Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** Trustees of the University of Pennsylvania

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 – 12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Gearline Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100054874

6. **Project Number and Title of Research Project:** 8-Identifying Genetic Determinants of Mammographic Density

7. **Start and End Date of Research Project:** 7/1/2011-12/31/2014

8. **Name of Principal Investigator for the Research Project:** Katrina Armstrong, MD, MSCE

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 120,721.53

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Chodosh, Lewis	Professor	1% Yr.2	$2476.48
Chen, Chien Chung	Mngr. Res. Project	3.5% Yr.2	$3,942
Keller, Brad	Research Associate	36% Yr.2	$16,900
Alvarez, James	Res. Investigator	1%Yr.2	$1,111.21
Smith, Judith	Mgr.Res.Proj.B	2%Yr.2	$11,302.95
Sterner, Christopher	Mgr.Res.Proj.A	13%Yr.4	$9,561.51

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Armstrong, Katrina	Principle Investigator	1%
Belka, George	Manager Research Project	5%, Yr. 4
Maidment, Andrew	Associate Professor	1% Yr 2
Kontos, Despina	Assistant Professor	1% Yr 2

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
Radiographic-Histological Correlates of Dense Breast Tissue	☐NIH ☐ Other federal (specify:_____) X Nonfederal source (Susan G. Komen Foundation)	12/2013	$180,000	$180,000

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes__X_____ No_____

If yes, please describe your plans:

We plan to apply for funds from the Department of Defense Breast Cancer Research Program to continue to the research described in this proposal to validate a rat model for identifying genetic determinants of mammographic density.

12. Future of Research Project. What are the future plans for this research project?

If funding can be obtained, we plan to extend the work proposed in this research project with the goal of validating methods for measuring genetic effects on mammographic density in rats that would enable elucidation of the genetic loci that determine this phenotype in mammals. This will require development of sufficiently robust imaging protocols and quantification methods to measure intra-animal, inter-animal and inter-strain variation in microCT attenuation with a high degree of reproducibility. If successful, we would pursue a large-scale genetic analysis of mammographic density among offspring from a backcross between an F1 hybrid of a low-density/low-risk rat strain with a high-density/high-risk rat strain.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				1
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White				1
Black				
Asian				
Other				
Unknown				
Total				**1**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No__X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes__X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This health research project enhanced the quality of research at the University by nucleating an inter-disciplinary group of scientists, including computational biologists, medical physicists, and cancer biologists, to focus on an important problem in cancer. This would not have happened but for the funding supplied by this project, and this will lead to ongoing research of importance to the population.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Identifying Genetic Determinants of Mammographic Density

Mammographic density is one of the strongest known predictors of breast cancer risk, but its basis is largely unknown. The realization that mammographic density is primarily determined by genetic factors, rather than reproductive endocrine events, suggests that the genetic pathways that control mammographic density may reflect entirely novel pathways and mechanisms of breast cancer susceptibility. Identifying the genes that control mammographic density would be greatly facilitated by a validated animal model for the effect of genetics on mammographic density. The objective of this pilot project was to validate a rat model for the genetic effects of mammographic density on breast cancer risk using in vivo and ex vivo imaging in combination with inbred strains of rats with differing intrinsic susceptibilities to breast cancer.

This study used microCT to radiographically image mammary tissue from different inbred strains of rats that differ in their intrinsic susceptibility to breast cancer. The specific aims of this pilot project were to: 1) develop reproducible in vivo and ex vivo microCT measures of mammographic density in rats; 2) determine intra-animal and inter-animal variability in microCT mammographic density within a rat strain; 3) determine inter-strain variability in mammographic density to identify strains with high versus low density; and 4) identify tissue-based correlates of mammographic density. These validation studies would enable a large-scale genetic analysis of mammographic density among offspring from a backcross between an F1 hybrid of a low-density and a high-density rat strain.
The rationale for this project was that identifying the genetic basis for mammographic density has the potential to identify entirely novel genetic pathways that contribute to the determination

of breast cancer susceptibility. As a proof-of-principle to identify genetically determined molecular and cellular correlates of mammographic density, this study attempted to validate a novel rat model for mammographic density by radiographically imaging and histologically analyzing mammary tissue from different inbred strains of rats that differ in their intrinsic susceptibility to breast cancer.

Animal handling, tissue harvest, and morphological analysis was conducted in Dr. Lewis Chodosh's laboratory in the Department of Cancer Biology. MicroCT imaging was carried out in Dr. Andrew Maidment's laboratory in the Division of Medical Physics within the Department of Radiology. MicroCT image analysis and quantification was carried out in Dr. Despina Kontos' laboratory in the Compuational Breast Imaging Group in the Department of Radiology.

Generation of rat mammary gland samples and preparation for imaging

The first phase of this mammographic density study was to determine the feasibility of the study with respect to several variables related to tissue harvesting, storage and placement during the imaging protocol. Rat mammary glands from the Lewis strain were harvested at different stages of rat mammary gland development and imaged ex-vivo. Rat mammary glands were collected from three juvenile (3-wk old) rats, three adult (16-wk or older) rats, two lactating (day 10 of lactation) rats, and two pregnant (day 13 and 17 of pregnancy) rats, as well as 3 rat mammary tumors and adipose tissue. Rats were maintained on a light/dark cycle of 12 hr (7am-7pm light/7pm-7am dark) on lab diet 5058. All glands were stored at 4°C until imaged.

A number of different options were examined to determine how best to prepare glands for visualization by micro-CT. First, unfixed number 4 mammary glands were adhered to the side wall of a 50ml conical tube and embedded in 0.5% agarose in 1x PBS). This method was abandoned due to poor visibility of the gland along the wall of tube when imaged. Second, number 4 mammary glands were fixed on a polystyrene slide overnight in 4% PFA and the slide with the affixed gland was embedded in 0.5% agarose in 1x PBS the following day. This method was abandoned due to difficulty distinguishing the boundary between the gland and the agarose on imaging. Third, we fixed number 4 mammary glands on polystyrene slides overnight in 4% PFA, which was replaced with PBS the following day. The gland was then sandwiched between another slide and stored until imaged. At imaging, PBS was removed and a PBS-dampened sponge was placed within the tube behind the slide and mammary gland to maintain proper humidity in tube. This method was abandoned due to pooling of PBS along the edge of gland in the sandwiched plates, causing bright edges along the outside of the gland. The fourth and final method evaluated was identical to the third method, with the exception that at imaging, both the PBS and the slide used to sandwich the gland were removed. This was determined to be the best method to capture images and was the method chosen for subsequent studies collecting and visualizing mammary glands.

Generation of microCT Images

Based upon the above pilot studies, we imaged mammary glands according to the following protocol. Rat mammary glands were imaged on a Scanco Medical Viva CT 40 microCT scanner. The peak voltage was set at 45 kVp, with 200 projections obtained per complete rotation of the x-ray tube. The image volume was reconstructed using a standard cone-beam conventional back-projection algorithm with an isotropic (x-, y-, z-) resolution of 76 µm.

Mammary glands were removed from storage in phosphate-buffered saline (PBS), and placed in the microCT sample holder (see Figures 1 and 2). Glands were placed on a plastic slide that was held in place in the center of the field of view. To improve reproducibility of imaging analysis, a hemicylindrical calibration phantom compartment was incorporated beneath the mammary gland-containing compartment. This phantom, which was constructed specifically for this study, was comprised of two materials approximating the x-ray attenuation of pure human adipose tissue and pure human fibroglandular tissue, respectively. In addition, a dampened sponge containing PBS was also placed beneath the mammary gland compartment to prevent drying of the tissue during scan time (typically 2 hours). After scanning was completed, mammary glands were returned to storage in PBS.

Quantitative Analysis of Rat microCT Images

To segment the mammary gland from the microCT images, a custom-made semi-automated software algorithm was developed that utilizes information about the geometry of the specimen preparation within a user-defined region of interest. Briefly, mammary gland specimens were placed between two rigid slides that separate the specimen container into three compartments (Figures 1 and 2). A critical aspect of this study was the ability to reproducibly determine x-ray attenuation through the mammary gland with sufficient precision and accuracy to enable discrimination between different strains of rats.

A user-defined bounding box was used to identify the region of interest (ROI) in the image in which the gland was placed, ideally excluding most of the other two compartments. Following ROI placement, the remainder of the algorithm was fully-automated. First, the plates separating the compartments were identified via a set of empirically determined thresholds in the initial and final 5 slices of the microCT (i.e. where there is no mammary tissue with similar gray-level intensity to the plates). Second, a rigid-body approximation of the plates was interpolated through the remaining slices of the microCT image based on fitting using a combination of linear regression and morphological dilation. After the segmentation of the dividing plates, the central compartment of the microCT images, which contained the mammary gland bounded by an air interface, was identified. The mammary gland was then computationally separated from air via a standard 2-class fuzzy c-means (FCM) clustering algorithm. Figure 3 (left) shows a coronal view of the segmented gland seen in Figure 2.

We next obtained an estimate of glandular density by quantifying the gray-level intensity distribution of the gland, as gray-levels are proportional to x-ray attenuation. We used the mean gray-level intensity as a measure of glandular density. In addition, we provide a calibrated density estimate based on a linear-transformation of the mean gray-level value of the image, scaled to the mean gray-levels of the two calibration materials present in each micro-CT image. A histogram of the distribution of gray-levels of the segmented gland is shown in Figure 3 (right) compared to the adipose and fibroglandular calibration phantom.

Effect of developmental stage on mammographic density

We next asked whether mammographic density varies with developmental stage of the animal. We removed the inguinal mammary glands from juvenile, adult, pregnant, and lactating Lewis rats. As a control, we also removed three mammary tumors from Lewis rats. Glands were

imaged to determine how density changes with developmental stage. Representative carmine-stained images, CT images, and quantification are shown in Figure 4.

Mammographic density in contralateral glands from the same animal
We next wished to determine whether microCT attenuation, as measured for each animal, was a consistent physical property, since this property would be essential for mapping of inherited variation in mammary density. That is, we wished to address the relative contribution of intra-animal variation, as well as inter-animal variation, in microCT attenuation in order to assess the biological significance of observed differences in inter-strain variation in microCT attenuation. Specifically, we predicted that intra-animal variation in measured micro-CT attenuation would be relatively small compared to inter-animal variation, as is observed in mammographic density evaluated in women.

To address this, we performed microCT scanning on paired right and left inguinal mammary glands harvested from 17 individual rats. microCT images from all 38 imaged glands were quantified as above. This analysis confirmed that there was a statistically significant correlation in mammographic density between the right and left inguinal mammary glands harvested from the same animal, suggesting that this is an intrinsic property of the animal (Figure 5 and Table 1).

While this result was encouraging, it was notable that the correlation between left and right mammary gland density within animals was only moderate ($r=0.58$). In contrast, the correlation between left and right mammographic breast density in women is substantially high ($r>0.85$). This suggests either that significant variation exists in right versus left mammary glands in rats or, more likely, that our sample preparation and/or imaging protocols may introduce variability into micro-CT measurements. If true, this would degrade our ability to make precise microCT attenuation estimates for individual animals problematic.

Inter-strain differences in mammographic density
Following the above feasibility and optimization studies, an initial pilot study was performed to determine whether quantitative differences in mammographic density, as ascertained by micro-CT, might be able to be identified between different rat strains. Initially, we analyzed microCT images from two inbred strains of rats, including eight Brown Norway rats and seven Copenhagen-strain glands. For each strain, rats were sacrificed at 12 weeks of age for microCT. Following microCT, glands were carmine stained and photographed. Carmine-stained morphological images were compared against micro-CT scans.

A preliminary analysis comparing the microCT-determined mammary gland density of Brown Norway and Copenhagen rats suggested that, while there may be significant variation in the intensity/apparent-density seen within a strain, these two inbred rat strains appeared to exhibit statistically significant, detectable differences in both raw and calibrated gland intensity (Figure 6, paired t-test, $p=0.003$). Moreover, that intensity alone had a strong capacity to distinguish between strains (Figure 6; logistic regression AUC>0.9, $p<0.01$).

Having developed a method to reproducibly measure mammographic density in rats, we next wished to ask whether the differences in breast cancer susceptibility of different inbred rat strains

is reflected by differences in mammographic density. Inguinal mammary glands were collected from five different strains of inbred rats: Brown Norway, Copenhagen, F344, Lewis, and Wistar Furth rats. As above, for each strain rats were sacrificed at 12 weeks of age for microCT.

During the study period, we completed the computational analysis of 54 micro-CT images from 29 individual rats representing 5 inbred strains of rats: Brown Norway, Copenhagen, F344, Lewis and Wistar Furth glands. Analysis of data obtained from one mammary gland per rat indicated that each of the different rat strains exhibited significantly different micro-CT attenuation and that this inter-strain difference was observed to be larger than the difference between individual animals of the same strain (ANOVA $p<0.0001$; Figure 7 and Table 2). Brown Norway rats exhibited the highest mammographic density, while F344 rats had the lowest mammographic density. This, in turn, suggests that: (a) individual strains of inbred rat possess differing amounts of "dense" epithelial content, which is consistent with the central hypothesis and goal of this pilot project; and (b) the greater inter-strain variation versus inter-animal variation observed supports the feasibility of the projects predicated upon the hypothesis that x-ray attenuation coefficients in mammary glands from different strains may differ significantly. Consequently, these studies would enable a large-scale genetic analysis of mammographic density among offspring from a backcross between an F1 hybrid of a low-density and a high-density rat strain.

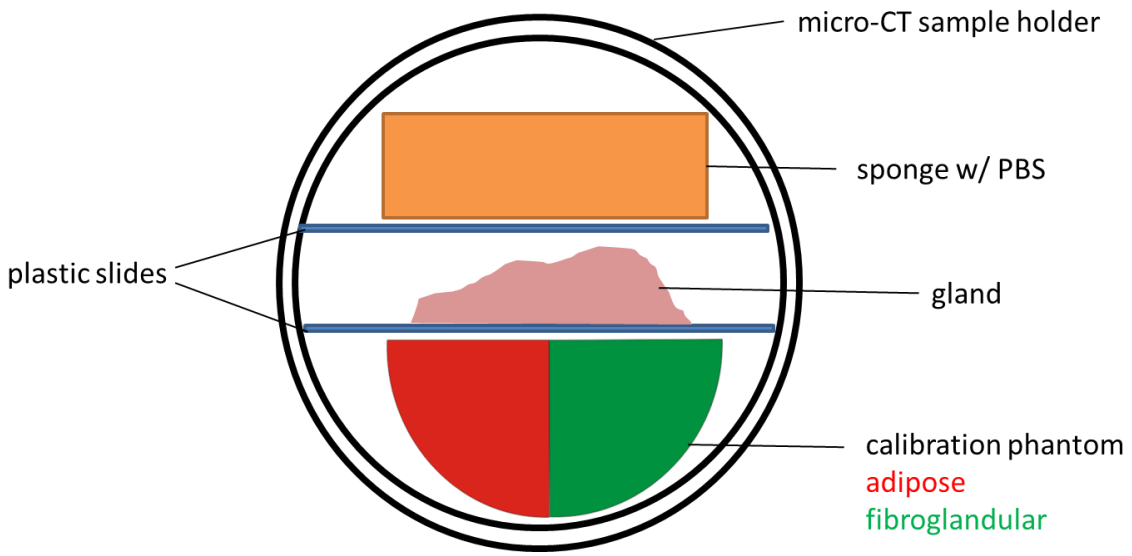


Figure 1. Cross-sectional view of micro-CT sample holder containing the sample, calibration phantom and sponge.

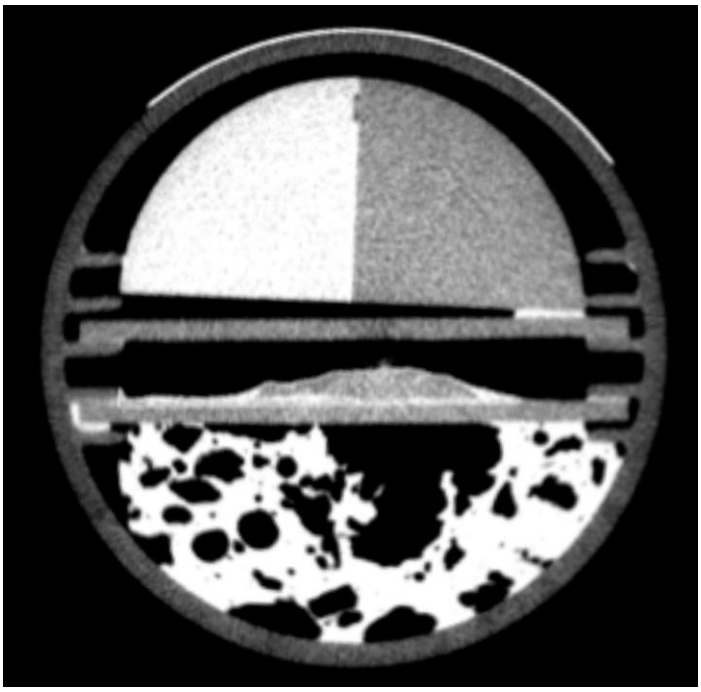


Figure 2. Example of a mammary gland specimen placed between two plates and imaged with calibration materials below and sponge to maintain hydration above.

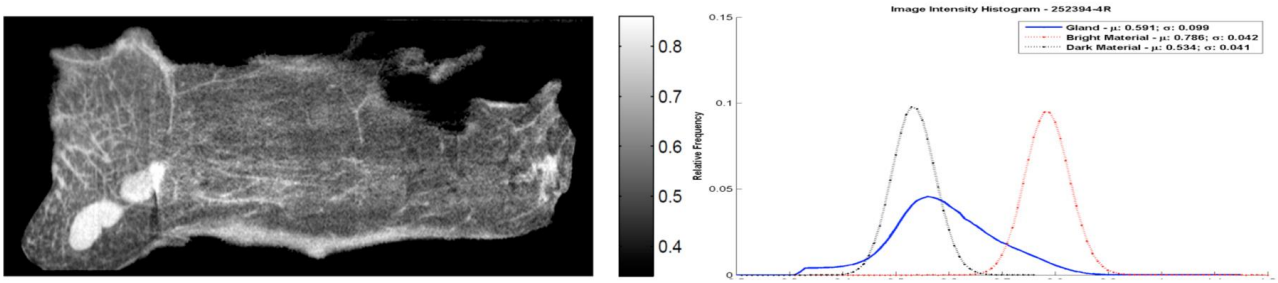


Figure 3. Example of a mammary gland specimen in coronal microCT view and the corresponding gland grey-level histogram (blue).

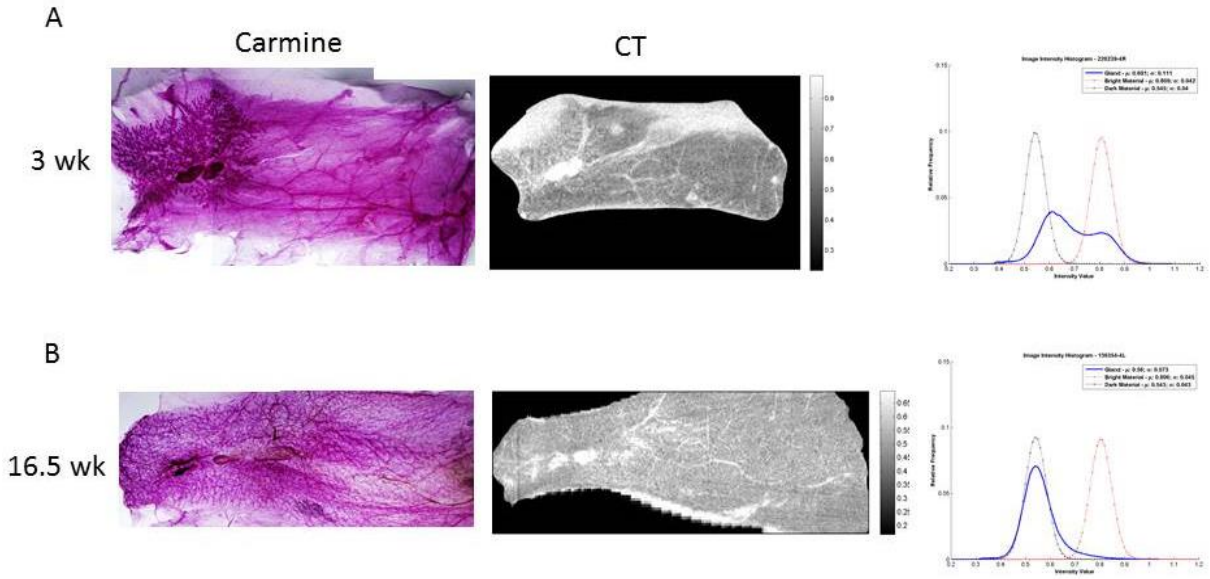


Figure 4. Representative Carmine-stained whole mounts (left), microCT images (middle), and quantification (right) from 3 week old and 16.5 week old Lewis rats.

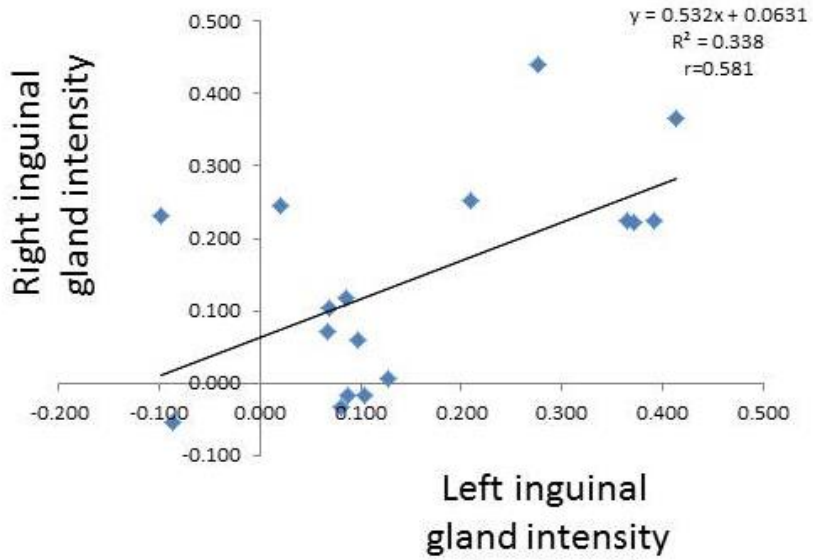
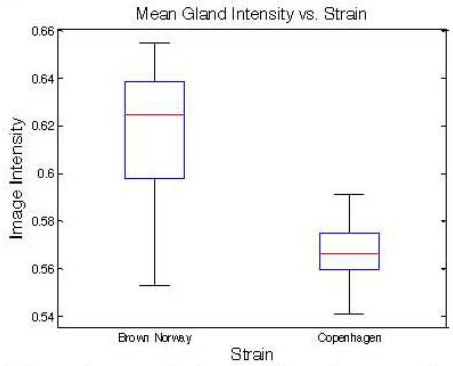


Figure 5. Quantification of intra-animal variations in mammographic density. Left and right inguinal glands from 17 rats were imaged by CT. There was a significant correlation in mammographic density between contralateral glands from the same rat.

Case #	Strain	Gland Intensity	Calibrated Intensity
252391-4L	Brown Norway	0.643	0.413
252391-4R	Brown Norway	0.634	0.366
252392-4L	Brown Norway	0.553	0.100
252393-4L	Brown Norway	0.616	0.365
252394-4L	Brown Norway	0.634	0.391
252394-4R	Brown Norway	0.591	0.225
252395-4L	Brown Norway	0.606	0.276
252395-4R	Brown Norway	0.655	0.441
252422-4L	Copenhagen	0.566	0.088
252423-4L	Copenhagen	0.562	0.127
252423-4R	Copenhagen	0.541	0.007
252424-4R	Copenhagen	0.577	0.147
252426-4R	Copenhagen	0.591	0.230
252427-4L	Copenhagen	0.569	0.097
252427-4R	Copenhagen	0.559	0.059



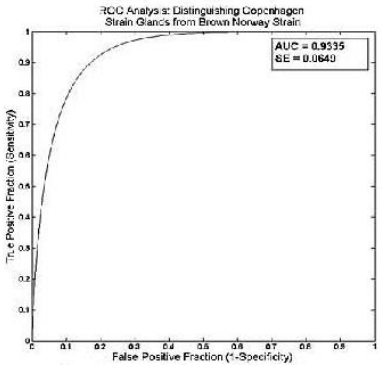


Figure 6: Results for the Brown Norway and Copenhagen strain mammary gland specimens with corresponding box-plots and ROC curve.

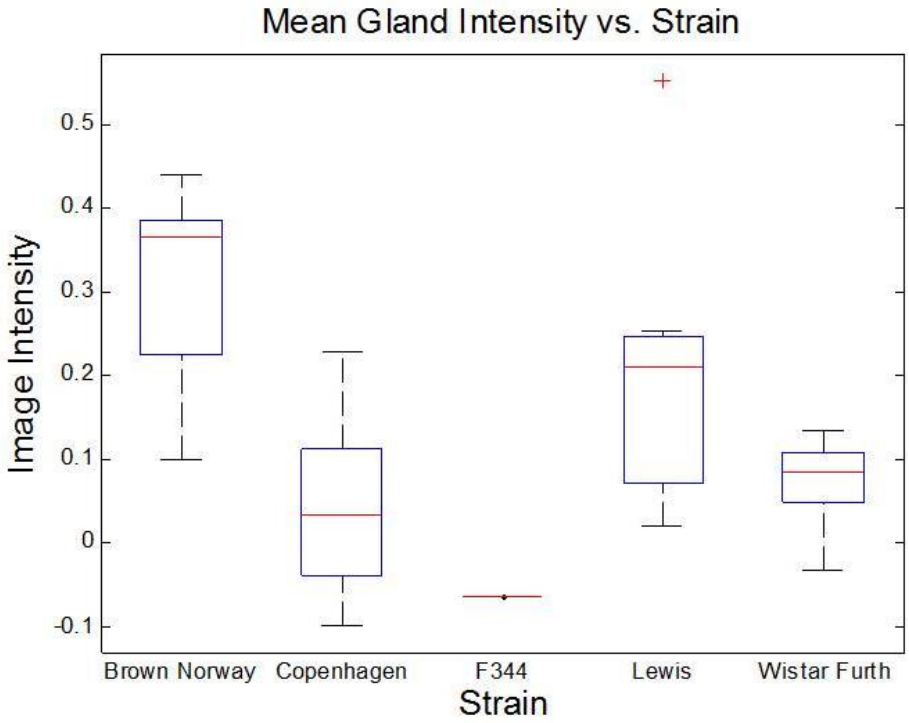


Figure 7. Quantification of inter-strain variations in mammographic density. Mammographic density was measured in 12 Brown Norway, 12 Copenhagen, 1 F344, 12 Lewis, and 12 Wistar-Furth rats. There was a significant variation in the mammographic density between rat strains (ANOVA $p<0.0001$).

Case	Strain	Normalized Mean - L	Normalized Mean - R
252391	Brown Norway	0.413	0.366
252393	Brown Norway	0.365	0.225
252394	Brown Norway	0.391	0.225
252395	Brown Norway	0.276	0.441
252396	Brown Norway	0.371	0.222
252422	Copenhagen	0.088	-0.017
252423	Copenhagen	0.127	0.007
252425	Copenhagen	-0.087	-0.055
252426	Copenhagen	-0.098	0.230
252427	Copenhagen	0.097	0.059
136354	Lewis	0.067	0.072
136355	Lewis	0.020	0.245
136359	Lewis	0.209	0.253
136366	Wistar Furth	0.105	-0.016
136368	Wistar Furth	0.085	0.117
136369	Wistar Furth	0.080	-0.033
136371	Wistar Furth	0.069	0.104

Table 1. microCT density scores for paired inguinal mammary glands after calibrating with a density-calibration phantom.

	Brown Norway (N=5)	Copenhagen (N=4)	F344 (N=5)	Lewis (N=9)	Wistar Furth (N=6)
Brown Norway (N=5)		p=0.0012	p=0.0003	p=0.0189	p=0.0006
Copenhagen (N=4)			p=0.6962	p=0.0245	p=0.0110
F344 (N=5)				p=0.0183	p=0.0030
Lewis (N=9)					p=0.1863
Wistar Furth (N=6)					

Table 2. Different inbred strains of rats exhibit different microCT density scores after calibrating with a density-calibration phantom.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X__ No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04),

the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X____ No_____

If yes, please describe your plans:

If we can obtain funding to perform additional analyses, we would hope to publish our findings.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Identifying the genes that control mammographic density would be enabled by a validated animal model in which mammographic density can be related to genetic background and breast cancer risk. While animal models have proven to be extraordinarily powerful tools for understanding human disease, there is currently no animal model for mammographic density and no studies in animal models that have attempted to relate variation in mammographic density with differences in breast cancer susceptibility. However, in order to perform such a mapping study, critical proof-of-principle experiments are required to validate the model and

quantitative approach. This project is intended to enable those studies. We anticipate that the studies proposed in this project will provide a validated rat model for the genetic determination of mammographic density and breast cancer risk. As such, this research project has the potential to provide a unique and important tool for mapping genes that control breast cancer susceptibility. The identification of such genes, in turn, has the potential to lead to the development of more effective molecularly targeted agents for the treatment or prevention of breast cancer.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:

Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Lewis A. Chodosh	Professor and Chairman of Cancer Biology
eRA COMMONS USER NAME (credential, e.g., agency login) chodoshl	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Yale University	B.S	1981	Biochemistry
Harvard Medical School	M.D.	1989	Medicine
Massachusetts Institute of Technology	Ph.D.	1989	Biology, Dr. Phillip Sharp
Massachusetts General Hospital	Medicine	1991	Medicine Residency
Massachusetts General Hospital	Endocrinology	1994	Endocrine Fellowship
Harvard Medical School	Postdoc	1994	Breast Cancer, Dr. Philip Leder

A. Personal Statement

I am a physician-scientist in the Departments of Cancer Biology and Medicine in the Division of Endocrinology, Diabetes and Metabolism. I have extensive experience in the use of molecular, cellular, *in silico*, and animal model systems to study the function of key regulatory molecules in cancer biology, including the generation and analysis of transgenic and knockout mouse models of breast cancer, protein biochemistry, molecular biology, molecular genetics, cell biology, endocrinology, animal physiology, small animal imaging, genomics and computational biology. As a physician, I have expertise in internal medicine and endocrinology. I serve as Chairman for the Department of Cancer Biology, Associate Director of the Abramson Cancer Center, and Director of Cancer Genetics for the Abramson Family Cancer Research Institute. In addition, I am Editor-in-Chief of *Breast Cancer Research*, an advisor to the Nurses' Health Studies I and II, and Principal Investigator for an NCI UO1 that supports one of the sites of the NCI Mouse Models of Human Cancers Consortium. I have trained numerous graduate students and postdoctoral researchers in scientific methods and approach, and routinely teach undergraduates, graduate students, medical students and medical residents.

B. Positions and Honors

Current Positions and Employment

2005- Director, Cancer Genetics, Abramson Family Cancer Research Institute
2005-2012 Program Leader, Breast Cancer Program, Abramson Cancer Center
2006- Professor, Departments of Cancer Biology, Cell & Developmental Biology, and Medicine
Division of Endocrinology, Diabetes & Metabolism, Univ. of Pennsylvania School of Medicine
2006- Associate Director for Basic Science, Abramson Cancer Center
2008- Chairman, Department of Cancer Biology, University of Pennsylvania School of Medicine
2012- Co-Director, 2-PREVENT Breast Cancer Translational Center of Excellence, ACC

Honors (selected)

2002-	American Society for Clinical Investigation
2002	AACR-Sidney Kimmel Symposium for Cancer Research Scholar Award
2003-2009	Co-Chair, AACR Special Conference on Advances in Breast Cancer Research
2008-2012	J. Samuel Staub Professor of Cancer Biology
2008-	Association of American Physicians
2013	Department of Cancer Biology, Teaching Award

C. Selected Peer-reviewed Publications (selected from 128 peer-reviewed publications)

29. Magnitsky S, Belka GK, Sterner C, Pickup S, Chodosh LA and Glickson JD. Lactate detection in inducible and orthotopic Her2/neu mammary gland tumours in mouse models. *NMR in Biomedicine*, 48:353-64, 2012.
30. Chen CC, Stairs DB, Boxer RB, Belka GK, Horseman ND, Alvarez JV and Chodosh LA. Autocrine prolactin induced by the Pten-Akt pathway is required for lactation initiation and provides a direct link between the Akt and Stat5 pathways. *Genes & Development*, 26:2154-68, 2012.
31. Yeh ES, Belka GK, Vernon AE, Chen CC, Jung JJ and Chodosh LA. Hunk negatively regulates c-myc to promote Akt-mediated cell survival and mammary tumorigenesis induced by loss of Pten. *Proceedings of the National Academy of Sciences USA*, 110:6103-8, 2013.
32. Alvarez JV, Pan TC, Ruth J, Feng Y, Zhou AY, Pant D, Grimley JS, Wandless TJ, Demichele A and Chodosh LA. Par-4 down-regulation promotes breast cancer recurrence by preventing multinucleation following targeted therapy. *Cancer Cell,* 24:30-44, 2013.
33. Feng Y, Pan TC, Pant DK, Chakrabarti KR, Alvarez JV, Ruth JR and Chodosh LA. SPSB1 promotes breast cancer recurrence by potentiating c-MET signaling. *Cancer Discovery,* 4:790-803. 2014.
34. Lee JV, Carrer A, Shah S, Snyder NW, Wei S, Venneti S, Worth AJ, Yuan ZF, Lim HW, Liu S, Jackson E, Aiello NM, Haas NB, Rebbeck TR, Judkins A, Won KJ, Chodosh LA, Garcia BA, Stanger BZ, Feldman MD, Blair IA and Wellen KE. Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. *Cell Metabolism,* 20:306-319, 2014.
35. Payne AW, Pant DK, Pan TC and Chodosh LA. Ceramide kinase promotes tumor cell survival and mammary tumor recurrence. *Cancer Research*, 21:6352-63, 2014.
36. Alvarez JV, Belka GK, Xu M, PanTC, Sudarshan S, Chen CC, Young B, Notarfrancesco K, Circiumaru V, Alavi A, Karp J, Acton P and Chodosh LA. Oncogene pathway activation in mammary tumors dictates [^{18}F]-FDG-PET uptake. *Cancer Research*, 2014 [Epub ahead of print].