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TITLE: Gene-Gene and Gene-Environment Interactions in the Etiology of Breast Cancer

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14. ABSTRACT

The purpose of this proposal is to evaluate gene-gene and gene-environment interactions in the etiology of breast cancer in two ongoing case-control studies, the Shanghai Breast Cancer Study (SBCS) and the Nashville breast health Study (NBHS) and in a proposed case-control study, the Breast Cancer in West Africa Study (BCAWS). An allelic variant of UGT1A1 (allele*28) was identified as a risk factor for postmenopausal breast cancer in Chinese women. A proposal was submitted and funded for the BCAWS study through U54-CA9140801 Adunyah (PI). Samples and surveys acquired from the BCAWS study are currently being analyzed.

15. SUBJECT TERMS
Breast cancer, genetic risk, environmental risk, risk factors, genotype

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Introduction

Background: Breast cancer is the most common form of cancer (other than skin) and a leading cause of cancer mortality among women in the United States. The two known major susceptibility genes, BRCA1 and BRCA2, do not explain a significant proportion of familial breast cancer. Most breast cancer has a complex multifactorial etiology. It is still unclear the number and nature of genetic variants that predispose women to breast cancer, and the interplay between these variants and environmental factors. Recent studies have not shown any consistency in the association of known genetic polymorphisms and breast cancer risk. Most of these studies have had small sample sizes and subjects have been predominantly Caucasians. Studies to detect interactions typically require large sample sizes. Differences in behavioral and cultural attitudes, ethnicity, economic status, and lifestyle influences among different groups of women require further study to determine how these factors contribute to enhancing or reducing breast carcinoma risk.
Objective: The main objective of this proposal is to evaluate the contributions of gene-gene and gene-environment interactions in the etiology of breast cancer in three studies of breast cancer risk (one ongoing and two proposed). The **Specific Aims** of this study are to (1) evaluate the gene-gene and gene-environment interactions in the study cohort of the Shanghai Breast Cancer Study, (2) to evaluate the role of genetic factors, gene-gene interaction, and gene-environment interaction in the study cohort of the Nashville Breast health Study, and (3) to investigate the association of breast cancer with lifestyle factors and environmental exposures and evaluate the role of genetic factors, gene-gene interaction, and gene-environment interaction in the breast Cancer in West Africa Study (BCWAS).

BODY

Statement of Work

Task 1. To collate phenotype and genotype information on the on-going Shanghai Breast Cancer Study (SBCS) participants and conduct analyses of the Shanghai Breast Cancer Study for two-level gene-gene and gene-environment interactions, (Months 1-24):

From Annual Report #1: "The SBHS conducted its first round of recruitment of 1,459 breast cancer cases and 1,560 age-frequency matched controls in Shanghai from August 1996 to March 1998. The SBHS is presently conducting a second phase of recruitment of participants to increase its study sample size to 3,000 breast cancer cases and 3,000 controls."

a. Collation and editing of phenotype information of all SBCS participants from already completed in person interviews into a database (Months 1-3).

From Annual Report #1: "We completed the collation into a database and edited the available phenotype information of 1,459

cases and 1,556 controls in preparation for data analyses as proposed within the first three months of the first year."

b. Collation of genotype information of all the SBCS participants for all the genes that genotyping assays have been completed (Months 4-6).

From Annual Report #1: "The genotyping work has been ongoing and has been completed for five functional polymorphisms. The first polymorphism we selected for study in this CDA was the UGT1A1*28 polymorphism. Uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) is involved in catalyzing estrogen, the hormone that plays a central role in the etiology of breast cancer. A common polymorphism [$A(TA)_6TAA$(allele*1) to $A(TA)_7TAA$ change(allele*28)] in the TATA-box of the promoter region of the UGT1A1 gene has been reported to have possible influences on the transcription of this gene. The UGT1A1 genotyping was completed in Dr. Zheng's laboratory at Vanderbilt University Medical Center, Nashville, TN (VUMC). Genotyping data were obtained from 1,047 (87.8%) cases and 1,082 (82.6%) controls that had blood samples. The major reasons for incomplete genotyping were insufficient DNA and unsuccessful polymerase chain reaction (PCR)."

c. In collaboration with Dr. Wei Zheng, conduct analyses and prepare manuscripts of the association of estrogen metabolic genes with breast cancer in the Shanghai Breast Cancer Study (Months 7-18).

The PI of this CDA (Dr. Adegoke) completed the analyses for investigating the association of UGT1A1*28 polymorphism with the risk of breast cancer among 1,047 breast cancer cases and 1,082 community controls in the sBCS and the evaluation of the relationship of UGT1A1 genotypes with plasma levels of estrone, estrone sulfate, estradiol, testosterone, and sex hormone binding globulins (SHBG) among 372 postmenopausal controls that were measured for these molecules. The results of these analyses were presented at the American Association for Cancer Research (AACR) "Molecular and Genetic Epidemiology of Cancer" (AACR Special Conferences in Cancer Research) Meeting, held from January 18-23, 2003, in Waikoloa, HI (Appendix I). In addition, a manuscript was prepared and published entitled: **"**Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1(UGT1A1) and risk of breast cancer**"**. It was published in the journal *Breast Cancer Research and Treatment* in 2004 (Appendix II).

Task 2. To actively participate in the fieldwork and conduct of the Nashville Breast Health Study and conduct analyses of the NBHS, (Months 1-48):

Dr. Adegoke wrote, in June 2003 that "The pilot phase of the NBHS is ongoing. We submitted a funding proposal to the National Cancer Institute for the full study phase in March 2003. Due to the recruitment successes recorded so far in the pilot phase, the target enrollment for the NBHS has been increased from 1,000

incident breast cancer cases to 1,500 incident breast cancer cases."

The aims of the NBHS are:

1) To recruit 1500 incident breast cancer cases and 1500 frequency-matched controls in Nashville;
2) To conduct a phone interview to obtain information on NSAID use, well-done meat intake, and other lifestyle factors;
3) To collect exfoliated buccal cell samples through mouth rinsing and extract DNA from these samples;
4) To perform genotyping assays for the polymorphisms of the following genes: *CYP2C9, UGT1A6, UGT1A1, CUP1A1, CYP1B1, NAT1, NAT2, SULTA1, UGT1A1, GSTA1, GSTM1, GSTP1, GSTT1, and COMT.* The polymorphisms of these genes are summarized in Table B1 and discussed in Sections B1 to B7.
5) To perform statistical analysis to evaluate the hypotheses described above;
6) To store DNA samples for future study of other genetic factors.

a. In collaboration with Dr. Wei Zheng, conduct analyses and prepare manuscripts of the NBHS (Months 36-48).

Due to Dr. Adegoke's sudden illness in April of 2004, task 2b was not, and will not be able to be completed with the inclusion of Dr. Adegoke in the work.

Task 3. To undergo intensive training in cancer biology, advanced genetic and molecular epidemiology and statistical genetics, (Months 6-15):

- **a. Audit course in Advanced Genetics: Biochemistry and Cell Biology taught by Dr. Scott Hiebert, Wayne Wahls, and Graham Carpenter at Vanderbilt University Medical Center (VUMC) (Months 6-10).**
- **b. Audit course in Cancer Biology taught by Drs. Graham Carpenter, Roy Jensen, and Earl Ruley at VUMC (Months 11-15).**
- **c. Audit course in Cellular and Molecular Basis of Pathology taught by Dr. Gregory Sephel at VUMC (Months 11-15).**
- **d. Audit course in Human Genetics taught by Drs. Jonathan Haines and James Sutcliffe at VUMC (Months 11-15).**
- **e. Audit course in Molecular Aspects of Cancer Research taught by Dr. Graham Carpenter at VUMC (Months 11-15).**

From Annual Report #1 "On review of this training task by Drs. Adegoke and Zheng, it was determined that Dr. Adegoke had had formal training that involved much of the subject matter covered in the courses that he had proposed to audit under this task. His training as a physician, and a doctoral epidemiologist with focus in cancer epidemiology has provided him with some of the required background for this CDA. In addition, he has attended many relevant courses, workshops, and conferences in the past

three years that have further served as training for him for this CDA. However, he will be auditing the Cancer Biology course taught by Drs. Graham Carpenter, Roy Jensen, and Earl Ruley at VUMC in the second year of this award and, the Molecular Aspects of Cancer Research taught by Dr. Graham Carpenter in the third year of this award. It has been deemed necessary that he takes both courses at different times to allow him enough time to participate on his other tasks on this award." Dr. Adegoke became ill late in the second year of his CDA so this task, as written, would not have been completed.

Task 4. To conduct a case-control study of breast cancer risk factors in West Africa, the Breast Cancer in West Africa Study (BCWAS) (Months 24-48):

Annual Report #1: "Though this task was proposed for the third and fourth year of this award, it was commenced in the first year. Drs. Adegoke and Zheng made two preliminary visits to West Africa in March 2002 (Dr. Adegoke) and June 2002 (Drs. Adegoke and Zheng) to finalize collaboration plans, discuss study protocol development, and start off plans for this study. Dr. Zheng's trip to West Africa with Dr. Adegoke underscore his strong commitment, not only to the success of this proposed study, but also to Dr. Adegoke's development into an independent investigator."

a. Develop and submit a grant proposal for institutional funding or Cancer Center funding of pilot study of breast cancer risk in West Africa (Months 24-28).

"A pilot project proposal for the BCWAS was developed and submitted for competitive funding on the Comprehensive Meharry Medical College/Vanderbilt-Ingram Cancer Center Cancer Research Partnership (1 U54-CA9140801) funded by the National Cancer Institute. Our pilot proposal was awarded a two-year funding starting from May 2003 with the following objectives: a) to develop the protocol for the recruitment of participants and selection of appropriate controls; b) to develop and validate culturally appropriate questionnaires for the collection of demographic, lifestyle history, medical history, reproductive history, family history, and anthropometrics measurements data; c) to develop protocols for the collection, shipment to the United States, and storage of blood samples, cheek cells, and urine samples at Meharry Medical College; d) to determine the response rates for cases and controls by study site and identify strategies to increase participation as required in the larger study; and e) to analyze the preliminary data collected." (Appendix III).

b. Develop recruitment strategy, develop manual of procedure for the sites, visit West Africa to meet with collaborators, and ship supplies to commence pilot study of BCWAS (Months 28-32).

This task was completed and administration of the questionnaires and collection of samples commenced during this time period. The protocol is presented in Appendix IV and the Questionnaire for

c. Begin the pilot study of BCWAS with a total of 100 cases and 100 controls from 5 study sites at 20 cases and 20 controls from each site (2 sites in Ghana and 3 sites in Nigeria) (Months 33-36).

Logistical problems required abandonment of the Ghana site for now. The current collaborations are with one group in Nigeria and one group in Kenya. Our goal is to acquire a total of 200 cases and 200 controls from the sites, collectively. Currently we have 36 cases and 42 controls from Nigeria and 76 cases and 4 controls from Kenya. During the time of Dr. Adegoke's illness, the U54 funding period for the BCAWS project ran out. Supplemental funding was awarded to Dr. Marshall by the MMC Clinical Research Center, for continued acquisition of samples and shipment to MMC and processing and genotyping of the samples. The BCWAS (Breast Cancer in West Africa Study) study became the BCAWS (Breast Cancer in African Women Study) as the replacement of the Ghana sites with the Kenya sites resulted in this study no longer targeting women in west Africa, but included an eastern location as well.

d. In collaboration with senior colleagues, submit a grant to conduct the full phase study of the BCWAS with a total recruitment of 1,500 cases and 1,500 controls (Months 37-40).

Due to Dr. Adegoke's illness, and the logistical problems that resulted in the need to restructure the format for sample delivery to MMC (vs Dr. Adegoke traveling to the sites and bringing the samples back himself), this is delayed while we accumulate the remainder of the samples for the pilot project. We are in communication with both sites in Africa and our collaborators are excited to start sample collection again. We expect that sample collection for the pilot will be completed by June 2007. We plan to submit a grant to conduct the full phase study in August of 2007.

e. In collaboration with Dr. Wei Zheng and West African collaborators, start the full phase of BCWAS after necessary amendments have been made to study protocol as suggested by the results of the pilot study, and ship supplies to study sites (Months 41-48).

For months 50-60, we have almost completed (to be completed July 6, 2007) the preliminary cleaning of the survey data from the pilot study. This will allow us to identify weaknesses or redundancies in the survey that need to be addressed in order to begin the larger study. The acquisition and shipping of samples has been working quite well so we do not anticipate any large changes with the study protocol itself.

Task 5. To conduct a cohort study, The West African women's Health Study (WAWHS), (Months 36-48):

a. Identify potential West African collaborators, develop recruitment strategy, draft manual of procedures, and determine logistic requirements (Months 36-42).

b. In collaboration with senior colleagues and West African collaborators, submit a grant to conduct a cohort study of 30,000 women in West Africa, the West African Women Health Study (WAWHS) (Months 43-48).

We are currently in months 56-60 and are in the very early stages of discussing future work with potential collaborators in Uganda and South Africa. This continues to change the focus from only West Africa to a broader range best described as Sub-Saharan Africa.

Key Research Accomplishments

- Participation in the analyses of the SBHS data. (Adegoke)
- Participation in the conduct of the pilot phase of the NBHS. (Adegoke)
- Participation in the development and submission of the full-study proposal for the
- NBHS. (Adegoke)
- Submission and funding, through a U54 award to MMC and VICC, of the BCWAS pilot proposal. (Adegoke)
- Development of collaborations with researchers in Nigeria and Kenya. (Adegoke)
- Development of appropriate questionnaires, with these collaborators for assessing behavioral and genetic risk factors contributing to breast cancer in African women. (Adegoke)
- Nigerian and Kenyan collaborators begin pilot phase of BCAWS study. (Adegoke)
- Acquisition of financial support, through the MMC-CRC, to complete sample acquisition and analysis for BCAWS. (Marshall)
- Commenced critical analysis of questionnaire for further development. (Marshall)

Research Accomplishments Timeline

- **January 2003:** Presented the study, "Genetic polymorphisms

in uridine diphosphoglucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer" at the American Association for Cancer Research molecular epidemiology meeting in Waikoloa, Hawaii.

- **March 2003.** Participated in the submission of full-study funding proposal resubmitted to the NCI.

- **May 2003:** Funding obtained to conduct the pilot project of the BCWAS for 2 years starting immediately (Meharry Medical College and Vanderbilt-Ingram Cancer Center).

- **November 2003:** First shipment of collection supplies to Nigeria.

- **December 2003:** Finalization of survey.

- ***April 2004*** *While traveling to Africa for meetings with collection sites, Dr. Adegoke becomes seriously ill. This results in the rest of the timeline being shifted back by approximately 1-2 years.*

- **June 2004:** Publication of "Genetic polymorphisms in uridine diphosphoglucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer." Breast Cancer Research and Treatment 85:239-245. Authors: **OJ Adegoke**, XO Shu, Y-T Gao, Q Cai, J Breyer, J. Smith and W. Zheng. This manuscript results in the completion of Task 1.

- **July 2005:** Received 72 blood samples from Nigeria and survey data in the form of an EXCEL workbook. (U54 funded project resulting from CDA support.)

- **February 2006:** 39 blood samples arrive from Kenya along with survey data in the form of an EXCEL workbook.

- **September 2006:** Re-established contact with Nigerian and Kenyan collaborators in regards to the continuation of this study to which they are agreeable.

- **March 2007:** Begin DNA extraction of blood samples for genotype analysis (MMC-CRC funding for extraction and analysis).

- **April 2007:** 41 blood samples arrive from Kenya along with survey data.

- **April-May 2007**: Survey data quality control is begun (will complete in July).

Reportable Outcomes

1) Recipient of AACR-HBCU Faculty Scholar Award for Cancer Research to attend the "Frontiers in Cancer Prevention Research", AACR Special Conferences in Cancer Research. Boston, MA. October 14-18, 2002.

2) Recipient of AACR-HBCU Faculty Scholar Award for Cancer Research to attend the "Molecular and Genetic Epidemiology of Cancer", AACR Special Conferences in Cancer Research. Waikoloa, Hawaii. January 18-23, 2003.

3) Recipient of AACR-HBCU Faculty Scholar Award for Cancer Research to attend the 94th Annual Meeting of the American Association for Cancer Research (AACR), Washington D.C. July 11-14, 2003.

4) **Adegoke OJ**, BeLue R, Gebretsadik T, Ahmed NU. Breast self examination and clinical breast examination in Metropolitan Nashville Health District. "Frontiers in Cancer Prevention Research", AACR Special Conferences in Cancer Research, Boston, MA. (Abstract #B122, October 16, 2002).

5) **Adegoke OJ**, Shu XO, Smith J, Yu H, Jin F, Cai Q, Gao YT, Zheng W. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. "Molecular and Genetic Epidemiology of Cancer", AACR Special Conferences in Cancer Research, Waikoloa, HI. (Abstract #A11, January 20, 2003).

6) Proposal for pilot project of the Breast Cancer in West Africa Study submitted to the MMC/VICC Comprehensive Cancer Center Research Alliance (1 U54-CA9140801) in February 2003. Funded in May 2003.

7) Proposal for Molecular and Genetic Epidemiology of Breast Cancer Training Program at Meharry Medical College (BC022334) submitted to Department of Defense (U.S. Army) in August 2002. (Investigators: Olufemi Adegoke (PI), Wei Zheng, Ana Grau, Carlos Arteaga, Qiuyin Cai). Not funded.

8) Proposal for full-phase of The Nashville Breast Health Study (RO1 CA100374) re-submitted to NIH in March 2003. (PI: Wei Zheng). The proposal was ultimately funded (5RO1 CA100374-03).

9) **Adegoke OJ**, Shu XO, Gao Y-T, Cai Q, Breyer J, Smith J and Zheng W. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. *Breast Cancer Research and Treatment*. 85: 239-245, 2004.

10) Proposal to Meharry Medical College Clinical Research Center submitted and funded (November 2006) to support genotype analysis of samples collected for the BCAWS study. Human subjects protocol approved by MMC (December 2006). D.R. Marshall (PI).

11) D.R. Marshall, Submission of Pfizer proposal to bring a Visiting Professor of Oncology to MMC for a 3-day visit with workshops. This was awarded to our department and we are bringing Dr. Stephanie Jeffrey (Stanford). I would not have had the knowledge to prepare this submission without the DOD CDA experience.

12) Seminar, Surgery Grand Rounds at MMC, February 14, 2007. "Breast cancer: Populations, People and Proteins.

13) D.R. Marshall (PI), Submission of DOD Concept Award Proposal (Feb. 2007), "Inflammation, MHC, Race/Ethnicity and Breast Cancer". Not Funded.

14) D.R. Marshall (PI), Submission of pilot study LOI to the MMC CRC (April 2007) for genetic analysis and generation of primary breast cancer cell lines. Status: a full proposal was requested that was submitted in May 2007. Projects are currently under review.

15) Submission of DOD Synergy Award for Breast Cancer Research (June 2007). "The multipotent influence of Vitamin D on breast cancer risk". D.R. Marshall (PI), primary submitter with Dr. Alecia Malin-Fair (PI), co-submitter.

Conclusion

The first year of this CDA afforded Dr. Adegoke the opportunity to expand on his understanding of the molecular epidemiology of breast cancer through attendance and presentations at many cancer research meetings. The result was that Dr. Adegoke was able to acquire a significant amount of experience, fairly quickly, towards his development as an independent investigator. Results from the investigation of the association of UGT1A1*28 polymorphism with the risk of breast cancer in Chinese women participating in the SBHS did not indicate a significant role for this polymorphism in the risk of breast cancer. However, in the analysis of hormone levels and UGT1A1 genotype among postmenopausal controls, we observed progressively lower blood levels of estrone (E1) and estrone sulfate (E1-S), and higher levels of testosterone and sex-hormone binding globulins (SHBG) with increasing presence of *28 allele compared to the wild *1/*1 genotype. Our observations suggest that genetic polymorphism in the UGT1A1 gene is related to the blood levels of SHBG and estrogens and may thus be related to the risk of postmenopausal breast cancer in Chinese women. Efforts are currently underway to conduct similar analyses for the Caucasian and African-American populations in the NBHS and to the African population in the BCWAS when these studies have recruited large enough numbers of participants.

Conclusions: "So what" category.

The identification of genes and environmental factors that result in the development or severity of breast cancer is critical to the understanding, and ultimately, treatment of the disease. This award has supported the development of an infrastructure that will be very valuable to the identification of factors that result in the development of aggressive breast cancer in women of all genetic backgrounds. The fundamental foundation of this type of work is based on groups of populations with overlapping, yet still reasonably separated genetics and also genetically similar groups with differing lifestyles. The development of good working relationships with collaborators in is one arm of this type of study. Further refinement of the study questionnaires and site protocols will strengthen this aspect of a study that can stand on its own in Africa as well as being part of a more comprehensive study that can include African American and Caucasian American people as well.

References

1. Adegoke OJ, Shu XO, Smith J, Yu H, Jin F, Cai Q, Gao YT, Zheng W. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. "Molecular and Genetic Epidemiology of Cancer", AACR Special Conferences in Cancer Research, Waikoloa, HI. (Abstract #A11, January 20, 2003)

2. Adegoke OJ, Shu XO, Gao Y-T, Cai Q, Breyer J, Smith J and Zheng W. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. Breast Cancer Research and Treatment. 85: 239-245, 2004.

List of people receiving monetary compensation from DAMD17-02-1-0482.

1. Dr. Dana Marshall
2. Dr. Joan Smith
3. Mr. Cleo Carter

Appendices

Genetic Polymorphisms in Uridine Diphospho-glucuronosyltransferase 1A1 (UGT1A1) and Risk of Breast Cancer

Olufemi J. Adegoke[1,2], Xiao-Ou Shu[2], Jeffrey Smith[2], Herbert Yu[3], Fan Jin[4], Qiuyin Cai[2], Yu-Tang Gao[4], and Wei Zheng[2] Meharry Medical College, Nashville, TN[1]; Vanderbilt University Medical Center, Nashville, TN[2]; Yale University School of Medicine, New Haven, CT[3]; Shanghai Cancer Institute, Shanghai[4].

Uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) is involved in catalyzing estrogen, the hormone that plays a central role in the etiology of breast cancer. A common polymorphism [A(TA)6TAA (allele *1) to A(TA)7TAA change (allele*28)] in the TATA-box of the promoter region of the UGT1A1 gene has been reported to have possible influences on the transcription of this gene. We investigated the association of this UGT1A1 polymorphism with the risk of breast cancer among 1047 breast cancer cases and 1082 community controls in the Shanghai Breast Cancer Study, a population-based case-control study. Approximately 12.5% of cases and 13.0% of controls carried the variant allele *28. Overall, no difference was observed between cases and controls in the distribution of UGT1A1 genotypes. Among postmenopausal women, a nonsignificant reduced risk for breast cancer was observed for women who carry one *28 allele (odds ratio [OR] = 0.7, 95% confidence interval [CI] = 0.5-1.1). The risk, however, was slightly elevated among subjects who carry two copies of this allele (OR = 1.5, 95%CI = 0.5-4.3); but the sample size was small in this group. No appreciable modifying effect of lifestyle factors was noted on the association between UGT1A1 polymorphism and breast cancer risk. We also evaluated the relationship of UGT1A1 genotypes with plasma levels of estrone, estrone sulfate, estradiol, testosterone, and sex hormone binding globulins (SHBG) among 375 postmenopausal controls that were measured for these molecules. The geometric means of estrone sulfate (p-trend = 0.02) and estradiol (p-trend = 0.02) were significantly lower in those heterozygous for *28 allele and homozygous for *28 allele than those homozygous for the *1 allele. On the other hand, the mean for SHBG was significantly higher among women who carried one or two copies of the *28 allele than those homozygous for the *1 allele (p-trend <0.01). The results from this study suggest that genetic polymorphism in the UGT1A1 gene is related to the blood levels of SHBG and estrogens and may thus be related to the risk of postmenopausal breast cancer in Chinese women.

Breast Cancer Research and Treatment **85**: 239–245, 2004.

Report

Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer

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Key words: breast cancer, etiology, genetic susceptibility, polymorphism, UGT1A1

Summary

Uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) is involved in catalyzing estrogen, the hormone that plays a central role in the etiology of breast cancer. A common polymorphism [A(TA)$_6$TAA (allele *1) to A(TA)$_7$TAA change (allele *28)] in the TATA-box of the promoter region of the *UGT1A1* gene has been reported to be associated with a reduced transcription of this gene. We investigated the association of this polymorphism with the risk of breast cancer among 1047 breast cancer cases and 1083 community controls in the Shanghai Breast Cancer Study, a population-based case-control study. Approximately same proportion of cases (12.5%) and controls (13.0%) carried the variant allele *28 in the Chinese population ($p = 0.32$). When stratified by age, carrying the *28 allele was associated with an increased risk of breast cancer among women aged less than 40 years (odds ratio [OR] = 1.7; 95% CI = 1.0–2.7) but not among women 40 years old and over (OR = 0.8; 0.7–1.1). Only a few women were homozygous for the *28 allele, precluding a detailed gene-dose association analysis. Additional analyses showed that, the elevated risk associated with the *UGT1A1*28* allele among young women was primarily seen in women who had a later menarche, short menstrual years, absence of family history of breast cancer, low waist-to-hip ratio, or low body-mass index. These results suggested that the *28 allele in the *UGT1A1* gene may be associated with an increased risk for breast cancer among Chinese women under age 40. No significant associations were observed with *28 allele and breast cancer risk by estrogen receptor/progesterone receptor status.

Introduction

Endogenous levels of sex-steroid hormones are believed to play an important role in the etiology of female cancers, including breast and endometrial cancer [1]. Prospective studies have shown that breast cancer cases present higher serum levels of estrogens compared to controls [2–4]. Uridine diphospho-glucuronosyltransferases (UGTs) catalyze the glucuronidation of estrogen and many other endogenous and exogenous compounds, giving rise, in most cases, to water-soluble and less reactive metabolites [5]. Uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1), is a major member of the UGT1 family [6]. A common polymorphism [A(TA)$_6$TAA (allele *1) to A(TA)$_7$TAA change (allele *28)] in the TATA-box of the promoter region of the *UGT1A1* gene has been reported to have possible influences on the transcription of this gene [2, 6–8]. Subjects who carry the variant *28 allele have a significant decrease in expression of enzymatic activity of hepatic UGT resulting in higher serum bilirubin levels, compared to those homozygous for the common *1 allele [7, 9, 10]. Heterozygosity (*1/*28) and homozygosity (*28/*28) for this polymorphism is clinically associated with Gilbert's Syndrome characterized by

a hereditary, mild, unconjugated hyperbilirubinemia resulting from impaired hepatic bilirubin clearance and otherwise normal liver function [11]. However, the role of *UGT1A1* polymorphism in breast cancer risk is still unclear. A recent study by Guillemette et al. observed an increased breast cancer risk in premenopausal African–American women who carried the *28 allele; however, similar results have not been shown in Caucasian women [2, 8]. Since estrogen metabolism appears to vary by race [12], and UGTs are part of the complex pathway of sex-steroid inactivation [1, 13], it is conceivable that polymorphic variations in UGTs may be one of the genetic factors accounting for racial differences in susceptibility for breast cancer [2, 14].

Methods

Study population

Our study population consisted of women aged 25–64 years, participants of the Shanghai Breast Cancer Study (SBCS), a population-based case-control study initiated in 1996 in Shanghai, People's Republic of China [15–18]. Eligible cases were women who were diagnosed with breast cancer from August 1996 to March 1998, aged 25–64 years, and permanent residents of urban Shanghai who had no prior history of breast cancer. Subjects with breast cancer were identified through a rapid-case-ascertainment system supplemented by the population-based Shanghai Cancer Registry. Of the 1602 eligible subjects identified, in-person interviews were completed for 1459 (91.1%) of them. Approximately 7% of the eligible subjects refused to participate, 1% died before the interview, while 1% could not be located for interviewing. Two senior study pathologists reviewed tumor slides to confirm breast cancer diagnoses for all the case subjects.

Controls were randomly selected from the female residents of Shanghai and were frequency matched to cases by 5-year age intervals. The number of control subjects in each age-specific stratum was determined in advance according to the most recent data on the age distributions of the breast cancer patients available from the Shanghai Tumor Registry. The Shanghai Resident Registry, which keeps registry cards for all adult residents in urban Shanghai, was used to randomly select control subjects. In-person interviews were completed for 1556 (90.3%) of the 1724 eligible control subjects identified. A hundred and sixty-eight potential control subjects were excluded from the study, including 166 refusals (9.6%), and 2 either died or had a prior cancer diagnosis (0.1%).

Information on demographic factors, menstrual and reproductive histories, hormone use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer was collected using a structured questionnaire. Subjects were all measured for their waist and hip circumferences, current weight, and heights. Blood samples were also obtained from 1193 (81.8%) of the cases and 1310 (84.2%) of the controls for the genotyping assays in this study.

Genotyping method

Genomic DNA was extracted from buffy coat fractions using the Puregene® DNA isolation Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. DNA concentration was measured by PicoGreen® dsDNA Quantitation Kit (Molecular Probes, Eugene, OR). UGT1A1 promoter region $A(TA)_6TAA \rightarrow A(TA)_7TAA$ polymorphism was detected by fluorescent amplimers on an ABI 3700 automated DNA sequencer. The primers for the PCR reaction were: forward: 5′-CAACAGTATCTTCCCAGC-3′ and reverse: 5′-gtgtcCACGTGACACAGTCAAAC-3′. Forward primer was labeled with HEX. Each 2.2 µl of PCR mixture included 0.1 unit AmpliTaq Gold DNA polymerase, 1 × Buffer II, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 335 nM of each primer, and 1 ng DNA. Thermal cycling conditions were as follows: 95°C × 10 min followed by 10 cycles of 94°C × 15 s, 55°C × 15 s, and 72°C × 30 s, 20 cycles of 89°C × 15 s, 55°C × 15 s, and 72°C × 30 s with a final extension step of 72°C × 10 min. Allele fragment size estimation was accomplished using the internal size standard Genescan 400HD ROX and the Local Southern algorithm of GENESCAN software. Editing of alleles was performed in GENOTYPER. Allele binning and adjustment of run mobility according to control alleles of CEPH 1347-02 were accomplished by custom software.

The laboratory staff was blinded to the identity of the subjects. Control samples (water, CEPH 1347-02 DNA, as well as blinded and unblinded duplicate DNA samples) were included among genotyping assays. The *UGT1A1* genotypes determined for the control samples yielded no discrepancies. Genotyping data were obtained from 1047 (87.8%) cases and 1083 (82.7%) controls that had blood samples. The major reasons for incomplete genotyping were in-

sufficient DNA and unsuccessful polymerase chain reaction (PCR).

Statistical analyses

Characteristics of participants were compared by χ^2 statistics (categorical variables) and by *t*-test (continuous variables). Odds ratios (ORs) and 95% confidence intervals (CI) were used to assess the relationship between UGT1A1 genotype and breast cancer risk. Unconditional logistic regression was used to adjust for potential confounding factors. Stratified analyses by conditions related to endogenous estrogen exposure, such as age at menarche, total years of menstruation, age at first live birth, body mass index (BMI) and waist-to-hip ratio (WHR) were performed to evaluate potential interactive effects of these variables with UGT1A1 genotype in the risk of breast cancer. All statistical tests were two-sided. The Statistical Analyses Software (SAS) statistical package, version 8 was used for all analyses (SAS Institute, Inc.).

Results

The distribution of demographic characteristics and major breast cancer risk factors among 1047 breast cancer cases and 1082 controls are shown in Table 1. More cases than controls reported ever having being diagnosed with a breast fibroadenoma ($p < 0.01$) while a nonsignificant higher number of cases than controls reported a family history of breast cancer among first-degree relatives ($p = 0.13$). The mean ages at menopause and at first live birth were significantly higher in cases than controls but the mean age at menarche was significantly lower in cases compared to controls. Menstrual years were significantly longer in cases than controls. Cases and controls were comparable in age, education, and history of ever having a live birth. In addition, cases had a higher mean BMI and mean WHR. Cases also had a lower proportion of subjects that were postmenopausal and have been physically active in the past 10 years. These results were virtually identical to the analyses based on all of the subjects included in the main study [19].

The allele frequencies for *UGT1A1* polymorphism and the estimated ORs for the association of *UGT1A1* genotypes and breast cancer risk by age are presented in Table 2. We observed an allele frequency of 0.128 for *UGT1A1*28* in our study population. There was no statistically significant difference in the distribution of the *UGT1A1*28* allele frequency between cases and controls. After adjusting for age, we observed a nonsignificant increase in breast cancer risk of 20% (95% CI = 0.6–2.2) in subjects homozygous for the *UGT1A1*28* allele compared to subjects homozygous for allele*1. However, among women aged less than 40 years, a statistically significant positive association was seen for subjects that carried any *UGT1A1*28* allele compared to subjects homozygous for allele *1 (OR = 1.7; 95% CI = 1.0–2.8).

We also evaluated the relationship between breast cancer and the genotypes by estrogen/progesterone receptor status (ER/PR) (Table 3). Although elevated

Table 1. Distribution of demographic characteristics and major breast cancer risk factors in cases and controls who were genotyped for the *UGT1A1* polymorphism: the SBCS

Subject characteristic	Cases ($n = 1047$)	Controls ($n = 1083$)	*P*-value[d]
Age (years) (mean ± SD)	47.6 ± 8.0	47.1 ± 8.7	0.21
Education (%)			
Elementary school or no formal education	11.8	15.0	
Middle or high school	44.7	43.1	
College or above	43.5	42.0	0.12
Breast cancer in first-degree relatives (%)	3.6	2.5	0.13
Ever had breast fibroadenoma (%)	9.9	4.9	<0.01
Age at menarche (mean ± SD)	14.5 ± 1.6	14.7 ± 1.7	<0.01
Ever had a live birth (%)	95.0	96.0	0.27
Number of live births[a] (mean ± SD)	1.5 ± 0.8	1.5 ± 0.9	0.16
Age at first live birth[a] (mean ± SD)	26.9 ± 4.1	26.2 ± 3.9	<0.01
Postmenopausal (%)	31.9	36.2	0.04
Age at menopause[b] (mean ± SD)	48.3 ± 4.6	47.4 ± 5.0	0.02
Menstrual years[c] (mean ± SD)	30.4 ± 5.5	29.2 ± 5.9	<0.01
Physically active past 10 years (%)	19.5	26.0	<0.01
BMI (mean ± SD)	23.5 ± 3.4	23.2 ± 3.4	0.03
WHR (mean ± SD)	0.81 ± 0.06	0.80 ± 0.06	<0.01

[a] Among parous women.
[b] Among postmenopausal women.
[c] Menopausal age or age at interview in premenopausal women – age at menarche.
[d] From χ^2-test (categorical variables) or *t*-test (continuous variables).

Table 2. Allele frequencies of the *UGT1A1* polymorphism and estimated ORs for the association between *UGT1A1* genotypes and breast cancer risk: the SBCS

UGT1A1 polymorphism	Cases ($n = 1047$) n (%)	Controls ($n = 1083$) n (%)	OR[a] (95% CI)	OR[b] (95% CI)
Allele frequency				
*1	1832 (87.5)	1882 (87.0)		
*28	262 (12.5)	282 (13.0)		
	$\chi^2 = 0.22$, df = 1, $p = 0.32$			
Genotype frequency				
*1/*1	807 (77.1)	820 (75.8)	1.0 (reference)	1.0 (reference)
*1/*28	218 (20.8)	243 (22.4)	0.9 (0.7–1.1)	0.9 (0.8–1.2)
*28/*28	22 (2.1)	20 (1.8)	1.1 (0.6–2.1)	1.2 (0.6–2.2)
Trend test	$p = 0.60$			
Women <40 years				
*1/*1	109 (72.2)	179 (81.4)	1.0 (reference)	1.0 (reference)
*28/any	42 (27.8)	41 (18.6)	1.7 (1.0–2.8)	1.7 (1.0–2.8)
Women ≥40 years				
*1/*1	698 (77.9)	641 (74.3)	1.0 (reference)	1.0 (reference)
*28/any	198 (22.1)	222 (25.7)	0.8 (0.7–1.0)	0.8 (0.7–1.1)

[a] Adjusted for age.
[b] Adjusted for age, prior history of breast fibroadenoma, physical activity, and WHR.

Table 3. Association between breast cancer risk and *UGT1A1* genotypes by estrogen and progesterone receptor status: the SBCS

ER/PR status[a]	All subjects				Age <40 years				Age ≥40 years			
	*1/*1		*28/any		*1/*1		*28/any		*1/*1		*28/any	
	N	OR[b]	N	OR[b]	N	OR[b]	N	OR[b]	N	OR[b]	N	OR[b]
Number of controls	820		263		179		41		641		222	
ER+/PR+	282	1.0	90	1.0 (0.8–1.3)	33	1.0	12	1.6 (0.8–3.3)	249	1.0	78	0.9 (0.7–1.2)
ER−/PR−	135	1.0	45	1.0 (0.7–1.5)	22	1.0	9	1.8 (0.8–4.2)	113	1.0	36	0.9 (0.6–1.4)
Others	107	1.0	27	0.8 (0.5–1.2)	12	1.0	5	1.5 (0.5–5.0)	95	1.0	22	0.6 (0.4–1.1)
Unknown	283	1.0	78	0.9 (0.6–1.1)	42	1.0	16	1.6 (0.8–3.2)	241	1.0	62	0.7 (0.5–1.0)

[a] The cases in every stratum were compared with the whole control group.
[b] Adjusted for age.

ORs of breast cancer associated with the *28 allele were seen regardless of ER/PR status among women younger than 40 years, none of the ORs were statistically significant, perhaps due to small numbers. Similarly, *28 allele was unrelated to the risk of any type of breast cancer among older women.

Table 4 shows the association between breast cancer risk and UGT1A1 genotypes by factors related to endogenous estrogen exposure. In comparing the presence of *28 allele to the homozygotes for the wild allele (*1/*1) among women less than 40 years old, statistically significant associations between breast cancer risk and UGT1A1 genotype were observed among women with age at menarche ≥14 years (OR = 2.1; 95% CI = 1.2–3.9), menstrual years <30 years (OR = 1.7; 95% CI = 1.0–2.8), absence of family history of breast cancer (OR = 2.6; 95% CI = 1.4–4.7), WHR < 0.80 (OR = 1.8; 95% CI = 1.0–3.5), or BMI < 23.0 (OR = 1.8; 95% CI = 1.0–3.5). Because no women aged less than 40 years had a menstrual duration of 30 or more years, we further evaluated the relationship of genotype and breast cancer by

Table 4. Association between breast cancer risk and *UGT1A1* genotypes by factors related to endogenous estrogen exposure: the SBCS

Variables	All subjects				Age <40 years				Age ≥40 years			
	*1/*1		*28/any		*1/*1		*28/any		*1/*1		*28/any	
	Cases/control	OR[a]	Cases/control	OR[a]	Cases/control	OR[a]	Cases/control	OR[a]	Cases/control	OR[a]	Cases/control	OR[a]
Age at Menarche[b]												
<14 years	253/205	1.0	72/72	0.8 (0.5–1.2)	41/63	1.0	11/16	1.0 (0.4–2.4)	212/142	1.0	61/56	0.7 (0.5–1.1)
≥14 years	554/615	1.0	168/191	1.0 (0.8–1.2)	68/116	1.0	31/25	2.1 (1.2–3.9)	486/499	1.0	137/166	0.8 (0.7–1.1)
Menstrual years[b,c]												
<30 years	323/415	1.0	111/117	1.2 (0.9–1.6)	109/178	1.0	42/41	1.7 (1.0–2.8)	214/237	1.0	69/76	1.0 (0.7–1.5)
≥30 years	482/404	1.0	129/145	0.7 (0.6–1.0)	0/0	–	0/0	–	482/404	1.0	129/145	0.7 (0.6–1.0)
Menstrual years[c,d]												
<22 years	39/105	1.0	19/23	2.3 (1.1–4.7)	37/88	1.0	16/19	2.0 (0.9–4.2)	2/17	1.0	3/4	6.1 (0.7–52.6)
≥22 years	766/714	1.0	221/239	0.9 (0.7–1.1)	72/90	1.0	26/22	1.4 (0.7–2.7)	694/624	1.0	195/217	0.8 (0.6–1.0)
Age at first livebirth[b]												
<26 years	312/363	1.0	90/111	0.9 (0.7–1.3)	37/74	1.0	13/14	1.9 (0.8–4.4)	275/289	1.0	77/97	0.8 (0.6–1.2)
≥26 years	495/457	1.0	150/152	0.9 (0.7–1.2)	72/105	1.0	29/27	1.6 (0.9–2.9)	423/352	1.0	121/125	0.8 (0.6–1.1)
Physical activity												
No	652/619	1.0	191/183	1.0 (0.8–1.2)	101/158	1.0	35/35	1.6 (0.9–2.7)	551/461	1.0	156/148	0.9 (0.7–1.1)
Yes	156/202	1.0	49/80	0.8 (0.5–1.2)	8/21	1.0	7/6	3.3 (0.8–13.4)	148/181	1.0	42/74	0.7 (0.4–1.1)
Family history of breast cancer												
No	502/595	1.0	156/194	1.0 (0.8–1.2)	64/135	1.0	32/26	2.6 (1.4–4.7)	438/460	1.0	124/168	0.8 (0.6–1.0)
Yes	305/225	1.0	84/69	0.9 (0.6–1.3)	45/44	1.0	10/15	0.7 (0.3–1.6)	260/181	1.0	74/54	1.0 (0.6–1.4)
WHR[b]												
<0.80	356/418	1.0	112/143	0.9 (0.8–1.2)	62/126	1.0	22/24	1.8 (1.0–3.5)	294/292	1.0	90/119	0.8 (0.5–1.0)
≥0.80	451/402	1.0	128/120	0.9 (0.7–1.3)	47/53	1.0	20/17	1.4 (0.6–2.9)	404/349	1.0	108/103	0.9 (0.7–1.2)
BMI[b]												
<23.0	380/431	1.0	125/138	1.0 (0.8–1.3)	71/136	1.0	26/27	1.8 (1.0–3.5)	309/295	1.0	99/111	0.9 (0.6–1.2)
≥23.0	427/389	1.0	115/125	0.8 (0.6–1.1)	38/43	1.0	16/14	1.3 (0.6–3.0)	389/346	1.0	99/111	0.8 (0.6–1.1)

[a] OR adjusted for age.
[b] Median value among all participants.
[c] Menopausal age or age at interview in premenopausal women – age at menarche.
[d] Median value among participants aged <40 years.

menstrual years by using their median menstrual years (22 years). Though not statistically significant, the *28 allele was associated with an increased risk of breast cancer in young women with menstrual years less than 22 years (OR = 2.0; 95% CI = 0.9–4.2).

Discussion

Several earlier smaller studies had observed that the frequency of the *UGT1A1*28* allele was lower among Asians, particularly the Chinese, compared to other ethnic groups such as the African–Americans, Caucasians, and Indians [20–24]. The frequency of 0.130 we observed for the *UGT1A1*28* allele in our Chinese study population is similar to the 0.160 reported for Asians in the study by Beutler et al. [24]. Although there was no overall difference between cases and controls in the distribution of UGT1A1 genotypes in our study, among women younger than 40 years we observed a significant 60% increase in risk for breast cancer associated with the presence of *UGT1A1*28* allele. Similarly, in a recent study, Guillemette et al. observed a 1.8-fold elevated risk for

breast cancer (95% CI = 1.0–3.1) in premenopausal African–American women with presence of low activity UGT1A1 alleles [2]. However, in another study conducted within the Nurses' Health Study (NHS) cohort, a study of predominantly Caucasian women, Guillemette et al. did not observe an association between UGT1A1 polymorphism and breast cancer risk [8]. The reasons for this discrepancy remain unclear. It is possible that racial variations in genes responsible for estrogen metabolism may contribute to some of the differences observed from previous studies. In stratified analyses, we found that the association with the *28 allele appeared stronger among women with late menarche (≥14 years), and shorter menstrual duration (<30 years), which suggests that this allele may contribute to the risk of developing breast cancer in women who have had shorter period of estrogenic stimulation. Interestingly, our results also showed that elevated risks for breast cancer in women less than 40 years old carrying the *28 allele were seen primarily among those who were at a low risk of breast cancer, such as those who have no family history of breast cancer, had WHR < 0.80 or BMI < 23.0. We have no readily available explanation for this observation and hope that future studies could re-evaluate these findings. No significant associations were observed with *28 allele and breast cancer risk by ER/PR status.

One of the strengths of this of study is that most of the participants of this study are of the same ethnic group, Han Chinese (>98%). Another is the high participation rate for eligible subjects (>91%) thus resulting in a low likelihood of confounding by ethnicity or selection bias. In addition, this study is one of the largest studies to have evaluated the role of UGT1A1 polymorphism in the risk of breast cancer.

In conclusion, the results from this study suggest that *UGT1A1*28* genetic polymorphism in the *UGT1A1* gene is associated with an increased risk for breast cancer in Chinese women under 40-years-old. This finding warrants further investigation in future studies conducted in other ethnic populations.

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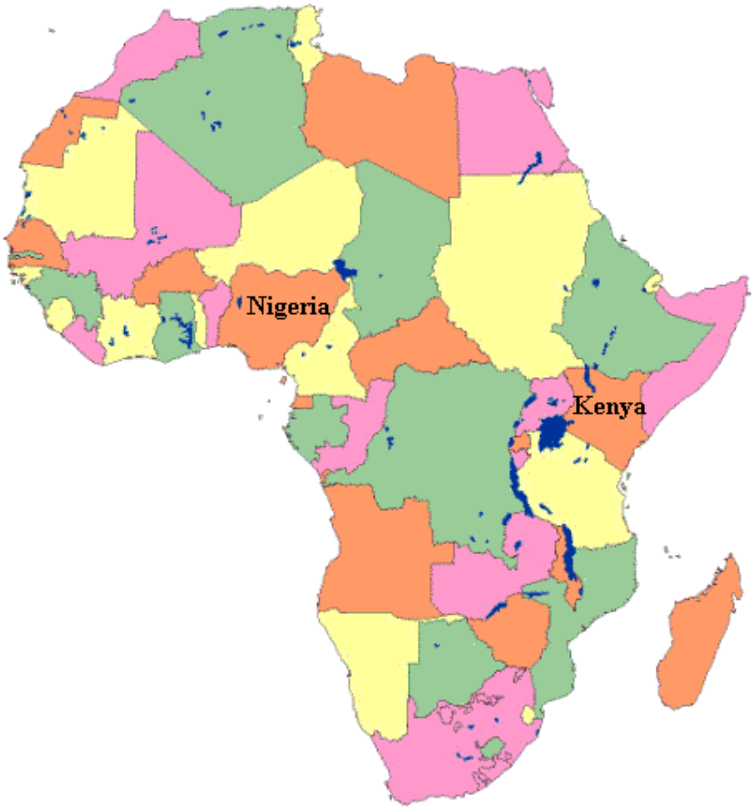
U54 Abstract.

In West Africa, the ancestral population of a large proportion of African Americans, the incidence rates of breast cancer have more than doubled over the past two decades. This increase has not been explained by improved diagnostic methods. Most breast cancer has a complex, multi-factorial etiology. The two known major susceptibility genes, BRCA1 and BRCA2, do not explain a significant proportion of familial breast cancer. Cumulative evidence suggests genetic factors and their interaction with lifestyle factors may play an important role in breast cancer etiology. In addition to the well-defined high penetrance genes, over two-dozen genes have been hypothesized to increase the susceptibility to breast cancer. Of note are polymorphic genes that are involved in: a) DNA repair, b) carcinogen metabolism, c) estrogen metabolism (or sex hormone biosynthesis), d) regulation of cell proliferation and apoptosis, e) immuno pathway, and f) iron metabolism pathway.

Our long-term goal is to conduct a large molecular epidemiologic study in West Africa to evaluate genetic factors and their interactions with lifestyle risk factors in the risk of breast cancer. We propose in this proposal to conduct a two-year pilot case-control study of 200 cases of breast cancer and 200 controls aged 25 to 64 years to evaluate the feasibility of conducting the larger study. This study will be conducted in Kenya and Nigeria. From each study site, we propose to recruit 100 breast cancer cases and 100 age frequency-matched population-based controls.

In this pilot study we propose to: 1) develop the protocol for the recruitment of participants and selection of appropriate controls, 2) develop and validate culturally appropriate questionnaires for the collection of demographic, lifestyle history, medical history, reproductive history, family history, and anthropometrics measurements data, 3) develop protocols for the collection, shipment to the United States and storage of blood samples, cheek cells, and urine samples at Meharry Medical College, TN, 4) determine response rates for cases and controls by study site and identify strategies to increase participation as required in the larger study, and, 5) analyze preliminary data collected. The results from this pilot project will provide clues to refine future hypotheses for our larger study that will provide insight into the underlying causes for the increasing risk for this malignancy among African American women, other ethnic minorities and underserved populations in the U.S. and other parts of the world.

Breast Cancer in African Women Study (BCAWS)



PROTOCOL MANUAL

Breast Cancer in African Women Study (BCAWS) Protocol

Overview

A. Overall Goal

This proposed population-based case-control study of breast cancer would be conducted among African women of Nigeria and Kenya where the incidence rates of breast cancer have more than doubled over the past two decades. The two known major susceptibility genes, *BRCA1* and *BRCA2*, do not explain a significant proportion of familial breast cancer. Most breast cancer has a complex, multi-factorial etiology. Our long-term goal is to conduct a large molecular epidemiologic study to evaluate lifestyle risk factors, genetic factors, gene-gene, and gene-environment interactions in the risk of breast cancer in women from the West African sub-region. However, in this pilot project, we will conduct a two-year case-control study of 200 breast cancer cases and 200 controls aged 25 to 64 years to evaluate the feasibility of conducting the larger study in West Africa and to provide clues to refine our hypotheses for our larger study.

B. Specific Aims

1. To develop the protocol for the recruitment of participants and selection of appropriate controls.
2. To develop and validate culturally appropriate questionnaires for the collection of demographic, lifestyle history, medical history, reproductive history, family history, and anthropometrics measurements data.
3. To develop protocols for the collection, shipment to the United States, and storage of blood samples, cheek cells, and urine samples at Meharry Medical College.
4. To determine the response rates for cases and controls by study site and identify strategies to increase participation as required in the larger study.
5. To analyze the preliminary data collected.

The aims of this project will be accomplished in two phases:

Phase I: We will conduct a two-year case-control study of 200 breast cancer cases and 200 controls aged 25 to 64 years to evaluate the feasibility of conducting a larger study in Africa and provide clues to refine our hypotheses for our larger study.

Phase II: The full scale study will begin following a positive result from the pilot study. At this time we will submit a RO1 proposal for a larger study and focus, in addition to several other low-penetrance genes, seven most promising polymorphic genes

Project Personnel

Name	Institution/Organization	Role on Project	Email
Nashville, TN, USA			
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Marian Ladipo	Meharry Medical College	Study coordinator/Research associate	mladipo@mmc.edu
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Nairobi, Kenya			
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Dr. W. Mwanda Ph.D	University of Nairobi	Co-investigator/Kenya site	
Dr. Biakikah	University of Nairobi	Co-investigator/Kenya site	
Dr. Joel Ochieng	University of Nairobi	Co-investigator and Site coordinator/Kenya site	

Institution/Organization Contact Information

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BCAWS Policies

Specific policies agreed upon by all participating in the BCAWS study will govern the activities that take place at the field sites as well as between the institutions. These policies relate to the informed consent of study participants; accessing information from their medical records; privacy of collected data; the regulations for BCAWS DNA access, storage, and use; and the referral protocol for medical conditions detected during the blood pressure measurement, blood collection, urine collection, and cheek cell collection.

A. Informed consent (see appendix A for informed consent documents)
   All individuals participating the BCAWS study will be asked to complete a series of questionnaires, to give samples of blood and urine, and to have blood pressure and anthropometric measurements taken. The informed consent will also authorize the Principal Investigator to share the specimens and the data with collaborators at other centers. The form clearly states that participation is strictly voluntary and that participation may be withdrawn at any time.
B. Standards of DNA Analysis and Use

Upon receipt of a specimen shipment, the receiving site is to promptly return the shipment verification form to the sending site. The samples are to be used only for the analyses approved by the BCAWS principle investigator.

All blood samples will be identified by the 3 digit site number and a 3 digit subject number. For example a blood sample at the Ile-Ife site would be identified by **001 +** *a 3 digit subject number*. At the Nairobi site blood samples would be identified by **002** + *a 3 digit subject number*. In the rare event that a participant wishes to withdraw from the study, the project coordinator for the appropriate site should address this immediately by doing the following:

1. Retrieve all hard copy data files
2. Contact the laboratory manager to stop any and all analyses
3. Refer to the shipment and tracking logs to determine if participant samples have shipped to other centers for analyses.
4. If samples have been shipped to other centers, the center laboratory manager should be contacted to stop any and all analysis

Any unused portion will be destroyed along with the hard copy data on the participant.

C. Referral Policies

Medical Evaluation

Patients whose results meet referral criteria will be contacted and advised of the findings. Referrals are usually made to personal physicians in the community. The site investigators will establish the referral criteria.

DNA ANALYSIS

The BCAWS study DNA referral policy is in compliance with the recommendations made by the American College of Medical Genetics (1995) on storing Genetic materials. In addition, the issues raised by the workshop convened by the National Center for Human Genome Research and the Centers for Disease Control and Prevention (1995) regarding obtaining informed consent for genetic research on stored human tissue samples will act as a guide to the investigators of the BCAWS study. If during the course of DNA analysis a deletererious gene that will affect the health of a participant is found, the Project Coordinator or Principal Investigator will make an attempt to contact the individual. This does not in any way hold BCAWS investigators responsible for further testing to confirm diagnosis or for providing medical advice or treatment. The site personnel will facilitate communication with the individual and the health care provider, if applicable, on findings from the DNA analysis.

I. Eligibility

a. Cases are women diagnosed with breast cancer, aged 25 to 64 and will be identified from the Ife-Ijesha Cancer Registry based at the Obafemi Awolowo

University Teaching Hospital Complex, Ile-Ife (OAUTHC), and from the surgical clinics and the pathology department of the OAUTHC.

b. Controls are women with no history of ANY cancer and will be randomly selected from the population of the city in which the study is located. Controls will be matched by 5-year age group and ethnicity to cases.
c. Exclusionary Criteria
    1. Cases and Controls must not be pregnant at the time of the study
    2. Men
    3. Children
    4. Women with ANY cancer

II. Recruitment of Subjects

a. Obtaining informed consent

Signed informed consent will be obtained from all participants for this study and future possible related studies for which samples and information from the participants may be used. No clinic procedure will be used until a study participant signs the consent form. If the study participant is unable to write, a fingerprint will be obtained. The informed consent will authorize the Principal investigator to share the specimens and data with the collaborators at the other centers. The form will state that participation is strictly voluntary and that the participant can withdraw from the study at any time. The study participant should read and understand the accompanying pages, which describe the nature of the study, the research principles, the expected length of involvement in the study as well as the procedures, risks, and benefits before signing the consent form. If the participant indicates that she wants the interviewer to read the information aloud, the interviewer must do this in the appropriate language/dialects of the participant. The interviewer should make a good fate effort to answer any questions asked by the participant. The interviewer must also sign the form as a witness to the participant's signature or thumbprint.

b. Clinic Procedures

After the informed consent has been obtained from the participant, the following will take place during the clinic visit:

1. Demographic/ Medical History - A trained field team member should administer this questionnaire. Questions will include information on demographics, lifestyle history, medical history, reproductive history, dietary history, and family history.
2. Anthropometrics Measurements
3. Blood Pressure Measurements – These readings will be taken using the provided aneroid sphygmomanometer. Two pulse and BP measurements will be taken and recorded carefully and legibly on the questionnaire.
4. Blood Collection – Twenty-five mls of venous blood will be collected per participant during clinic examination and put in one 10 ml lavender top, two 5 ml red top for serum and one 5ml green top. Only experienced phlebotomists will draw blood from all consenting participants.

5. Urine Collection – Spot urine will be collected from all participants in the urine collection kit provided.
6. Cheek Cell Collection – Exfoliated cheek cells will be collected as an additional source of DNA for genotyping by using approximately 5 mls (one tablespoon) of commercial mouthwash.
7. Saliva Collection – Saliva collection kits with instructions will be used for saliva sample collection.

Permission to bring human specimens into the US will be obtained from US Customs and Centers for Disease Control (CDC) in Atlanta, Georgia. All shipping containers should be marked "Human Blood Specimens – for Diagnostic Purposes Only". The total number of vials per person should be shipped in two batches.

III. Data Collection and Management

A. Overview

Research data is only as good as the procedures and instruments utilized to collect the information. Therefore, we have implemented a system of quality control to standardize data collection. The primary goal of the quality control (QC) procedure is to ensure the quality and comparability of collected data among the field staff and attending physicians. To achieve this goal, all study procedures are standardized, including forms, training, certification, survey, and sampling procedures.

B. Data Entry and Storage Quality Control

Quality assurance often depends more on diligence than on complex methodology. The US Coordinating Center (CC) will continually receive data (hard copy and Electronic) from Nigeria and Kenya sites. All data submitted to the CC will be edited rigorously, through a number of straightforward methods that will identify problems. We will examine univariate distributions, compare study results with external sources, and monitor the amount of data that is either missing or has failed edit checks. We will also perform bivariate and multivariate edits on the data to detect additional problem areas. We will use bivariate plots of variables known to be correlated (e.g., weight and height) to identify possible error in data collection and entry.

To reduce error rates, data are entered directly from the data collection forms. The interviewer checks all data items before the participant leaves the clinic. The completed forms are returned to the Site Coordinator who rechecks the forms and numerically codes the handwritten fields. Only after all forms are checked and all fields are properly coded do the data entry begin. A trained data entry operator enters data items once at the site. Then, a second entry will be done in the United States. The database program used for data is Microsoft Excel. The Microsoft Excel database created should be password protected by the personnel responsible for data entry in order to maintain the integrity of the data. The two files are then compared to verify data. The data manager will go through as many iterations necessary until the two data files are identical. After

data entry, a validation and logic check program are conducted to detect any outliers. All computer files are password protected and each file has its own unique password.

IV. Laboratory Methods

Red Top (Serum)
After collecting specimen gently invert tube 5 times to mix the clot activator with the blood. Allow tube to clot 30 minutes to 1 hour in a vertical position. Spin tubes at ≥ 3000 rpms to separate the serum from the clot. Pipette serum into 4 2ml tubes. Transfer clot into 2 separate 2ml tubes.

Lavender and Green Top (Plasma)
After collecting specimen spin immediately at ≥ 3000 rpms for 10 minutes. If not possible, place on ice or store in ice to 4°C and spin ASAP.

i. Pipette plasma into 2-3 2ml tubes. Be sure not to obtain too much because you don't want to take the next layer, which is the buffy coat.
ii. Pipette buffy coat into 2 2ml tubes. In your effort to obtain the entire buffy coat you may take some RBCs along with your sample. This is acceptable.
iii. Pipette the remaining red blood cells (RBCs) into 2 or 3 2ml tubes.

Labeling
Label all transport tubes by:
a. Subject ID (For example **001**123. **001** represents the site number and 123 the subject number.)
b. Type of Sample (e.g. Plasma, WBC, RBC, Buffy Coat)
c. For Lavender or Green top sample sources, clearly specify source of sample as Green (G) or Lavender (L).

V. Storage and Shipment Policy

When available, all vials should be stored at –86°C. If not immediately available, items should be stored at –20 to –40°C for a maximum of 2 to 3 months. Properly labeled vials should be stored sequentially in the provided cryogenic cardboard freeze-storage boxes by type of specimen (ie., plasma, DNA and Urine samples should be stored in separate boxes). The corresponding ID for each specimen type should be entered into the space provided in the storage inventory forms. A copy of this form should accompany all shipment to the United States.

As a backup, one aliquot from each tube should remain in the study site. The remaining frozen urine, plasma, and buffy coat should be shipped frozen in dry ice packed in the large polyfoam shipping containers provided to each site. The containers should be completely covered (filled to the top) with dry ice before sealing the box with the provided shipment tapes. This will ensure that specimen remain frozen on arrival in the US anywhere from 3 to 5 days. It is therefore important to completely fill the shipment box with dry ice. Preferably, shipment should be made at the beginning of the week

(Monday or Tuesday) to avoid weekend delays. Most customs offices are closed on Sundays.

Laboratory Methods at Meharry Medical College and Vanderbilt

Once the samples arrive from Kenya, they will be stored in a locked freezer Meharry Medical College. Marian Ladipo will then transport the samples from Meharry Medical College to Dr. Wei Zheng's lab at Vanderbilt University. The head of the laboratory personnel at Vanderbilt has a doctoral degree in toxicology with over 15 years experience. In the lab at Vanderbilt University, DNA will be isolated from the blood and saliva samples collected from the subjects. The two known major susceptibility genes are *BRCA1* and *BRCA2*. We are going to look at those as well as other genes that have been found to have an association with breast cancer. Other genes, other than *BRCA1* and *BRCA2* have observed significant association to breast cancer risk, however these particular studies were not performed in African-American or African populations. We are going to look at a few of these genes too. These genes are *HRAS1*, *GSTM1*, *GSTP1*, *CYP1B1*, *CYP2D6*, *CYP19*, *VDR*, *Tp53*, *ER*, and *PR*. After selecting genes, genotyping will be performed. Genotyping is the procedure used to identify the specific sequence of genes in a sample of DNA. The procedure can be used to identify similarities or differences in the sequence of genes and is useful in establishing whether an individual may be genetically predisposed to a disease or condition. The genotyping process consists of utilizing the PCR reaction to amplify markers, and gel electrophoresis to estimate the size of the marker.

We have extensive experience in analyzing genetic polymorphisms of candidate genes in large epidemiological studies and have performed assays to determine over 300,000 genotypes in epidemiological association studies over the past five years. For single nucleotide polymorphisms (SNPs), various techniques have been used in our lab, including PCR-RFLP, allele specific oligonucleotide, SSCP, direct sequencing, and TaqMan allelic discrimination. Currently, the TaqMan assay is the standard approach in our lab for genotyping, as it is robust and cost-efficient for moderate-scale genotyping operations. For each SNP detection, a pre-developed TaqMan assay reagent (PDAR) kit (Assay-on-Demand and Assay-by-Design reagents), containing one pair of PCR primers and one pair of fluorescent TaqMan probes will be purchased from Applied Biosystems (Foster City, CA). According to the standard TaqMan protocol developed by ABI, each 5μL PCR contains 3 ng of genomic DNA, 180 nM primers, 50nM probes, and 2.5μL of TaqMan Universal PCR master mix (ABI) and TaqGold DNA polymerase in a 384-well plate. Amplification conditions are: 50°C, 2 min; 95°C, 10 min; followed by 40 cycles of 94°C, 15 sec and 62°C, and 1 min in a PTC-200 Peltier Thermal Cycler (MJ Research, Inc., Waltham, MA). Fluorescence in each well will be measured after PCR using an ABI 7900HT sequence detection system. The SDS 2.1 software with the 7900HT instrument will be used for data acquisition, allele calling, and data exporting.

Genotyping for the STR polymorphisms will be performed by detection of fluorescent amplimers on an ABI 3700 automated DNA Analyzer. One primer will be labeled with FAM. Each 4 μl of PCR mixture includes 0.5 unit AmpliTaq Gold DNA polymerase, 1x Buffer II, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 335 nM of each primer, and 2 ng DNA. Thermal cycling conditions are as follows: 95°C x 10 min; followed by 10

cycles of 94°C x 15 s, 55°C x 15 s, and 72°C x 30 s, 20 cycles of 89°C x 15 sec, 55°C x 15 sec, and 72°C x 30 sec; with a final extension step of 72°C x 10 min. Allele fragment size estimation is accomplished using the internal size standard Genescan 400HD ROX and the Local Southern algorithm of GENESCAN software. The number of repeats within a repeat allele is confirmed by direct sequencing using BigDye Terminator Chemistry on an ABI 3700 automated DNA Analyzer.

The urine collected will be used to examine biomarkers, a cellular or molecular indicator of exposure, disease, or susceptibility to disease. For example, we could use urine samples to evaluate the intake level of phytoestrogens. This assay will be conducted using the LC/MS system. 0.25 mL centrifuged, clear urine will be incubated with beta-glucuronidase and arylsulfatase in the presence of triethylamine acetate buffer for one hour at 37^{o}C followed by extraction with ethyl ether, evaporation of the organic solvent and redissolving in a methanol acetate buffer mixture. Liquid chromatography photo diode array multiple generation mass spectrometry (LC/PDA/MS) analysis will be performed in the negative mode after injection of 20 L. This system consists of a Spectra-Physics, Inc., designed quaternary solvent delivery liquid chromatography system with multiple channel diode-array detection and a quadrupole ion trap mass spectrometer model 'Advantage' equipped with an Z-electrospray ionization unit (Thermo Finnigan Corpor., San Jose, CA). When levels exceed the MS calibration curve, analytes will be monitored at 200-400 nm with photo-diode array detection and will be quantitated with this method as described previously. Quantitation will be performed using peak areas after adjustment for internal standard recovery. The intra- and inter-assay variability of this method is small, with mean coefficient of variation (CV) less than 10% for all phytoestrogens and their metabolites combined. The CVs are smaller for major phytoestrogens and their metabolites. Approximately 1 ml of urine sample will be needed for this assay.

Subjects' identities will be disconnected from information about them. All samples will be identified by the 2 digit site number and a 3 digit subject number. After the lab work has been completed, the remnant of the samples will be destroyed.

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VN001 _ _ _

BREAST CANCER IN AFRICAN WOMEN STUDY (BCAWS)

QUESTIONNAIRE

I am one of the researchers working on a health survey organized by the Obafemi Awolowo University Teaching Hospital, IlE-Ife as part of the Breast Cancer in West Africa Study (BCAWS). This survey focuses on the effects of environment and lifestyle factors on women's health. We are inviting you to participate in this study, which will ask you some questions related to your lifestyle and your disease history. We will keep all survey information confidential, i.e. without your permission, we will not let anybody see the information about you except the researchers who are directly participating in the study. We appreciate your cooperation and help.

Study site: _____ [__]__]__]

Hospital ID (if applicable): _____

Study ID: _____ [__]__]__]__]__]__]

Case …….1 OR Control …..2 [__]

Tumor Site: _____ [__]__]__]__]

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_ _

Interviewer's name: _____ [__]__]

Date of the interview : _____year _____month _____day [__]__] [__]__] [__]__]

Interview start time: _____ morning …..1 afternoon …..2
[__]__]__]__]__]

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PART ONE BACKGROUND INFORMATION

A1. Name: _____

A2. Nickname: _____

A3. House Number_____ A31.Street/Suburb name _____

A32. Town: _____ A33. LGA/District: _____

A34. State/Region: _____ A34. Village name:_____

A35. Landmark_____

A36. Phone_____

A4. Work Place: _____ Address of work place: _____

A5. In order to facilitate our long-term follow-up of your health status, Please provide a contact person's name, home address, phone number, working unit,
etc._____
_____

A6. Sex: Female …..1 Male …..2 [__]

A7. What is your date of birth?
[__]__] [__]__] [__]__]
Year month day

A8. What is your place of birth? _____

A9. At what age did you begin to live permanently in your present city/town/village?
[__]__]__]
Age: _____years old.

A10. What is your current marital status? [__]
1……..Single
2……..Married or cohabit (If yes, ask A11)
3……..Widowed
4……..Separated
5……..Divorced

A11. When did you get married? (This refers to the first marriage if you have been married more than once.)

Year: _____ Month: _____

A12.How many children have you had?

How many alive? [A121]

How many deceased? [A122]

A13. What is your educational level? (please read the following categories to the participants) [__]

1……..….Have never received formal education
2……...Primary/Elementary school
3…….... Junior secondary school
4……..... Senior secondary school
5…… …….. Technical high school
6…….....….. Polytechnic/ College of Education
7…….. University or above
8…….. Others
9……..... Unknown

A.14. How many people (including yourself) lived together in your household last year? [__]__]

A15. What was your family's annual income in Naira (including all sources) last year? [__]

1..... Less than 10000.
2.....10000 less than 50000.
3.....50000-less than 100000
4.....100000-less than 250000
5.....250000-less than 5000
6.....500000 or more.

A16. What tribe do you belong to? [__]

1……Yoruba
2……Ibo
3……Hausa
4……Other _____[A164]
5……Does not know.

A17. What tribe does/did your natural mother belong to? [__]

1……Yoruba
2……Ibo
3……Hausa
4……Other _____[A164]
5……Does not know.

A18. What tribe does/did your natural father belong to? [__]

1……Yoruba
2……Ibo
3……Hausa
4……Other _____[A164]
5……Does not know.

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PART TWO DISEASES HISTORY

B1. At the following ages, how many times did you receive a chest X-ray (excluding those you received in the last year)?

Age (years)	Number of X-ray examinations							
	0	1-2	3-4	5-9	10-14	15 or more	Unknown	Coded number
A. <15	1	2	3	4	5	6	9	
B. 16-20	1	2	3	4	5	6	9	
C. >20	1	2	3	4	5	6	9	

B2. Excluding the past year, have you ever received chest radiotherapy?

1…….Yes
2…….No
9…….Unknown

B2a1. If yes, when did you receive it? Year_____
B2a2. Reason for the therapy:_____
[__]___]__]

B3. Has your doctor ever told you that you had any of the following breast diseases, such as, lobular proliferation of mammary gland, fibroadenoma, mammary gland cyst, etc (not including the last year)?

Disease	a.Yes	No	Unknown	b. Age diagnosed	c.Left	Right	Both	Unknown	d. Removed by surgery? Yes	No
1. Lobular	1	2	9		1	2	3	9	1	2
proliferation	1	2	9		1	2	3	9	1	2
2.fibroadenoma	1	2	9		1	2	3	9	1	2
3.breast cyst	1	2	9		1	2	3	9	1	2
4.fibrocystic	1	2	9		1	2	3	9	1	2
fibroadenoma	1	2	9		1	2	3	9	1	2
5.breast abscess	1	2	9		1	2	3	9	1	2
6.other	1	2	9		1	2	3	9	1	2
(specify)										

Admission number: _____
Hospital of surgery_____

Admission date: _____year _____month _____day
Discharge date: _____year _____month _____day
Surgery date: _____year _____month _____day

B4. Have you ever had a Mastectomy (excluding the past year)? [__]

1. Yes
2. No (Ask B5)

B41. If yes, specify the reason _____
[__]__]__]

B42. The type of the surgery was:

1. Left breast mastectomy
2. Right breast mastectomy
3. Both breast mastectomy
4. [__]

B5. Has your doctor ever told you that you have a tumor or cancer? (excluding the past year) [__]

1. Yes
2. No (Ask B6)

3. Unknown (Ask B6)

If yes:

B51. Please specify the type and site of the tumor or cancer: _____

[__]__]__]__]

B52. At what age were you first diagnosed? _____years old [__]__]

B6. Do you have any ear wax? [__]

1. Yes
2. No
3. Unknown

B6a. What is your earwax type? [__]

1. Dry and fragile 1
2. Wet, sticky and brown 2
3. Mixed 3
4. Unknown 4

B7. What is your hair type? [__]

1. Oily 1
2. Dry 2
3. Normal 3
4. Unknown 4

B8. Have you ever had fibroids? [__]

1. Yes
2. No
9. Unknown

B81. If yes to B8, what age were you when diagnosed? [__]

B82. How was diagnosis made? [__]

1. Ultrasound
3. Gynecologic physical examination only
4. Both
9. Unknown

B83. Has the fibroid(s) been removed? [__]

1. Yes
2. No
9. Unknown

B84. If fibroids removed, when was surgery? Month [__]__] Year [__]__]

B9. What is your hemoglobin genotype? [__]__] Unknown [__]__]

PART THREE MENSTRUAL AND REPRODUCTIVE HISTORY

C1. Your blood type is _____ C2. Your last period was on: ___ ___ ___ ___ ___ ___
year month day

The following are some questions related to your menstrual, pregnancy and childbirth history.. With the information that you provide, we will be able to know more about the relationship between the reproductive characteristics and women's health.

C3. At what age did you have your first period (menarche)? _____ years old [__]__]
Have you ever had a period ……..96 (Ask C4)
Unknown……99

C4. Have you had regular periods for most of your life (excluding times of pregnancy, breast feeding, or contraceptive use)?
1. Yes
2. No
3. Sometimes [__]

C5. Excluding the time when you used / were using contraceptives, what is the length of your usual menstrual cycle (i.e. from the first day of one period to the first day of the next period)?

C51. From: _____ days C52. To: _____days [__]__] – [__]__]

C6. Have you ever had an abdominal surgery?

Type of surgery	Yes	No	Unknown	Reason for the surgery	Year of the surgery
1. hysterectomy	1	2	8		[__]__]__]__]
2. overiectomy (one side)	1	2	8		[__]__]__]__]
3. overiectomy (both sides)	1	2	8		[__]__]__]__]
4 .fallopian tube ligation	1	2	8		[__]__]__]__]
5. others	1	2	8		[__]__]__]__]

C7. Have you ever had any period in the past 12 months (do not include the periods caused by using female hormones)?
1. Yes (Ask C8)
2. No

C71. If no, what was the date of your last period? _____year _____month [__]__]__]__]
C72. If the last period was 12 months or earlier, the reason was:
Normal menopause 1 [__]
Surgery (hysterectomy/ovariectomy) 2
Breast feeding 3
Others (please specify) _____ 6

C8. How many times have you ever been pregnant (including live births, stillbirths, miscarriages, abortions, tubal or other ectopic pregnancies, and current pregnancy)?
Number of pregnancies _____ [__]__]
Never been pregnant 96

C9. If you have never been pregnant, the reason is: [__]
1. Never married (Ask Part 4)
2. Other reason: Explain _____
9. Unknown

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C10. Next, would you please tell me the ending date and the outcome of each of your pregnancies in sequence?

Pregnancy result	coded number	Pregnancy result	coded number
Live birth	1	Salpingocyesis or other ectopic pregnancies	5
Abortion	2	Being pregnant at present	6
Miscarriage	3	Others (please specify)	7
Stillbirth	4		

This form is very important, so please ask accurately.

Pregnancy ending date	Pregnancy outcome	Total weeks of pregnancy	If livebirth, breast-fed or not? Yes / No	If breast fed, how many months	Side of breast usually used: Left ↓ / Right / Both / Unknown ↓	Why didn't breast feed? 1.No milk 2.Disease 3.Unwilling 6.Other
1.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
2.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
3.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
4.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
5.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
6.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
7.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
8.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
9.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
10.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
11.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6
12.	[__]	[__]__]	1 2	[__]__]	1 2 3 8	1 2 3 6

Note: If you are pregnant at present or breast feeding, please fill in how many months you have been pregnant or breast feeding.

PART FOUR ORAL CONTRACEPTIVE AND HORMONE USE

D1. Have you ever taken oral contraceptives?

1. Yes
2. No (Ask D6)

Next, I will ask a few questions on all of the oral contraceptives that you have ever taken. (Please treat the different regiments as different drugs. If you stopped taking any oral contraceptives and then resumed, ask questions D1-D2 again)

~~D2. Name of the oral contraceptive~~	~~D3.~~ Age of first use	~~D4. Duration of use~~	~~D5. Reason for stopping use~~
~~1.~~_____[__]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~2.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~3.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~4.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~5.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~6.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~7.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~8.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~9.~~ .~~_____~~[- __]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~10.~~ ~~_____~~[__]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____

D^. Have you ever received contraceptive injections? [__]

1. Yes
2. No (Ask D11)

Please specify the following items. (If you stopped use and then resumed, please ask questions D7-D10 again.)

~~D7. Name of the shot~~	~~D8. Age of first use~~	~~D9. Duration of use~~	~~D10~~ Reason for stopping use
~~1.~~_____ [__]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~2.~~ .~~_____~~[__]__]	[__]__]	~~Month 1~~ Year 2 _____[__]__]__]	_____
~~3.~~ .~~_____~~[__]__]	[__]__]	~~Month 1~~	_____

		Year 2 _____[__]__]__]	
4. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
5. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
6. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____

D11. Have you ever used female hormones (oral, shots, or any other types) to alleviate menopause symptoms?

1. Yes [__]
2. No (Ask D16)

D12. Name of the drug	D13. Age of first use	D14. Duration of use	D15. Reason for stopping use
1._____ [__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
2. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
3. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
4. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
5. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____
6. ._____[__]__]	[__]__]	Month 1 Year 2 _____[__]__]__]	_____

D16. Have you ever used any hormones (oral or shots) over a long period (at least a month) because of other reasons: such as to treat acne, sterility, mulleriosis, etc.

1. Yes [__]
2. No
9. Unknown

If yes, specify:

D17. Name of the drug	D18. The reason for use	D19. Age of use	D20. For how long did you use hormones?
1._____ [__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]
2. ._____[__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]
3. ._____[__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]
4. ._____[__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]
5. ._____[__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]
6. ._____[__]__]	_____	[__]__]	Month 1 Year 2 _____[__]__]__]

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PART FOUR PHYSICAL ACTIVITY

Now, I would like to ask you some questions about your physical activity at different times. Please repeat the questions E1-E5 for different ages.

E. Age ranges	What 5 physical Activities do you Participate in Most often (trek To the market; work on farm; go to gyms; play sports like football, etc.)?	How many minutes per day or week did you do this activity?	How many years did you participate in this activity?	Compared with other women Of your age, the time you Spent on this activity was? 1. more than average 2. a little more than average 3. about average 4. a little less than average 5. less than average 9. unknown	When you did perform These activities, you 1. sweated every time 2. sweated most of the time 3. normally did not sweat 8. never did Any of these activities
E1. Age <13	E11. 1. _____[__]__] 2. _____[__]__] 3. _____[__]__] 4. _____[__]__] 5 _____[__]__]	E12. Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__]	E13 [__]__] [__]__] [__]__] [__]__] __]__]	E14. [__] [__] [__] [__] [__]	E15 [__] [__] [__] [__] [__].
E2. Age 13-19	E21. 1. _____[__]__] 2. _____[__]__] 3. _____[__]__] 4. _____[__]__] 5. _____[__]__]	E22. Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__]	E23. [__]__] [__]__] [__]__] __]__] [__]__]	E24. [__] [__] [__] [__] [__]	E25. [__] [__] [__] [__] [__]
E3. Age 20-49	E31. 1. _____[__]__] 2. _____[__]__] 3. _____[__]__] 4. _____[__]__] 5. _____[__]__]	E32. Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__]	E33. [__]__] [__]__] [__]__] [__]__] [__]__]	E34. [__] [__] [__] [__] [__]	E35. [__] [__] [__] [__] [__]

E4. Age ≥50	E41. 1. _____[__]__] 2. _____[__]__] 3. _____[__]__] 4. _____[__]__] 5. _____[__]__]	E42. Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__]	E43. [__]__] [__]__] [__]__] __]__] [__]__]	E44. [__] [__] [__] [__] [__]	E45. [__] [__] [__] [__] [__]
E5. During the last 10 years	E51. 1. _____[__]__] 2. _____[__]__] 3. _____[__]__] 4. _____[__]__] 5. _____[__]__]	E52. Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__] Day1 _____ Week2 [__]__]	E53. [__]__] [__]__] [__]__] [__]__] [__]__]	E54. [__] [__] [__] [__] [__]	E55. [__] [__] [__] [__] [__]

E6. Please repeat the question for the different ranges of age.

E6. During this period, how many hours did you spend on the following activities?	a. Age 13-19	b.During the last 10 years
E61. Housework	[__]__] hours	[__]__] hours
E62a. Which floor did you live on?	[__]__] floor	[__]__] floor
E62b. Which floor was your office on?	[__]__] floor	[__]__] floor
E63. How many minutes did you walk every day (including walking to work, .shopping, sending or picking up children, etc)?	[__]__]__]minutes	[__]__]__]minutes
E64. How many minutes did you bike every day (including riding to work, .shopping, sending or picking up children, etc)?	[__]__]__]minutes	[__]__]__]minutes
E65a. Did you live next to an electric power plant?	1. Yes 2. No 9. Unknown [__]	1. Yes 2. No 9. Unknown [__]
E65b. How far from the plant to your house or apartment?	[__]__]__]meters	[__]__]__]meters
E65c. How many years did you live there?	[__]__]years	[__]__]years

PART SIX DIETARY HISTORY

Now I would like to ask about your dietary habits over the past 5 years. If there were changes in the past year, please tell me the habits

before the changes. I will first read to you some types of food. Would you please tell me if you ate these foods

and how much you ate

in genera per day, per week or per month over the past 5 years? We know it's hard to estimate the exact amount and the number of times you ate a food, but the estimated amounts generally reflect your dietary intake, and those numbers will be of great help to us.
F.

Cereals, Breads, Snacks

Food	**Not at all** *Never*	*Every Year Rearly*	*Every Month Sometimes*	**Every week** *Often*	*Everyday Almost Always*	*Quantity Portion Size*
1. White Bread, Rolls	1	2	3	4	5	
2. Wheat/Rye Bread	1	2	3	4	5	
3. Bagel	1	2	3	4	5	
4. Dumpling(Flour/Chinese)	1	2	3	4	5	
5.Pancakes	1	2	3	4	5	
6. Pizza.	1	2	3	4	5	
7.Pita, Soft Taco	1	2	3	4	5	
8. Enchilada	1	2	3	4	5	
9. West African bread	1	2	3	4	5	
10. Corn Chips	1	2	3	4	5	
11. Potato Chips	1	2	3	4	5	
12. Plantain ripe fried (dodo)	1	2	3	4	5	
13. Biscuit, crackers	1	2	3	4	5	
14.Porridge (Ogi,Akamu,Okababa)	1	2	3	4	5	
15. Oatmeal	1	2	3	4	5	
16. Others	1	2	3	4	5	

Meat, Fish, Eggs

Food	**Not at all** *Never*	*Every Year Rarely*	*Every Month Sometimes*	**Every week** *Often*	*Everyday Almost Always*	*Quantity Portion Size*
17. Chicken, Hen	1	2	3	4	5	
18. Turkey/ Roast Turkey	1	2	3	4	5	
19. Smoked Poultry	1	2	3	4	5	
20. Duck	1	2	3	4	5	
21. Beef/Steak, Roast beef	1	2	3	4	5	
22. Lamb, Goat	1	2	3	4	5	
23. Pork, Pork Chop, Ham	1	2	3	4	5	
24. Smoked Pork	1	2	3	4	5	
25. Bush meat	1	2	3	4	5	
26. Pig foot, Cow leg, skin	1	2	3	4	5	
27. Kidney, Liver, Gizzard	1	2	3	4	5	
28. Tripe	1	2	3	4	5	

29. Cow/Pig/Goat intestines, tripe	1	2	3	4	5	
30. Oxtail	1	2	3	4	5	
31. Meat balls/ Meatloaf	1	2	3	4	5	
32. Sausage	1	2	3	4	5	
33. Hotdog	1	2	3	4	5	
34. Bacon	1	2	3	4	5	
35. Can Fish. Tuna, Sardine	1	2	3	4	5	
36. Can Salmon (Geisha)	1	2	3	4	5	
37. Dark fish, White fish	1	2	3	4	5	
38.Ice fish, Frozen fish	1	2	3	4	5	
39.Smoked ice fish	1	2	3	4	5	
40. Dry fish	1	2	3	4	5	
41. Stock Fish	1	2	3	4	5	

42. Fresh Salmon	1	2	3	4	5	
43.Shrimp, Lobster	1	2	3	4	5	

Meat, Fish, Eggs

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
44. Large Snail	1	2	3	4	5	
45. Scallop, Oysters	1	2	3	4	5	
46. Periwinkle, little snails	1	2	3	4	5	
47. Fish in bundle	1	2	3	4	5	
48. Grilled fish	1	2	3	4	5	
49. Processed Meats (salted, dried)	1	2	3	4	5	
50. Salted Fish	1	2	3	4	5	
51.Crabs	1	2	3	4	5	
52. Eggs	1	2	3	4	5	
53. Others	1	2	3	4	5	

Mixed Dishes and Soups

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
54. Ogho (Owo) soup	1	2	3	4	5	
55. Mushroom soup	1	2	3	4	5	
56. Fresh fish soup	1	2	3	4	5	
57. Dried fish soup	1	2	3	4	5	
58. Meat soup	1	2	3	4	5	
59. Plain vegetable	1	2	3	4	5	
60. Vegetable soup	1	2	3	4	5	
61. Groundnut soup (Gbegiri)	1	2	3	4	5	
62. Groundnut Tomato	1	2	3	4	5	
63. Groundnut Tomato Vegetable	1	2	3	4	5	
64. Plain Egusi	1	2	3	4	5	
65. Egusi Tomato	1	2	3	4	5	
66. Egusi Vegetable	1	2	3	4	5	
67. Egusi Tomato Vegetable	1	2	3	4	5	
68. Egusi Okra	1	2	3	4	5	
69. Okra soup	1	2	3	4	5	

70. Okra Tomato	1	2	3	4	5	
71. Okra Vegetable	1	2	3	4	5	
72. Okra Tomato Vegetable	1	2	3	4	5	
73. Ogbolo Tomato	1	2	3	4	5	
74. Ogbolo Okra	1	2	3	4	5	
75. Ogbolo Vegetable	1	2	3	4	5	
76. Ewedu Tomato	1	2	3	4	5	
77. Banga (Palm Sauce)	1	2	3	4	5	
78. Banga Tomato	1	2	3	4	5	
79. Banga Egusi	1	2	3	4	5	
80. Ogho (Owo)	1	2	3	4	5	
81. Pepper Soup Tomato	1	2	3	4	5	
82. Pepper Soup Native	1	2	3	4	5	

Other Ingredients Mixed Dishes and Soups

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
83. Pumpkin leaf	1	2	3	4	5	
84. Bitter leaf	1	2	3	4	5	
85. Water leaf	1	2	3	4	5	

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86. Red Leaf	1	2	3	4	5	
87. Coco Yam Leaf	1	2	3	4	5	
88. Sweet Potato Leaf	1	2	3	4	5	
89. Igbure	1	2	3	4	5	
90. Efo soko	1	2	3	4	5	
91. Efo riro						
92. Efo tete	1	2	3	4	5	
93. Mustard	1	2	3	4	5	
94. Baobab	1	2	3	4	5	
96. Groundnut oil	1	2	3	4	5	
96. Palm Oil	1	2	3	4	5	
97.Others	1	2	3	4	5	

Dairy Products

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
98. Milk	1	2	3	4	5	
99. Chocolate Milk	1	2	3	4	5	
100. Ice cream	1	2	3	4	5	
101. Cheese	1	2	3	4	5	
102. Butter	1	2	3	4	5	
103. Yoghurt	1	2	3	4	5	
104. Others	1	2	3	4	5	

Vegetables and Grains

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
105. Corn (boiled)	1	2	3	4	5	
106. Corn (grilled)	1	2	3	4	5	

107. Okra (raw)	1	2	3	4	5	
108. Okra (cooked)	1	2	3	4	5	
109. Green Beans	1	2	3	4	5	
110. Green Peas	1	2	3	4	5	
111. Casava leaves	1	2	3	4	5	
112. Potato	1	2	3	4	5	
113. Moimoi (steamed bean cake)	1	2	3	4	5	
114. Ekuru	1	2	3	4	5	
115. Akara (frien bean cake)	1	2	3	4	5	
116. Foo foo corn	1	2	3	4	5	
117. Tomatoes	1	2	3	4	5	
118. Gari (Eba)	1	2	3	4	5	
119. Amala 9Yam flour)	1	2	3	4	5	
120. Elubo lafu	1	2	3	4	5	
121. Kpokpo gari	1	2	3	4	5	
122. Cassava/Yuka boiled	1	2	3	4	5	
123. Fufu	1	2	3	4	5	
124. Starch Cassava/Potato	1	2	3	4	5	
125. Banku	1	2	3	4	5	
126. Ghana fufu (plantain/cassava)	1	2	3	4	5	
127. Semolina	1	2	3	4	5	
128. Cocoyam/Taro Malanga	1	2	3	4	5	
129. Carrots	1	2	3	4	5	
130. Celery	1	2	3	4	5	
131. Onions (red)	1	2	3	4	5	
132. Onions (green)	1	2	3	4	5	
133. Sweet potato	1	2	3	4	5	
134. Ginger	1	2	3	4	5	

135. Lettuce						
136. Cabbage	1	2	3	4	5	
137. Others	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	

Fruits

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
138. Apples	1	2	3	4	5	
139. Pears	1	2	3	4	5	
140. Apricots	1	2	3	4	5	
141. Peaches	1	2	3	4	5	
142. Nectarines	1	2	3	4	5	
143. Plums	1	2	3	4	5	
144. Bananas	1	2	3	4	5	
146. Kiwi	1	2	3	4	5	
147. Guava	1	2	3	4	5	
148. Avacado Pear	1	2	3	4	5	
149. Little pear	1	2	3	4	5	
150. Cantelope	1	2	3	4	5	
151. Orange Melon	1	2	3	4	5	
152. Honey Dew	1	2	3	4	5	
153. Papaya	1	2	3	4	5	

154. Mango	1	2	3	4	5	
155. Ogbolo fruit	1	2	3	4	5	
156. Strawberries	1	2	3	4	5	
157. Grapes/Berries	1	2	3	4	5	
158. Cherry	1	2	3	4	5	
159. Agbalumo	1	2	3	4	5	
160. Awin	1	2	3	4	5	
161. Wallnut (Awusa 0	1	2	3	4	5	
162. Groundnuts	1	2	3	4	5	
163. Lemon	1	2	3	4	5	
164. Watermelon	1	2	3	4	5	
165. Red melon	1	2	3	4	5	
166. Pinapple fresh	1	2	3	4	5	
167. Canned fruits	1	2	3	4	5	
168. Applesauce	1	2	3	4	5	
169. Others	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	

Spices, Sauces and Condiments

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
170. Pepper	1	2	3	4	5	
171. Chili/Hot	1	2	3	4	5	
172. Salt	1	2	3	4	5	
173. Vitamins and Supplements	1	2	3	4	5	
174. Others	1	2	3	4	5	
175. Honey	1	2	3	4	5	
176. Syrup	1	2	3	4	5	
177. Brown Sugar	1	2	3	4	5	

178. Artificial Sweetners	1	2	3	4	5	
179. White sugar	1	2	3	4	5	
180. Iru	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	
	1	2	3	4	5	

Beverages, Alcohol, etc.

Food	**Not at all** *Never*	***Every Year*** *Rarely*	***Every Month*** *Sometimes*	**Every week** *Often*	***Everyday*** *Almost Always*	***Quantity*** *Portion Size*
	1	2	3	4	5	
1	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	

1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	
1.	1	2	3	4	5	

FF1a. When you eat fried or baked pork chops, pork ribs or steaks, you normally prefer that:

Entire surface is brown with a slight burnt flavor	1	[__]
Majority of the surface is brown	2	
Little surface is brown	3	
Surface is not brown, bone still has bloody color	4	
Never eat	6	
Don't Know	9	

FF1b. When you eat fried or baked pork or beef, you normally prefer that:

Entire surface is brown with a slight burnt flavor	1	[__]
Majority of the surface is brown	2	
Little surface is brown	3	
Surface is not brown, bone still has bloody color	4	
Never eat	6	
Don't Know	9	

FF2. When you eat fried or baked chicken, duck, or goose, you normally prefer that:

Entire surface is brown with a slight burnt flavor	1	[__]
Majority of the surface is brown	2	
Little surface is brown	3	
Surface is not brown, bone still has bloody color	4	
Never eat	6	
Don't Know	9	

FF3. When you eat fried or baked fish, you normally prefer that:

Entire surface is brown with a slight burnt flavor	1	[__]
Majority of the surface is brown	2	
Little surface is brown	3	
Surface is not brown, bone still has bloody color	4	
Never eat	6	
Don't Know	8	

FF4. Over the past 5 years, approximately how many times did you eat fresh vegetables (any kind)?

1.....Per day

2.....Per week _____times [__]__]__]

FF5. Over the past 5 years, how many times have you eaten fresh fruits (any kind; per day, per week or per month?

1.....Day
2.....Week _____times [__]__]__]
3.....Month

FF6. How much of the following oils did your family consume per month (50g)?

1. Vegetable oil: _____ (50g) [__]__]__]
2. Soy bean oil: _____ (50g) [__]__]__]
3. Groundnut oil: _____ (50g) [__]__]__]
4. Lard: _____ (50g) [__]__]__]
4. Brown (bleached) sugar: _____ (50g) [__]__]__]

FF7. How many people live in your household, including yourself?

_____persons [__]__]

FF8. Do you often store foods in the refrigerator? [__]

Yes.....1
No.....2 (Ask F34)

FF9. In which year did you first buy a refrigerator? _____ [__]__]

FF9a. How many years have you used a refrigerator? _____ [__]__]

FF10. In the past 5 years, have you ever taken continuously the following vitamins at least three times a week for two months
(If there were changes in the past year, please refer to the use before the changes)?

~~Name FF10...~~	~~Vitamin A~~	~~Vitamin C~~	~~Vitamin E~~	~~Vitamin B~~	~~Multiple Vitamins~~	~~Others (specify)~~
1. Ever took?	Yes 1 No 2 [___]	Yes 1 No 2 [___]	Yes 1 No 2 [___]	Yes 1 No 2 [___]	Yes 1 No 2 [___]	Yes 1 No 2 [___]
~~2.How many~~ months totally did you take the vitamins?	[__]__]__] month(s)	[__]__]__] month(s)	[__]__]__] month(s)	[__]__]__] month(s)	[__]__]__] month(s)	[__]__]__] month(s)
~~3. When you~~ took vitamins, how many times per week did you take them?	[__]__]__] day(s)	[__]__]__] day(s)	[__]__]__] day(s)	[__]__]__] day(s)	[__]__]__] day(s)	[__]__]__] day(s)

FF11. When you were sick, did you often take or receive vitamins?

Yes	1	[__]
No	2	
Unknown	9	

PART SEVEN PERSONAL HABITS AND LIFESTYLE

Now I would like to ask a few questions about your smoking habits.

G1. Have you ever smoked at least one cigarette per day, for more than 6 months, consecutively? [__]

Yes.....1
No.....2 (AskG7)

G2 At what age did you start to smoke at least one cigarette per day? [__]__]

_____years old

G3 Do you smoke regularly now? [__]

Yes.....1
No.....2 (AskG5)

G4. How old were you when you quit smoking? _____ years old [__]__]

G5. How many years in total did you smoke or how many years have you smoked so far? (Excluding the years when you did not smoke) _____ years

[__]__]

G6. When you smoked, on average, how many cigarettes did you smoke per day?

[__]__]__]

_____cigarettes/day

G7. Did you ever smoke other kinds of tobacco products? [__]

Yes.....1
No.....2

G8. Did you ever drink alcohol, such as beer, fruit wine, palm wine, liquor, etc., at least one time per week for more than six months continuously?

Yes.....1 [__]
No.....2 (Ask G14)

G9. How old were you when you started to drink alcohol regularly? _____years old [__]__]

G10. Do you still drink alcohol regularly? [__]

Yes.....1 (Ask G11)
No.....2

G10a How old were you when you stopped drinking alcohol regularly? [__]__]

_____years old

G11. When you drank one of the following alcoholic beverage, how much did you drink per time? [__]

A.	Liquor	_____ cup or none (96)	[__]__]
B.	Illicit gin	_____ cup or none (96)	[__]__]
C.	Wine	_____ cup or none (96)	[__]__]
D.	Beer	_____ cup or none (96)	[__]__]
E.	Palm wine	_____ cup or none (96)	[__]__]

G13. How many years in total have you drunk or how many years have you drunk frequently? (Excluding the years when you did not drink) _____years
[__]__]

G14. Did you ever drink tea regularly (change the tea leaves at least twice a week for more than 3 months continuously)?
Yes.....1 [__]
No.....2 (Ask G21)

VN001 _ _ _

G15. At what age did you start to drink tea regularly? _____years old [__]__]

G16. How many years in total have you drunk tea so far (excluding the years when you did not drink)?
_____ years [__]__]

G17. What kinds of tea did you normally drink? [__]
Green tea.....1
Black tea.....2
Other kinds (please specify).....6

G18. Did you ever drink coffee regularly? [__]
Yes.....1
No.....2 (Ask G21)

G19. At what age did you start to drink coffee regularly? _____years old [__]__]

G20. How many years in total have you drunk tea so far (excluding the years when you did not drink)? [__]__]
_____ years

G21. What kinds of coffee did you normally drink?
Instant .....1 [__[
Brewed 2
Other kinds (please specify).....6

G22.Did you ever eat kolanut regularly? [__]
Yes.....1
No.....2 (Ask G23)

G23. At what age did you start to eat kolanut regularly? _____years old [__]__]

G24. How many years in total have you eaten kolanut so far? [__]__]
_____years

G25. What kinds of kolanut did you normally eat? [__]
Obi (hausa type) 1
gbanja..... 2
Other kinds (please specify).....6

G26. Did you ever eat orogbo regularly? [__]
Yes.....
No.....2 (Ask G27)

G27. At what age did you start to eat orogbo regularly? _____years old [__]__]

G28. How many years in total have you eaten orogbo so far? [__]__]
_____ years

G29. Did you ever use snuff regularly? [__]

Yes.....

No.....2 (Ask G27)

G30. At what age did you start to use snuff regularly? _____years old [__]__]

G31. How many years in total have you used snuff so far? [__]__]

_____ years

G32. Next, I would like to ask you a few questions on the household use of electric appliances in the past 5 years.

Name of the appliance	A. Ever used? Yes No Occasionally	B. How many hours did you use it per day or week? day week month	kitchen living room hall bedroom other (specify)
1. refrigerator	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
2. TV set	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
3. stereo system	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
4. microwave oven	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
5. electric rice cooker	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
6. washing machine	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____
7. hair dryer	1 2 3	1 2 3 [__] [__]__] [__]__] min	1 2 3 4 6 [__] _____

PART EIGHT EMPLOYMENT HISTORY

I would like to ask about all jobs you have ever had which have lasted longer than three years, throughout your lifetime(including the jobs of farming, the jobs you had after retirement). Different positions and different responsibilities should be considered as different types of jobs. Military service should also be considered as a job.

~~H1-H6 must be repeated for each job.~~

	~~(01) the most~~ recent job	~~(02) the job that~~ Proceeds the one on the left	~~(03) the job that~~ Proceeds the one on the left	~~(04) the job that~~ Proceeds the one on the left
~~H1. Name of the job.~~ (starting with the most recent one)	[__]__]__]	[__]__]__]	[__]__]__]	[__]__]__]
~~H2. Name of the~~ work place				
~~H3. In which year did you~~ start the job	~~19~~ [__]__]	~~19~~ [__]__]	~~19~~ [__]__]	~~19~~ [__]__]
~~H4. In which year did you~~ end the job	~~19~~ [__]__]	~~19~~ [__]__]	~~19~~ [__]__]	~~19~~ [__]__]
~~H5. About how many~~ hours did you stand or walk per day at this job?	___, ___hours [__],[__]	___, ___hours [__],[__]	___, ___hours [__],[__]	___, ___hours [__],[__]
~~H6. Your job can be~~ Classified as:	~~Heavy physical~~ work……1 Medium physical work……2 Light physical work……3 Non-physical work……4	~~Heavy physical~~ work……1 Medium physical work……2 Light physical work……3 Non-physical work……4	~~Heavy physical~~ work……1 Medium physical work……2 Light physical work……3 Non-physical work……4	~~Heavy physical~~ work……1 Medium physical work……2 Light physical work……3 Non-physical work……4

H7. Did you ever go to the countryside and work on a farm?

Yes……1
No……2 (Ask Part Nine)

H71. Where was the farm? _____
]__]__]__]__]

H72. What was your major responsibility? _____
[__]__]__]

H8. Were you ever exposed to pesticides or herbicides at your work or in daily life?
(refer to at least ten days a year)

Yes……1
No……2 (Ask Part Nine)

H9. In which year were you first exposed to pesticides or herbicides? 19[__]__]

H10. How many years in total were you exposed to pesticides or herbicides? [__]__]

H11. Usually how many days/months per year were you exposed to pesticides or herbicides?

Day 1
Week 2
]__]__]__]__]
Month 3

H12. Please tell me the names of the pesticides that you were ever exposed to. _____ [__]__]
_____ [__]__]
_____ [__]__]

PART NINE FAMILIAL CANCER HISTORY

VN001 _ _ _

Next I would like to ask a few questions about your first-degree relatives, which includes your parents, your brothers and sisters, and your children.

111. How many daughters do you have? _______ daughters [__]__]
112. How many sons do you have? _______ sons [__]__]
113. How many sisters do you have (you are not included)? _________ [__]__]
114. How many brothers do you have (you are not included)? _________ [__]__]

12. Among your blood related relatives including your parents, sisters and brothers and your children, has anybody ever been diagnosed with a cancer or a malignancy?

Yes..................... 1
No.......................2 (Ask Part Ten)
Unknown............3 (Ask Part Ten)

13. Which relative? Initials Relationship	14. Which type of cancer has she/he had?	15. How old was he/she when he/she was diagnosed?	16. Birth date	17. Is he/she still living? 1. Yes 2. No Date of Birth
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]
1.______ __________ [__]__]	____________ [__]__]__]	[__]__]	[__]__]	1. Yes 2. No [__]__]

PART TEN PHYSICAL DEVELOPMENT AND BODY MEASUREMENT

Next I would like to ask a few questions on your weight and height:

Compared to your peers, what was your height and weight during childhood and adolescence?

Period	J1. Height 1. Shorter 2. A little shorter 3. Average 4. A little taller 5. Taller 5. Unknown	J2. Weight 1. Shorter 2. A little shorter 3. Average 4. A little taller 5. Taller 5. Unknown
At about 10 years old	[__]	[__]
At about 15 years old	[__]	[__]
At about 20 years old	[__]	[__]

J3. What was your height when you were 20 years old? _____cm.

What was your weight during the following time periods?

Period	J4. Weight
Around age 20	[__]__]__]
Around age 30	[__]__]__]
Around age 40	[__]__]__]
Around age 50	[__]__]__]
Around age 60	[__]__]__]
In the past year	[__]__]__]

PART ELEVEN ANTHROPOMETRIC MEASUREMENT

K1. This part is to measure the height, weight, waistline and hipline of the interviewees. To insure the accuracy of the measurements, the interviewees are required to wear only one layer of clothes. If this is refuse, estimate the actual values and record the clothes of the interviewees wore in the remark column.

Remarks: Measure each participant at least twice? If the difference between the first two measurements exceeds the tolerated difference, please take a third measurement.

Instruction for anthropometric measurements:

1. Waist circumference: Let the participant stand straight, breath evenly; wear a single layer of clothes, and no belt or tight pants. Put a cloth tape horizontally 1 (one) inch above the navel, read the number to nearest one decimal point.

2. Hip circumference: Let the participant stand straight, breath evenly; wear a single layer of clothes, and no belt or tight pants. Put a cloth tape horizontally between the waist and leg and move the tape up and down between the waist and leg. Take the measurement at the level of maximum extension of the buttocks, read the number to nearest one decimal point.

3. Sitting height: Let the participant sit straight on a chair with a flat surface, keeping the head and shoulders in a horizontal position, and measure the distance between the top of the head and the surface of the chair.

Finally, to end the interview session, can you please answer the question below? Thanks.

L1. Did you find any of the questions embarrassing?

1. Yes [__]
2. No
3. Sometimes

PART TWELVE INTERVIEWER POSTSCRIPT

M1. How did the participants cooperate?

Very well	1	[__]
Well	2	
Average	3	
Bad	4	

M2. The accuracy of the responses:

Very accurate	1	[__]
Accurate	2	
Some parts inaccurate	3	
Very inaccurate	4	
Hard to evaluate	5	

M3. The evaluation of the quality of the whole interview:

High	1	[__]
Generally reliable	2	
Not very reliable	3	
Unsatisfactory	4	

M4. Which parts are not reliable?

	Reliable	Generally reliable	Not reliable	
Part one	1	2	3	[__]
Part two	1	2	3	[__]
Part three	1	2	3	[__]
Part four	1	2	3	[__]
Part five	1	2	3	[__]
Part six	1	2	3	[__]
Part seven	1	2	3	[__]

M5. Interview site

Hospital	1	[__]
Working place	2	
Participant's home	3	
Others	4	

M6. Participant's signature: _____

M7. The time when the interview ended: morning/afternoon _____ minutes past _____

M8. How long did the interview last? _____minutes

Breast Cancer in African Women Study (BCAWS) Storage Inventory

Study Site_____ Specimen Type _____
Box Number_____ Approx. Vol._____
Date_____

November 13, 2006

Proposal to CRC: Gene-gene and gene-environment interactions as risk factors for breast cancer in women of diverse genetic backgrounds.

Principal Investigator: Dana R Marshall, Ph.D. Associate Professor, Department of Surgery, x6549, dmarshall@mmc.edu
Co-investigator: Regina S. Offodile, M.D. Assistant Professor, Department of Surgery, x5673, roffodile@mmc.edu

Background:

African American women have a lower incidence of breast cancer than Caucasian American women yet they are more likely to die from their disease (1). When breast cancer is divided into pre- and post-menopausal disease, the incidence is actually higher in African American women and it is here that the bulk of the disparity in mortality is accounted for (2). The incidence of breast cancer in Subsaharan Africa has been increasing over the last three decades and despite the differences in many environmental risk factors between these two demographic groups, there are strong correlations between many of the cellular and molecular aspects of the disease that distinguish African and African American women, as a single group, from Caucasian women (3,4). Our **hypothesis** is that there is an underlying genetic component that explains why African women, and women of African descent, are more likely to present with high grade, late stage, hormone receptor negative tumors that have already metastasized.

A pilot study addressing the increasing breast cancer incidence in African women (Breast Cancer in African Women Study or BCAWS) was initiated by Dr. Olufemi Adegoke, a Meharry Medical College (MMC) faculty member, in 2002. Dr. Adegoke had a stroke in early 2004 and was not able to return to work. Despite this tragic event, the collaborators in Africa, and faculty and staff at MMC, continued to work towards acquiring and shipping blood, buccal and urine samples from Nigerian and Kenyan women to MMC. We currently have 78 case, and 40 control samples stored here that have not been analyzed. Additionally, the Kenya site just informed me that they have 45 additional samples to ship to us. The goal for this pilot study is 100 cases and 100 controls from Nigeria and Kenya. The Africa sites have IRBs in place for collecting samples. I am preparing one for us as this was to be put in place once there were samples here to work on.

The MetroGeneral Breast Health Clinic patients are a new, and important, addition to this study in which we are addressing ethnic and racial disparities in breast cancer mortality. Dr. Offodile will direct the acquisition of samples from women who visit the clinic. She has developed a human subjects protocol for consenting patients and an environmental risk survey. A realistic goal for recruiting from the breast health clinic is 30 cases and 30 controls over a one year period.

Our objectives in this project are:

(1) Analyze pre-existing lifestyle and behavioral risk surveys from the BCAWS study.
(2) Genotype samples from the BCAWS study for prevalence of risk polymorphisms.
(3) Analyze lifestyle and behavioral risk surveys from MetroGeneral Breast Health Clinic study.
(4) Genotype samples from the MetroGeneral Breast Health Clinic study for prevalence of risk polymorphisms.
(5) Co-analyze genotype data from all cases vs all controls for further clarification of trends that are specific for African women or women of African descent relative to what is reported in the literature for other races or ethnicities.