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Breast Density Assessment by Dual Energy X-ray Absorptiometry in Women and Girls

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Increasing evidence suggests that breast cancer risk is determined early in life. Mammographic density has been used as a biomarker for breast cancer risk because of its strong association with breast cancer. However, use of this screening method is contraindicated in young women and girls because the risk of X-ray based mammograms outweighs potential benefits in that age group. In contrast, Dual Energy X-ray Absorptiometry (DXA) has extremely low radiation and is commonly available. The specific aims of this project among adult women and adolescent girls, who will be recruited as mothers and daughters, will be to 1. Correlate breast density measured by DXA with mammographic density among adult women; 2. Compare the association of known breast cancer risk factors with breast density from DXA scans to their association with mammographic density; 3. Assess DXA breast density by Tanner stage of breast maturation among adolescent girls; 4. Relate DXA breast density to other observable measures of pubertal maturation, e.g., height and menarche; and 5. Examine the relation between breast density measured by DXA in mothers and daughters. During this year, we have recruited 19 mother-daughter pairs plus one additional daughter and obtained DXA scan images from all participants. We project to recruit the remaining 80% (81 mother-daughter pairs) and complete data collection by the end of next year. Upon completion of the study, our multiethnic sample will generate a pilot data on the DXA scan as a method to evaluate breast cancer risks in women and young girls from various ethnic groups.
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(4) Introduction
Increasing evidence suggests that breast cancer risk is determined early in life. However, use of mammography as a screening method is contraindicated in young women and girls because the risk of X-ray exposure outweighs potential benefits in that age group. In contrast, Dual Energy X-ray Absorptiometry (DXA) has extremely low radiation and is commonly available. The rationale of this project is that predictors of breast cancer risk may be observed during pubertal development in girls. Our hypotheses are as follows:
1. DXA imaging can provide a valid assessment of breast density in adult women and in young girls.
2. DXA assessed breast density is associated with indicators of pubertal maturation and with known breast cancer risk factors.
3. Due to its strong genetic component, breast density obtained from the DXA scans is correlated between mothers and adolescent daughters.

The specific aims of this project among adult women and adolescent girls, who will be recruited as mothers and daughters, are to:
1. Correlate breast density measured by DXA with mammographic density among adult women.
2. Compare the association of known breast cancer risk factors with breast density from DXA scans to their association with mammographic density.
3. Assess DXA breast density by Tanner stage of breast maturation among adolescent girls.
4. Relate DXA breast density to other observable measures of pubertal maturation (e.g., height and menarche).
5. Examine the relation between breast density measured by DXA in mothers and daughters.

We aim to recruit a total of 100 adult women with daughters between 8-16 years of age. Based on the ethnic distribution of Hawaii’s population, we expect that approximately half of the study subjects will be of Asian (primarily Japanese, Chinese, and Filipino) and Pacific Islander descent.

(5) Body
During this year, we have accomplished the following tasks as outlined in the approved Statement of Work.

Task 1. Study plan and procedures.
Task 1.a. Study manual. Last year through earlier this year, we faced a lower-than-expected participant enrollment rate. To improve our recruitment efforts, we made modifications to the participant eligibility criteria that include: 1) expanding BIRADS to include categories 1-3; 2) expanding mothers’ age group to 30 years and older; and 3) dropping oral contraceptive use. These modifications and related revisions to the study protocol and study forms were approved by all IRBs. Moreover, we developed a study flyer and follow-up letters to send to Kaiser members who did not reply to our initial recruitment letters. Subsequently, we received Kaiser and UH CHS approvals; however, we did not seek DOD approval, as participant enrollment improved during the 2nd quarter. We reached our goal of recruiting 100 mother-daughter pairs in the 3rd quarter.

Task 1.b. Subcontracts. We continued collaborative efforts with the University of California at San Francisco (UCSF) and with Kaiser Permanente Hawaii under subcontracts established during the first year.

Task 1.c. DXA procedures.
DXA procedure was established and outlined in the DXA Operator’s Manual during the first year. No modifications were made this year.

Task 1.d. Plan recruitment strategy with Kaiser. We made modifications to participant eligibility criteria as previously described in Task 1.a. Revised criteria are described in the approved Study Protocol.

Task 1.e. Train DXA technician. Training of a licensed x-ray technician at the Clinical Research Center, University of Hawaii was completed during the first year. No additional training was required this year.
Task 1.f. Establish study database.
A password-protected, study database was created during the first year to be accessed by authorized study personnel for participant data entry and analysis. This database will be merged with both mammographic density data and DXA scan data for further analysis.

Task 1.g. Purchasing study supplies. Additional twenty gift cards were purchased this year. A total of 220 cards have been purchased.

Task 2. Subject recruitment. This year, we mailed 3,491 invitation letters through Kaiser to eligible mothers and daughters, making it a total of 3,915 letters mailed through Years 1 and 2. Among those who responded, we enrolled 102 mothers and 112 daughters and completed our recruitment in the second half of this year.

Task 3. Conduct study visits. Study visits were conducted by trained research staff members at the Clinical Research Center including a licensed x-ray technician. These staff members were trained in explaining the study procedures, performing final eligibility check, obtaining informed consent/assent, administering the study questionnaire, and collecting anthropometric measurements. In addition, the x-ray technician had previously been trained in conducting Tanner-stage assessments of pubertal development (breast and pubic hair), and has performed the assessments of all 112 girls participated in our study.

Task 4. Perform DXA scans. A licensed x-ray technician who received training for this study performed scans of the whole body and breasts on a Lunar Prodigy DXA Bone Densitometer (DF+12484). DXA scans were obtained from all participants. Scanned images were saved on a password-protected USB memory drive each day as directed by the DXA Operator’s Manual. At the end of each month, these images were burned to a CD, protected by password, and mailed to University of California at San Francisco, Breast Bone Density Group (UCSF BBDG) via FedEx by the project coordinator.

Task 5. Perform Breast Density Analysis. After all mothers and daughters have completed study visits, we obtained screening mammogram films from Kaiser and non-Kaiser clinics. We have obtained 94 mothers’ mammogram films so far, and we are currently in the process of obtaining the remaining 8 mothers’ films. These mammogram films were scanned on a Kodak LS80 digitalizer into a password-protected computer. All personal identifiers were removed from the scanned images, which were then saved with study participant ID numbers. After delineating total and dense breast areas, the percentage density was calculated as the ratio of the dense area to the total area. All mammograms for one woman were assessed during the same session. DXA scan images of all participants have been sent to UCSF BBDG and are currently being analyzed for density measures. We are expecting to receive the results from UCSF shortly.

Task 6. Data Management and Analysis. All participants’ data have been entered into a password-protected, study database including demographic data, health and menstrual/reproductive information, anthropometric measures, and Tanner stages for girls. Using SAS Version 9.1, we calculated the summary statistics to describe our participant-recruitment (Figure 1), as well as the demographics of the final study population (Table 1), which consisted of 102 mothers and 112 daughters. Mean age of the mothers was 47.0±4.4 years, and that of the daughters’ was 13.4±1.7 years. Their self-reported ethnicities reflected the diverse population of Hawaii (Table 1). Tanner stage assessments of the breast and pubic hair indicated a successful recruitment of girls from all 5 levels of pubertal development (Table 2). During these analyses, we checked for data entry errors in the database. We will conduct further statistical analysis when density measures from mammography and DXA scans become available for all participants.
(6) Key Research Accomplishments
   None to date.

(7) Reportable Outcomes
   None to date.

(8) Conclusions
   This year, we completed participant recruitment and DXA scans. We are currently working on finishing mammographic density analysis while waiting to receive DXA image analysis results from UCSF. When both of these become available, we will further proceed with statistical data analysis including correlation coefficients, general linear models, and mixed models. Such analysis will generate pilot data on the DXA scan in mother-daughter and provide more information about the heritable aspects of breast density from an ethnically-diverse population. Moreover, the current study will develop a technique that will provide a unique tool to screen for breast cancer risk in early life and aid in developing prevention strategies.

(9) References
   No articles cited.

(10) Appendices
   None.

(11) Supporting Data
   Figure 1: Recruitment and Study Population (Page 7)
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   Table 2: Daughters’ Tanner stage distribution (Page 8)
Figure 1. Recruitment and Study Population

3915 Invitation letters sent

304 Mothers with daughters expressed interest in study

166 Ineligible mothers with daughters due to:
   - 50 No mammograms
   - 42 Age
   - 18 Biological mother/daughter not available
   - 18 History of breast cancer/surgery or abnormal mammogram/biopsy
   - 12 Breast implants
   - 10 Not interested/unable to contact
   - 9 Daughter with no breast development
   - 4 Off island
   - 3 Health problems

138 Eligible mothers with eligible daughters scheduled study visits

102 Mothers with daughters completed study:
   - 90 mothers with one daughter
   - 11 mothers with 2 daughters
   - 1 mother with no daughter

304 Mothers with daughters expressed interest in study

138 Eligible mothers with eligible daughters scheduled study visits

102 Mothers with daughters completed study:
   - 90 mothers with one daughter
   - 11 mothers with 2 daughters
   - 1 mother with no daughter

166 Ineligible mothers with daughters due to:
   - 50 No mammograms
   - 42 Age
   - 18 Biological mother/daughter not available
   - 18 History of breast cancer/surgery or abnormal mammogram/biopsy
   - 12 Breast implants
   - 10 Not interested/unable to contact
   - 9 Daughter with no breast development
   - 4 Off island
   - 3 Health problems
Table 1. Participants’ demographic Information

<table>
<thead>
<tr>
<th>Ethnic category</th>
<th>Japanese</th>
<th>White</th>
<th>Hawaiian &amp; other pacific</th>
<th>Other Asian</th>
<th>Other††</th>
<th>Total</th>
<th>P value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>22</td>
<td>32</td>
<td>18</td>
<td>25**</td>
<td>5</td>
<td>102</td>
<td>--</td>
</tr>
</tbody>
</table>

| Age (years)     | 49.0±4.6  | 47.4±4.3 | 46.0±4.5  | 45.8±4.0 | 45.0±2.7 | 47.0±4.4 | .08       |
| BMI (kg/m²)     | 26.0±5.6  | 26.6±5.8 | 26.9±5.6  | 30.2±5.7 | 34.2±5.8 | 27.5±5.9 | .01       |

<table>
<thead>
<tr>
<th>Mothers</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche (years)</td>
<td>11.9±1.1</td>
<td>12.9±1.6</td>
<td>11.8±1.3</td>
<td>13.3±2.0</td>
<td>13.2±1.9</td>
<td>12.5±1.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Age at first child birth (years)</td>
<td>30.5±6.0</td>
<td>30.0±5.4</td>
<td>29.8±5.7</td>
<td>25.7±5.8</td>
<td>27.1±4.2</td>
<td>29.1±5.8</td>
<td>.05</td>
</tr>
</tbody>
</table>

| Number of children | 2.4±1.0 | 2.5±1.0 | 2.2±0.6  | 3.0±1.4 | 2.4±1.1  | 2.5±1.0 | .13       |

| Menstruation Yes/No† (N) | 10/12 | 13/18‖ | 8/10 6/19 | 1/4 | 38/63 | .59 |

| Hormone (estrogen) use (%)‡‡‡ | 32 | 16 | 22 | 21 0 21 | .52 |

| Overweight/obese (%) | 36/14 28/25 | 33/44 | 36/20 20/80 | 32/28 | -- |

| N               | 25       | 35    | 29            | 16 | 7 | 112 | --       |

<table>
<thead>
<tr>
<th>Daughters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.6±1.6</td>
<td>13.5±1.6</td>
<td>13.0±1.7</td>
<td>12.9±2.1</td>
<td>14.3±1.3</td>
<td>13.4±1.7</td>
<td>.23</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>50.6±11.4</td>
<td>52.4±13.6</td>
<td>58.2±17.3</td>
<td>51.1±11.5</td>
<td>64.2±15.0</td>
<td>15.0±54.1</td>
<td>14.3</td>
</tr>
</tbody>
</table>

| Height (cm)      | 154.0±6.3 | 158.6±9.5 | 159.9±8.7  | 154.2±7.7 | 160.3±4.3 | 157.4±8.4 | .03       |

| Reached menarche (N) | 18 | 23 | 21 | 12 5 79 | .96 |

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* Including 10 Chinese, 13 Filipino, 1 Korean and 1 Other Asian
†† Including 1 Black, 2 Hispanic, 1 Native American and 1 Other.
‡‡‡ N = 100. Based on self-reported hormone (estrogen) use. Excluded 2 mothers who reported IUD and Lupron.
§ Menstruation status was determined based on the last menstrual period within the past 60 days.
‖ Excluding 1 mother with missing last period date
** Including 1 mother (Other Asian) whose daughter refused to participate.
*** P value among the 5 ethnic categories.

Table 2: Daughters’ Tanner stage distribution

<table>
<thead>
<tr>
<th>Tanner Stage</th>
<th>N</th>
<th>Tanner Stage</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>17</td>
<td>0-2</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>Total 110*</td>
<td></td>
</tr>
</tbody>
</table>

*Two daughters refused to complete Tanner pubic-hair assessment.