

Protocol for Collaboration on Studies of Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers

VERSION 9/2006

I. Introduction

The purpose of this document is to outline the collaborative efforts of The University of California, Irvine (Susan Neuhausen, PI; R01-CA074415) and the University of Pennsylvania (Timothy Rebbeck, PI; R01-CA102776). While these represent independent scientific investigations, these projects overlap in terms of participating centers, data and analytical methods. Therefore, we are coordinating the execution of these studies in order to maximize efficiency.

II. Specific Aims

Both centers have unique but complementary study aims, addressing the role of modifier genes and exposures on cancer risk, considering BRCA1/2 mutation status. These aims, as stated in the original grants, are as follows.

A. University of California, Irvine Aims

1. To screen Single Nucleotide Polymorphisms (SNPs) in genes involved in the insulin-growth factor signaling pathway and determine the minimal number of SNPs for genotyping that will maximize haplotype information. We will screen SNPs (and identify new SNPs if needed) in *IGF1*, *IGF1R*, *IGFBP1*, *IGFBP3*, *IRS1*, and *SHBG* in a set of 100 unrelated women with breast cancer (50 Caucasian and 50 African American) and identify haplotype blocks by linkage disequilibrium (LD) analysis. Haplotype-tagging SNPs, including those with known function, will be selected for genotyping in Aim 3.
2. To continue enrollment of *BRCA1/2* mutation carriers in order to expand our existing cohort. This involves: a) enrolling additional family members in previously studied families carrying deleterious mutations; b) enrolling African-American women with breast cancer, testing for *BRCA1* and *BRCA2* mutations, and expanding families found to have deleterious mutations; and c) obtaining DNA and information from eligible mutation carriers from our collaborators. This cohort includes both affected and unaffected mutation carriers who will serve as cases and controls, respectively.
3. To genotype SNPs selected in Aim 1 in our cohort of *BRCA1/2* mutation carriers. We will genotype eligible affected and unaffected mutation carriers from within the larger cohort described in Aim 2 with SNPs selected in Aim 1.
4. 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-only design, we will evaluate the association of the SNPs with age at diagnosis and pathologic stage and grade of breast cancer. Using a nested case-control design, we will evaluate the association of the SNPs with breast cancer status. Furthermore, we will investigate whether the modifying effects of the genes differ between *BRCA1* and *BRCA2* mutation carriers. A secondary outcome will be ovarian cancer, as we recognize that we have limited power for detection of associations with ovarian cancer.

B. University of Pennsylvania Aims

1. Use a case-only study of BRCA1/2 mutation carriers to evaluate whether candidate DNA repair genes alter the characteristics of breast cancers and therefore may be involved in the natural history of breast tumors in these women.

Hypothesis 1.1. Candidate genes or other risk factors affect the age at breast cancer diagnosis.

Hypothesis 1.2. Candidate genes or other risk factors affect breast tumor characteristics including stage.

2. Use a nested case-control study to evaluate whether candidate DNA repair genes are associated with altered breast cancer risk.

Hypothesis 2.1. Candidate genes alone, or upon interaction with one another, or with exposures influence breast cancer risk.

Hypothesis 2.2. The risk modifying effects of candidate genes and exposures differ by BRCA1/2 or by mutation location within each gene.

3. Use a nested case-control study to evaluate whether candidate DNA repair genes predict cancer site.

Hypothesis 3.1. Candidate genes alone, or upon interaction with one another, or with exposures predict risk of breast vs. ovarian cancer.

II. Participating Centers

Table 1 lists the centers that are participating in the studies outlined here. We will likely expand the number of centers as other investigators have expressed an interest in participating.

Table 1: Participating Centers

<u>Center ID</u>	<u>Center</u>
AU	Austria
BA	Baylor-Houston
BI	Beth Israel
BU	Baylor-Dallas
CH	City of Hope
CR	Creighton
DF	Dana Farber
DU	Duke
EH	Northwestern
FC	Fox Chase
GU	Georgetown
LA	UCLA
MC	Mayo Clinic
SW	UT Southwestern
UC	Chicago
UP	Penn
UT	UT/UCI
WC	Women's College , Sunnybrook (Toronto)

III. Participant Eligibility

Participants, data, and biosamples used in the Irvine and Penn studies will be the same. We are studying affected and unaffected carriers of disease-associated BRCA1 or BRCA2 mutations.

IV. Data Acquisition and Sharing

Detailed descriptions of data acquisition protocols and data sharing will be supplied by each center. The general protocol for eligibility as well as data/sample transmission are provided in an attached document ("Penn-Irvine Eligibility Criteria.doc) and summarized below. Initially, Penn will send a list of all participants currently in our databases. For each participant, we will indicate whether we have questionnaire data or DNA, whether updated data are required, or if a DNA sample is needed. We will work with each center to assess what new or updated data will be sent back to Penn for processing using the following steps:

Step 1. Steps for Determining Eligibility: Each center will identify all women tested positive for BRCA1/2 mutations, and provide information needed to determine eligibility to Penn. Eligible participants will include women over the age of 18 with documented disease-associated inherited mutations in BRCA1 or BRCA2 who have never been diagnosed with cancer at any site or were diagnosed with only breast or ovarian cancer within five or three years, respectively, of their clinic ascertainment. Penn will identify eligible participants and let the site know who is eligible. Subjects will be eligible for inclusion in the sample even if they do not have complete questionnaire data.

Step 2. Send data and samples for eligible subjects: Any format of data with a codebook or data dictionary (preferable format: merged PROSE/Modifier Codebook,) can be sent to Penn. Complete data can be sent, but only the data outlined in the MAGIC study data dictionary will be included in the final data set. Two equal DNA aliquots should be sent to 1) Penn and 2) UC. 5ug of DNA should be sent to **each** center.

❖ The address to send the DNA to for Penn is:

c/o Amy Walker
421 Curie Blvd
BRB 2/3
RM 531
University of Pennsylvania
Philadelphia, PA 19104

Special instructions: Dry ice overnite is good and printed freezer-proof labels is preferred-esp the printer generated part. Monday thru Friday for arrival and an e-mail for a heads-up appreciated. **Please e-mail a completed Modifier DNA Info Template.xls (see below for a sample and the sample excel file) along with their lab IDs to ttran@cceb.med.upenn.edu and tklingne@cceb.med.upenn.edu (215-898-5853) and the Fed EX Tracking number the day they are sent out.**

The address to send the DNA to for UCI is:

c/o Susan Neuhausen
 Rm 218 Sprague Hall
 839 Health Science Court
 University of California Irvine
 Irvine, CA 92697

special instructions: for UCI contact please **also cc Linda Steele** at steelel@uci.edu ((949) 824-5603) with the sample ID numbers (UID, CID, FAM , EGO, DNA concentration and volume) along with their lab IDs. It is important to retain the subject's UID throughout the study.

DNA samples for MODIFIERS Study

From:	<i>Name of the Center or Lab of origin</i>	Contact:	<i>Enter contact emails at origin</i>
To:	<i>Name of the Center or Lab of destination</i>	Contact:	<i>Enter contact emails at destination</i>
Date Sent:	<i>Enter date here</i>		
Tracking #:	<i>Enter tracking number if known</i>		

Lab ID	UID*	Family ID	Individual ID	Concentration (ng/μl)	Volume (μl)	Amount (μg)	BRCA: 1 or 2	Additional Info or Comment
Example 1	XX0070701569	707	1569	20	250	5.0	1	
Example 2	YY0090100023	901	23	63	100	6.3	2	

Step 3. Clean and finalizing data: Data will be processed and merged into common data set (by Penn). Error and range checking will be undertaken. Unmappable data, apparent data errors, and other data questions will be returned to each center for clarification, and a final data set will be generated.

IV. Database

A common, shared database will be maintained at Penn. The goal of having a unified database will be to optimize data sharing, data checking, generating of analysis samples, and controlling inclusion in various research studies to avoid overlap and double-publishing. This relational database will contain the following tables:

Table a: Common variables: universal ID number (UID), date of birth, mutation type (BRCA1 or BRCA2), mutation, ethnicity, other demographic characteristics; family history, occurrences of cancer, dates of diagnosis; occurrences of surgery

(including prophylactic surgery) and dates of surgeries; etc. A variable or variables denoted studies to which each individual belongs should be included.

Table b: Irvine/Penn-specific Questionnaire variables: reproductive history, exposure history, etc.

Table c: Genotype data: results of genotypes generated in fulfillment of various study aims.

V. Payment

A. Invoicing Procedures

Payment will be made upon invoice for data and biosamples submitted from each center. To simplify the paperwork and minimize the number of invoices each center will have to provide, each center should submit their invoice to Penn for processing. Penn in turn will submit an invoice to Irvine to obtain their funds for the shared costs.

Invoices should be sent to: Timothy R. Rebbeck, Ph.D.
Center for Clinical Epidemiology and Biostatistics
904 Blockley Hall
423 Guardian Drive
Philadelphia, PA 19104

B. Payment Amounts

Payments to each center will be made according to the type of contribution, as outlined in Table 2. We have devised this payment schedule in order to use grant funds judiciously and to provide sufficient payment for the work to be done. Costs have been calculated based on the data or samples that will actually be provided for a particular participant (Table 2).

Table 2: System of Payments for Questionnaire Data and DNA Contribution
Appendix 1: Eligibility Criteria and Ascertainment for Irvine/Penn Studies

(This table includes PROSE invoicing as well)

Task	Biosamples				Questionnaire Data				
	Group	Blood Draw (specifically for Modifiers)	DNA Extraction from Blood or Cell Line	Aliquoting and Shipping Two 5 µg* DNA samples	Baseline Data (aka "PROSE")	Additional Questionnaire Information (for Modifiers) OR Follow Up information for either study	Questionnaire Administration (specifically for these studies)	Transmission of Existing Data and medical records	Payment Amount per Eligible Subject
Specific Tasks	1	X							\$100
	2		X						\$50
	3			X					\$50
	4				X				\$50
	5					X			\$50
	6						X		\$50
	7							X	\$50
Example of payment for Combined submission tasks	8	X	X	X	X	X		X	\$350
	9		X	X	X	X		X	\$250
	10	X	X	X					\$200
	11		X	X					\$100
	12			X	X	X		X	\$200
	13			X				X	\$100
	14				X	X		X	\$150
	15				X			X	\$100
	16					X		X	\$100

1. General Description of Study Participants

The goals of our participant identification process are to maintain our ability to apply consistent inclusion/exclusion criteria, and completely enumerate the cohort from which the study sample is drawn. Eligible study participants will be identified from among BRCA1/2 mutation carriers. We will achieve uniform inclusion of eligible participants and enumerate the cohort from which the study sample is drawn by undertaking the steps shown in Figure 1.

2. Steps for Determining Eligibility

Each center will identify all women tested positive for BRCA1/2 mutations, and provide the following data needed to determine eligibility to Penn:

Center ID
Family ID
Ego
Year of Birth
Year of Ascertainment (i.e., first appearance for clinical or research participation)
Cancer Diagnosis (i.e. Breast, Ovarian, Melanoma etc...)
(please include all diagnoses)
Date of all Cancer Diagnoses
Gender
BRCA1 or BRCA2 mutation

Using these data, Penn will then identify eligible participants.

Note: The following information about inclusion/exclusion criteria is provided for your information only. Each center should NOT apply any inclusion/exclusion criteria when submitting the initial set of BRCA1/2 mutation carriers for evaluation at Penn. It is important that all carriers be submitted to Penn for evaluation to avoid selection biases and to allow enumeration of the entire cohort from which eligible study subjects were drawn.

3. Eligibility Criteria (to be determined at Penn):

All study participants must meet the following eligibility criteria for study entry:

- 1) Female;
- 2) At least 18 years old
- 3) Positive test for disease-associated BRCA1/2 mutation; and
- 4) Informed consent provided for participation in this research.
- 5) A questionnaire must have been administered to each participant. This questionnaire can be the local center's data collection instrument, but participants who have never been administered a questionnaire will not be eligible for participation.

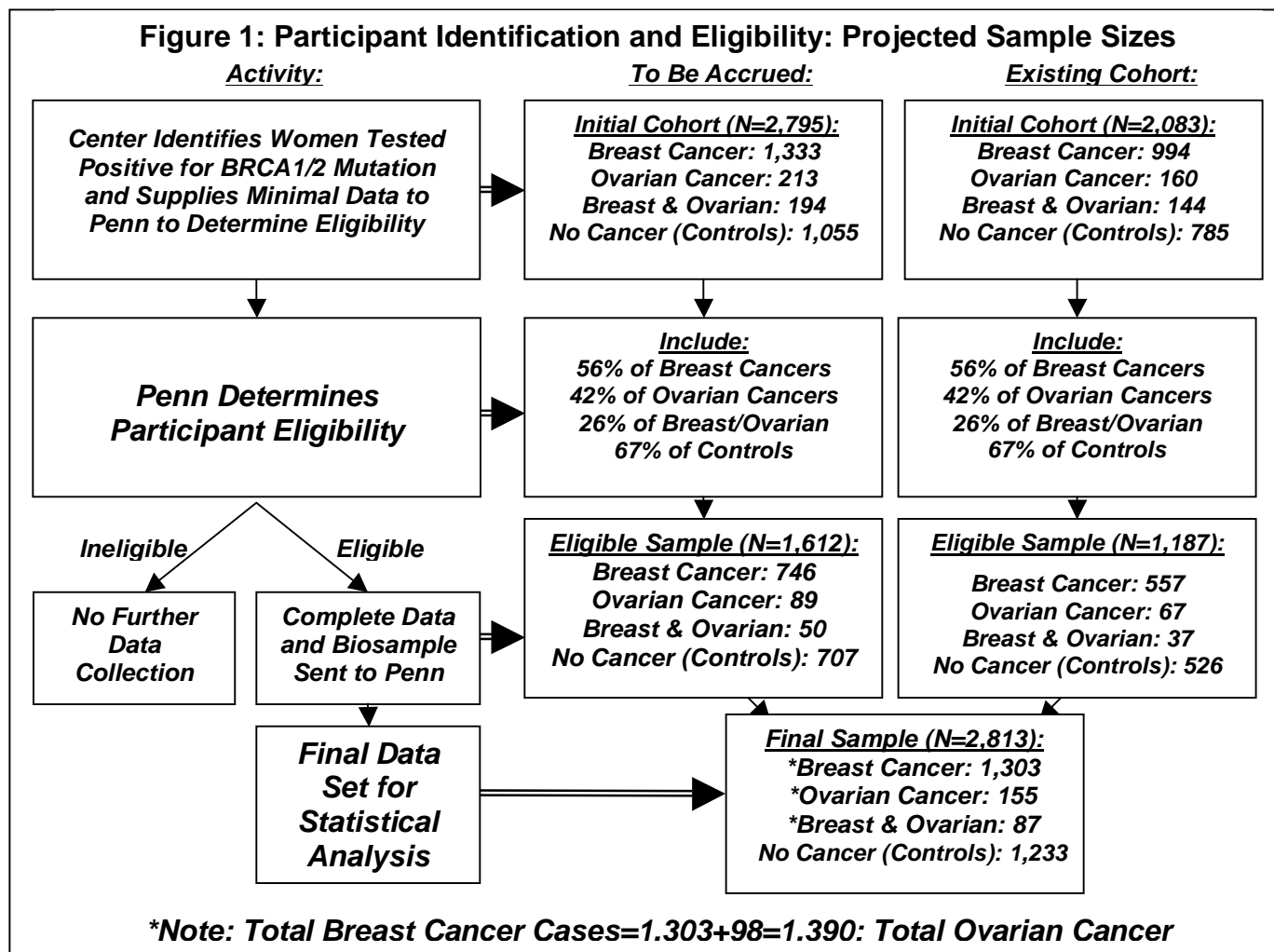
4. Exclusion Criteria

Participants will be excluded if they have:

- 1) Inherited a BRCA1/2 variant without known disease association (i.e., missense variants that are considered polymorphisms or that likely are "not deleterious") and no other disease-causing mutation; or
- 2) Germline mutations in any of the following genes with clinical confirmation of an associated familial syndrome: MSH2, MLH1 (Muir-Torre Syndrome), TP53 (Li-Fraumeni Syndrome), PTEN (Cowden Syndrome), STK11 (Peutz-Jeghers Syndrome).

Cancer cases will be excluded if:

- 1) They are non-incident (diagnosis of cancer must be no earlier than five years prior to ascertainment for breast cancer cases or three years prior to ascertainment for ovarian cancer cases), or
- 2) They have previously been diagnosed with a tumor at another site.



5. Detailed Case and Control Descriptions (from grant proposal)

a. Breast Cancer Cases

Breast cancer cases will be identified through high risk referral clinics at each center. Cases of invasive breast cancer or ductal carcinoma *in situ* (DCIS) will be included if they have been diagnosed after the time of their ascertainment, or if they were diagnosed within five years prior to their study ascertainment. This limitation in time from diagnosis is meant to minimize survival bias by excluding women who may have been diagnosed with breast cancer a long time ago, but are now just coming to clinics for genetic testing. Women who have undergone bilateral prophylactic mastectomy who developed subsequent breast cancers will be excluded as breast cancer cases. Women who have undergone bilateral prophylactic oophorectomy who developed subsequent breast cancers will be excluded as breast cancer cases because risk of breast cancer is reduced after oophorectomy (Rebbeck 2002). Similarly, women who have had a prior ovarian cancer before their breast cancer will also be excluded because their treatment usually involves

oophorectomy. Women who have an ovarian cancer diagnosis after their breast cancer diagnosis will not be excluded as breast cancer cases. While most ovarian cancers are diagnosed after breast cancer in our study subjects, the majority of women with both cancers would be excluded because their breast cancer would not be considered “newly diagnosed” under our inclusion criteria. Therefore, we anticipate that few eligible breast cancer cases will be included who also have a personal ovarian cancer history. Cases will not be selected on the basis of family history (i.e., all participants will be included regardless of their family history), although eligibility for genetic testing usually involves knowledge of family history to determine carrier probabilities.

b. Ovarian Cancer Cases

Ovarian cancer cases will be identified through high risk referral clinics at each center. Cases of ovarian cancer will be included if they have been diagnosed after the time of their ascertainment, or if they were diagnosed within three years of the time of their study ascertainment. This limitation in time from diagnosis is meant to minimize survival bias by excluding women who may have been diagnosed with ovarian cancer a long time ago, but are now just coming to clinics for genetic testing. Women who have undergone bilateral prophylactic oophorectomy who developed subsequent ovarian (i.e., peritoneal) tumors will be excluded as ovarian cancer cases. Women who have a breast cancer diagnosis after their ovarian cancer diagnosis will not be excluded as ovarian cancer cases. Even though most ovarian cancers are diagnosed after breast cancer in our study subjects, potentially eligible ovarian cancer cases with a history of breast cancer may still be excluded if their breast cancer would not be considered “newly diagnosed” to minimize survivor biases. Therefore, we anticipate that few women will be included who have both breast and ovarian cancer. Cases will not be selected on the basis of family history (i.e., all participants will be included regardless of their family history), although eligibility for genetic testing usually involves knowledge of family history to determine carrier probabilities.

c. Controls

We propose to use an incidence density sampling design to select controls from our existing cohort of female *BRCA1/2* mutation carriers. All eligible controls will meet the general inclusion/exclusion criteria described above. Using the incidence density sampling design, women will be eligible controls for a breast cancer case if they have no history of breast cancer and have not undergone prophylactic oophorectomy or mastectomy at or before the time of the matched cases’ breast cancer diagnoses. Similarly, women will be eligible controls for an ovarian cancer case if they have no history of ovarian cancer and have not undergone prophylactic oophorectomy at or before the time of the matched cases’ ovarian cancer diagnoses. Controls will be matched to cases on gene (i.e., *BRCA1* or *BRCA2*) and frequency matched on year of birth in five year age windows. As described below, analyses will be undertaken that include both *BRCA1* and *BRCA2* mutation carriers, as well as stratified analyses of *BRCA1* and *BRCA2* mutation carriers separately.