimated from the same model). Case/case comparisons using data from the three clinic-based sites (New York, Fox Chase (Philadelphia), and Utah and analyzed by family set with complement these analyses.

Hypothesis

Breast cancer is a heterogeneous entity. Many risk factors for breast cancer are modest in nature. We hypothesize that some risk factors will only be related to certain subgroups of cases defined by histologic subtype, estrogen and progesterone receptor (ER/PR) status, stage and grade. By separating cases into more homogeneous subgroups defined by pathologic criteria, we hope to understand how risk factors relate to the multiple underlying pathways.

Application ID: B-EX-0403-01 **Status:** Approved, Ongoing

Principal Investigator: Tilley, Wayne D. **Year:** 2003

Institution: The University of Adelaide, Hanson Institute

Title: *The Role of the Androgen Receptor in Early-Onset Breast Cancer*

Abstract:

Androgens suppress the growth of breast cancer cells in culture, and when used in vivo have an efficacy comparable to tamoxifen. However the masculinizing side effects of androgens have limited their clinical utility. A better understanding of the androgen signaling pathways in breast cancer cells would facilitate the development of novel androgen receptor (AR)-based strategies that regulate breast cancer growth but avoid the side effects of androgens. In this proposal we will determine whether AR signaling plays a protective role in breast cancer, in a well-characterized, population-based cohort of 800 breast cancer patients with early onset disease. First we will investigate whether expression of AR, by immunohistochemical staining, in breast tumors is associated with more aggressive disease, as measured by standard predictors of prognosis, tumor size, grade, stage (including nodal status), and ER and PR immunohistochemistry, and poorer outcome, as measured by mortality and recurrence. Secondly, we will determine whether the AR, as an X-linked gene, is selectively X-inactivated in breast tumors such that less active alleles are preferentially expressed. The length of the polyglutamine tract in the AR protein alters its activity, with shorter repeats having greater activity than long repeats. For tumors expressing AR from cases who are heterozygous for upper and lower tertile of polyglutamine repeat length, we will use methylation analysis of microdissected DNA to determine whether those with selective X-inactivation of the shorter, more active AR allele, are more likely to present with more aggressive disease and have poorer outcome. These studies will provide important information about the role of the androgen receptor in breast cancer. The results we obtain will provide new insight for the development of AR-based strategies to regulate breast cancer growth, thereby increasing the therapeutic options available to breast cancer patients.

Specific Aim(s):

Aims

Aim 1: To determine the levels of expression of the AR in malignant breast tumors and relate this to the aggressiveness of the disease at diagnosis and outcome.

Aim 2: To determine whether women whose breast tumors express a shorter, more active, AR-CAG allele have more aggressive disease at diagnosis than women whose tumors express a longer allele, and worse outcome.

Hypothesis

We hypothesise that androgen receptor signaling is protective against breast cancer cell growth in vivo.

Application ID: B-SF-0403-02 **Status:** Approved, Ongoing

Principal Investigator: Whittemore, Alice **Year:** 2003

Institution: Stanford University School of Medicine

Title: *A Study of Oral Contraceptives and Ovarian Cancer Risk among Carriers of BRCA1 or BRCA2 Mutations*

Abstract:

Women who carry deleterious mutations of the genes BRCA1 or BRCA2 are at increased risk of developing ovarian cancer [Struewing et al., 1997; Ford et al., 1998]. Oral contraceptive use is associated with reduced ovarian cancer risk in the general population [Whittemore et al., 1992; LaVecchia et al., 1999]. It is important to know if a similar risk reduction holds for mutation carriers. Previous investigations have addressed this question with conflicting results [Narod et al., 1998, 2001; Modan et al., 2001]. Resolution of this issue is important because oral contraceptive use at early ages may increase the risk of breast cancer in mutation carriers [Ursin et al., 1997; Grabick et al., 2000; Narod et al., 2002].

We need these data because it is so difficult to obtain cases and controls simultaneously matched on mutation type (BRCA1 vs. BRCA2), country of residence, and year of birth for adequate power to detect the hypothesized reduced risk.

We aim to evaluate the associations between ovarian cancer risk and oral contraceptive use in women who are carriers of deleterious BRCA1 or BRCA2 mutations.

We wish to gather data on ovarian cancer status, oral contraceptive use, and reproductive history among carriers for a matched case-control analysis. Cases and controls have been ascertained from the United Kingdom Consortium for Clinical Cancer Research (UKCCCR) Ovarian Cancer Register, Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab), Gilda Radner Familial Ovarian Cancer Registry at the Roswell Park Cancer Institute in Buffalo, NY, and Risk Assessment Program at the Fox Chase Cancer Center in Philadelphia, PA. However, we need more carrier cases and matched controls to adequately address this issue.

Specific Aim(s):

Hypothesis

The use of oral contraceptives for at least one year reduces the risk of ovarian cancer among women who carry mutations of BRCA1 or BRCA2. The risk of ovarian cancer decreases as duration of use of oral contraceptives increases.

Application ID: B-NY-1202-01 **Status:** Approved, Ongoing

Principal Investigator: Ahsan, Habibul **Year:** 2002

Institution: Columbia University Mailman School of Public Health

Title: *CHEK2*1100delC Allele and Breast Cancer in Families Who Do Not Harbor Mutations in the BRCA1/2 Genes*

Abstract:

The human checkpoint kinase 2 (CHEK2, also known as CHK2), the human ortholog of yeast Cds1 and Rad53, encodes a cell-cycle checkpoint kinase that plays a role in DNA repair processes involving BRCA1 and p53 and is thus a candidate gene for familial breast cancer and Li-Fraumeni Syndromes (LFS). Although several germline missense mutations in the CHEK2 gene have been reported in LFS families who are negative for p53 mutations, they were not associated with breast cancer. One particular deletion mutation (1100delC) in the CHEK2 gene, which was first identified in a p53-wildtype LFS family with 4 breast cancers, has recently been shown to increase the risk of breast cancer in North American and European families who are negative for BRCA1 and BRCA2 mutations as compared to controls from the same countries. This frameshift mutation on codon 366 (due to deletion of a single base), which causes premature termination at codon 381, clearly abrogates the kinase activity of the encoded protein.

Specific Aim(s):

Hypothesis

We hypothesize that the CHEK2*1100delC allele is associated with breast cancer in families who do not harbor mutations in the BRCA1 and BRCA2 genes. Using a family-based design, we propose to examine this hypothesis among 1,612 BRCA1 and BRCA2 negative families from 5 North American centers participating in the NCI's Breast Cancer Family Registry (BCFR) for whom genomic DNA and questionnaire and family data have already been collected.

Application ID: B-TO-1202-03 **Status:** Approved, Ongoing

Principal Investigator: Andrulis, Irene **Year:** 2002

Institution: Cancer Care Ontario

Title: *Germline Alleles of the Human PS-2 Gene and Breast Cancer*

Abstract:

Important insights into the mechanisms of malignant growth have been gained through the identification and characterization of genes that are common targets of genetic alterations in tumor tissues. In a study of genes differentially expressed in axillary node-negative breast cancer, we identified several genes that mapped to chromosomal regions that frequently undergo loss of heterozygosity (LOH) and may be the sites of tumor suppressor genes. One of these was found to be Presenilin 2 (PS-2), one of two presenilins implicated in familial Alzheimer's disease (FAD). Because functional studies have also suggested a role for the presenilins in Notch signaling and cancer-related pathways, we examined primary human tumors for mutations in PS-2. Using SSCP analysis and direct DNA sequencing of colon and breast tumors, we identified two missense alterations (R62H variant and R71W mutation) that occur in the amino hydrophilic domain of the PS-2 protein. Corresponding normal tissue was available for the colon cancer cases and the changes were found to be germline alterations.

Using biochemical assays and a C. elegans in vivo assay for Notch signaling, we have found that the mutant and variant are less efficient than WT PS-2. In contrast, they did not cause an increase in A peptide, a major component of the amyloidogenic deposits in the brains of AD patients. Furthermore, both R62H and R71W alterations compromised the ability of PS-2 to impeded cellular growth in PS-2(-/-) mouse embryonic fibroblasts (MEF). We determined that these missense variants lead to a more rapid degradation of PS-2 full-length protein. In support of this biochemical finding, the inefficiency the variants to function in C. elegans and PS-2(-/-) MEF could be compensated by increasing the level of expression, indicating that the mutant and variant have a quantitative effect on PS-2 protein functions.

In a pilot study of 271 colon cancer cases, the alterations were found to occur more frequently in cases under the age of 35 (Cochran-Armitage trend test; p=0.009), suggesting that they may be associated with an early onset of colorectal carcinogenesis. We have identified 4 alterations in 174 axillary node-negative breast cancers and 4 alterations in blood DNAs from 124 breast cancer cases under the age of 45. The pilot study did not have sufficient power to detect an age effect in the breast cancer cases. In addition, we examined 394 non-cancer controls under the age of 55 and found the R62H variant in 3 individuals (0.8%), but did not detect the R71W mutation in any of the 394 non-cancer controls. Based on combination of the biochemical, functional, and genetic data, we hypothesize that these PS-2 alleles alter the function of the protein and may confer a moderate risk of susceptibility to cancer.

We propose a Registry-wide study to validate our original observations and to determine whether PS-2 alterations play a role in hereditary breast cancer. A similar collaborative study is proposed for the Colon CFR.

Specific Aim(s):

1. To determine the frequency of the variant and the mutant PS-2 alleles in breast cancer cases from the Breast CFR and to test whether there is an association of the mutant allele and breast cancer using cases and controls.

2. To investigate whether there are any age effects of the PS-2 alleles in breast cancer.

3. To examine whether other PS-2 alleles are associated with breast cancer using the Breast CFR.

Application ID: B-TO-0402-02 **Status:** Approved, Ongoing

Principal Investigator: Boyd, Norman **Year:** 2002

Institution: The Ontario Cancer Institute

Title: *The Genetic Epidemiology of Breast Cancer: A Case-Parental Control Study of Candidate Genes and Risk of Premenopausal Breast Cancer and Breast Density Phenotype*

Abstract:

The goal of this study is to find genetic determinants of breast cancer risk. One of the strongest risk factors for breast cancer, mammographic density, is a phenotypic trait that is determined by environmental and genetic factors. One recent twin study determined that the heritability of breast density is at least 75%. Mammographic density is a continuous trait that is likely determined by multiple genes and phenotypic variation in mammographic density is likely the result of normal genetic variation and environmental factors. It may be possible to find genes other than BRCA1 and BRCA2 that are involved in familial breast cancer by looking for genes that influence breast density. Also, the genes that influence breast density may increase breast cancer risk. Examining the results of studies assessing the observed association between lipoproteins and breast cancer yields likely candidate genes for breast density and breast cancer. One particular fasting lipoprotein profile has been observed among women at high risk of breast cancer as measured by previous incidence of the disease, family history of breast cancer or increased mammographic density, which cannot be explained by measured environmental factors including dietary fat. Genes controlling lipoprotein metabolism, either directly or indirectly, may contribute to both breast density and breast cancer risk. The following candidate genes have been selected as the most likely to explain the association between lipoprotein metabolism and risk of breast cancer: apolipoprotein E, apolipoprotein CIII, hepatic lipase, seven alpha hydroxylase, paraoxonase, lipoprotein lipase, growth hormone, growth hormone receptor, growth hormone releasing hormone, and growth hormone releasing hormone receptor. To test for an association between the candidate genes and breast cancer and breast density, a population based case-parental control study has been designed, which maximizes power and eliminates confounding due to population stratification. To complete this study, 289 families will be required from the Toronto and Australian sites of the CFR; the Toronto site has supplied DNA for 113 families. Cases are premenopausal women diagnosed with incident invasive breast cancer (ICD 174) before age 50. For the family to be eligible, at least one parent must have DNA available for genotyping. Genotyping will be carried out using standard PCR-based methods. Mammographic density will be measured by standard computer assisted methods. Statistical analyses will be carried out using the transmission disequilibrium test, which is appropriate for both categorical outcomes such as breast cancer and continuous outcomes such as mammographic density. Finally, the study as designed is both cost-effective and is a highly appropriate method to test candidate genes for a linkage with breast cancer risk. This study is a collaborative effort between the Australian and Canadian sites of the CFRBCS.

*Original application B-TO-1200-01S

Specific Aim(s):

Hypothesis:

Genes involved in the regulation of lipoprotein metabolism may be candidates for increased risk of breast cancer, and these genes may explain some of the variation in breast density phenotype measured mammographically.

Purpose: To determine whether common polymorphisms of the lipoprotein metabolism regulatory genes, including genes involved in growth hormone signalling which influence lipoprotein metabolism, are linked to breast cancer and the breast density phenotype in premenopausal women. The genes to be tested are the apolipoprotein E (APO E), apolipoprotein CIII (APO CIII), hepatic lipase (HL), 7 alpha hydroxylase (CYP7), paraoxonase (PON1), lipoprotein lipase (LPL), pituitary growth hormone (GH1), growth hormone receptor (GHR), and growth hormone releasing hormone (GHRH) and its receptor (GHRHR).

Specific Hypotheses to be tested:

1. Common polymorphisms of genes involved in lipoprotein metabolism may be linked with familial breast cancer in premenopausal women. The genes to be tested are apo E, apo CIII, HL, CYP7, PON, LPL, GH1, GHR, GHRH,and GHRHR. Refer to Appendix 1 for detailed information on the selected polymorphisms.

2. These same polymorphisms may be linked with phenotypic variation in mammographic density in premenopausal women.

*Original application B-TO-1200-01S

Application ID: B-TO-1202-01 **Status:** Approved, Ongoing

Principal Investigator: Knight, Julia **Year:** 2002

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital

Title: *Potential Modification of the Effect of Alcohol on Breast Cancer Risk by Variation in Genes Involved in Alcohol Metabolism*

Abstract:

There is considerable evidence that alcohol is a modest risk factor for breast cancer. Alcohol metabolism is at least partially genetically determined by a number of genes coding for enzymes involved in the process. These genes are polymorphic with functional consequences. This system (alcohol and genes involved in alcohol metabolism) appears to be a good candidate for potential gene-environment interaction with respect to breast cancer risk. A few, mostly small, studies have considered this, but the issue is by no means resolved. A very large study that can examine a number of polymorphisms, a variety of alcohol parameters, and potential confounders such as ethnicity and other breast cancer risk factors would be useful. Such an investigation would contribute to public health by 1) helping to identify subgroups who may be at higher risk from the harmful effects of alcohol and 2) providing data to support potential biological mechanisms which will help to rule out confounding as an explanation for the modest association seen between alcohol intake and breast cancer. The Breast Cancer Family Registry (BCFR) with DNA and extensive risk factor data, including information on alcohol consumption, from unrelated cases and controls as well as sister sets provides an excellent opportunity to examine this question. We propose to carry out a case

control study and a study of sisters within the BCFR to address the question of whether functional, or likely functional, polymorphisms in genes involved in alcohol metabolism modify the effect of alcohol on breast cancer risk. This is a particularly important area to address as alcohol intake is one of the few potentially modifiable breast cancer risk factors.

Specific Aim(s):

Hypothesis

Alcohol is becoming an established risk factor for breast cancer. Pooled analyses have consistently shown an association between alcohol intake and breast cancer risk. However, the risk elevation is relatively small, approximately a 10% increase in risk per drink per day. There are number of genes that code for enzymes involved in alcohol metabolism. Some of these genes are known to be polymorphic with known functional consequences. It is possible that the relationship between alcohol and breast cancer may be modified by genotype, leading to greater risks associated with alcohol in a subgroup of the population.

Application ID: B-TO-0802-02 **Status:** Approved, Ongoing

Principal Investigator: Knight, Julia **Year:** 2002

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto

Title: *VDR Genetic Variants and Breast Cancer*

Abstract:

There is considerable evidence for an antiproliferative effect of vitamin D and its analogues on epithelial cells. In animal models, the vitamin D receptor (VDR) plays a role in normal breast development and an analog of vitamin D can prevent chemically-induced breast tumours. There is also some evidence from epidemiologic studies that sunlight or increased vitamin D levels may protect against breast cancer. The evidence relating to dietary vitamin D, including supplements, is limited. Although sunlight is the primary source of vitamin D, dietary vitamin D still contributes significantly to vitamin D levels, particularly in winter. It likely plays a greater role in geographic areas with limited sun exposure than in those with plentiful sun exposure. The effect of dietary vitamin D may also vary depending on VDR genotype. We have been conducting a case control study of VDR genetic variants and breast cancer in the Ontario site of the Breast Cancer Family Registry (BCFR). We propose to examine the relationship of dietary vitamin D and breast cancer in a case control analysis using data from all three population-based sites of the BCFR. In particular, we propose that the effect may differ among the three sites, Ontario, Northern California, and Australia, as exposure to ultraviolet light from the sun varies. In addition, we wish to carry out a pilot study to examine possible interaction between dietary vitamin D and VDR genotype in Ontario, where the VDR genotyping has been completed. The BCFR provides the advantage of consistently collected dietary data across geographic sites with varying sunlight exposure.

Specific Aim(s):

The primary hypothesis is that increased intake of dietary vitamin D does protect against breast cancer development and that the magnitude of the effect will depend on average ultraviolet light exposure from sunlight, determined by geographic location. A secondary hypothesis is that the effect of dietary vitamin D varies with either the FokI or polyA genotype.

Application ID: B-UT-1202-01-A1 **Status:** Approved, Ongoing

Principal Investigator: Neuhausen, Susan L. **Year:** 2002

Institution: University of California, Irvine

Title: *The IGF Pathway and Breast Cancer Risk in African Americans*

Abstract:

We propose a comprehensive analysis of genes in the insulin-like growth factor signaling pathway, a pathway integrally involved in cellular proliferation, and genes of proteins that cross-talk with this pathway. We will use powerful new approaches that combine multiple linked variants in a single gene to form haplotypes and that allow evaluation of multiple genes in a single mechanistic pathway. We will study female African-American breast cancer cases and population-based controls. Few studies have been conducted in African Americans, even though they often present at a younger age and have more aggressive cancer at presentation. We will study genetic variants in IGF1, IGF1R, IGFBP1, IGFBP3, IGFBP5, IRS1, SHBG, IGFBP2, IGFBP4, IGFBP6, IGFBP7, IRS2 PI3K, SHC, MTOR, MMP-7, ESR1, ESR2, INSR, INS, and mtG10398A. To accomplish our objectives, we will first identify the Single Nucleotide Polymorphisms (SNPs) in these genes either through sequencing in 50 unrelated African Americans or obtaining in silico sequencing information. Our objective is to mark the common variation across the genes while minimizing genotyping in the larger cohort. Haplotype-tagging SNPs, including all those with known function, will be selected. We will genotype affected (cases) and unaffected (matched controls) for SNPs selected in Aim 1. Lastly, analyses will be done to evaluate the role of the genotypes with breast cancer development, age at diagnosis, stage, tumor size, and lymph node status. Our focus is African American women who, because of their higher risk of developing aggressive cancer at an early age, are an essential group in which to better identify risk factors for developing this disease. This information can be used to target women for prevention or treatment strategies.

Application ID: B-TO-1202-04 **Status:** Approved, Ongoing

Principal Investigator: O'Malley, Frances **Year:** 2002

Institution: Cancer Care Ontario

Title: *Breast Cancer in BRCA2 Mutation Carriers: Histopathological Phenotype and Investigation of Cell Cycle*

Abstract:

Studies have consistently described differences between BRCA1 associated breast cancer and sporadic breast cancers without germline mutations in this gene. However, the literature on breast cancers in BRCA2 mutation carriers is less prolific and so far, a consensus has not been reached on a distinct histopathologic phenotype for these cancers. Likewise, the expression profile of these tumors for estrogen receptor (ER), progesterone receptor (PR), and HER2/neu has not been clearly elucidated. BRCA2 functions, in part, to repair double stranded DNA breaks. In its absence, somatic mutations accumulate. It has been hypothesized that somatic mutations at critical cell cycle control checkpoints must accompany BRCA2 mutations in tissues for tumor initiation and progression.

The objective of this study is two-fold: (1) to perform detailed histopathologic review of BRCA2 associated breast cancers and to determine their biomarker profile for ER/PR and HER2/neu, and (2) to investigate at a molecular level, mutations in known cell cycle control checkpoints, namely p53 and cyclin D1.

Breast cancers in BRCA2 mutation carriers and age-matched controls will be accrued from all sites of a Cooperative Family Registry for Breast Cancer Studies. All breast cancers will be reviewed by pathologists who are part of this Cooperative group. The participating pathologists have developed a uniform pathology data form that is used across all 6 sites. Paraffin tumor blocks collected at all sites will be used to construct tissue microarrays (TMA) from this cohort. Once constructed, the TMA blocks will be analyzed for ER, PR, HER2/neu, p53, and cyclin D1 utilizing immunohistochemical and fluorescence in situ hybridization methodology, where appropriate. As well, single strand conformation polymorphism (SSCP) and direct sequencing will be performed to study p53 mutations in these tumors.

Specific Aim(s):

We hypothesize that breast cancers in BRCA2 mutation carriers are of higher grade and are more likely to be ER and PR positive and negative for HER2/neu protein overexpression than breast cancers in sporadic controls. At a molecular level, we expect that BRCA2 associated tumors will demonstrate a high frequency and unique spectrum of p53 mutations and that high cyclin D1 levels within these tumors will also be detected.

The specific objectives of this study are:

1. To determine if a distinct histopathologic phenotype of BRCA2 associated breast cancers exists and to determine the biomarker profile for ER/PR expression and HER2/neu overexpression in these tumours.

2. To investigate molecular alterations in known cell cycle control checkpoints, namely p53 and cyclin D1.

Application ID: B-TO-1202-05 **Status:** Approved, Ongoing

Principal Investigator: Ozcelik, Hilmi **Year:** 2002

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital

Title: *Variants of Cell Cycle Pathway and Breast Cancer Risk*

Abstract:

Single Nucleotide Polymorphisms (SNPs) have been implicated as risk alleles in different genetic diseases including breast cancer. Searching for disease causing SNPs is an ambitious task due to the complex nature of many phenotypes of interest and the enormous number of SNPs to be analyzed. Innovative strategies may help to target the molecular studies to a group of SNPs that may be more likely to confer disease risk. A fraction of SNPs are non-synonymous (ns) resulting in amino acid substitutions in the coding regions of genes. Although the majority of nsSNPs are harmless, it is estimated that a fraction will affect the structure and function of the proteins. Their effect may be modest at the functional level, but important nonetheless. These nsSNPs may directly alter or modify cancer risk induced by other factors, when they occur within genes known to function in biological pathways relevant to that disease. We have developed a computational model to investigate the functional effects of a set of 169 nsSNPs from over 53 genes involved in cell cycle pathway. We have determined potentially functional SNPs among this set by investigating the evolutionary conservation of SNP sequences using SIFT alignment program. Additionally the effects of SNPs on physicochemical properties of amino acids and phosphorylation status of the protein have been investigated. The outputs of these computational prediction tools are evaluated to rank the nsSNPs according to their likelihood of being deleterious. In this study we propose to use the strengths of the Cooperative Familial Registries of Breast Cancer Studies and a throughput SNP-genotyping 5′ nuclease (TaqMan) technology, to make a significant inquiry into the possible roles of genetic variants in cell cycle genes on the risk of breast cancer. We propose to study the contribution of the selected SNPs using a case control design. The positive results will be validated using the family-based design. Identification of novel breast cancer predisposition alleles will help us to expand our knowledge of the critical molecular events underlying breast etiology and pathogenesis, leading toward improved strategies for the management of breast cancer. The results of this study will provide important conclusions regarding the specific role of cell cycle variants in modulating breast cancer risk.

Specific Aim(s):

Aims

1. To study the association of functional cell cycle SNPs in population-based series of women affected with breast cancer and controls frequency matched by age.

2. To validate the positive associations of cell cycle SNPs using the family based approach.

3. To study the interactions of genes/proteins with potentially functional SNPs from the same pathway.

Hypothesis

Variation, due to SNPs, in the function of cell cycle proteins contributes to breast cancer risk. We hypothesize that the collective study of gene variants within the same pathway may provide important knowledge on understanding the role of cell cycle genes in the etiology of cancer. Although these allelic variants may individually confer a small effect on the overall breast cancer risk, their common occurrence in the population could contribute to a large proportion of breast cancer cases.

Application ID: B-TO-1002-01S **Status:** Approved, Ongoing

Principal Investigator: Ozcelik, Hilmi **Year:** 2002

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto

Title: *Prediction of Deleterious Breast Cancer Alleles Based on Protein Structure and Function Using Bioinformatics*

Abstract:

Single Nucleotide Polymorphisms (SNPs) have been implicated as risk alleles in different genetic diseases including breast cancer. Searching for disease causing SNPs is an ambitious task due to the complex nature of many phenotypes of interest and the enormous number of SNPs to be analyzed. Innovative strategies may help to target the molecular studies to a group of SNPs that may be more likely to confer disease risk. A fraction of SNPs are non-synonymous (ns), resulting in amino acid substitutions in the coding regions of genes. Although the majority of nsSNPs are harmless, it is estimated that a fraction will affect the structure and function of the proteins. Their effect may be modest at the functional level, but important nonetheless. These nsSNPs may directly alter or modify cancer risk induced by other factors when they occur within genes known to function in biological pathways relevant to that disease. We propose a computational model to investigate the functional effects of a set of 1,000 nsSNPs from more than 500 genes involved in DNA repair, cell cycle, angiogenesis, and carcinogen metabolism pathways. The nsSNPs will be investigated using: (1) Web-based public databases containing information related to the structure and important amino acid residues of proteins, and (2) Web-based computational tools designed to predict the effect of amino acid substitutions on the protein structure and function. The prediction model investigates the: (1) sequence-conservation in related proteins, (2) structural alteration in the secondary and tertiary conformation, (3) effect on stability, (4) alteration in critical contact sites with other proteins, (5) effect on protein-phosphorylation sites, and (6) changes in the physicochemical characteristics including charge, volume, and polarity of the amino acids. The outputs of these computational prediction tools will be evaluated to rank the nsSNPs according to their likelihood of being deleterious. We will further study the top ranking 50 nsSNPs to validate their status and estimate their allelic frequencies in the population using direct sequencing and single strand conformation polymorphism (SSCP) analysis. The current proposal will allow the development of a model to establish a valuable prioritized resource of potentially functional SNPs. The association of these potentially deleterious SNPs with breast cancer risk will be investigated as a future goal by taking advantage of resources provided by breast cancer registries. The identification of novel breast cancer predisposition alleles will help us to expand our knowledge of the critical molecular events underlying breast etiology and pathogenesis, leading toward improved strategies for the management of breast cancer.

Application ID: B-TO-1202-02 **Status:** Approved, Ongoing

Principal Investigator: Rommens, Johanna M. **Year:** 2002

Institution: The Hospital for Sick Children

Title: *Genetic Studies of Mammographic Density*

Abstract:

The long term goal of the proposed research is to increase the understanding of the etiology of breast cancer (1) by investigating the genetic determinants of mammographic density, one of the strongest known risk factors for breast cancer. Percent mammographic densities (PMD) refers to variations in the breast among women that reflect differences in tissue composition. Women who have breast density (>75%) have a risk of breast cancer 4-6 times greater than women of the same age with negligible measured breast density (2, 3). Twin studies indicate that the heritability of PMD is 63% (4). PMD is a continuous trait with a unimodal normal distribution and is thus likely to be influenced by allelic variation in multiple genes. We propose to determine new genetic factors as well as evaluate suspected candidate genes that determine variation in PMD. The high heritability of PMD provides an excellent opportunity to identify contributing genes; information of this quantitative trait can be gained from both females with and without breast cancer. Genetic mapping of PMD loci and identification of the genes involved will allow for the testing of their influence on risk of breast cancer.

Our specific aims are:

1) To collect PMD data from 1,846 female sibpairs who have participated in the population- based Breast CFRs in Ontario, California and Australia.

2) To identify chromosomal regions linked to PMD using these sibpair families by performing a genome-wide scan.

3) To fine map loci linked to PMD and identify the underlying genes using additional genetic markers and linkage and association analyses. Candidate genes will be identified based on annotated genome sequence and/or genomic sequence analysis and will be prioritized based on recognized biological features.

4) To assess variants of candidate genes involved in estrogen metabolism for association with PMD.

5) To establish if and how genes affect PMD variation influence susceptibility to breast cancer. Variants found to be associated with PMD will be tested for association with breast cancer in the same families.

Specific Aim(s):

Hypothesis

Since PMD is highly heritable we hypothesize that major genes influence this quantitative trait and that they can be mapped using a genome-wide linkage approach. Such loci will be fine-mapped to identify the genes responsible. Because PMD is a major risk factor for breast cancer, we hypothesize that some of the genes that influence PMD are also susceptibility genes for breast cancer.

Application ID: B-SF-0602-01S **Status:** Approved, Ongoing

Principal Investigator: Whittemore, Alice **Year:** 2002

Institution: Stanford University School of Medicine

Title: *Risk of Site-Specific Cancers and Death in First-Degree Relatives of Population-based Incident Cases of Breast and Ovarian Cancer*

Abstract:

Many women have mothers, sister, or daughters with cancers of breast or ovary. These women need to know about their risks of cancer and other fatal events. Little is know about the risks among women whose cancer-afflicted relatives are BRCA-negative. Moreover, women whose afflicted relatives have breast cancer may have higher breast cancer risks than women whose relatives have ovarian cancer (or vice-versa). If so, such differences in risk would provide evidence for the presence or breast and ovarian cancer genes other than BRCA1 and BRCA2.

Specific Aim(s):

Aim

We propose to compare the risks of site-specific cancers and all-cause mortality among first-degree relatives of population-based incident breast cancer cases to that among first-degree relatives of population-based incident ovarian cancer cases. Data from incident cases of breast cancer (hereafter called breast cancer probands) and their relatives will be obtained from the Northern California component of the CFRBCS. Data from incident cases of ovarian cancer (ovarian cancer probands) and their relatives will be obtained from the Northern California Familial Registry of Ovarian Cancer (FROG).

Our hypotheses are:

1) relatives of breast cancer probands and ovarian cancer probands without BRCA1 or BRCA2 mutations have elevated mortality and site-specific cancer risks;

2) relatives of breast cancer probands (both with and without BRCA mutations) have higher breast cancer risks and lower cancer risks than do relatives of ovarian cancer probands.

Application ID: B-NY-0601-02S **Status:** Approved, Ongoing

Principal Investigator: Bagiella, Emilia **Year:** 2001

Institution: Columbia University

Title: *Validation of BRCAPRO in Ashkenazi Women*

Abstract:

The primary objective of this study is to use the Ashkenazi portion of the sample from the Cooperative Family Registry for Breast Cancer Studies (CFRBCS) to validate the BRCAPRO program developed by Parmigiani et al. for predicting BRCA1 and BRCA2 carrier status from family disease history.

Secondary objectives are:

to compare estimates from the BRCAPRO program with estimates from an analogous algorithm developed by Hopper et al. to predict the probability of carrying a BRCA1 or BRCA2 mutation in Ashkenazi Jewish women;

to evaluate if the information from relatives other than first or second degree improves the prediction of carrier probabilities;

to evaluate if the information about occurrence of cancers other than breast or ovary improves the prediction of carrier probabilities.

The primary analysis will focus on whether the expected estimated probabilities are consistent with the observed status. The test statistics will be a normalized sum, across subjects, of the difference between the true status and the estimated probability. Logistic models will be used to evaluate the impact of greater than second degree relatives and other cancers on the estimated carrier probability.

Specific Aim(s):

1. Use the BRCAPRO program to estimate the probability of BRCA1 and BRCA2 carrier status in the Ashkenazi portion of the CFRBCS subjects.

2. To quantify the prediction accuracy of the estimated rates from the BRCAPRO program in the Ashkenazi population.

3. To compare the BRCAPRO estimates with estimates obtained from an analogous algorithm developed by Hopper et al., for predicting carrier probabilities in Ashkenazi women.

4. To evaluate if the information from relatives other than first or second degree improves the prediction of carrier probabilities.

5. To evaluate if the information about occurrence of cancers other than breast or ovary improves the prediction of carrier probabilities.

Application ID: B-AU-1201-02 **Status:** Approved, Ongoing

Principal Investigator: Chenevix-Trench, Georgia **Year:** 2001

Institution: Queensland Institute of Medical Research

Title: *DNA Repair Gene Variants in Early Onset Breast Cancer*

Abstract:

A substantial proportion of familial aggregation of breast cancer is likely to be due to "low-risk" genetic factors. Candidates for “low-risk” genetic factors are common but subtle functional variants in genes likely to be involved in cancer predisposition. Genes of interest include those mediating a range of functions such as steroid hormone metabolism, cell cycle control and DNA repair. To date many studies have investigated the role of genes involved in steroid metabolism, but the role of DNA repair genes is largely unknown. We intend to carry out a two-phase study of early onset breast cancer using material already collected by the three population-based sites in Australia, California and Canada from the Cancer Family Registry for Breast Cancer Studies (CFRBCS). For DNA repair gene polymorphisms in which an association with breast cancer risk is found in the case-control comparison in Phase 1, we will extend the study to a family-based design in Phase 2. If associations with breast cancer risk are confirmed using these complementary methods, it will provide strong evidence that the association is not a false positive due to population stratification or multiple comparisons. We will focus exclusively on non-synonymous, non-conservative SNPs and all genotyping will be performed with the ‘TaqMan’ technology. Unconditional logistic regression will be used for the analysis of Phase 1, and modified segregation analysis and likelihood-based approaches for Phase 2. The large scale of this study allows us to test both recessive and dominant models with substantial power to detect moderate effects, and the two Phase study will allow us to draw definite conclusions about the role of non-synonymous, non-conservative DNA repair gene SNPs in the etiology of early onset breast cancer.

Specific Aim(s):

Hypothesis

Certain allelic variants of genes involved in DNA repair confer increased risks of breast cancer in young women.

Specific Aims

Aim 1: to genotype non-conservative, non-synonymous coding region polymorphisms in a population-based series of women affected with breast cancer before the age of 40, already assembled by the CFRBCS, and controls frequency-matched for age, and thereby identify polymorphisms in low-risk predisposition genes acting in different DNA repair pathways that could be associated with risk of early onset female breast cancer - Phase 1.

Aim 2: to genotype relatives in the nuclear families, already assembled by the population-based sites of the CFRBCS, for any polymorphisms found to show an association with breast cancer risk in Phase 1 in an attempt to replicate the finding by family-based analyses that will provide an essentially independent validation free from stratification bias – Phase 2.

Application ID: B-AU-1201-01 **Status:** Approved, Ongoing

Principal Investigator: Chenevix-Trench, Georgia **Year:** 2001

Institution: Queensland Institute of Medical Research

Title: *Genetic Modifiers of BRCA1 and BRCA2*

Abstract:

Mutations in BRCA1 and BRCA2 are not fully penetrant, suggesting that other genetic or environmental factors modify the expression of disease-causing mutations in BRCA1 and BRCA2. Identification of genetic modifiers, if validated in independent studies, will eventually allow more accurate assessment of penetrance, expression and age of onset in carriers. Some modifier genes have been reported in studies of candidate genes in BRCA1 and BRCA2 carriers, but only the effect of the 3' UTR single nucleotide polymorphism (SNP) in RAD51 has been confirmed in independent studies. The literature on association studies, aimed at identifying low risk genes that contribute to the risk for 'sporadic' breast cancer, is fraught with positive findings that cannot be replicated in subsequent independent studies. It is therefore imperative that studies of 'low risk' modifying genes are not accepted or considered for clinical use until they are replicated. However, it is also imperative that such replication studies be conducted with equal or greater power than the original study. We have collected DNA samples from 557 BRCA1 and BRCA2 female carriers from various Australian sources (mainly kConFaB), and also the EMBRACE study in the UK and we wish to access the CFRBCS carriers as a replication set for any positive findings that we obtain from the Australian/UK carrier set. SNPs will chosen from genes involved in DNA repair, hormone metabolism and detoxification of carcinogens. Genotyping will be performed by the 'TaqMan' method. Multivariate analyses will use multiple logistic regression to test for any associations between particular genotypes and cancer, while taking into account potential confounding effects of measured breast cancer risk factors and using survival analyses to determine whether a given DNA variant influences diseased free survival. Stratification by ethnicity will be used to compare the effects in Ashkenazi and non-Ashkenazi carriers.

Specific Aim(s):

1) That genetic modifiers of BRCA1 and BRCA2 expression exist that affect age of onset, penetrance and type of cancer.

2) That some of these genetic modifiers will be genes involved in DNA repair, hormone metabolism and detoxification of carcinogens, and will include polymorphisms in highly penetrant susceptibility genes such as BRCA1 and BRCA2.

Application ID: B-TO-0801-01 **Status:** Approved, Ongoing

Principal Investigator: Cotterchio, Michelle **Year:** 2001

Institution: Cancer Care Ontario

Title: *Mouse Mammary Tumour Virus (MMTV) and Human Breast Cancer*

Abstract:

It has long been known that mice infected with the Mouse Mammary Tumour Virus (MMTV), an insertional mutagen, develop breast cancer. Recently, well conducted human studies detected an MMTV-env DNA sequence in 40% of human breast cancers, but in less than 4% of normal breast tissue or blood. These studies employed PCR techniques using primers that were specific to the MMTV-env gene and not significantly homologous to any human endogenous retrovirus sequences or other human gene. Furthermore, expression of MMTV was detected in 66% of these MMTV-positive breast tumours and in none of the MMTV-negative tumours or normal breast tissues, and the entire MMTV provirus has been identified in human breast cancers. The intriguing evidence to date suggests that the etiology of a subgroup of human breast cancers may have MMTV involvement, and this warrants further investigation. The proposed pilot study will explore whether certain epidemiological variables are correlated with MMTV-positive breast tumours. One hundred breast cancer patients participating in the Ontario Familial Breast Cancer Registry will be randomly selected for this study. DNA will be isolated from slides of microdissected paraffin-embedded breast tumour tissue. We will use the 250 base-pair PCR primers and similar conditions to those in the previous MMTV publications that worked with paraffin samples. Positives will be confirmed by Southern blotting of the PCR products using an internal oligonucleotide probe as well as by sequencing the purified PCR products. Following the laboratory analysis of the breast tumour tissue blocks, women with breast tumours that have MMTV DNA detected will be considered MMTV-positive and the remaining breast cancer patients, with no MMTV detected, will be considered MMTV-negative. Epidemiologic factors associated with the risk of MMTV-positive (vs. negative) breast cancer will be examined univariately using Chi-square statistics and by computing crude odds ratio (OR) estimates and approximate 95% confidence intervals. Multivariate logistic regression will be used to estimate the OR estimates associated with the risk of MMTV-positive breast cancer compared to MMTV-negative breast cancer. The purpose of this pilot study is to generate hypotheses based on patterns seen in the data that will then be further evaluated in a full grant proposal.

Specific Aim(s):

AIMS OF THE PROPOSED PILOT STUDY

1) To refine the laboratory methods needed to specifically detect MMTV DNA sequences using our PCR machines and parafn-embedded breast tumour tissue samples from Ontario women, and to attempt to replicate the previously published finding that MMTV DNA is detected in ~40% of human breast cancer
tumours.

2) To explore the association between various epidemiologic factors (e.g., family history, parity) and MMVpositive breast cancer (vs. MMTV-negative breast cancer) using breast tumour tissue samples and data collected by the Ontario Familial Breast Cancer Registry (OFBCR).

Examples of specifc hypotheses:

a) ifMMTV is passed on through mechanisms such as breast-feeding or the
genetic transmission of pro viruses (1) or if mice infect directly (8), then it is expected that breast cancer patients with MMTV-positive tumours would be more likely to have a first degree relative with breast cancer (vs. MMV-negative)~ b) ifMMTV is pregnancy-related (6), parity may be associated with MMTV-positive breast cancer~ c) since the MMTV promoter responds to estrogen and progesterone (1) we might expect that hormonerelated variables may be associated with MMTV -positive breast cancer.

Application ID: B-AU-1101-01S **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2001

Institution: University of Melbourne

Title: *Validation of LAMBDA Model for Predicting BRCA1 and BRCA2 Founder Mutation Status in Ashkenazi Women Using the CFRBCS*

Abstract:

Currently the only models available for estimating the probability that a Jewish woman carries an ancestral mutation in BRCA1 or BRCA2, based on her personal and family cancer history, have either been derived from a statistical model (Parmigiani et al ., 1998), based on data from mostly non-Ashkenazi individuals (Shattuck-Eidens et al ., 1997), or have been derived using a large data-set that did not have many individuals with highly predictive features of mutation status (Hartge et al ., 1999). This means that, currently, genetic clinic staff do not have a reliable and easy formula for predicting mutation carrier status in Ashkenazi Jewish women attending their clinics. Such a formula would allow clinicians to determine better the appropriateness of testing of individuals attending their clinics, and allow them to identify the individual within a family at highest probability of carrying a mutation. Genetic testing could, therefore, be targeted towards those individuals at highest probability of carrying a mutation, thus improving the efficiency of these services.

The LAMBDA model was developed by Apicella and Hopper (2001), using data from 240 Australian Jewish women who enrolled in the Australian arm of the CFRBCS and 184 British Jewish women who enrolled in a London based study of the Jewish ancestral mutations in BRCA1 and BRCA2. The formula for predicting mutation carrier status derived from this model was developed from a substantial number of Jewish women with in some cases a quite high probability of carrying an ancestral mutation (due to either a personal or family cancer history of breast or ovarian cancer). In addition, the information derived from the large Washington study (Hartge et al) on American Jewish women at lower probability of carrying an ancestral mutation was included in deriving the model.

The formula derived from this model (known as the LAMBDA score) has been represented in an easy-to-use format that can be used by genetic clinic staff within their clinic practice, and is easily converted to a probability using a simple conversion table.

Internal consistency checks of the model and formula have been conducted . It now remains for the model to be tested against an independent data-set to see whether it can accurately predict mutation carrier status. This proposal, therefore, is to use the North American CFRBCS Ashkenazi data for validation of the LAMBDA model.

Specific Aim(s):

Hypothesis

To use CFRBCS Ashkenazi personal and family cancer history data to validate the LAMBDA model for predicting BRCA1 and BRCA2 Ashkenazi ancestral mutation carrier status in Ashkenazi Jewish women.

Application ID: B-TO-0601-01S **Status:** Approved, Ongoing

Principal Investigator: Ozcelik, Hilmi **Year:** 2001

Institution: Samuel G, Lunenfeld Research Institute, Mount Sinai Hospital

Title: *Cloning and Characterization of Expanded Trinucleotide-Repeat Containing Sequence(s): Identification of Candidate Breast Cancer Predisposition Gene(s)*

Abstract:

Molecular genetic and epidemiological studies of familial breast cancer have demonstrated that germline mutations in either BRCA1 or BRCA2 genes are associated with a high risk of developing breast and/or ovarian cancer. However, there is now compelling evidence that other yet-to-be-identified predisposition genes contribute to breast cancer risk. In a previous study, we have hypothesized that genetic anticipation occurs in some breast cancer families and that, as shown in other genetic diseases, it is the result of intergenerational instability of trinucleotide repeats in susceptibility genes. Analysis of breast cancer cases and population controls using the Repeat Expansion Detection (RED) system has provided strong evidence for the presence of CAG-repeat expansion in a considerable portion of breast cancer cases. Approximately 3.4% of cases in our study have been shown to have undergone CAG-repeat expansion, which may influence the function or the intrinsic properties of nearby gene(s).

In the current study, we propose to identify candidate breast cancer predisposition gene(s). The sequence(s) flanking the CAG-repeat expansions will be cloned using lambda cloning vector system and DNA obtained from breast cancer cases with CAG-expansions. We will apply enrichment steps to our cloning strategy to increase the efficiency of identifying CAG-repeat containing clones. Using the cloned sequence data, we will map the sequences to specific loci. This will help to identify gene(s) within that region that may be influenced by these expansions. We will determine the allelic frequency of identified repeats using microsatellite analysis. This will provide information about the prevalence of these variants in the population and their potential contribution to the disease. Using the public sequence databases containing the information provided by the Human Genome Project, we will determine complete gene sequence(s) containing or flanking these CAG-repeats. When located within or near transcribed sequences, the expanded repeat can have an effect on either the gene transcript or the gene product, which may manifest in disease. When located in the intronic regions, it may interfere with the proper processing of the gene transcript. Therefore, we will identify the position of CAG-repeat(s) in relation to the genes by studying the genomic structure of the genes involved with these repeats. This will help us understand the influence of CAG-repeat expansions on the normal function of the identified gene(s).

Our proposed approach has the potential to allow the rapid identification of novel breast cancer predisposition gene(s) that will provide benefits for women with breast cancer, as well as the potential for insights into the pathobiology of this devastating disease. The identification of genes with unstable repeat expansions will open new avenues in the study of the molecular genetics of breast cancer.

Specific Aim(s):

Specific aims of the current work:

To identify breast cancer predisposition genes, functions and/or intrinsic properties of which areaffected by trinucleotide repeat expansions. Cloning will be carried out using DNA from 8 breastcancer cases with CAG-repeat expansions. The cloned sequences will be used to identify completegenes. The properties of these genes and their interaction with the CAG-repeat expansions will bestudied.

Hypothesis of the current work:

The hypothesis of the proposed work is that the trinucleotide repeat expansions exist in breastcancer, and are associated with candidate breast cancer predisposition gene(s). Our hypothesis isbased on the previous preliminary work that CAG-repeat expansion occurs in a considerableportion of breast cancer cases.

Technical objectives of the current work:

1.Cloning of gene sequences with trinucleotide repeat (CAG) expansion.
2.Identification and characterization of gene(s) containing or flanking expanded trinucleotiderepeats (CAG)

Application ID: B-TO-0401-01 **Status:** Approved, Ongoing
Principal Investigator: Ozcelik, Hilmi **Year:** 2001
Institution: Samuel G. Lunenfeld Research Institute, Mount Sinai Hospital
Title: *Study of DNA repair SNPs in Breast Cancer Using DNA Pooling*

Abstract:

In this study, we propose to explore the potential contribution of SNPs in genes involved in DNA repair to breast cancer risk. Deficiencies in the DNA repair process have long been hypothesized to increase breast cancer risk and augmented cancer incidence is a feature of inherited diseases caused by defects in DNA damage recognition and repair. The contribution of common genetic variations to cancer risk is an emerging focus in the study of genetics and disease. SNPs occur frequently in the genome and may potentially affect the gene function. Thus, when SNPs occur in genes relevant to breast cancer, they may be associated with an increase in breast cancer risk. The presence of an enormous number of SNPs in the genome reinforces the need to develop DNA pooling strategies to effectively reduce the cost and time of experimentation without compromising the efficacy. In this proposal, we will focus on up to 60 commonly occurring DNA repair SNPs with potential functional significance. By combining innovative application of DNA pooling with Single Strand Conformation Polymorphism methodology, we will be able to compare the allelic frequencies of these SNPs in breast cancer cases, familial and sporadic, versus population controls. Access to the population-based Ontario Familial Breast Cancer Registry will further strengthen the approach taken in this proposal. This study has the potential to identify SNPs in the DNA repair pathway contributing to the risk of breast cancer. This will provide further insight into breast cancer etiology, leading toward improved strategies for the management of the disease.

Specific Aim(s):

Specific Aims

1. To develop methodologies to quantitate alleles in a pooled DNA sample.

2. To study the association of SNPs in the DNA repair pathway with breast cancer risk using pooled DNA samples in women with breast cancer (with and without family history) and women without breast cancer.

3. To examine whether any association differs between women under age 50 and women aged 50 and older in women with and without family history and in women without breast cancer.

Hypothesis

The hypothesis of the proposed work is that DNA repair gene variants affect the risk of breast cancer development. We hypothesize that the collective study of gene variants within the same pathway may provide important knowledge on understanding the role of DNA repair genes in the etiology of cancer. Although these allelic variants may individually confer a small effect on the overall breast cancer risk, their common occurrence in the population could contribute to a large proportion of breast cancer cases.

There are a large number of candidate SNPs, which makes it difficult to identify ones that are more likely to contribute to breast cancer risk. In this proposal we intend to develop and apply a pooling method where potential disease-associated SNPs can be identified much more efficiently.

Application ID: B-NY-0401-02S **Status:** Approved, Ongoing
Principal Investigator: Terry, Mary Beth **Year:** 2001
Institution: Columbia University
Title: *Early Determinants of Breast Cancer Risk*

Abstract:

The research component of this career development award seeks to add to the understanding of how fetal and infant exposures (FIEs) might influence breast cancer risk later in life. In breast cancer research, a number of reports now suggest an influence of the intrauterine environment on the daughter's breast cancer risk later in life. The first of these reports were studies of maternal age, birth order, and twinning, all of which are determinants of the intrauterine environment and are easily queried and reliably recalled in epidemiologic studies. Although studies examining these factors have not always been consistent, they motivated more recent study of other factors that either reflect or influence fetal and infant development. I propose to investigate three of these FIEs: birthweight, maternal preeclampsia, and cerebral asymmetry. These three factors may be related through a common pathway such as intrauterine hormone exposure, or they may have independent effects on breast cancer risk. Two of these FIEs (birthweight, preeclampsia) have been the focus of increasing interest, while the third (cerebral asymmetry) is a less studied, but is an intriguing FIE.

During the course of the award, I plan to pursue refined and novel approaches to examine the relation of these factors to breast cancer risk and examine the potential intervening pathways through child and adolescent development. I will conduct three different studies, in each case enhancing a unique data base established by one or more of my mentors. The first study (Years 1-3) will use the resources of the Metropolitan New York (NY) Registry of Breast Cancer Families (PI Dr. Ruby Senie, co-PI Dr. Regina Santella); an ongoing study of women at high risk of breast cancer. This study will be one of the first to examine FIEs, specifically among women at high risk for breast cancer and to use sister pairs discordant on breast cancer status for tight control of confounding. The other two studies make use of other parent projects and, therefore, are not described here.

Specific Aim(s):

Study 1 uses data from the Metropolitan NY Registry of Breast Cancer Families (n=300 sister pairs discordant on breast cancer status) to examine the relationship between FIEs and breast cancer risk in a sample of women at high risk for breast cancer. I will test the following hypotheses:

Aim 1a: High birthweight will be associated with an increased breast cancer risk. If an association is demonstrated, two different pathways between birthweight and breast cancer risk will be explored: one involving age at menarche, and another independent of age at menarche. In addition, effect modification of these pathways by HER2/neu genotype status will be investigated.

Aim 1b: Maternal preeclampsia will be inversely associated with breast cancer risk. As an extension to this analysis, I will examine whether a common polymorphism of methylenetetrahydrofolate (MTHFR) (C677T), an indicator of increased risk of preeclampsia, is inversely associated with breast cancer.

Aim 1c: Markers of cerebral asymmetry will be associated with increased breast cancer risk. Two markers will be examined: left handedness and the A1 allele of the dopamine D2 receptor (DRD2) gene.

Secondary Aims: In addition to the primary aims, this career development award will plans to examine additional markers that are relevant to events along the life course important to breast cancer risk. These markers will include: CYP3A4 and age at menarche, and interactions between polymorphisms in CYP3A4, AIB1, IGF1, and reproductive factors (including use of oral contraceptives). These genes are important both as they pertain to early life risk factors (e.g., age at menarche) and risk for premenopausal breast cancer.

Application ID: B-NY-0401-01S **Status:** Approved, Ongoing

Principal Investigator: Terry, Mary Beth **Year:** 2001

Institution: Columbia University

Title: *Alcohol Intake, Alcohol Metabolism, and Breast Cancer Risk Among High-Risk Women*

Abstract:

Regular alcohol use has been associated with a 30 to 40 percent increase in breast cancer risk in both case-control and cohort studies. Some studies suggest that the effect of alcohol is even stronger among those women at high-risk of breast cancer. Despite the consistency of the association, little is known about the relevant time periods of risk and about possible biological mechanisms. This innovative study aims to develop a model to address changes in alcohol use patterns over the lifetime and will investigate whether these associations are modified by a woman's genotype for alcohol dehydrogenase 3 (ADH3) and Cytochrome P4502E1 (CYP2E1), two alcohol metabolizing genes. We aim to further models and mechanisms examined in cell lines and in animal studies by examining the influence of genotype on breast cancer susceptibility from alcohol use in humans.

Genotyping results from 300 breast cancer cases and 300 unaffected sisters of the cases will be combined with questionnaire data from the Metropolitan New York Registry of Breast Cancer Families. The hypothesis that fast metabolizers of alcohol, as measured by the ADH3 1-1 genotype and c1/c1 CYP2E1 genotype, have a higher risk of breast cancer will be tested.

Specifically, we hypothesize that:

Aim 1: Moderate alcohol use will increase the risk of breast cancer in women with a family history of breast cancer. The effect of modification by body size, HRT, menopausal status, and folate intake will be examined. The overall average lifetime consumption as well as different ages will be investigated.

Aim 2: Among women with a family history of breast cancer, women with the ADH3 1-1 genotype will have an increased risk of breast cancer from alcohol intake relative to women without this genotype, who consume similar amounts of alcohol.

Aim 3: Among women with a family history of breast cancer, women with the c1/c1 CYP2E1 genotype will have an increased risk of breast cancer from alcohol intake relative to women without this genotype, who consume similar amounts of alcohol.

Application ID: B-AU-0000-00-AS **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 2000

Institution: University of Melbourne

Title: *Australian Jewish Women's Experiences with a Breast Cancer Gene Testing Program*

Abstract:

Background: Stated Preference Discrete Choice Modelling (SPDCM) is a theoretically valid technique for eliciting preferences for the attributes of health services. To date, most SPDCM studies have focussed on measuring preferences for different treatment options and/or models of care delivery.

In this study, SPDCM was used to measure preferences for aspects of the agency relationship between genetic counsellors and clients. That is, we elicited clients' preferences for the different attributes, or functions, of genetic counselling and testing for breast cancer gene mutations.

Methods: Questionnaires were sent to 339 female participants of the Australian Jewish Breast Cancer Study. Women were asked to complete simple ranking and discrete choice questions to determine the relative importance of the different attributes of genetic counselling. Attributes of genetic counselling were developed through consultation with clinical geneticists, counsellors, and clients.

Results: Participants consistently valued the provision of genetic and cancer risk information much more highly than other attributes of genetic counselling. Most placed lower importance on preparation for genetic testing, and on direction in making decisions about genetic testing. There was some evidence that preferences varied systematically with personal characteristics.

Conclusions: Genetic counselling and testing for breast cancer gene mutations has the potential to provide significant benefits for clients, including improved: cancer prevention, surveillance and screening strategies; psychological well-being; and ability to make informed choices. However, the extent to which some, or all, of these benefits are realised in practice will be influenced by whether genetic counselling is consistent with clients' preferences.

Application ID: B-TO-1200-01 **Status:** Approved, Ongoing

Principal Investigator: Ozcelik, Hilmi **Year:** 2000

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital Ontario

Title: *The Role of Single Nucleotide Polymorphisms of the DNA Repair Pathway in Breast Cancer Risk*

Abstract:

The aggregation of breast cancer occurrences in a family may be attributed to a common environmental habitat and risk conferring inheritance factors. Breast cancer susceptibility genes, BRCA1 and BRCA2, have been shown to explain only a small portion of familial aggregation of breast cancer. An emerging concept in genetics emphasizes the contribution of "low-risk" genetic variations to breast cancer risk. Single nucleotide polymorphisms (SNPs) occur frequently in the regulatory and coding regions of genes and can potentially affect the function or intrinsic properties of the genes. Thus, such SNPs may be associated with a smaller increase in breast cancer risk or modify the cancer risk induced by other factors when they occur in genes relevant to breast cancer. Despite the low penetrance associated with such alleles, their common occurrence in the population could contribute to a significant proportion of breast cancer in the population. The cellular DNA is continually subject to exogenous and endogenous damaging agents including chemical mutagens and radiation. These can produce DNA lesions, which, if not repaired, can result in infidelity of replication, mutations, and cancer. Cellular DNA repair pathways remove these lesions, maintaining the integrity of cellular DNA. Deficiencies in the DNA repair process have long been hypothesized to increase cancer risk including breast cancer and augmented cancer incidence is a feature of inherited diseases caused by defects in DNA damage recognition and repair. In this study, we propose to use the strengths of the population-based Ontario Familial Breast Cancer Registry and new high throughput technologies to make a major enquiry into the possible roles of common variants in DNA repair pathway genes on risk of breast cancer. Instead of focusing on a few SNPs, we selected 22 SNPs in 11 genes using word wide Web databases. These SNPs occur commonly (15% and higher) and are located in the coding and the regulatory regions of these genes. We have designed a case-control study to analyze 420 cases and 420 population controls from the Ontario Familial Breast Cancer Registry using the high-throughput microarray and mass spectrometer approach. The results of this study will help us to understand the contribution of DNA repair pathway to the risk of breast cancer and help us to select important candidates for future studies to investigate their role in breast cancer in the context of gene-gene and gene-environmental interactions. This proposal will provide basis for future studies with multigenic approach, which goes beyond investigating the cancer risk associated with individual alleles to determine the impact of multiple alleles affecting cancer risk in concert.

Specific Aim(s):

Specific Aim

To study the association of 22 potentially functional SNPs in 11 genes of the DNA repair pathway in breast cancer using high-throughput technologies microarray and MALDI-TOF mass spectrometer analysis. This case-control study will be carried out using a population-based sample of 420 breast cancer cases and 420 population controls.

Hypothesis

The hypothesis of the proposed work is that DNA repair gene variants affect the risk of breast cancer development. We hypothesize that collective study of gene variants from the same pathway may provide important knowledge on understanding the role of DNA repair genes, as well as the pathway on the disease. Although these allelic variants may have a small effect on the overall breast cancer risk, their common occurrence in the population could contribute to a large proportion of breast cancer cases.

Application ID: B-TO-0499-01 **Status:** Approved, Ongoing

Principal Investigator: Andrulis, Irene **Year:** 1999

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital , Toronto, Canada

Title: *BRCA1 and BRCA2 Genotyping Analysis of Families in the CFRBCS*

Abstract:

Knowledge of the germline BRCA1 and BRCA2 status of individuals in the CFRBCS is an essential first step in utilizing the Registry for molecular epidemiological studies of genotype-phenotype correlations, gene-gene and gene-environment interactions. Identification of specific mutations will lead to refinement of models to predict the probability of carrying a mutation based on personal and family histories of breast, ovarian and other cancers and may lead to the development of better genetic tests for cancer predisposition. In addition, information on BRCA1 and BRCA2 status will allow us to identify a valuable cohort of families to search for new genes that predispose to breast and ovarian cancer, and to study both retrospectively and prospectively the cancer risks, and potential modifiers of risk, for mutation carriers. Thus, the work described in this proposal is an essential step in answering the key question: what can be done to reduce the risk of cancer in individuals who have inherited a mutation in BRCA1 or BRCA2?

This proposal is submitted by Dr. Andrulis and the PIs and the Molecular Characterization Working Group members of the population-based sites of the CFRBCS (Ontario, NCCC and Australia). Our plan is to use a centralized testing approach to complete the BRCA1 and BRCA2 mutational analysis of 4050 population-based probands in the CFRBCS to address the following specific aims.

The present proposal complements the NIH/NCI-funded grant of Dr. Frederick Li and colleagues to genotype case probands from two of the CFRBCS population-based sites (Australia, NCCC) for BRCA1. Here we propose to determine the BRCA2 genotypes of CFRBCS case probands from all registry sites, and the BRCA1 and BRCA2 genotypes of case probands from the other population-based site, Ontario. In addition for case probands who are carriers, we will genotype all DNA samples from relatives for the “family-specific” mutation. This work will enable us to accomplish the following specific aims.

1) Determine the prevalences of germline BRCA1 and BRCA2 mutations in population-based samples of breast cancer cases from the US, Canada and Australia.

By completing both BRCA1 and BRCA2 mutation testing of case probands in the three population-based familial breast cancer registries, we will be able to calculate with precision: (i) the percentage of breast cancer in the population that is caused by mutations in BRCA1 and BRCA2, and (ii) how this percentage depends on the age at diagnosis of the case proband, and characteristics of the family cancer history, including ages at onset, numbers of affected relatives and relationships among affected relatives.

2) Estimate the penetrances of germline BRCA1 and BRCA2 mutations and compare these estimates across:

a) genes (BRCA1 vs. BRCA2)
b) mutation type (the two Ashkenazi Jewish founder mutations vs. all others)

We will estimate the risk of breast and other cancers for carriers of BRCA1 and BRCA2 mutations. We will evaluate penetrance by analyzing phenotype data from all first-degree relatives of case probands. This analysis will use all available information on the genotypes of relatives.

3) Examine existing models for predicting the probability that a person carries a BRCA1 or BRCA2 mutation based on his/her personal and family history of breast and/or ovarian cancer.

These studies will enable us to expand the usefulness of the CFRBCS resource for studies addressing: genotype-phenotype correlations, genetic and environmental modifiers of risk in carriers, gene discovery studies, and in the long term, clinical interventions and prognosis.

Application ID: B-TO-0899-02 **Status:** Approved, Ongoing

Principal Investigator: Esplen, Mary Jane **Year:** 1999

Institution: Mount Sinai Hospital, Toronto

Title: *Development of a Measure of Self-Concept in BRCA1 and BRCA2 Gene Carriers*

Abstract:

Relevance

A new & validated measure of self-concept for the clinical population of BRCA1 & BRCA2 carriers has theoretical, clinical & research relevance. It will contribute to the literature on psychosocial impact of predictive DNA testing for BC and has potential use as a clinical screening tool that could enhance the provision of DNA test results. Information on a person's self-concept associated with carrier status would also generate research questions & further our understanding of the impact of predictive testing on an individual's sense of self and well-being and the associated link with adjustment to receiving genetic information. The value of a self-concept scale may provide new information on how genetic test results contribute to self-definitions, both current and future in relation to the impact of genetic testing results. The information on specific self-descriptors could be used to generate counselling intervention strategies that can be empirically tested. Improved counselling approaches to risk communication will promote the prevention & early detection of BC through improved surveillance & the adoption of preventive techniques . The scale would also provide a useful outcome measure (e .g . in

the assessment of change in self-concept over time) for future trials.

Specific Aim(s):

Hypothesis

The main objectives are to:

(1) develop a reliable and valid scale to measure the impact of having a genetic mutation on a woman's self-concept in BRCA1 and BRCA2 carriers, and

(2) identify the specific cognitions (e.g., self-schemas) associated with being informed of BRCA1 and BRCA2 mutation carrier status.

This application requests funding for the development of the instrument. A subsequent application will request funding to rigorously evaluate and validate the measure through larger samples of BRCA1 and BRCA2 carriers from multisite cancer genetic centres.

Application ID: B-NY-0499-03 **Status:** Approved, Ongoing

Principal Investigator: Offit, Kenneth **Year:** 1999

Institution: Memorial Sloan-Kettering Cancer Center

Title: *Genetic Polymorphisms as Modifiers of Breast Cancer Risk in Women With Germline BRCA Mutations*

Abstract:

We and others have shown that 2.5% of unselected individuals of Ashkenazi descent carry one of three specific mutations (BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT). The breast cancer risk associated with germline mutations in BRCA1 and BRCA2 is the subject of active investigation. Evidence suggests that the products of BRCA1 and BRCA2 function as components of a DNA damage response pathway. Cells lacking functional BRCA1 are deficient in the transcription-coupled repair of DNA damage induced by oxidative stress. BRCA2-deficient cells are defective in the repair of double-strand DNA breaks, such as those induced by ionizing radiation and certain chemotherapeutic agents. According to this model, factors that further compromise the DNA damage response are likely to increase the risk of breast cancer in BRCA heterozygotes. The central hypothesis of this proposal is that the predisposition to breast cancer conferred by a germline mutation in BRCA1 or BRCA2 is subject to modulation by genetic cofactors. We propose a case-control study utilizing the accumulated resources of the Cooperative Family Registry for Breast Cancer Studies. Cases will be female individuals of Ashkenazi descent affected with breast cancer who have germline mutations in either BRCA1 or BRCA2. Controls will be female relatives of cases who also have germline BRCA mutations, but who have not developed breast or ovarian cancer. Samples will be analyzed BRCA1 185delAG, BRCA1 5382insC, BRCA2 6174delT, abnormalities in DNA repair pathways: BLMASH, FAC (Fanconi's anemia), polymorphisms in pathways of oxygen free radical generation and detoxification: Superoxide dismutase (SOD A allele v. V allele), Glutathione-S-transferase (GSTM1, GSTT1), polymorphisms in enzymes involved in metabolism of environmental carcinogens and steroid hormones: CYP1A1, CYP17, N-acetyl transferase 1 and 2, and polymorphisms in signal transduction pathways: APC I1307K; TGF-BR1 6A/10A. Based on polymorphic frequencies of these mutations in this population, the study should have at least 90% power at 5% significance level (two-sided) to determine associations between FAC or BLM heterozygosity and cancer status with a relative risk of at least 13.4 in the presence of 400 individuals, and at least 6.7 in the presence of 1000 individuals. The study will also be adequately powered to discern the reported relative risk of 2.6 due to APC I1307K among BRCA heterozygotes. We have also calculated estimates of minimum detectable RR with control genotype frequencies of 0.5%, 2%, and 5%. The demonstration that the breast cancer risk in BRCA heterozygotes may be modified by polymorphisms in other genes would be of practical importance in the risk stratification of such women and, potentially, for intervention studies to reduce the incidence of cancer in this population.

Specific Aim(s):

The specific aim of this proposal is to determine whether the predisposition of breast cancer conferred by a germline BRCA mutation is subject to modulation by other genetic factors, such as an APC, BLM or FAC mutation, or polymorphisms influencing the levels of DNA-damaging species within the cell.

The basic premise is that BRCA mutation alone does not account for the entire risk for breast cancer. The breast cancer risk is elevated in the presence of other genetic factors, in addition to a BRCA mutation. By evaluating the relative risks of other genetic factors we hope to be able to refine the ability to predict breast cancer risk in individuals with a BRCA mutation. This could provide benefits for cancer prevention initiatives.

Application ID: B-TO-1299-01 **Status:** Approved, Ongoing

Principal Investigator: Ozcelik, Hilmi **Year:** 1999

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital , Toronto

Title: *Microarray Technology to Study the Role of Genetic Polymorphisms in Breast Cancer Risk*

Abstract:

It has long been hypothesised that genetic variation is responsible for observed differences in cancer risk and susceptibility amongst the human population. Mutant alleles of dominant highly penetrant breast cancer genes, including BRCA1 and BRCA2, do not occur frequently, and hence account for only a small proportion of breast cancer cases. On the other hand, several studies have suggested an association between low penetrant alleles and breast cancer risk. Although the contribution of low penetrant alleles to the individual breast cancer risk is relatively small, they can contribute to a large proportion of breast cancer cases in the population because the risk-conferring alleles of these genes are common.

At present, the candidate gene approach remains the most logical and practical way of identifying these risk enhancing, but low penetrant variants (also known as low penetrant alleles or genetic polymorphisms) involved in breast cancer. Identification of these low penetrant alleles is challenging, requiring a very large number of individuals (including those with breast cancer) and very large number of genetic markers. The identification of these genetic markers is ongoing and will be available in the future. One major obstacle to such an approach, however, has been a lack of technology suited to genotyping the large populations required for these studies. Consequently, many studies have focused effort on only a few genes at a time, and even in these cases, the analysis was limited to relatively small sample sizes. This is an exciting time because the recent emergence of high throughput microarray technology has made large scale genotyping studies much more efficient and manageable. In short, microarray technology permits the genotyping of a large number of genes simultaneously.

The objective of the proposed work is to identify low penetrant, yet commonly occurring, genetic polymorphisms, which contribute to the risk of developing breast cancer. We propose to exploit the high throughput power of microarrays to screen 32 candidate genetic polymorphisms simultaneously in a well-defined population containing a large number of subjects. We have access to the Ontario Familial Breast Cancer Registry (OFBCR), a large population-based breast cancer registry. We also have support from the established microarray facility of the Ontario Cancer Institute in Toronto. Given the opportunity to perform large-scale genotyping with this technology, we have selected polymorphisms in genes shown to function in biological pathways previously implicated in breast cancer development, because intuitively, these are more likely to have an effect on breast cancer risk. Our list of genes includes those involved in cell proliferation, carcinogen metabolism, and the immune system.

In summary, we have proposed an innovative approach to assess the contribution of a large number of different variants of genes implicated in cancer related pathways to breast cancer risk. This will be the first large population-based study to examine the effect of many different variants on breast cancer risk simultaneously. The proposed work will serve as a foundation for future studies designed to determine the effect of diverse combinations of genetic variants and interactions with environmental factors on an individual's breast cancer risk. Such studies will ultimately lead to improved strategies to prevent, detect, and treat breast cancer as we more fully understand the various mechanisms leading to breast cancer in the population.

Specific Aim(s):

HYPOTHESIS/RATIONALE/PURPOSE

The hypothesis of the proposed work is that low penetrant allelic variants in a number of genes effect the risk ofbreast cancer development. Although these allelic variants may have a small effect on the overall breast cancerrisk, their common occurrence in the population can account for a large proportion of breast cancer cases. Toassess the role of these allelic variants in breast cancer risk, it is important to be able to achieve large-scalegenotyping on large population-based samples.We propose to investigate the potential association between 32different genetic polymorphisms and breast cancer risk using the emerging high throughput microarraytechnology.

TECHNICAL OBJECTIVES

1.To establish methodologies for application of microarray technology for large-scale genotyping of geneticpolymorphisms.

2.Applying high throughput microarray technology to investigate potential associations between geneticpolymorphisms of candidate genes and breast cancer risk in a population based study.

Application ID: B-AU-1299-01 **Status:** Approved, Ongoing

Principal Investigator: Phillips, Kelly-Anne **Year:** 1999

Institution: Peter MacCallum Cancer Institute

Title: *A Population-Based Study of Prognosis in Breast Cancer Patients with Germline Mutations in BRCA1 or BRCA2*

Abstract:

Germline mutations in two breast cancer (BC) predisposition genes, BRCA1 and BRCA2, account for a substantial proportion of hereditary BCs and for up to 5% of all BC cases. Paradoxically, somatic mutations in these genes are extremely rare in BC. This observation, along with the distinct histologic phenotypes described for BRCA-associated BCs and observed differences in the frequency of somatic mutations of other prognostically important genes in these tumors, gives biologic plausibility to the suggestion that they may be fundamentally different in terms of their prognosis, compared with BCs occurring in non-carriers. Determination of such a difference in prognosis would have significant implications for the treatment and counseling of women affected by this important subtype of BC.

All published studies, which have examined the prognosis of BRCA1- and BRCA2-associated BCs, have had important methodologic problems including the use of non-systematically collected retrospective data from opportunistically-sampled multiple-case families, incomplete follow-up, and inadequate sample size. Here, we propose to study the prognostic effect of germline mutations in BRCA1 and BRCA2 in a population-based cohort of women with BC who are enrolled in the Australian Breast Cancer Family Study (ABCFS). The use of a population-based cohort of women from which both cases and controls are derived is a major strength of the study, as it avoids the problems of ascertainment bias that have occurred in other studies of this nature. A subgroup of individuals in the ABCFS (those enrolled between 1996 and 1999) are enrolled in the Cooperative Family Registry for Breast Cancer Studies (CFRBCS). In this proposal, we request access to relevant demographic, pathology, and genotyping data on all probands in the CFRBCS who have been ascertained via the ABCFS. A similar study is underway in population-based Ontario cases in the CFRBCS, which will provide the opportunity to perform pooled analyses of the data to address related questions requiring a larger sample size.

The primary hypothesis is that women with early-stage BC due to germline mutations in either BRCA1 or BRCA2 will have a worse prognosis, as measured by distant disease-free survival, than those without mutations in these BC predisposition genes.

The specific objectives of this proposed research are:

1) To determine the prognostic effect of germline mutations in BRCA1 and BRCA2 (considered together and separately) on distant recurrence (primary endpoint), locoregional recurrence, and death from BC, both before (primarily) and after (secondarily) consideration of traditional prognostic factors.

2) To provide data for pooled analysis in an international collaboration with the Ontario Cancer Genetic Network (OCGN) in Canada, in order to address related questions requiring a larger sample size. Such questions include the influence of the site of germline BRCA1 or BRCA2 mutation on BC prognosis, and the influence of type of adjuvant systemic therapy (e.g., anthracycline-containing versus anthracycline-free regimens) on distant recurrence and death in mutation carriers.

3) To extend the current CFRBCS and ABCFS databases, adding clinical treatment and outcome data to the currently available demographic, family history, mutation status and epidemiology data on each woman. This will provide a unique resource with which to address future research questions (such as the impact of modifier genes on outcome in BRCA1 and BRCA2 mutation carriers, and the influence of currently undefined low penetrance genes on BC prognosis).

We propose to examine the prognostic effect of germline mutations in BRCA1 and BRCA2 in a population-based inception cohort of women diagnosed with BC between late 1991 and late 1998, ascertained from the Victorian and New South Wales State Cancer Registries in Australia via the ABCFS. This includes a subgroup of women (those enrolled in the ABCFS between 1996 and 1999) who are included in the CFRBCS. Women were recruited to the ABCFS within six months of their initial BC diagnosis. This delay is due to the time taken for data regarding new cancer diagnoses to reach the cancer registries and for permission to be sought from the treating surgeons for their individual patients to be approached for the ABCFS. In Victoria and NSW, about 3% of new cases of BC are metastatic at diagnosis. A small number of women, therefore, die within six months of diagnosis and are not included in the population-based sample. This is unlikely to significantly bias the current study, as women with metastatic disease at the time of diagnosis are not eligible. Women will be grouped into three categories: (1) those with a mutation in BRCA1, (2) those with a mutation in BRCA2, and (3) those without a mutation in BRCA1 or BRCA2.

Application ID: B-AU-1299-02 **Status:** Approved, Ongoing

Principal Investigator: Spurdle, Amanda Barbara **Year:** 1999

Institution: Queensland Institute of Medical Research

Title: *Variation in DNA Repair Genes and Breast Cancer Risk*

Abstract:

We propose to genotype a population-based series of women with breast cancer and matched controls, ascertained through the Australian Breast Cancer Family Study (ABCFS), for known variants in candidate low-risk predisposition genes acting in the DNA repair pathways. The availability of extensive epidemiological and family data will allow us to determine if specific genotypes of putative predisposition genes interact with certain environmental exposures. We will attempt to confirm positive findings by replicating relevant studies in a separate sample of population-based cases and controls collected through the NIH-funded Co-operative Family Registry for Breast Cancer Studies (CFRBCS). The results will increase our understanding of the aetiology of breast cancer, and will have important clinical implications with respect to both counseling and disease prevention.

Specific Aim(s):

Hypothesis

BRCA1 and BRCA2 are the major, known, predisposing breast cancer genes for familial breast and ovarian cancer, but are rarely mutated in breast cancer patients with no obvious family history. Since the BRCA1 and BRCA2 genes are believed to be involved in DNA repair, variation in other genes of the DNA repair pathway may contribute to breast cancer susceptibility in the general population.

Application ID: B-NY-0499-02 **Status:** Approved, Ongoing

Principal Investigator: Wolff, Mary **Year:** 1999

Institution: Mount Sinai Hospital, New York

Title: *The Role of Dietary Phytoestrogens and BRCA Status in Relation to Breast Cancer Among Ashkenazi Women*

Abstract:

The risk of breast cancer ranges from 76 to 87 percent among carriers of BRCA1 or BRCA2 mutations who are from families with multiple cases of breast and ovarian cancer. Experimental and epidemiologic evidence suggests that exogenous hormonal factors may modify the risk of cancer among these women.

The overall goal of this project will be to determine whether dietary phytoestrogens alter risk for cancer among BRCA mutation carriers.

We propose a pilot study where the specific aims are:

(1) to determine phytoestrogen content for foods in the Diet Questionnaire used by the Metropolitan New York Registry, and

(2) to assess the variability of phytoestrogen intake among a small number of BRCA mutation carriers and noncarriers with and without cancer to guide

the study design for a larger investigation on this topic.

Breast cancer risk conferred by BRCA positivity may be modulated by hormonally active dietary constituents, namely phytoestrogens and antioxidants and by dietary fiber constituents that alter estrogen transport in the gut.

We will study diet among individuals already screened for the three common mutations found in the BRCA1 and BRCA2 genes among the Ashkenazi population or the BCRFS. We will compute the usual reported dietary intake of phytoestrogens among antioxidants of individuals with and without mutations and with and without these patients from the diet questionnaire. Dietary phytoestrogens and cancer will be compared. Other relevant factors including reproductive history, hormone use, and age will be adjusted in the analysis. At a later date, if BRCA status information becomes available on an adequate number of relatives, we will expand our strategy to incorporate the additional information.

Basic knowledge about these mitigating risk factors will: (1) aid our understanding of how genetic factors work, (2) provide the basis for more detailed studies, and (3) eventually lead to prevention and intervention.

Application ID: B-EX-1298-02 **Status:** Approved, Ongoing

Principal Investigator: Goldgar, David **Year:** 1998

Institution: International Agency of Research on Cancer

Title: *A Collaborative Genomic Search for Additional Breast Cancer Susceptibility Loci*

Abstract:

A proportion of breast cancer cases, particularly those occurring at a young age, are due to the inheritance of susceptibility genes, giving rise to families with multiple cases of the disease. Two such genes, BRCA1 and BRCA2, have now been mapped and cloned. These genes appear to account for most families with six or more cases of breast cancer, particularly those whom also contain ovarian cancer cases. The majority of families with five or fewer cases of breast cancer, however, do not appear to be due to BRCA1 or BRCA2, indicating that further susceptibility genes remain to be identified. We have previously initiated a genome-wide search for one or more additional breast cancer susceptibility loci. To maximize our chances of successfully identifying at least one such locus, it will be important to analyze a large number of families in order to have sufficient power to detect a locus under genetic heterogeneity. We will add the CFRBCS families to our current set as well as new families from other collaborators to obtain a well-defined, powerful set of families that are not due to either BRCA1 or BRCA2. These families will be tested using a panel of 400microsattelite markers with the resulting data to be analyzed by both parametric and non-parametric linkage analysis.

Hypothesis: The hypothesis that is to be tested using the material requested from the CFRBCS (and elsewhere) is that one or more novel breast cancer susceptibility loci exist and can be localized using a genomic search approach in families with multiple cases of breast cancer.

Aim(s): Two genes, BRCA1 and BRCA2, jointly explain the large majority of families with: (1) the breast-ovarian cancer syndrome, (2) at least one case of male breast cancer, or (3) at least six cases of breast cancer diagnosed under 60. Roughly 60% of the families with four or five cases of breast cancer diagnosed under 60 and no cases of ovarian cancer are not accounted for by these genes. The aim of this proposal is to map and identify additional breast cancer predisposition genes by means of linkage analysis, using the requested CFRBCS families in addition to families previously collected by our collaborating group in this particular group of families.

Application ID: B-AU-0598-01 **Status:** Approved, Ongoing

Principal Investigator: Hopper, John **Year:** 1998

Institution: University of Melbourne

Title: *Population-Based Estimation of the Penetrance of Protein Truncating Mutations in BRCA1 and BRCA2*

Abstract:

The aim of this study is to estimate the average age-specific cumulative risk of breast cancer (i.e., the prevalence) of protein-truncating mutations in BRCA1 and BRCA2, weighted by the observed distribution of such mutations in a sample of women with breast cancer, sampled on a population basis through state cancer registries.

The question being asked is whether the estimate of prevalence based on a population-based sample of women with breast cancer is less than that derived from ad hoc samples of families with multiple cases of breast cancer, chosen for linkage studies with the aim of discovering “high-risk” breast cancer genes. (In fact, these genes are “anti-breast cancer genes,” and only women with mutated forms of the genes are at increased risk of breast cancer.)

The Australian Breast Cancer Family Study (ABCFS) uses a case-control-family design, in which family members of cases and controls are studied systematically. Consequently, the age-specific risk of (breast) cancer will be estimated by analysis of the (breast) cancer history of the relatives of those cases found to be carriers of protein-truncating mutations in BRCA1 or BRCA2.

There is considerable controversy about the penetrance of mutations in BRCA1 and BRCA2. Most of the information to date has come from analysis of multiple-case families such as those of the Breast Cancer Linkage Consortium (BCLC). These atypical families were selected on an ad hoc basis, specifically for use in linkage studies aimed at detecting genes associated with breast cancer. There are two problems with these estimates. First, the mutations occurring in multiple-case families may be unrepresentative in that they may be associated with a higher risk of breast cancer. Second, because the families are not selected in a systematic manner, it is not possible to make an appropriate adjustment for their ascertainment without conditioning out a large amount of information. Hence, the estimates are imprecise, and in practice, have been observed to be unstable (Easton et al., 1997). To date, the best estimates of breast cancer risk to age 70 are: 71% (95% c.i. 53%-82%) for BRCA1 (Narod et al., 1995), and 84% (95% c.i. 43%-95%) for BRCA2 (Ford et al., 1998).

Application ID: B-TO-0598-03 **Status:** Approved, Ongoing

Principal Investigator: Knight, Julia **Year:** 1998

Institution: Mount Sinai Hospital, Toronto

Title: *Genetic Susceptibility to Risk Factors for Breast Cancer*

Abstract:

Women with breast cancer are likely to be a heterogeneous population with respect to the etiology of their disease. Some may have disease that is due largely to nongenetic risk factors (low genetic risk). Those with a family history of breast or ovarian cancer, early onset, multiple primaries, or from certain populations, are more likely to have a genetic component to their disease (high and moderate genetic risk). Some of these latter women may in fact be carriers of known mutations in BRCA1 and BRCA2. Among those in whom genetics likely played a major role in their disease, including BRCA1 and BRCA2 carriers, nongenetic risk factors (e.g., diet and oral contraceptives) may still be involved in determining either age of onset or whether disease occurs at all, because the penetrance of currently known genetic mutations is less than 100%. Risk factors may operate early in life or in adulthood and may be associated with different levels of risk in different women.

In this proposal, we focus on the hypothesis that the magnitude of risk associated with known or suspected adult risk factors varies among different groups within the population of women with breast cancer. Specifically, we define: a high genetic risk group, which includes those with onset <36, or multiple breast and/or ovarian primaries with the first occurring <40, or a family history (see the definition of familial below) of breast and/or ovarian cancer with one case <40; a moderate genetic risk group including those with multiple breast and/or ovarian primaries <40 or a family history of breast and/or ovarian cancer with all cases <40; and a low genetic risk group (all other cases). We wish to observe whether risk magnitude varies when each group is compared separately to population controls, ultimately testing for statistical differences between groups.

It is also of great interest to know whether known or suspected risk factors play a role in the development of breast cancer in BRCA1 and BRCA2 mutation carriers. Therefore, we also propose a subgroup analysis of these women who will be identified by testing those in the high or moderate genetic risk groups defined above.

Application ID: B-NY-0597-03 **Status:** Approved, Ongoing

Principal Investigator: Kabat, Geoffrey **Year:** 1997

Institution: State University of New York at Stonybrook

Title: *Biomarkers of Estrogen Metabolism and Women at Differing Risks of Breast Cancer*

Abstract:

Alternative pathways in the metabolism of estrogen have been hypothesized by Bradlow and others to be associated with breast cancer risk. Bradlow has proposed that 16(alpha)-hydroxyestrone increases the risk of breast cancer, whereas 2-hydroxyestrone is protective. However, to date, little is known about variation in estrogen metabolism by family history of breast cancer. This pilot study will use data from the Metropolitan New York Registry of Breast Cancer Families to determine whether urinary estrogen metabolites or specific polymorphisms thought to play a role in estrogen metabolism differ between affected and nonaffected sisters from high-risk families. The following hypotheses will be tested: (1) the ratio of 2-OHE1/16 -OHE1 (estrogen metabolite ratio) is lower in affected sisters compared to nonaffected sisters from high-risk families enrolled in the Registry; (2) women who are below the median for 2-OHE1/16 -OHE1 and who have a high-fat/low cruciferous vegetable intake are at increased risk of breast cancer compared to women who have only one risk factor, who in turn are at increased risk compared to women who have neither risk factor; and (3) polymorphisms of cytochrome P450 enzymes involved in estrogen metabolism (specifically, CYP1A1, CYP17, and CYP19) differ between affected and nonaffected sisters. Study subjects will comprise 200 affected sister-nonaffected sister pairs from different families. Where urine samples are available from multiple affected and/or nonaffected sisters within a family, the affected-nonaffected pair closest in age will be selected. The two estrogen metabolites will be measured in urine by EIA in Dr. Bradlow's laboratory. Determination of genotypes for CYP1A1, CYP17, and CYP19 will be performed by PCR and restriction enzyme analysis in Dr. Santella's laboratory. The Wilcoxon signed rank test will be used for the bivariate comparison of the estrogen metabolite ratio between affected and nonaffected sisters. Conditional logistic regression will be used to estimate the risk of being affected, with adjustment for potential confounding variables. Based on sample size calculations, 200 sister pairs will provide adequate power to test the hypotheses. This pilot study will provide important data on the association of estrogen metabolism and polymorphisms potentially involved in estrogen metabolism, with breast cancer in a high-risk population.

Specific Aim(s):

Hypotheses

1. The ratio of 2-hydroyxestrone/16alpha-hydroxyestrone (estrogen metabolite ratio) is lower in affected sisters compared to nonaffected sisters from high-risk families enrolled in the Registry.

2. Women who are below the median for 2-hydroxyestrone/16alpha-hydroxyestrone and who have a high fat/low cruciferous vegetable intake are at increased risk of breast cancer compared to women who have only one risk factor, who in turn are at increased risk compared to women who have neither risk factor.

3. Polymorphisms of cytochrome P450 enzymes involved in estrogen metabolism (specifically, CYP1A1, CYP17, and CYP19) differ between affected and nonaffected sisters.

Application ID: B-SF-0597-01 **Status:** Approved, Ongoing

Principal Investigator: Li, Frederick **Year:** 1997

Institution: Dana-Farber Cancer Institute

Title: *BRCA1 Mutations Spectrum and Genotype-Phenotype Analysis*

Abstract:

A growing number of publications, from various groups and using various test systems, suggest that BRCA1 may be involved in the regulation of cell proliferation and differentiation and that expression is influenced by ovarian hormones. We hypothesize that exposure to hormones such as oral contraceptives (OCs) or hormonally related events such as age at first full term pregnancy, may interact with BRCA1 in a manner that affects the penetrance of this gene. Specifically, we hypothesize that OCs may interact with BRCA1 to increase the risk of breast cancer among carriers of BRCA mutations relative to carriers of BRCA1 mutations who do not use OCs and subjects who do not have a BRCA1 mutation, but use OCs. (In our study of 68 multiple-case families with bilateral breast cancer, we used 17q haplotypes to infer BRCA1 status and observed results suggesting a possible OC-BRCA1 interaction). We view these as very preliminary results with relatively small numbers that warrant further investigation. We also hypothesize that an earlier age at first full term pregnancy will increase (rather than decrease) the risk of breast cancer among subjects with a BRCA1 mutation. Consequently, age at first full term pregnancy should be earlier among breast cancer cases with a BRCA1 mutation compared to breast cancer cases without this mutation. The hypothesized effects for ovarian cancer are not as clear (e.g., OCs may increase, decrease, or have no effect on the penetrance of BRCA1 mutations for ovarian cancer, depending on the underlying biological model). We believe that it is very important to investigate these hypotheses in the same study using similar methodology for both breast and ovarian cancer, because the effects we observe may conceivably be different for breast versus ovarian cancer.

This R01-funded study will use a high-throughput method to test the DNA of probands from the California and Australian centers for BRCA1 mutations. This project will test this method and also provide this mutation information to the CFRBCS. In addition, the grant will validate the mutation findings from a sample of probands from Utah, Ontario, New York, and Philadelphia. This grant will provide BRCA1 mutation status for about 800 probands in California. The NCCC has been involved in the development of this proposal and will work with Dr. Li throughout the funding period.

Specific Aim(s):

Specific Aims of this R01 are to analyze DNAs of 2,036 breast cancer probands from the California and Australia CFRBCS sites and the relatives of carriers to estimate:

1. population-based germline BRCA1 mutation frequency;
2. BRCA1 penetrance; and
3. using risk factor data, identify genotypephenotype correlations.

Application ID: B-AU-0803-02 **Status:** Approved, Inactive

Principal Investigator: Cui, Jisheng S. **Year:** 2003

Institution: University of Melbourne

Title: *Segregation Analyses of Early-Onset Breast Cancer in Population-Based Families*

Abstract:

We propose to conduct a statistical analysis which is aimed at estimating the magnitude of the unmeasured genetic risks of breast cancer, after excluding the measured genetic risks, based on the population-based NCI Breast Cancer Family Registry data. These risks are given in the form of the genetic relative risks for each mode of genetic inheritance. In combination with the population incidence of breast cancer, we can estimate the penetrance of the unmeasured risk, after adjusting for the disease status of the mother, if appropriate, and the age of onset of the proband and the mother. In a previous analysis, Cui et al. (Am J Hum Genet 2001a) fitted a two-locus model and suggested that, after excluding the known BRCA1 and BRCA2 mutation carriers and their relatives at the time of analysis, there might be residual dominantly inherited risk and a substantial recessively inherited risk of early-onset breast cancer.

Recently, Cui et al. (2003a) conducted an updated segregation analysis and excluded further 9 families with a mutation in BRCA1 or BRCA2. They found that, apart from BRCA1 and BRCA2, there might no longer be other substantial dominantly inherited risks for breast cancer. A highly penetrant

recessively inherited risk for early-onset breast cancer still remains. This has also been suggested in a recent study of breast cancer risks in case relatives (Dite et al., 2003).

All studies mentioned above were conducted using the population-based data from the Australian Breast Cancer Family Registry. We wish to use similar analysis techniques to analyse the population-based family data that are collecting by the other two NCI breast cancer registries (Northern California Cancer Center, Cancer Care Ontario) to see if the same or different conclusions can be made from analyses of separate and pooled family data sets.

Specific Aim(s):

Hypothesis

There are both dominantly and recessively inherited high risks of breast cancer evident in the families of woman diagnosed with breast cancer before the age of 40 years.

After excluding known BRCA1 and BRCA2 mutation carriers and their relatives, there is little or no evidence for a residual dominantly inherited risk for breast cancer

The recessively inherited risk for breast cancer remains after excluding BRCA1 and BRCA2 mutation carrier and their relatives.

Application ID: B-SF-1102-01S **Status:** Approved, Inactive

Principal Investigator: Gomez, Scarlett **Year:** 2002

Institution: Northern California Cancer Center

Title: *Racial/Ethnic Patterns and Sociodemographic Correlates in Treatment for Early-Stage Breast Cancer*

Abstract:

Despite NIH endorsements, there remains substantial geographic, socioeconomic, and ethnic variation in the US in the use of breast-conserving surgery (BCS) for early-stage breast cancer. Specifically, research has shown that certain Asian subgroups are more likely than other racial/ethnic groups to undergo mastectomy. Currently, little is known about the joint influences of socioeconomic, immigration/acculturation, cultural, and clinical factors on the observed racial/ethnic differences in the use of BCS. Using epidemiologic interview data from the Northern California Family Registry for Breast Cancer, we propose to 1) characterize the racial/ethnic patterns in treatment for localized breast cancer, in particular focusing on variations within Asian subgroups; and 2) identify clinical, sociodemographic, and immigration/acculturation characteristics associated with the racial/ethnic treatment patterns. Despite the relative wealth of research on BCS and factors associated with its use, most of the research has focused on white and black women. The proposed research would contribute much needed information on the issues associated with treatment choice in other racial/ethnic groups.

Specific Aim(s):

We will use the epidemiologic data collected from probands enrolled in the NorthernCalifornia Family Registry for Breast Cancer to address the following specific aims:

1.Characterize the racial/ethnic differences in treatment for early-stage breast cancer(i.e., localized disease that has not penetrated beyond the breast tissue and do not involveregional lymph nodes), in particular focusing on variations within the Asian subgroups.

2.Identify the clinical characteristics of the tumor and sociodemographic, immigration/acculturation, and diagnosing hospital characteristics that contribute to theracial/ethnic treatment patterns.

Application ID: B-SF-0802-01 **Status:** Approved, Inactive

Principal Investigator: Li, Frederick **Year:** 2002

Institution: Dana-Farber Cancer Institute

Title:

Abstract:

In 1999, we received an R01 grant to analyze 2,036 DNA specimens collected by the three population-based centers in the Cooperative Family Registry for Breast Cancer Studies (CFRBCS). To date, 860 samples have been completed, and the work wil be completed within budget and timeline. We have detected 13 germline BRCAl mutations among these samples. According to the BICS Database, 26 alterations designated as unclassified varants were found. There were also 10 "unclassified varant/polymorphisms", as well as 28 alterations not found in the Database. Some of these alterations were found in one sample only, whereas others were found in multiple samples. Many of these varants are likely to be rare polymorphisms, whereas others may have deleterious effects that predispose to breast and other cancers. To date, relatively little attention has been given to the study of unclassified variants so opportunities exist for important discoveries. In this study, we propose to analyze the demographic characteristics of breast cancer probands with unclassified variants in BRCA1/2, and compare these findings with the corresponding data for BRCA1/2 mutation carers and normals. Our hypothesis is that a substantial fraction of probands with unclassified varants actually have deleterious BRCA mutations. As a first step in testing this hypothesis, we wil examine CFRBCS probands at NCCC with unclassified variants for their personal and family histories of breast cancer and ovaran cancers focusing on numbers of affected relatives and ages at diagnosis, by generation. The same analyses will be made for CFRBCS cases with BRCA mutations and "normal" results. We postulate that the cancer histories of cases with unclassified varants will be less striking than probands with germline BRCAl mutations, but stronger than personal and family histories of cancer among BRCAl normals. By quantifying the magnitude of these differences, we wil

estimate the proportion of unclassified variants that are likely to deleterious mutations. If a substantial proportion of those with unclassified variants are likely to be BRCA1 cariers, functional assays wil be used to identify unclassified variants that change BRCA1 fuction in laboratory experiments.

Specific Aim(s):

Hypothesis

A substantial proportion of BRCAl and 2 genetic alterations that are presently called "unclassified variants" are actually deleterious mutations.

Application ID: B-SF-0601-01S **Status:** Approved, Inactive

Principal Investigator: Glaser, Sally **Year:** 2001

Institution: Northern California Cancer Center

Title: *Epstein-Barr Virus (EBV) as a Causal Factor in Breast Cancer: A Molecular-Epidemiologic Study of Women at High Risk for EBV-Related Malignancy*

Abstract:

Hypothesis

This study will explore the question of an etiologic role for EBV in breast cancer by using state-of-the-art molecular technology to look for EBV in tumors from patient subgroups at risk for viral association based on epidemiologic patterns in EBV-related malignancies and breast cancer. The study will address four hypotheses: 1) As EBV-related malignancies and breast cancer are both more common in families and are linked to HLA type, suggesting a genetic modulation of immune response to EBV that alters cancer risk, EBV will be associated with breast cancers in women at elevated genetic risk (i.e., positive family histories identified by the NCCC Breast CFR). 2) As parity may decrease risk for EBV-related malignancies, perhaps due to a transient effect of parity on EBV-related carcinogenesis, and for breast cancer, breast cancer patients of reproductive age who are nulliparous will be more likely to have EBV in their tumors than patients who are parous. 3) As EBV-related cancers are more likely to occur in economically less developed areas, likely reflecting effects of early childhood poverty on immune function, and as EBV prevalence in breast cancer was reported to be higher in areas of high risk for nasopharyngeal carcinoma but also low economic development, we hypothesize that EBV should be associated with breast cancer in women from less developed countries (i.e., Mexico and central America). 4) As EBV predicts poorer survival in older women with Hodgkin=s disease and was found in breast cancers with poorer prognosis in one study, EBV will predict poorer relative survival of older women following breast cancer.

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Application ID: B-NY-0601-01S **Status:** Approved, Inactive

Principal Investigator: Juo, Suh-Hang Hank **Year:** 2001

Institution: Columbia University

Title: *BRCA1/2 and Other Genetic Factors Among Chinese and Korean American Breast Cancer Families in New York City*

Abstract:

Compared to white women, Chinese and Korean women are at a lower risk of breast cancer. However, the age at diagnosis in the latter two groups is younger than that in whites (Menon et al. 1992). The incidence of breast cancer has dramatically increased in Chinese women in Asia (Jin et al. 1999) and also increases when Asian women immigrate to Western countries (Zielger et al. 1993).

Asian Americans have received less attention than other major ethnic groups in cancer research studies. Research on the genetics of breast cancer in Asian Americans is very limited. BRCA1/2, accounting for more younger than older cases, have not been well studied in these two populations. Nor are there data on the prevalence of BRCA1/2 mutations in Chinese or Korean breast cancer families. In order to address this gap in our knowledge of the impact of genetics in breast cancer among Asian Americans, we propose to conduct research with the two major Asian subgroups in New York City: Chinese Americans and Korean Americans. We will first detect the BRCA1/2 mutations in Chinese/Korean breast cancer families to determine the proportion of BRCA1/2 families in our sample. The non-BRCA1/2 Chinese/Korean families will be used for novel gene discovery. We believe our pilot study and future full-scale collaborative studies will provide invaluable data on the epidemiology and population genetics among Chinese and Korean breast cancer families. This project will also provide Dr. Juo with an opportunity to expand his career in cancer genetics.

Specific Aim(s):

Hypothesis

We hypothesize that novel breast cancer susceptibility genes that are mapped to chromosomes 13g21-22 (Kainu et al. 2000) and 2q regions (unpublished data), which have recently been reported by Dr. Juo andhis colleagues in different populations, will be found in some BRCA1 and BRCA2 negative Chinese and Korean breast cancer families participating in the NY Registry.

Application ID: B-NY-0899-01 **Status:** Approved, Inactive

Principal Investigator: Hurley, Karen **Year:** 1999

Institution: Mount Sinai Medical Center, New York

Title: *Factors Associated with BRCA 1 / 2 Testing among Ashkenazi Jewish Women in Three Countries*

Abstract:

The population frequency of three founder genetic mutations (185delAg and 5382insC in BRCA1 and6174delT in BRCA2) in women of Ashkenazi (Eastern European) Jewish descent is approximately 2.5%, compared to .3% in the general population. Efforts to improve counseling services for Ashkenazi Jewish women who are trying to make decisions about genetic testing for susceptibility to cancer and managing their cancer risk must build on knowledge accumulated through mainstream research in a way that also addresses issues that are specific to these women. These issues include: increased probability of being agene carrier, truncated family histories that make risk estimation more difficult, and concerns about social stigma. These population- specific factors exert their influence within the context of general health beliefs such as perceived risk, and institutional factors such as access to care (universal coverage vs. fee-for-serviceor risk-based insurance) that vary by region and country. Understanding the factors that go into decision-making about genetic testing for BRCA1/2 mutations will provide a sound, scientific basis for future intervention studies.

We propose a pilot project with the following aims:

1) to describe uptake of genetic testing among Ashkenazi Jewish women seeking counseling in the U.S., Canada and Australia about genetic risk for cancer;

2) to identify factors associated with uptake of genetic testing;

3) to examine regional and national differences in uptake and associated factors within this ethnic group; and

4) to demonstrate the feasibility of carrying out a multi-site prospective psychosocial study with this population.

Predictions are that: 1) most, but not all, Ashkenazi Jewish women seeking counseling about genetic riskfor cancer will pursue testing; 2) perceived risk of breast cancer, anxiety about developing cancer, feelings of stigma and concerns about confidentiality of results will be associated with uptake of genetic testing; and3) women in the U.S. will report greater perceived risk, higher levels of cancer anxiety, and greater concerns about confidentiality than Australian and Canadian women.

Application ID: B-EX-0499-02 **Status:** Approved, Inactive

Principal Investigator: Pike, Malcolm **Year:** 1999

Institution: University of Southern California Medical School

Title: *GnRH Agonist Chemoprevention of Breast Cancer*

Abstract:

There is overwhelming evidence for the role of ovarian hormones in the etiology of breastcancer.'Epidemiological studies have found that early menopause reduces breast cancer risk. Bilateraloophorectomy has a larger effect; a 65 to 75% decrease in risk with oophorectomy around age 35. Breastcell proliferation is substantially higher before than after menopause; peak mitotic activity is in the mid-luteal phase of the menstrual cycle and is consistent with a mitogenic effect of estrogen andprogesterone. Total breast cell proliferation in women using oral contraceptives (OCs) appear similar tothe rates in women with normal cycles. This finding is consistent with the epidemiological data whichshow either no effect, or a small increased risk of breast cancer, with OC use? In contrast, OCs have asubstantial protective effect against both endometrial and ovarian cancer; 'OCs inhibit cell proliferationin these tissues, but not in breast epithelium. OCs have the same ovarian cancer protection in mutBRCA1.

OCs inhibit ovarian steroidogenesis and reduce serum E2 and progesterone (Prg) levels to close topostmenopausal levels. OCs would be associated with a substantial reduction in breast cancer risk were itnot for the fact that they also contain considerable amounts of synthetic analogs of E2and Prg. Ahormonal regimen, which uses a GnRHA to suppress ovarian function, needs to include only very lowdoses of sex-steroids. The amount of add-back estrogen could be similar to that used for estrogenreplacement therapy (ERT), and the add-back progestin could be given intermittently. Thus, the dose ofboth sex-steroids would be much lower than in OCs, and lower than at any time in the menstrual cycle.Such a regimen should reducelifetimebreast cancer risk by almost one third if used for 5 years, by morethan 50% if used for 10 years, and by 70% if used for 15 years.

On mammograms, fat appears as a radiologically lucent area, whereas connective and epithelialtissue appear as mammographic `densities'. Measured mammographic densities show a breast cancer riskgreater than that of almost all other breast cancer risk factors.'8Postmenopausal women have reducedmammographic densities compared with premenopausal women of the same age.'We have argued thatmammographic densities are related to breast epithelial cell proliferation. At least part of the lowereffectiveness of mammographic screening in younger women is because they have mammographicallydenser breasts.

The risk of breast cancer in mutBRCAI individuals varies substantially between and withinfamilies. It is likely that at least some of the variability in mutBRCA1 breast cancer risk is due to otherfactors. In mouse mammary cells,BrcalmRNA is increased by stimulation with estradiol andprogesterone. BRCA1 appears to be a hormonally responsive growth regulating gene. These results suggest that hormones play an important role in the development of breast cancer in women with aBRCA1 mutation, although this has not been shown directly. This study will show that breast tissue ofmutBRCAI carriers responds to lower sex-steroid hormone levels in a manner similar to that of women with wtBRCA1 (see Preliminary Studies).

Chronic administration of a GnRHA produces paradoxical suppression of follicle-stimulatinghormone (FSH) and luteinizing hormone (LH) release and

hence suppression of ovarian function. Inpremenopausal women the use of GnRHA is limited by the significant negative effects of sex-steroiddeficiency. The use of a GnRHA with add-back ultra-low-dose sex-steroids is predicted to prevent thenegative effects of the GmRHA. The long-term use of GnRHA has also been shown to reduce serum Tby as much as 50%.

The goal of this program is to develop a daily transmucosal drug product consisting of thegonadotropin-releasing hormone agonist (GnRHA) deslorelin (D), with partial replacement of estradiol(E2) and testosterone (T). The product is designed for the long-teen treatment of premenopausal women,who are at known high risk of breast cancer on the basis of (1) family history and (2) a BRCA1 mutationto achieve a substantially reduced breast (and ovarian) cancer risk, and to enhance the efficacy ofpremenopausal mammographic screening.

Specific aims of this study are to demonstrate the reduction of mammographic densities with thecurrent intranasal drug regimen of Balance Pharmaceuticals, Inc., and to demonstrate the efficacy of theintranasal E2plus T, in the presence of sufficient D to suppress ovarian function, in preventing signs andsymptoms of hormone deficiency. The testosterone dose is aimed at simply replacing the ovarian Tproduction blocked by D.

This study is to develop pilot experience and to verify the reduction of mammographic densities(surrogate endpoint biomarker). Mammographic densities will be quantitated at baseline, after 6 and 12months of treatment and after 12 months off study. This step is necessary prior to proposing anyrandomized study.

Twenty of the 25 treated subjects will have a known BRCA1 mutation. Five additional subjects athigh risk of breast cancer on the basis of family history, but with wild type BRCA1 or unknown BRCAIstatus, will also be included, so that merely being in the study does not identify the subject as having amutBRCA1 germline mutation.

Application ID: B-NY-1298-01 **Status:** Approved, Inactive

Principal Investigator: Bernstein, Jonine **Year:** 1998

Institution: Mount Sinai Medical Center, New York

Title: *A Comparison of BRCAl Mutations found in DNA from Paraffin-Embedded Breast Tumor Tissue with Mutations in DNA from Lymphocytes: A Feasibility Study*

Abstract:

Using DNA isolated from fixed, paraffin- embedded tissue blocks is an expeditious way to conduct genetic epidemiologic studies, as blood or fresh tissue may be unavailable . One very small study suggested mutations detected from archived ovarian tumor tissue blocks may provide unreliable DNA sequence data . The purpose of this feasibility study is to determine whether, among the same subjects, BRCA1 mutations found in DNA isolated from paraffin-embedded breast tissue differ from BRCA1 mutations found in blood.

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Application ID: B-TO-0598-01 **Status:** Approved, Inactive

Principal Investigator: Darlington, Gerarda **Year:** 1998

Institution: Cancer Care Ontario

Title: *Study Design Issues for Investigating Both Genetic and Environmental Risk Factors in a Case-Control Study*

Abstract:

With emerging information regarding genetic mutations linked to breast cancer, the simultaneous investigation of genetic and environmental factors is possible. Specifically, the existence of the Cooperative Family Registry for Breast Cancer Studies (CFRBCS) will make studies of this type feasible.

One approach to address questions of this type is the case-control design. While in traditional epidemiologic studies controls are randomly selected from disease-free individuals in the population from which cases were selected, other alternatives for genetic epidemiologic studies have been considered. In particular, advantages and disadvantages of using sibling or cousin controls have been addressed when the focus of study is the effect of candidate genes (Witte et al ., 1998).

If the question of interest in a case-control study is to measure the independent effects of genetic and environmental risk factors, then the use of family controls may, because of overmatching, result in bias in the estimation of environmental effects. For some design choices (i .e . use of pseudosibs as controls) only interactions between genetic and environmental factors can be evaluated.

For breast cancer research, the nature and extent of this potential bias can be investigated using data from the Ontario centre of the CFRBCS. In this proposal, we will use data from breast cancer cases and their family members to derive realistic parameters for a theoretical study of bias and efficiency issues associated with using family controls versus population controls.

The CFRBCS is crucial resource for studying the combined effects of genetic and epidemiologic risk factors. Certain study designs may preclude the estimation of environmental effects and should be avoided if these variables are of interest. This methodologic study will quantify bias and efficiency problems that may result from using family controls in case-control studies of breast cancer. Use of data from the CFRBCS will allow methodologic study

with relevant parameters and will allow exploration of feasibility of various design alternatives.

Application ID: B-PH-1298-01 **Status:** Approved, Inactive
Principal Investigator: Johnson, Robert **Year:** 1998
Institution: Coriell Cell Repositories
Title: *Cell Cycle Control and DNA Repair in Breast Cancer*

Abstract:

Highly penetrant genes such as BRCA- 1 and BRCA-2 which are strongly associated withbreast cancer account for only five percent of cases, while inherited mutations in the ataxiatelangiectasia and Li-Fraumeni genes together probably account for a similar number. Themajor problem is, therefore, to identify other genes of lower penetrance whose mutationsmay contribute to breast cancer. One common thread linking the gene products of BRCA-1and 2, ATM and p53 is their participation in DNA repair and/or in checkpoint control of thecell cycle after DNA damage. Using the nomenclature of Kinzler & Vogelstein (1997),these genes act either as caretakers (BRCA-1 & 2), or as caretakersandgatekeepers(ATM, p53); the dual role of the latter may make them particularly critical in any genomedestabilization that leads to breast cancer.Moreover, reports suggest that in up to 40 percent of breast cancer cases there is an increased susceptibility of normal peripheral bloodcells to ionizing radiation, implying the involvement of additional genes withcaretaker/gatekeeper roles that protect the genome from damage (Helzlsouer et al 1995;Scott et al 1996, 1998).

Specific Aim(s):

The hypothesis to be tested, therefore, is that, in addition to cells expressing BRCA-1 or 2,or ATM mutations, a significant fraction (perhaps up to 30 per cent) of peripheral blood-derived cell lines from breast cancer patients collected in the Cooperative Family Registrywill show enhanced genome damage after X-irradiation. Since the multicomponentmechanisms of repair and cycle control that operate during S-phase to allay the effects ofionizing radiation damage include BRCA-1 and 2, ATM and p53 gene products, it is also hypothesized that the postulated mutations in new candidate genes responsible for theexaggerated sensitivity are likely to be components of these signalling/repair mechanisms whose major role is to repair and otherwise protect the S-phase genome from disruption.

Application ID: B-EX-1298-03 **Status:** Approved, Inactive
Principal Investigator: Kushi, Lawrence **Year:** 1998
Institution: University of Minnesota
Title: *Interaction of Dietary Factors and Estrogen Metabolites and Estrogen- and Carcinogen-Metabolism Genes with Risk of Breast Cancer*

Abstract:

Hypothesis

Various dietary factors aside from dietary fat have been proposed to be associated with breast cancer risk . Among factors that may increase risk, intake of meat, particularly well-done meat with high levels of heterocyclic amines, has been suggested. Factors such as retinol, cruciferous vegetables, and soy have been proposed to decrease breast cancer risk. Although there has been interest in these factors, findings have generally been inconsistent or inadequately studied. It is suggested that the risk of breast cancer that may be attributable to these factors may be modified by genotype of relevant carcinogen- or estrogen-metabolizing enzymes. This study proposes to examine whether intake of these and related dietary factors differs among women with differing genotypes of several such enzymes, and whether dietary factors are associated with differences in the ratio of 2-hydroxy to 16alpha-hydroxy El metabolites in urine. Possible interactions between dietary factors and these biological parameters on breast cancer risk will also be examined.

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Application ID: B-NY-1298-02-A1 **Status:** Approved, Inactive
Principal Investigator: Oddoux, Carole **Year:** 1998
Institution: New York University Medical Center
Title: *Genetic Susceptibility to Breast Cancer Among Ashkenazi Jews*

Abstract:

This project will test the basic hypothesis that a given microsatellite marker allele in linkage disequilibrium with a cancer-predisposing mutation occurs with greater frequency among the cases than among the controls.For this case-control study, we propose to analyze Ashkenazi Jewish women at high heritable risk for breast cancer in comparison to age-matched low risk family members as well as to population based controls who do not have a family history of breast cancer and therefore are at very low heritable risk. Each group will have approximately 200 women. In addition, we will determine allele frequencies in a group of 100 family controls that are more closely matched for age andenvironmental exposures. High-density (<1cM) genetic marker analysis will be performed on each of these women using polymorphic markers from regions known to be linked to the BRCA1 and BRCA2genes on chromosomes 17 and 13, respectively. Relative risks will be calculated for cases versus controls. In turn, these high-risk markers will be correlated with the presence of mutations known to be a higher frequency in the Ashkenazi Jewish population (BRCA1 185delT and 5382insC and BRCA2 6174de1T). If other high- risk chromosomes (i.e., not associated with known mutations) are identified, these will be sequenced to identify novel mutations in the BRCA1/2 genes. Should such mutations be identified, their frequencies will be determined in the high and low risk panels and relative risks will be calculated. This study will test the feasibility of using association in a homogeneous population as a method for identifying genes that increase the risk for developing breast cancer. If successful, this method can be used for identifying susceptibility genes for breast cancer at other sites in the human genome.

Specific Aim(s):

This is a proposal to calibrate a method that could represent an important technique for identifying genes and mutations that increase susceptibility to cancer.The Ashkenazi Jewish founder mutations in the known breast cancer susceptibility genes BRCA1 and BRCA2 will be used as a model to test the utility of linkage disequilibrium (LD) for the identification of cancer predisposition genes and mutations.

1) The degree of LD will be determined as a function of genetic distance from the gene.

2) The degree to which the markers in aggregate and per marker can predict the presence/absence of Ashkenazi Jewish mutations will be examined. (This is analogous to the sensitivity, specificity and predictive value of clinical tests.

3) Do the markers show an association with breast/ovarian cancer when theAshkneazi Jewish mutations are negative?

4) If there is LD for the persons in #3, then can mutations in exons or regulatoryregions be identified?

Application ID: B-EX-0598-01 **Status:** Approved, Inactive

Principal Investigator: Wooster, Richard **Year:** 1998

Institution: Sanger Center

Title: *Mutations in the ATM Gene and Risk of Breast Cancer*

Abstract:

N/A

Specific Aim(s):

This study seeks to determine if having a germline mutation in the ataxia-telangiectasia (A-T) mutated (ATM) gene, predisposes a woman to breast cancer, and if so, to estimate the risk of breast cancer in muation carriers of given ages.

Application ID: B-TO-0997-02 **Status:** Approved, Inactive

Principal Investigator: Boyd, Norman **Year:** 1997

Institution: The Ontario Cancer Institute

Title: *Mammographic density and risk of breast cancer in carriers of BRCA 1 and 2 mutations*

Abstract:

A family history of breast cancer is an established risk factor that is in some families due to the inheritance of mutations in the BRCA1 and BRCA2 genes. Although mutations in these genes are associated with a striking increase in risk of breast and ovarian cancer, they are present in only a small proportion of cases with other affected relatives, and other factors must exist that contribute to familial aggregation of the disease. Further, although carriers of mutations in these genes are at marked increased risk of breast cancer, there is much variation in age at onset, and there are, among genetically predisposed individuals, some who do not develop breast or ovarian cancer at any age. Other factors, genetic and environmental, are therefore likely to exist that modify risk. In this application we propose to examine the role in familial and genetic predisposition to breast cancer of radiologically dense breast tissue, an appearance that we describe further below and refer to as “mammographic densities”, which we and others have in previous work found to be strongly related to risk of breast cancer, and are influenced by many of the reproductive and hormonal factors that influence risk of the disease. Further, as we show below, mammographic densities are common in the population, and there is evidence that they are heritable. Women with breast cancer and a family history of the disease will have more extensive mammographic densities than women with breast cancer without such a family history. In the research proposed here we will determine whether women with breast cancer who are carriers of mutations in BRCA1 or BRCA2 genes will have more extensive mammographic densities than women with breast cancer that are not carriers. Further we will determine whether mammographic densities influence risk of breast cancer among women with a family history of the disease, and among carriers of mutations in BRCA1 or BRCA2 genes.

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Application ID: B-EX-1196-12 **Status:** Approved, Inactive

Principal Investigator: Lewis, Cathryn **Year:** 1996

Institution: Guy's Hospital, United Medical and Dental School

Title: *Sib Pairs Analysis for Breast Cancer Susceptibility Genes*

Abstract:

Not on file.

Application ID: B-AU-0403-02 **Status:** Approved, Complete

Principal Investigator: Phillips, Kelly-Anne **Year:** 2003

Institution: Peter MacCallum Research Institute

Title: *Validation Of Self-Reported Breast Cancer Treatment Against Medical Records In The Cooperative Family Registry For Breast Cancer Studies*

Abstract:

Self-reported data is a potentially relatively inexpensive and convenient way to obtain data regarding breast cancer treatment from participants of large-scale epidemiological studies. A self-report treatment questionnaire has been developed for use within the CFRBCS, but has not been validated. As part of the CFRBCS BRCA prognosis study, we have abstracted treatment data from the medical records of all consenting living breast cancer cases. This affords the opportunity to compare the data derived from the self-report questionnaire with that abstracted from the medical record (the latter being considered the “gold-standard”), thus enabling a formal validation study of the self-report instrument.

Specific Aim(s):

Specific Aims

1) To determine the validity of self-report of initial breast cancer treatment, as documented in the CFRBCS treatment questionnaire, using data abstracted from the medical record as the “gold-standard”

2) To examine predictors of accuracy of self-report. Factors that will be examined include: type of treatment data sought (eg surgery versus hormonal therapy), time since cancer diagnosis, age, and socioeconomic status.

HYPOTHESES

1) That self-report of initial breast cancer treatment is inaccurate.

2) That predictors of more accurate self-report of breast cancer treatment include type of treatment data sought (eg surgical data more accurate than hormonal data), time since cancer diagnosis and socioeconomic status.

Application ID: B-AU-0502-01S-A1 **Status:** Approved, Complete

Principal Investigator: Dite, Gillian **Year:** 2002

Institution: University of Melbourne

Title: *Familial Risks Associated with Breast Cancer*

Abstract:

This study proposes to use data from the Australian Breast CFR in an analysis of familial aggregation of breast and other cancers in a cohort of first- and second-degree relatives of women with breast cancer and population-based controls. Particular attention will be focused on analysis of the familial aggregation of breast and other cancers that remains after exclusion of the relatives of known carriers of mutations in BRCA1 and BRCA2. It also aims to pilot analytic methods that could be used in a larger collaborative analysis using pooled data from both case and control families from the three population-based Breast CFRs.

The study will use family history data from first-and second-degree relatives of population-based case and control probands from the Australian Breast CFR who were aged under 60 years at the diagnosis of their breast cancer or at the time of their recruitment, respectively. It will also incorporate data

from a population-based, case-control-family study of breast cancer with data collected prior to the establishment of, but with almost identical study protocols as, the Breast CFRs.

Application ID: B-EX-0302-01S **Status:** Approved, Complete
Principal Investigator: Horsman, Douglas **Year:** 2002
Institution: British Columbia Cancer Agency
Title: *Validation of Automated Fluorescent SSCP as a Test Method for Mutation Analysis*

Abstract:

Numerous genetic mutation analysis methods are available and are being utilized by different institutions to test for inherited mutations of the BRCA1 and BRCA2 genes. One recent validation study has been undertaken by the Breast Cancer Family Registries (Breast CFR) institutions to assess the capability of a variety of laboratory techniques to detect known mutations in the BRCA1 gene. One of the test methods assessed was single strand conformation analysis (SSCP). One recent report has indicated that manual SSCP has a significant false negative rate for detection of mutations. Our Program has used a variation of SSCP (automated fluorescent SSCP or afSSCP) using fluorescence labeled DNA and gel electrophoresis analyzed on an ABI 377 sequence analyzer using Genescan software. To this point, we have had a high level of confidence in the ability of this mutation scanning technique to detect BRCA1 and BRCA2 mutations. We would like to reassess this capability using the 22 sample validation set that the Breast CFRs have recently studied.

Specific Aim(s):

Automated fluorescent SSCP is superior to manual SSCP for detection of inherited mutations in the BRCA1 and BRCA2 genes.

Application ID: B-NY-0802-01 **Status:** Approved, Complete
Principal Investigator: Santella, Regina M. **Year:** 2002
Institution: Columbia University
Title: *T7271G and IVS10-6TtG Mutations in BRCA1 and 2 Negative Families*
Abstract:

Mutations in BRCA1 and BRCA2 have been identified in only fraction of families in the Breast and Ovarian Cancer Family Registry. Thus, other genes likely contribute to risk in Registry participants. ATM is one candidate gene and two mutations, T7271G and IVS10-6T[tau symbol]G, have recently been identified in several multiple case families. This proposal is a joint submission for the three clinic-based sites, Columbia University, Fox Chase Cancer Center and Huntsman Cancer Institute, to analyze subjects who are negative for BRCA1 and BRCA2 mutations for the two mutations in ATM. Each site will carry out the laboratory analyses on their own subjects, but data will be reported jointly.

Specific Aim(s):

Hypothesis

We hypothesize that two mutations in the ATM gene, T7271G and IVS10-6T G, are associated with breast cancer in families who do not harbor mutations in the BRCA1/2 genes. We propose to examine this hypothesis among BRCA 1 and 2 negative families from the 3 clinic-based centers participating in the NCI's Breast and Ovarian Cancer Family Registry (CFR) for whom genomic DNA and questionnaire and family data have already been collected.

Application ID: B-SF-0602-02S **Status:** Approved, Complete
Principal Investigator: Whittemore, Alice **Year:** 2002
Institution: Stanford University School of Medicine
Title: *All-cause Mortality and Cancer Incidence in Relation to Ashkenazi Jewish Heritage among Individuals of Admixed Heritage*
Abstract:

Ashkenazi Jews in Europe experienced a bottleneck in population size during the Middle Ages, and this bottleneck has resulted in their having relatively high prevalence of certain mutations, called "Founder Mutations." Consequently, Askenazi Jews have increased prevalence of some rare and fatal conditions, including cancers of the breast and ovary due to mutations in BRCA1 and BRCA2. There has been considerable interest in detecting genes associated with diseases that vary across historically isolated populations by studying present-day admixture populations. Many US Jews are of mixed AJH and non-AJH, and the proposed analysis represents and exploratory first step to investigate the feasibility of gene detection by studying admixed populations. The work has potential applications to disease risks in other admixed racial or ethnic groups.

Specific Aim(s):

Aims

We aim to evaluate all-cause mortality and site-specific cancer incidence in relation to Ashkenazi Jewish heritage (AJIT) among individuals in the CFRBCS who are of AJH, of Caucasian non-AJH, and admixed with ancestors of both AJH and non-AJH.

Hypothesis

Our hypothesis is that there exists a gradient in mortality and cancer incidence rates with respect to extent of AHJ, with rates highest among individuals of pure AJH, intermediate among admixed individuals, and lowest among individuals of pure non-AJH.

Application ID: B-TO-1200-01S **Status:** Approved, Complete

Principal Investigator: Boyd, Norman **Year:** 2000

Institution: The Ontario Cancer Treatment Center

Title: *The Genetic Epidemiology of Breast Cancer: A Case-Parental Control Study of Candidate Genes and Risk of Premenopausal Breast Cancer and Breast Density Phenotype*

Abstract:

The goal of this study is to find genetic determinants of breast cancer risk. One of the strongest risk factors for breast cancer, mammographic density, is a phenotypic trait that is determined by environmental and genetic factors. A twin study determined that the heritability of breast density is at least 75%. Mammographic density is a continuous trait that is likely determined by multiple genes, and phenotypic variation in mammographic density is likely the result of normal genetic variation and environmental factors. It may be possible to find genes other than BRCA1 and BRCA2 that are involved in familial breast cancer by looking for genes that influence breast density. The genes that influence breast density may increase breast cancer risk. Examining the results of studies assessing the association between lipids and breast cancer yields likely candidate genes for breast density and breast cancer. A particular fasting lipid profile has been observed among women at high risk of breast cancer as measured by previous incidence of the disease, family history of breast cancer, or increased mammographic density, which cannot be explained by measured environmental factors including dietary fat. Genes controlling lipid metabolism, either directly or indirectly, may contribute to both breast density and breast cancer risk. The following candidate genes have been selected as the most likely to explain the association between lipid metabolism and risk of breast cancer: apolipoprotein E, apolipoprotein CIII, hepatic lipase, seven alpha hydroxylase, paraoxonase, lipoprotein lipase, growth hormone, growth hormone receptor, growth hormone releasing hormone and growth hormone releasing hormone receptor. To test for an association between the candidate genes and breast cancer and breast density a population based case-parental control study has been designed, which maximizes power and eliminates confounding due to population stratification. To complete this study 163 families will be required. Cases are premenopausal women aged 30 to 50, diagnosed with incident invasive breast cancer (ICD 174), with a family history of the disease. For the family to be eligible, at least one parent must have DNA available for genotyping. Genotyping will be carried out using standard PCR based methods. Mammographic density will be measured by standard computer assisted methods. Statistical analyses will be carried out using the transmission disequilibrium test, which is appropriate for both categorical outcomes such as breast cancer and continuous outcomes such as mammographic density. Finally, the study as designed is both cost-effective and highly appropriate method to test candidate genes for an association with breast cancer risk.

Specific Aim(s):

Genes involved in the regulation of lipid metabolism may be candidates for increased risk of breast cancer, and these genes may explain some of the variation in breast density phenotype measured mammographically.

Purpose: To determine whether common polymorphisms of the lipid metabolism regulatory genes, including genes involved in growth hormone signalling which influence lipid metabolism, are associated with breast cancer and the breast density phenotype in premenopausal women. The genes to be tested are the apolipoprotein E (APO E), apolipoprotein CIII (APO CIII), hepatic lipase (HL), 7 alpha hydroxylase (CYP7), paraoxonase (PON1), lipoprotein lipase (LPL), pituitary growth hormone (GH1), growth hormone receptor (GHR), and growth hormone releasing hormone (GHRH) and its receptor (GHRHR).

Specific Hypotheses to be tested:

1. Common polymorphisms of genes involved in lipid metabolism may be associated with familial breast cancer in premenopausal women. The genes to be tested are apo E, apo CIII, HL, CYP7, PON, LPL, GH1, GHR, GHRH, and GHRHR. Refer to Appendix 1 for detailed information on the selected polymorphisms.

2. These same polymorphisms may be associated with phenotypic variation in mammographic density in premenopausal women.

Application ID: B-TO-0499-02 **Status:** Approved, Complete

Principal Investigator: Andrulis, Irene **Year:** 1999

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital , Toronto

Title: *Investigation of Associated Somatic Molecular Genetic Abnormalities in Breast Carcinomas of Ashkenazi Jewish Women with 617delT Germline Mutation in BRCA2*

Abstract:

The specific objective of this proposal is to classify the families in the CFRBCS according to their BRCA1 and BRCA2 (BRCA1/2) genotypes to enhance the value of the Registry.

The CFRBCS contains families identified through population-based registries and high-risk clinics. To date mutational studies have focused mainly on families with a very strong history of breast and/or breast/ovarian cancers . Even though the BRCA1/2 genes have been extensively studied in highrisk families, investigators have failed to detect mutations in approximately 30% of the families. Thus, it is likely that there are additional breast cancer susceptibility genes. In addition to the high-risk families, there are also low and medium risk families with a history of breast/ovarian cancer in the CFRBCS. The decreased risk in such families may be explained by the presence of novel BRCA1/A2 mutations, mutations in other predisposition genes, or BRCA1/2 mutations identical to those observed in the high-risk group, whose phenotypic expression is influenced by modifier genes or environmental factors . We are far from identifying all of the different mutations in the BRCA1/2 genes, clarifying their effect on protein function, and determining how each contributes to the disease phenotype.

Specific Aim(s):

Knowledge of the germline BRCA1/2 status of the individuals in the CFRBCS is an essential first step in utilizing the Registry for molecular epidemiological studies including those to address genotype-phenotype correlations, gene-gene and gene-environment interactions . Identification of these specific mutations will lead to refinement of models to predict the probability of carrying a BRCAI/2
mutation based on personal and family histories of breast and/or ovarian cancer and to the development and implementation of better genetic tests for cancer predisposition. In addition, information on the BRCA 1 /2 status is necessary for further molecular and gene discovery studies.

Application ID: B-PH-0599-01-AS **Status:** Approved, Complete

Principal Investigator: Daly, Mary **Year:** 1999

Institution: Corielle Cell Repositories

Title: *Creation of a Permanent Genetics Resource at the CFRBCS X*

Abstract:

The Cooperative Family Registry for Breast Cancer Studies (CFRBCS) is an international multi-site cooperative consortium of investigators who collaborate to ascertain families at risk for breast cancer, collect pedigree information, epidemiological and clinical data, biological specimens from these individuals, and to provide these resources to the research community. Three of these sites are population-based cancer registries (Northern California Cancer Center, the University of Toronto, and the University of Melbourne); and three are high-risk registries (Fox Chase Cancer Center, Metropolitan New York Registry, and Huntsman Cancer Institute). From each individual participating in the Registry, sufficient blood is collected (30 mL) to permit the storage of frozen whole blood for DNA isolation, the preparation of plasma, and the isolation of lymphocytes. These lymphocytes, which are cryopreserved with 10% DMSO and stored in liquid nitrogen, may be used for nucleic acid preparation and/or the establishment of a cell line.

In the aggregate, from the material currently stored, the Registry would be able to provide between 100-250 micrograms/individual to investigators interested in this resource. Given the current requirements for genome-wide scans, it would seem that the Registry could provide DNA samples to a relatively few individuals.

In anticipating the resources required for gene discovery and characterization, it seems prudent to have a source for production of unlimited quantities of DNA available. The establishment of a cell line would provide this source for DNA. In addition to providing an unlimited supply of DNA, the establishment of cell lines will eliminate the need to resample individuals. This is extremely important because some individuals may have expired or refuse to be re-sampled. Having the cell line available also provides a source of other products such as RNA, which may be potentially important for generating screening assays as well as providing cultured cells for functional studies.

Furthermore, it is anticipated that cell lines from the CFRBCS participants will be extremely desirable because of the wealth of clinical and family history data that will accompany each sample. To this end, the following specific aims are proposed:

a) Enhance the resource by the establishment of lymphoblastoid cell lines from a subset of CFRBCS participants. At each participating site, lymphoblastoid cell lines will be established and stored for future genetic exploration by using Epstein-Barr virus transformation protocols and storage in liquid nitrogen.

b) Establish quality standards that will guarantee the success of a variety of gene discovery and gene characterization approaches. Once the cell lines are established, they will be tested for viability and sterility. Only those lines that are viable and are free from bacterial, fungal, and mycoplasma contamination will be accepted into the Registry.

In summary, transformation of cryopreserved lymphocytes from CFRBCS participants will provide an unlimited supply of nucleic acids from individuals for many studies, and, at the same time, eliminate the need to re-sample individuals.

Application ID: B-TO-0899-01 **Status:** Approved, Complete

Principal Investigator: Esplen, Mary Jane **Year:** 1999

Institution: Mount Sinai Hospital, Toronto

Title: *Development of a Group Counseling Intervention for BRCA1 and BRCA2 Carriers*

Abstract:

Relevance

Our proposed research has direct relevance for advancing knowledge on the communication and promotion of integration of genetic testing information to individuals at risk for cancer syndromes. Improved counselling techniques for those with a family history may impact on the prevention of BC through improved surveillance and preventive techniques, enhanced psychosocial functioning and well-being, facilitate the decision to undergo genetic testing among other family members in the future and may promote the adjustment to BC, should individuals "at risk" later develop the disease.

Specific Aim(s):

Hypotheses

(1) to develop, describe, and standardize a group intervention that incorporates principles of supportive-expressive therapy for individuals with a family history of BC who test positive for BRCA1 and BRCA2 and to examine the impact of this intervention on pre- and post-intervention measures of: (a) psychosocial functioning (cancer-specific distress, depression, anxiety and grief), (b) knowledge about BC risk/genetics, and (c) screening behaviors;

(2) to examine, in a descriptive fashion, the relationship between psychosocial factors, demographic characteristics, family functioning, and BC knowledge before and after the group intervention and to identify potential predictive factors of improvement in psychological adjustment, knowledge, and screening.

Application ID: B-PH-0499-01 **Status:** Approved, Complete

Principal Investigator: Godwin, Andrew **Year:** 1999

Institution: Fox Chase Cancer Center

Title: *Identification of Candidate Cancer Susceptibility Loci in Ashkenazi Jewish Breast Cancer and Breast-Ovarian Cancer Prone Kindreds*

Abstract:

The last decade has seen a virtual explosion in the description of genes that regulate the growth and differentiation of cells, determine response to endogenous and exogenous agents, and maintain the integrity of an individual's genetic constitution. In this aspect, two hereditary breast cancer genes, BRCA1 and BRCA2, have been cloned (1-3). Germline mutations in BRCA1 and BRCA2 account for the majority of families with both hereditary breast and ovarian cancer, but only 30 to 50% of hereditary breast cancer is found in kindreds. Therefore, other genes must exist that contribute to hereditary breast cancer and possibly sporadic forms of the disease as well.

First, we propose to apply the comparative genomic hybridization (CGH) technique to identify chromosomal sites that are commonly deleted in tumors from high-risk Ashkenazi Jewish families negative for a BRCA1 or a BRCA2 founder mutation. Comparative genomic hybridization is a valuable method for examining genetic imbalances within entire tumor genomes. We hypothesize that consistent genomic imbalances identified in different cancers (not only breast and ovarian tumors), within and between high-risk cancer families, will represent visible manifestations of a common event(s) critically involved in the development of these neoplasms. Second, we propose to refine the candidate regions identified by CGH. A lazer capture microdissection system will be used to isolate more pure populations of tumor cells and the corresponding DNA will be evaluated for evidence of consistent loss of heterozygosity (LOH). Third, panels of microsatellite repeat polymorphisms will be used to establish linkage to the regions identified by CGH and LOH analyses. DNA isolated from Ashkenazi families with a minimum of three confirmed cases of breast and/or ovarian cancer will be typed using markers spanning the region of interest. LOH data will be incorporated into the likelihood calculations. Overall, the studies proposed should enable us to identify chromosomal sites harboring candidate genes that predispose women (and likely men) to cancer.

Specific Aim(s):

Hypothesis

The ultimate goal of this proposal is to identify genes, in addition to BRCAI and BRCA2, that predispose to breast and/or ovarian cancer in Ashkenazi Jewish women. It is becoming increasingly clear that additional highrisk alleles must exist and that multiple approaches must be used to uncover them. We propose to use biological specimens from participants with a family history of breast cancer and breast-ovarian cancer collected through the CFRBCS to search for candidate loci housing known genes or currenly unidentified genes. Comparative genomic hybridization (CGH), allelic loss mapping, and linkage will be used in combination to identify new gene loci that predispose to these cancers. CGH will be used to identify recurrent chromosomal losses, gains, and DNA amplifications that contribute to the pathogenesis of hereditary breast cancer . We hypothesize that consistent genomic imbalances identified in tumors from breast cancer syndrome families will represent visible manifestations of molecular genetic events critically involved in the development of these neoplasms. Allelic loss mapping will be used to refine the location of candidate susceptibility genes, and genotyping of cancer-prone families will be used to establish potential association by linkage. Identification of as many genetic factors as possible will be important in ultimately determining a woman's lifetime risk of developing breast cancer.

To estimate the proportion of breast cancer families attributable to mutations in BRCAI or BRCA2, in a multicenter study we screened a large panel of North American Jewish breast cancer families. Among the 220 eligible families (i .e., a minimum of two women affected with breast cancer, at least one

of whom was diagnosed with breast cancer at age 50 or younger), 82 also contained cases of ovarian cancer. At least one woman with breast or ovarian cancer from each family was screened for the recurrent mutations. A total of 100 families (45.5%) carried BRCAI or BRCA2 mutations, including 71 families with the 185delAG mutation (32 .3%), 20 families with 5382insC (9.1%) and 9 families with the 6174de1T mutation (4 .1%). Overall, we found that of the 138 site-specific breast cancer families (no ovarian cancer), 40 (29%) carried one of the recurrent BRCAI or BRCA2 mutations. In comparison, we found that of the 82 breast-ovarian cancer syndrome families, 60 (73 .2%) carried a mutation (4) . Our studies are consistent with another report which found that 79% of high-incidence Jewish breast cancer families and 35% of high-incidence Jewish breast-ovarian cancer families had none of the three known founder mutations, indicating that more mutations or genes responsible for inherited breast and ovarian cancer remain to be found in this population (5) . In this aspect we have screened 50 BRCAI recurrent mutation negative individuals and have not identified any additional BRCAI truncating mutations, further suggesting that other genes must be involved (Daly and Godwin, unpublished data). Therefore, we propose to use several approaches to begin to identify additional loci that may house genes that predispose to breast cancer. Our focus will be on Ashkenazi breast cancer and breast-ovarian cancer syndrome families that have tested negative for founder BRCAI or BRCA2 mutations . We have targeted this population because they have been characterized (at least partially) in regards to their BRCAI and BRCA2 mutation status and are likely to be less heterogeneous in respect to the number of potential genes which contribute to hereditary forms of breast cancer as compared to other breast cancer syndrome families collected through the CFRBCS.

Application ID: B-TO-0499-03 **Status:** Approved, Complete

Principal Investigator: Knight, Julia **Year:** 1999

Institution: Mount Sinai Hospital, Toronto

Title: *Genetic Polymorphisms of the Vitamin D Receptor and Risk of Breast Cancer*

Abstract:

The primary aim of this proposal is to test whether certain specific common variants of the vitamin D receptor (VDR) gene are associated with an increased risk of breast cancer. VDR are expressed in normal breast tissue as well as in breast tumours. Vitamin D and its receptor have been shown to influence cell proliferation in breast cancer cell lines and common VDR polymorphisms have been associated with the risk of prostate cancer. We hypothesize that common, possibly functional, polymorphisms of the VDR gene such as the variable poly(A) repeat or the FokI polymorphism, are associated with breast cancer development. We plan to test this hypothesis with a case control study making use of the existing, population-based Ontario Familial Breast Cancer Registry (OFBCR) to test this hypothesis. The OFBCR collects cancer family history, risk factor information, and blood samples from women newly diagnosed with invasive breast cancer and from some of their relatives. Cases and related controls will be identified from the OFBCR. Because there is controversy about the effects on bias and efficiency that arise from using related controls versus population controls, we also wish to include an age-matched population control group as an additional control group to examine this methodologic issue. Population controls for the OFBCR are being identified as part of another funded study. We are requesting funding to test two VDR polymorphisms (poly(A) repeat using microsatellite analysis and FokI using single strand conformational polymorphisms analysis) in 310 women from the case group and each of the two control groups and to collect some population control blood samples. We will compare the case group to each control group separately adjust for known breast cancer risk factors in the analysis.

Even if the increase in risk is small relative to an established “breast cancer gene,” a common polymorphism could contribute to the development of a large proportion of breast cancer cases. Vitamin D is a micronutrient and alteration in its intake could affect breast cancer risk.

Specific Aim(s):

Study Aims

1) To test whether there is an association between VDR poly(A) and FokI genotypes and breast cancer risk in a population-based case control study, without and with adjustment for other risk factors. We will also test whether this association differs by ER status, tumour stage, or family history as a secondary
aim.

2) To compare results obtained in carrying out objective 1 using relative controls with those obtained using population controls to explore the effect of population stratification in a real example.

Hypothesis

We hypothesize that polymorphisms of the VDR gene affect breast cancer susceptibility. Vitamin D receptors are found in breast cells (both normal and malignant). They are associated with cell division, and have a possible effect on ovarian hormones and on the development of mammary lesions in mice. VDR
possibly interact with ER, and VDR polymorphisms are associated with the risk of developing prostate cancer. VDR polymorphisms, particularly the poly(A) repeat and the Fok I, which may have functional effects, could be associated with the risk of developing breast cancer . VDR polymorphisms could affect the development of breast cancer through an effect on cell division and/or through an effect on or interaction with estrogen and estrogen receptors. Identification of an association between VDR polymorphisms and breast cancer development would lead to further work to determine the mechanism(s) and may lead to the development of preventive measures in a population subgroup with increased susceptibility (i .e., those with particular VDR genotypes). Given that these polymorphisms are common in the population, the potential attributable proportion associated with them is high. If, as it appears in studies of prostate cancer, the population frequency of the long poly(A) allele is about 80% (based on the proportion in controls) and if carriers of this polymorphism have twice the risk of breast cancer compared to short allele homozygotes, the proportion of
breast cancer occurring in the population that is attributable to the long poly(A) allele is 44%. If the risk associated with breast cancer is as high as it appears to be in prostate cancer studies, the proportion of breast cancer attributable to the long poly(A) allele would be even greater than 44%. Therefore, VDR variants could play a significant role in breast cancer.

Application ID: B-TO-1299-02 **Status:** Approved, Complete

Principal Investigator: Knight, Julia A. **Year:** 1999

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital , Toronto

Title: *Genetic Variants of Antioxidant Enzymes and the Risk of Breast Cancer*

Abstract:

There is considerable evidence to suggest that oxidative DNA damage, particularly in the mitochondria, can lead to diseases of ageing including cancer. The antioxidant enzymes, SOD, CAT, and GPX form a critical component of the cellular defenses against oxidative damage. Two of these, MnSOD and GPX1, operate within the mitochondria where ROS-generating aerobic respiration takes place. Potentially functional genetic variants of some of these antioxidant enzymes, including those operating within the mitochondria, exist. It is plausible that these variants are related to cancer susceptibility. There is a body of evidence linking antioxidant enzymes specifically to breast cancer and one study has found an association between an antioxidant enzyme genetic polymorphism and breast cancer. These variants may be important because even at a genotype frequency of 20%, the attributable risk percent of premenopausal breast cancer in the population would be 29% with a relative risk of 3.0, as observed in one published study. Given the possibility of interaction with dietary antioxidants and oxidants, there is the possibility of dietary intervention in a susceptible subpopulation.

We will use a case control approach making use of the Ontario Familial Breast Cancer Registry (OFBCR) to test for an association between antioxidant enzyme polymorphisms and breast cancer development. In the currently proposed study, both population and relative controls will be used. A sample size of 300 cases and 300 population controls will be required, and approximately 300 siblings will be genotyped to conduct a sibling TDT analysis.

Specific Aim(s):

Objectives

1) To examine the association between three antioxidant enzyme gene polymorphisms (SOD2 val-9ala, GPX1 pro197leu, GPX1 GCG repeat) and breast cancer before age 55, including consideration of interactions with exposure to sources of oxidants and antioxidants, in a case control study.

2) To test for associations between the antioxidant enzyme gene polymorphisms listed above and breast cancer occurring within families of the breast cancer cases.

Application ID: B-AU-0499-01 **Status:** Approved, Complete

Principal Investigator: McCredie, Margaret **Year:** 1999

Institution: University of Otaga

Title: *Collaborative Group on Hormonal Factors in Breast Cancer*

Abstract:

The Collaborative Group on Hormonal Factors in Breast Cancer was set up in 1992 to bring together and, where appropriate, combine results of all epidemiological studies of the relationship between breast cancer and hormonal factors (particularly use of hormonal contraceptives, use of hormonal replacement therapy, and reproductive factors). Reports on breast cancer and hormonal contraceptives and hormonal replacement therapy have now been published. Preliminary results on reproductive factors have been presented and discussed at meetings of collaborators both in 1993 and 1995 and reports on the results are being prepared, but are not expected to be ready for circulation until at least 1999.

A survey of collaborators conducted in the spring of 1998 indicated widespread support for a second cycle of collaboration. A summary of the suggestions by collaborators about further work is attached.

Specific Aim(s):

Objectives: the second cycle of collaboration will utilize data already contributed to the collaboration to investigate the relationship between breast cancer incidence and:

i. family history of breast cancer;
ii. anthropometric factors, i.e., height and weight;
iii. consumption of alcohol and tobacco; and
iv. certain medical conditions and surgical procedures, i.e., benign breast disease, tubal ligation, hysterectome, and bilateral oophorectomy;
v. factors associated with cancers that are localized to the breast will be contrasted with factors associated with more disseminated disease.

Application ID: B-NY-0499-01 **Status:** Approved, Complete

Principal Investigator: Peacocke, Monica **Year:** 1999

Institution: College of Physicians and Surgeons of Columbia University

Title: *Germline Mutations in PTEN in Women with Breast and Endometrial Cancer*

Abstract:

Cowden's syndrome (CS) is an autosomal dominant disorder associated with skin lesions, thyroid disease, and an increased susceptibility to breast cancer. It has also been suggested that the CS is associated with an increased susceptibility to endometrial cancer. In the last year, germline mutations in a gene known as PTEN have been associated with at least a subpopulation of individuals with CS. As CS is frequently under-recognized in the community, the goal of this study is to determine the frequency of germline mutations in PTEN in a Registry of Breast Cancer Families in individuals with a family history of breast cancer and the presence of these two malignant tumors.

These studies will test the hypothesis that germline mutations in PTEN are asspciated with women presenting with both breast cancer and endometrial cancer.

Study subjects will be individuals in the Registry presenting with pathologically documented breast cancer and endometrial cancer. Using 200 nanograms of DNA, perimers flanking the nine exons of the PTEN gene will be used to amplify sequences from participating subjects. Once amplified, the PCR products of each of the nine exons will be subjected to automated DNA sequencing. Any sequence differences will be verified by resequencing a second PCR product, as well as sequencing of the reverse strand. Confirmation of the mutation at the genomic level will be by restriction digestion, where possible, ot allele specific hybridization, as described (Tsou, H.C., et al, 1997). At the end of the study we will then be able to ascertain the number and the types of germline mutations in this gene in this selected subpopulation of women with two primary tumors associated with CS.

Application ID: B-NY-0598-01 **Status:** Approved, Complete

Principal Investigator: Ahsan, Habibul **Year:** 1998

Institution: Columbia University Mailman School of Public Health

Title: *Estrogen- and Carcinogen-metabolism Genes and Risk of Breast Cancer*

Abstract:

Completion of full-term pregnancies, especially the first, reduces breast cancer risk. A large body of literature suggests that human chorionic gonadotropin (hCG), a glycoprotein hormone exclusively produced from placenta, mediates this protection. Since all women who complete pregnancies may not get the equal protection, it is likely that there are inherent variations in the protective effects of pregnancy which may result from variations in the placental hCG gene. Since placental genotype is the same as the genotype of the child born out of that pregnancy, we hypothesized that genetic variants in the offspring's hCG gene (i.e., the placental genotype during pregnancy) are associated with breast cancer risk of the mother. No prior studies have examined this hypothesis. Of the several hCG genes, the hCG5 gene is the most expressed hCG gene during pregnancy. We propose to evaluate our hypothesis by comparing the hCG5 genotypes among first-born children of cases and controls participating in the Metropolitan New York Registry (MNYR). Since 1995, as one of the six NCI-funded CFRBCS centers, MNYR has been collecting questionnaire data and biospecimens from breast cancer patients and their affected and unaffected family members. To date over 1,150 families are participating including more than 3,500 individuals. Using the unique existing resources assembled by the MNYR (i.e., questionnaire data and DNA samples on breast cancer patients and their unaffected family members) we propose to examine our novel hypothesis by comparing the hCG-5 gene variants between first-born children of 419 breast cancer case women and 687 control women. These 1,106 DNA samples (which have already been isolated and stored as part of MNYR) will be genotyped for the four hCG? 5 gene SNPs using fluorescent polarization technique. Logistic regression models will be used to estimate odds ratios for the association between offspring's hCG-5 gene variants and breast cancer adjusting for potential confounders. An association between offspring's hCG? 5 gene variants and breast cancer, if observed, will have two important implications. First, from the risk assessment point of view, it will suggest that the genotype of the father of a woman's child may affect her future breast cancer risk (through altered placental hCG function during pregnancy). Related to this, since placental genotypes are actually the genotypes of developing fetus, among parous women, their children's genotypes may be predictive the protective effect conferred by pregnancy. Second, from the clinical perspective, this study may provide further evidence supporting the role of hCG in preventing breast cancer among women and thus suggesting its potential use in future breast cancer prevention trials.

Specific Aim(s):

Hypothesis

This proposal is to examine the associations of genes that are involved in the metabolism of endogenous (e.g., estrogen) and exogenous (e .g., tobacco) carcinogens, with breast cancer risk. Several recent studies have shown that variants of these metabolism genes, associated with increased estrogen or bio-active carcinogens in the body, are associated with breast cancer risk (Feigelson et al ., 1997 ; Nedelcheva et al .. 1998 ; Lavigne et al ., 1997; Ambrosone et al ., 1996 ; Ishibe et al ., 1998 ; Helzlsouer et al ., 1998) . All of these studies (published or ongoing) have utilized a classical case-control design to examine the genotype-breast cancer association and have found promising results . In our ongoing studies, we are also examining these associations in population-based case-control studies in the Long Island Breast Cancer Study Project . We propose to examine these metabolism genes and their interaction with biologically meaningful exposure variables in breast cancer utilizing a family-based study-design (case-parental control) within the Cooperative Family Registry for Breast Cancer Studies project.

Application ID: B-TO-0598-02 **Status:** Approved, Complete

Principal Investigator: Goodwin, Pamela J. **Year:** 1998

Institution: Samuel G. Lunenfeld Research Institute, Mount Sinai Hospital

Title: *A Prospective Study of Prognosis in Heritable and Familial Breast Cancer*

Abstract:

The recent cloning of BRCA1 and BRCA2, two predisposition genes that account for 50-70% of all heritable breast cancer, has led to preliminary observations suggesting that breast cancer prognosis may be influenced by the presence of mutations in these genes. The biologic possibility of a prognostic effect is supported by in vitro and in vivo evidence. Although not entirely consistent, this evidence suggests that heritable breast cancer is associated with a number of adverse prognostic factors, but that the presence of these adverse factors may not translate into a worsened prognosis.

We propose to conduct a prospective study of the prognostic effects of these mutations and of familial breast cancer in general in the context of an inception cohort of Ontario women with breast cancer newly diagnosed between January 1, 1996 and December 31, 1998. Our research is being carried out in collaboration with the Ontario Cancer Genetics Network. Specifically, we will assemble three groups of women:

Group 1 – those with heritable breast cancer due to an identifiable mutation in BRCA1 or BRCA2.

Group 2 – those with familial breast cancer in whom a mutation in BRCA1 and BRCA2 has not been identified.

Group 3 – those with sporadic breast cancer.

Strict criteria will be used to define familial breast cancer. Baseline clinical and treatment data will be collected and all women will be followed prospectively for outcome events. A detailed pathology review will be performed centrally to ensure a highly standardized classification of all pathologic prognostic factors. The primary study outcome will be distant disease recurrence. The statistical analysis will first examine the association of BRCA1 and BRCA2 mutations with traditional prognostic factors. With continued follow-up, we will examine the direct prognostic effect of these mutations in relation to other familial breast cancer and to sporadic breast cancer. The latter represents our primary analysis. We will also examine overall prognostic effects of familial breast cancer. Sample size and power calculations demonstrate that we will have sufficient power to identify a relative risk of recurrence of 1.8 to 2.0 for mutations in BRCA1 and a relative risk of recurrence of 2.1 to 2.2 for mutations in BRCA2. These calculations assume a power of 80%, a type I error or 0.05, 2-tailed, enrollment of 210 women in each of the three study groups and an event rate of 20-25% at three years.

Our affiliation with the Ontario Cancer Genetics Network allows us to conduct this research in a very timely and cost efficient manner. We are currently requesting funding for the first three years of the proposed research. It is anticipated that an additional two years of funding (at a very modest level) will be required to accrue sufficient events so that a disease free survival analysis can take place.

Specific Aim(s):

Aims

The objectives of our research in a population based inception cohort of women with BC are:

1.) To examine the prognostic effect of germline BRCA1 and BRCA2 mutations on BC recurrence and death, before (primarily) and after (secondarily consideration of traditional prognostic factors. This is our primary objective.

2.) To examine the prognostic effect if family history on BC recurrence and death, before (primarily) and after (secondarily) consideration of traditional prognostic factors.

3.) To examine associations of traditional BC prognostic factors with germline BRCA1 and BRCA2 mutations and with family history.

Hypothesis

It is hypothesized that women with hereditary BC due to germline mutations in BRCA1 or BRCA2 will have worse prognosis than those with sporadic BC. Furthermore, it is hypothesized that those with familial BC in whom no mutations are identified will have a prognosis that is intermediate between those with heritable BC and those with sporadic BC.

Application ID: B-TO-0597-02 **Status:** Approved, Complete

Principal Investigator: Andrulis, Irene **Year:** 1997

Institution: Mount Sinai Hospital, Toronto

Title: *Studies of Hereditary Breast Cancer Genes and Their Associations With Somatic Molecular Alterations and Histopathological Features in Cases From the Ontario Familial Breast Cancer Registry (aka Ontario Familial Breast Cancer Registry Pilot Studies)*

Abstract:

Since the cloning of BRCA1 and BRCA2 genes, there have been a number of studies published that report on detection of mutations in these genes. The mutations, which have been reported, are predominantly frameshift or nonsense mutations, which result in truncations of the BRCA1 and the BRCA2 gene products and loss of function of the proteins. Single base substitutions that result in amino acid changes in the BRCA1 and BRCA2 genes have also been reported in families, although the frequency of these mutations is much lower than the truncation mutations. However, the frequency of missense mutations may be underestimated due to difficulty in detecting these changes relative to truncation mutations and inconclusive evaluation of the missense changes detected in families because the functions of BRCA1 and BRCA2 are not known. The analysis of both BRCA1 and BRCA2 is also complicated by the fact that the mutations observed are variable in nature and their location appears to be distributed throughout the large coding sequence, making the complete analysis of these genes very difficult. To date, mutational studies have focused mainly on families with a very strong history of breast and/or breast/ovarian cancers. In addition to these high risk families, there are also low and medium risk families with a history of breast cancer. The incidence of decreased risk in such families may be explained by the presence of novel BRCA1 and BRCA2 mutations, mutations in other predisposition genes, or BRCA1 and BRCA2 mutations identical to those observed in the high risk group, whose phenotypic expression is influenced by modifier genes or environmental factors.

Specific Aim(s):

Specific Aims

We propose to use the population-based Ontario Familial Breast Cancer Registry (OFBCR):

1) to determine the frequency and the types of BRCA1 and BRCA2 mutations in a population-based sample of breast cancer cases considered familial according to defined criteria and a sample of nonfamilial cases chosen from the same population;

2) to assess whether there are associations between family history of cancer and other characteristics and mutations in BRCA1 and BRCA2;

3) to estimate the penetrance of BRCA1 and BRCA2 mutations in this population-based sample;

4) to examine risk factors associated with being an affected carrier as opposed to an unaffected carrier and associated with age at onset in carriers;

5) to perform descriptive studies on the families in this population-based registry including the prevalence of gene mutations in these families.

Analysis will be done on the first 200 registered familial cases and, eventually, 100 registered nonfamilial cases in the OFBCR.

Hypotheses

The hypothesis of this pilot study is that different molecular alterations in breast cancer predisposition genes may be associated with different types of family history of cancer and penetrance. A second hypothesis is that epidemiologic risk factors play a role in the development of cancer in gene mutation carriers.

Application ID: B-TO-0597-01 **Status:** Approved, Complete

Principal Investigator: Andrulis, Irene **Year:** 1997

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital , Toronto

Title: *Investigation of Molecular Genetics Abnormalities in Breast Carcinomas of Ashkenazi Jewish Individuals with Germline BRCA1/2 Mutations*

Abstract:

Five to ten percent of breast cancer is hereditary. Germline mutations in the BRCA1 or BRCA2 tumor suppresser genes are now known to be associated with the majority of these breast cancers. It has recently been shown that mutations in these genes are common in the Ashkenazi Jewish population, with a carrier rate of about 2.5%. The BRCA1 and BRCA2 genes, however, are not mutated in sporadic breast cancer, suggesting the hypothesis that tumors associated with these germline mutations have a different pathway of molecular pathogenesis from sporadic cancers. We propose to investigate the frequency of alterations in the p53 and erbB-2 genes in tumors associated with germline mutations and compare them with sporadic tumors. One subgroup of patients from the same study, but without documented germline mutations, will be used as controls and will be matched for menopausal status and tumor stage. The results of the proposed research will contribute significantly to the understanding of the molecular pathogenesis of breast cancers associated with BRCA1 and BRCA2 germline mutations. It will provide a basis for ongoing research in this area, particularly examination of the role of other somatic gene abnormalities, the elucidation of which may ultimately contribute to the development of new treatment strategies and improved survival in women with this disease.

Specific Aim(s):

We propose to investigate the frequency of alterations in the p53 and erbB-2 genes in tumors associated with germline mutations and compare them with sporadic tumors.

Application ID: B-AU-0597-03 **Status:** Approved, Complete

Principal Investigator: Venter, Deon **Year:** 1997

Institution: Peter MacCallum Cancer Institute

Title: *The Molecular Pathology of Familiar and Sporadic Breast Cancer: Genetic Assessment of Disease Progression*

Abstract:

To determine the molecular events which accompany the development of specific subtypes of invasive breast cancer, by a molecular pathological assessment of tumor tissue at different stages of disease progression.

Hypothesis: It is likely that there are differences in the molecular pathogenesis of specific subtypes of breast cancer. Knowledge of such alterations could influence the choice of therapy and identify novel targets for gene-based drugs. We intend to examine the molecular alterations occurring in a population-based series of breast cancers at different stages of progression to elucidate genetic events, which play a significant role in the development of this disease. Such data can also help establish similar or differing genetic, environmental, and modifier gene interactions occurring in the different categories of breast cancer.

Application ID: B-TO-1196-10 **Status:** Approved, Complete

Principal Investigator: Andrulis, Irene **Year:** 1996

Institution: Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto

Title: *Molecular Epidemiology of Heritable Breast Cancer*

Abstract:

We propose to use the population-based Ontario Familial Breast Cancer Registry (OFBCR):

1) to determine the frequency and the types of BRCA1 and BRCA2 mutations in a population-based sample of breast cancer cases considered familial according to defined criteria and a sample of nonfamilial cases chosen from the same population;

2) to assess whether there are associations between family history of cancer and other characteristics and mutations in BRCA1 and BRCA2;

3) to estimate the penetrance of BRCA1 and BRCA2 mutations in this population-based sample;

4) to examine risk factors associated with being an affected carrier as opposed to an unaffected carrier and associated with age at onset in carriers;

5) to perform descriptive studies on the families in this population-based registry including the prevalence of gene mutations in these families.

Specific Aim(s):

Hypotheses

The hypothesis of this pilot study is that different molecular alterations in breast cancer predisposition genes may be associated with different types of family history of cancer and penetrance. A second hypothesis is that epidemiologic risk factors play a role in the development of cancer in gene mutation carriers.

Application ID: B-PH-1196-07 **Status:** Approved, Complete

Principal Investigator: Buetow, Kenneth **Year:** 1996

Institution: Fox Chase Cancer Center

Title: *Informatic Support Prototype for CFRBCS*

Abstract:

Not on file.

Application ID: B-AU-1196-11AS **Status:** Approved, Complete

Principal Investigator: Hopper, John **Year:** 1996

Institution: University of Melbourne

Title: *Technology Development for BRCA1 Mutation Testing*

Abstract: *(also listed as "Population-Based Estimation of the Penetrance of Protein-Truncating Mutations in BRCA1and BRCA2")*

This is a preliminary application for a proposal to organise and analyse data collected through the Ashkenazim Supplement to the CFRBCS, with the intent of obtaining estimates of the penetrance of the three mutations in BRCA1 or BRCA2 known to be common in people of Ashkenazi descent.

Families have been collected through "probands" selected on basis of personal or family history, and for being of Ashkenazi Jewish descent . (Note that the proband may not necessarily be affected). Therefore analysis must be performed conditional on family cancer history, and this may involve breast and/or ovarian cancers.

The most straightforward analysis is to restrict to families in which it is known a mutation has been found. In principle it would be possible to incorporate data on those families in which mutations were not found, but this would require one to model familial effects not due to BRCA1/2. In this analysis it is necessary to condition also on the mutation status of the first identified carrier — this may not necessarily be the "proband." The likelihood to be maximised is therefore of the form: L(D,CID,M, [gamma symbol]).

Application ID: B-AU-1196-11 **Status:** Approved, Complete

Principal Investigator: Hopper, John **Year:** 1996

Institution: University of Melbourne

Title: *Population-Based Estimation of the Penetrance of Protein-Truncating Mutations in BRCA1 and BRCA2*

Abstract:

This project has been superceded by #B-AU-0598-01AS, which now includes BRCA2.

There is considerable controversy about the penetrance of mutations in BRCA1 and BRCA2. Most of the information to date has come from the analysis of multiple-case families such as those of the Breast Cancer Linkage Consortium (BCLC). These atypical families were selected on an ad hoc basis specifically for use in linkage studies aimed at detecting genes associated with breast cancer. There are two problems with these estimates. First, the mutations occurring in multiple-case families may be unrepresentative in that they may be associated with a higher risk of breast cancer. Second, because the families are not selected in a systematic manner, it is not possible to make an appropriate adjustment for their ascertainment without conditioning out a large amount of information. Hence, the estimates are imprecise, and in practice, have been observed to be unstable. The aim of this study is to estimate the average age-specific cumulative risk of breast cancer (i.e., the prevalence) of protein-truncating mutations in BRCA1 and BRCA2, weighted by the observed distribution of such mutations in a sample of women with breast cancer sampled on a population basis through state cancer registries. The question being asked is whether the estimate of prevalence based on a population-based sample of women with breast cancer is less than that derived from ad hoc samples of families with multiple cases of breast cancer, chosen for linkage studies with the aim of discovering “high-risk” breast cancer genes. (In fact, these genes are “anti-breast cancer genes,” and only women with a mutated form of the genes are at increased risk of breast cancer).

The Australian Breast Cancer Family Study (ABCFS) uses a case-control-family design, in which family members of cases and controls are studied systematically. Consequently, the age-specific risk of (breast) cancer will be estimated by analysis of the (breast) cancer history of the relatives of those cases found to be carriers of protein-truncating mutations in BRCA1 or BRCA2.

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Application ID: B-SF-1196-01 **Status:** Approved, Complete

Principal Investigator: Koenig, Barbara **Year:** 1996

Institution: Stanford University

Title: *A Pilot Study of Social and Ethical Issues Confronting High-Risk Families*

Abstract:

This pilot study includes the following aims:

1) To describe how individuals in high risk families (including both men and women and social as well as genetic family members) understand and experience being at an elevated “genetic risk” for beast cancer; specifically, how they conceptualize the magnitude of risk in a lifetime, transmission patterns within families, the possibilities of controlling or preventing the development of cancer in the future, and the efficacy of genetic testing for lowering cancer risk;

2) To compare the unique “folk models” or popular understandings of genetic cancer risk with current scientific knowledge and to describe how folk models influence attitudes about genetic testing;

3) To compare the attitudes and beliefs of key California ethnic groups, particularly: (1) Latinos (focusing on Mexican Americans), (2) African Americans, (3) European Americans, and (4) Chinese Americans;

4) To describe how information about genetic risk of cancer is communicated, both within families and beyond the family, including individual’s understanding of the risks of disclosure of genetic illness to employers or insurers; and

5) To describe how individuals in high-risk families experience their recruitment into and on-going relationship with the NCCC Familial Registry for Breast Cancer.

The research identifies families from the NCCC database who may be at high risk. Qualitative, ethnographic methods will allow the focus on the family as the primary unit of analysis.

Application ID: B-SF-1196-04 **Status:** Approved, Complete

Principal Investigator: Spiegel, David **Year:** 1996

Institution: Stanford University

Title: *Reduction of Distress among Family Members of Women at High Risk for the BRCA-1 Gene Who Receive Cancer Risk Counseling and Group Psychotherapy (also titled, Needs Assessment in Women Whose Relatives Have Breast Ovarian Cancer)*

Abstract:

Early identification of high genetic risk cancers allow for modulation or even prevention of disease through altered health behaviors, aggressive surveillance, and medical/surgical interventions. Cancer risk assessment and genetic testing, however, raise considerable psychosocial, ethical, and legal problems for individuals, their families, and society. The genetic risk information presented is frequently complex. Risk related anxiety may interfere with the individual's ability to comprehend risk assessment and the ability to actively participate in their own cancer prevention. Furthermore, brad problems indicate a need for further research into their nature and evaluation of means of addressing them, with the goal of improving the presentation and utilization of genetic information about cancer risk, thereby enhancing early detection and more effective treatment of genetic cancers. This study conducts needs assessments in the relatives of patients with breast cancer. It will provide pilot data that will help form the basis for a larger randomized intervention protocol which will aim to address these needs by providing group intervention with women at risk for breast cancer.

Specific Aim(s):

The specific aims are to:

1) assess the psychosocial response of relatives of breast cancer patients to having a family member(s) with breast cancer,

2) assess the perception of breast cancer risk in relatives of breast cancer patients in relation to their desire to be tested, and

3) of those relatives who have undergone genetic testing and/or counseling, assessment will be made of their reaction to testing results and their related psychosocial needs.
