

AD _____

Award Number: W81XWH-04-1-0575

TITLE: Nutritional and Genetic Determinants of Early Puberty

PRINCIPAL INVESTIGATOR: Loic LeMarchand, M.D.

CONTRACTING ORGANIZATION: University of Hawaii
Honolulu, HI 96822

REPORT DATE: June 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20050916 137

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY		2. REPORT DATE June 2005	3. REPORT TYPE AND DATES COVERED Annual (15 May 2004 - 14 May 2005)	
4. TITLE AND SUBTITLE Nutritional and Genetic Determinants of Early Puberty			5. FUNDING NUMBERS W81XWH-04-1-0575	
6. AUTHOR(S) Loic LeMarchand, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Hawaii Honolulu, HI 96822 E-Mail: loic@crch.hawaii.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Past studies suggest that early menarche, growth velocity, and specific hormonal patterns during breast development may be critical in determining risk of breast cancer later in life. Nutritional factors during childhood and puberty, and inherited genetic factors are suspected to interact in modulating these early-life exposures. However, the biological processes involved remain poorly understood. We propose to test the relationships between nutrition, genetic factors, hormonal levels and early life events contributing to breast cancer risk in a unique cohort of 323 adolescent girls of Caucasian or Asian ancestry originally recruited for the Female Adolescent Maturation (FAM) Study. These girls were studied twice, 2 years apart, for dietary intake, body size and composition, sexual maturation, growth and bone density. Data collection will be extended by conducting a third examination and obtaining blood samples for DNA genotyping and hormone analysis. A cross-sectional sample of 140 additional girls will also be recruited. Most of the first year was devoted to developing protocols and study instruments. The study received all IRB approvals, including from the DOD Human Subjects Review Board in early December 2004. Recruitment started on 2/4/05. To date, 10 new girls from Kaiser, and 11 girls from the cohort have been recruited. Six girls have completed their 2 visits at Kapiolani Clinical Research Center and have blood and urine collection appointments scheduled. Data entry and analysis have not begun.				
14. SUBJECT TERMS Molecular epidemiology, nutrition, genetics, hormones				15. NUMBER OF PAGES 8
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

COVER.....	1
SF 298.....	2
Introduction.....	4
BODY.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	6
References.....	6
Appendices.....	7

INTRODUCTION

Past studies suggest that early menarche, growth velocity, and specific hormonal patterns during breast development may be critical in determining risk of breast cancer later in life (1-5). Nutritional factors (i.e., adiposity, physical activity and diet) during childhood and puberty, and inherited genetic factors are suspected to interact in modulating these early-life exposures (2, 6-15). However, the biological processes involved remain poorly understood. These gene-environment interactions may explain the remarkable increase in breast cancer incidence observed among US-born Asian women. We propose to test the relationships between nutrition, genetic factors, hormonal levels and early life events contributing to breast cancer risk in a unique cohort of 323 adolescent girls of Caucasian or Asian ancestry originally recruited for the Female Adolescent Maturation (FAM) Study (USDA NRI grant 99-00700). These girls were first studied in 1999-2000 at age 9-14 years and, again two years later in 2002-2003, for dietary intake, body size and composition, sexual maturation (Tanner staging), growth and bone density. Data collection will be extended by conducting a third examination and obtaining blood samples for DNA genotyping and hormone analysis at a time when most girls will have attained menarche. A cross-sectional sample of 100 additional girls will also be recruited.

BODY

Below are the research accomplishments as outlined in the approved Statement of Work through May 14, 2005.

Task 1. Plan Study and Procedures.

- a. Purchase supplies and equipment
 - a. Supplies for recruitment and visits, including gift certificates, copying of questionnaires, pregnancy test kits and mailing supplies have been purchased. Lab supplies for blood and urine collection have also been purchased.
- b. Create tracking database
 - a. A central database for recruitment and study visits has been created and maintained by the Lead Study Coordinator at the University of Hawaii. Study staff at Kapiolani Clinical Research Center and Cancer Research Center Hawaii tracks the portion of their study procedures, and send them to the Lead Study Coordinator monthly.
 - b. Information in the database include participants' first and last name, parents' first and last name, participants' birthdates, study ID number, response to the recruitment letter, study visits dates and dates of blood and urine collection.
- c. Wrote procedure manuals and finalize instruments
 - a. A study procedure manual has been completed and signed by the Principal Investigators. Study questionnaires and forms have been finalized.
- d. Train interviewer and Clinical Research Center technical staff
Training sessions have been held by the Lead Study Coordinator to train Clinical Research Center staff on:
 - a. Diet record collection. Two training sessions have been held to train interviewers on diet record data collection. They were trained to help participants estimate portion sizes in standard measurements as well as asking questions on cooking methods, types of foods (e.g. calcium fortified orange juice vs. regular orange juice etc.).
 - b. Anthropometry. Five training sessions have been held to train new staff on anthropometric measurement, and standardization between the new staff and the Clinical Research Center study coordinator is currently ongoing.
 - c. Consent/assent/questionnaires collection. The consenting process, study flow and standard probes have been established.

- e. Update subject addresses using available sources
 - a. Participant databases have been updated with the Kaiser database for addresses, and there has not been a need to access public data sources.
- f. Obtain Institutional Review Board approvals
 - a. IRB approvals have been obtained from two local IRBs (Hawaii Pacific Health Institute and Kaiser Permanente Health Institute) and from the Department of Defense Human Subjects Review Board.
- g. Identify potential subjects for the new recruitment
 - a. Kaiser has identified potential subjects from their database for potential recruitment. Members were identified based on ethnicity, age, gender, and living in the Honolulu area.

Task 2. Subject Recruitment and Data Collection

- a. Enroll FAM participants for the follow-up examination, 4 years after the baseline examination.
 - a. Recruitment letters have been sent to FAM participants for follow-up visits. Letters were first sent to the oldest girls, before they potentially leave Hawaii for college, and recruitment letters are now sent in the chronological order of their FAM 1 visit. To date, 56 letters have been sent out.
- b. Enroll new participants
 - a. Kaiser has started sending letters to potential new recruits, and the Kaiser Study Coordinator has been calling the girls who indicated interest in order to screen them for eligibility. To date, 10 new girls have been recruited.
- c. Schedule clinical exam and perform data collection
 - a. Five girls have completed visits 1 and 2
 - b. Two girls have completed visit 1 only and are scheduled for visit 2
 - c. Seven girls have appointments scheduled.
- d. Collect blood and urine samples
 - a. Three girls have completed a blood draw and two of these girls have completed 2 overnight urine collections. Four other girls who have completed the clinical study visits at Kapiolani have been scheduled for a blood and urine collection.
- e. Process biospecimen for storage
 - a. Three blood collections have been processed for storage.
 - b. Five urine samples have been processed for storage.

KEY RESEARCH ACCOMPLISHMENTS

Preliminary results from a feasibility study on 158 of the girls examined in 2002-2003 who donated a mouthwash sample show a direct association between the *CYP3A4 *1B* polymorphism with pubic hair Tanner stage ($p=0.01$), but no association of this variant with breast Tanner stage or age at menarche. This association is consistent with the role of CYP3A4 in the catabolism of testosterone and the involvement of this hormone in the early stages of puberty.

REPORTABLE OUTCOMES

An abstract was submitted to the Era of Hope 2005 Conference (see appendix) to be held in Philadelphia June 8-11. This abstract was accepted for both oral and poster presentations.

CONCLUSIONS

Further data should provide critical evidence in support of the causal involvement of specific biological pathways in early maturation and may reveal possible means for the early prevention of breast cancer through lifestyle modification.

REFERENCES

1. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. *Cancer Res* 1982;42:3232-9.
2. Bernstein L, Ross RK. Endogenous hormones and breast cancer risk. *Epidemiol Rev* 1993;15:48-65.
3. Henderson BE, Pike MC, Bernstein L, Ross RK. Breast cancer. In: Schottenfeld D, Fraumeni JFJ, eds. *Cancer epidemiology and prevention*. 2nd ed. New York: Oxford University Press, 1996:1022-39.
4. MacMahon B, Cole P, Brown JP, et al. Urine estrogen profiles of Asian and North American women. *Int J Cancer* 1974;14:161-7.
5. Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. *Cancer Causes Control* 1990;1:51-8.
6. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* 2000;92:1472-89.
7. Fischbein S. Onset of puberty in MX and DZ twins. *Acta Genet Med Gemellol* 1977;26:151-158.
8. Trelour SA, Martin NG. Age at menarche as a fitness trait: non additive genetic variance detected in a large twin sample. *Am J Human Genetics* 1990;4:137-148.
9. Feigelson HS, Coetzee GA, Kolonel LN, et al. A polymorphism in the CYP17 gene increases the risk of breast cancer. *Cancer Res* 1997;57:1036-65.
10. Kitagawa I, Kitagawa Y, Kawase Y, Nagaya T, Tokudome S. Advanced onset of menarche and higher bone mineral density depending on vitamin D receptor gene polymorphism. *Europ J Endocrinol* 1998;139:522-527.
11. Comings DE, Gade R, Muhleman D, Peters WR, MacMurray JP. The LEP gene and age at menarche: maternal age as a potential cause of hidden stratification in association studies. *Mol Genet Metab* 2001;73:204-10.
12. Stravou I, Zois C, Ioannidis JP, Tsatsoulis A. Association of polymorphisms of the estrogen receptor alpha gene with the age of menarche. *Hum Reprod* 2002;17:1101-5.
13. Lorentzon M, Lerentzon R, Nordstrom P. Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy Caucasian men: a cross-sectional and longitudinal study. *J Clin Endocrinol Metab* 2000;85:1666-71.
14. Raivio T, Huhtaniemi I, Anttila R, Siimes MA, Hagenas L, Nilsson C, Petterson K, Dunkel L. The role of luteinizing hormone-beta gene polymorphism in the onset and progression of puberty in healthy boys. *J Clin Endocrinol Metab* 1996;81:3278-82.
15. Kadlubar FF, Berkowitz GS, Delongchamp RR, Wang C, Green BL, Tang G, Lamba J, Schuetz E, Wolff MS. The *CYP3A4*1B* variant is related to the onset of puberty, a known risk factor for the development of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:327-31.

APPENDICES

1. Loïc Le Marchand, Rachel Novotny, John S. Grove, Rudolf Kaaks, Yihe G. Daida, Vinutha Viyajadeva. **Nutritional And Genetic Determinants Of Early Puberty.** Era of Hope 2005 Department of Defense Breast Cancer Research Program Meeting, 2005.

NUTRITIONAL AND GENETIC DETERMINANTS OF EARLY PUBERTY

Loïc Le Marchand, Rachel Novotny, John S. Grove, Rudolf Kaaks, Yihe G. Daida, Vinutha Vijayadeva

University of Hawaii and International Agency for Research on Cancer
loic@crch.hawaii.edu

Past studies suggest that early menarche, growth velocity, and specific hormonal patterns during breast development may be critical in determining risk of breast cancer later in life. Nutritional factors (i.e., adiposity, physical activity and diet) during childhood and puberty, and inherited genetic factors are suspected to modulate these early-life exposures. However, the biological processes involved remain poorly understood. These factors may explain the remarkable increase in breast cancer incidence observed among US-born Asian women.

We are investigating the relationships of nutrition, genetic factors and hormonal levels with early life events contributing to breast cancer risk in a unique cohort of 349 adolescent girls of Caucasian or Asian ancestry originally recruited for the Female Adolescent Maturation (FAM) Study. These girls were first studied in 1999-2000 at age 9-14 years and, again two years later in 2002-2003, for dietary intake, body size and composition, sexual maturation (Tanner staging), growth and bone density. Data collection is now being extended by conducting a third examination and obtaining blood samples for DNA genotyping and hormone analysis at a time when almost all girls will have attained menarche. A cross-sectional sample of 100 additional girls is also being recruited.

The aims include the testing of the associations of functional polymorphisms in genes involved in sex hormone metabolism, or coding for growth promoting hormones and related-proteins, with early menarche, early breast development, accelerated growth, higher bone density, and elevated sex and IGF-I hormones, which have all been associated with an elevated risk of developing breast cancer later in life. Another aim will test the associations of adolescent diet, physical activity, abdominal adiposity and pubertal stage with decreased levels of IGFBP-1, and elevated levels of estradiol, IGF-I, leptin, and C-peptide, a marker of insulin secretion. Preliminary results from a feasibility study on 158 of the girls examined in 2002-2003 who donated a mouthwash sample show a direct association between the CYP3A4 *1B polymorphism with pubic hair Tanner stage ($p=0.01$), but no association of this variant with breast Tanner stage or age at menarche. This association is consistent with the role of CYP3A4 in the catabolism of testosterone and the involvement of this hormone in the early stages of puberty.

This on-going study should provide critical evidence in support of the causal involvement of specific biological pathways in early maturation and may reveal possible means for the early prevention of breast cancer through lifestyle modification.

The U.S. Army Medical Research and Materiel Command under W81XWH-04-1-0575, the USDA NRI under grant 99-00700 and the Terry Fox Cancer Research Foundation supported this work.