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 717-783-2548.

1. **Grantee Institution:** Lincoln University

2. **Reporting Period (start and end date of grant award period):** January 1, 2010 – June 30, 2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Susan E. Safford, Ph. D.

4. **Grant Contact Person's Telephone Number:** 484-365-7512

5. **Grant SAP Number:** 4100050900

Project Number and Title of Research Project: 1-Polymorphisms in the 1,25D3-MARRS Receptor: Characterization and Association with Serum Vitamin D Levels

6. **Start and End Date of Research Project:** Jan 1, 2010-June 30, 2012

7. **Name of Principal Investigator for the Research Project:** Susan E. Safford

8. **Research Project Expenses**.

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

   $ 23037.91

   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	Position Title	% of Effort on Project	Cost
None			

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	Position Title	% of Effort on Project
Safford, S.	Principal Investigator	10%
Wilson, R.	Co-Principal Investigator	5%

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___X_____ No_____

If yes, please indicate the source and amount of other funds: RIMI mini-grant; $25,000

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_____ No___X_____

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 to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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: Robin Wilson and I are planning to write a grant to by June 2013, that uses these data with additional data both of us generated from other projects.

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

The research plan is to investigate the potential interactions between polymorphic variants of nVDR and CYP24A1, and the wild-type 1,25D_3-MARRS and to look for isoforms of 1,25D_3-MARRS that may be splice variants and are not genetically encoded. We will also look at these protein:protein interactions in at least two cell types, as 1,25D_3-MARRS involvement in specific functions seems to be tissue and age-dependent

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_____ No_____X_____

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

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

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

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

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.

The Principal Investigator participated in genomic research, which is a new field for her. She was able to obtain an additional cell line and recombinant DNA constructs for nVDR to pursue new lines of research at her home institution.

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__X_____ No_____

If yes, please describe the collaborations:

This project involved collaboration with Penn State College of Medicine in Hershey as they were the depository of the human samples used and this is where the PI received training in genomic research.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). 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. Provide detailed results of the project. 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.

Introduction and Specific Aims

The long range research goal of my laboratory is to understand the structure:function relationship of 1,25D_3-MARRS, a novel membrane binding protein for calcitriol [1,25$(OH)_2D_3$], the active form of vitamin D. Part of this research has involved studies to determine the frequency of specific polymorphisms within seven of the 13 exons that make-up 1,25D_3-MARRS. Polymorphisms can alter the structure and therefore the function of a protein. The current project focused on two of those seven exons. An investigation into the possible incidence of polymorphisms in exons of 1,25D_3-MARRS was of interest as several earlier studies have shown that specific polymorphisms in the nuclear vitamin D receptor (VDR) are associated with either increased or decreased risk of certain cancers (Holick *et al*., 2007; Roff and Wilson, 2008; Bai *et al*., 2009; Tamez *et al*., 2009). The results of these studies suggest that the correlations between increased or decreased cancer risks and 1,25$(OH)_2D_3$ may be associated with receptor polymorphisms and not serum 1,25$(OH)_2D_3$ levels. Another recent study showed that the anti-

proliferative effect of $1,25(OH)_2D_3$ continued even after knock down of VDR (Costa *et al*., 2009), suggesting that some of the anti-cancer actions attributed to $1,25(OH)_2D_3$ may be due to its interaction with a receptor other than the classical VDR. It is, therefore, possible that polymorphisms in the membrane-binding protein, $1,25D_3$-MARRS also influence $1,25(OH)_2D_3$ effects on cancer. Thus, one broad research objective of this study was to determine the frequency of specific polymorphisms in$1,25D_3$-MARRS, in a healthy population. Another broad effect was to study the interactions between two distinct variants of VDR and $1,25D_3$-MARRS in a lung carcinoma cell line (H1299). The effects of interactions between VDR and $1,25D_3$-MARRS or their response elements on specific gene promoters may provide necessary information to better design treatments for specific cancers. Tissue and blood samples from a healthy population that were earlier obtained as part of an ongoing study were available through the Pennsylvania State University College of Medicine. Serum vitamin D levels, incidence of polymorphisms in the VDRE of the CYP24A1 promoter, and frequency of occurrence of short and long forms of VDR have been measured on these samples.

The Specific Aims were:

1. To identify existing and novel polymorphisms in $1,25D_3$-MARRS and estimate their prevalence in a healthy population through screening a sub-sample (N=25) and genotyping (N=100)

2. To determine whether there are differences in transactivation of vitamin D-responsive genes related to polymorphisms in the $1,25D_3$-MARRS receptor.

Materials and Methods

Subjects

The subjects we used were a subset of ones used previously in Dr. Wilson's laboratory. Their description was published in Roff and Wilson (2008) and is briefly described here. We selected healthy individuals who classified themselves as at least 50% African American and who were recruited at Pennsylvania State University General Clinical Research Center during the winter and spring months of 2007. Each participant donated a venous blood sample that was used to prepare dried blood spot cards and for Ficoll separation with Ficoll-Paque PLUS (GE Healthcare) to obtain lymphocytes.

DNA isolation and sequencing to screen for 1,25D3-MARRS (ERp57; PDAI3) polymorphisms in exons 1 and 6

Genomic DNA was isolated from dried blood spot cards from 25 randomly selected African American participants using the QIAamp DNA Micro Kit (QIAGEN). A literature search was conducted to identify single nucleotide polymorphisms (SNPs) found in $1,25D_3$-MARRS, and several from exons 1 and 6 were identified as asynchronous and likely to be responsible for functional differences (Table 1). A 926-bp region encompassing exon 1 and a 220-bp region encompassing exon 6 of $1,25D_3$-MARRS were PCR-amplified using Phusion DNA polymerase (New England Biolabs) and primers for each exon as shown in Table 2. The PCR products were run on 1% agarose gels in tris borate EDTA buffer containing 0.1% Syber-Safe DNA stain (Gibco). The desired PCR products were gel-isolated using the QIAquick Gel Extraction Kit (QIAGEN), run on agarose gels as described above to verify DNA recovery, and sequenced using the forward PCR primer at the University of Pennsylvania School of Medicine DNA Sequencing Facility by automated cycle sequencing.

SNP genotyping for new polymorphisms
Custom TaqMan SNP Genotyping assays (Applied Biosystems) were designed for literature-identified SNPs when PCR and sequencing failed to identify any SNPs in the test population. Quantitative real-time PCR was conducted at the Functional Genomics Core Facility at the Penn State College of Medicine using 10 ng of genomic DNA per assay.

Table 1. Known Polymorphisms of Exons I and VI of 1,25D_3-MARRS.

Exon #	Nucleotide Position	Nucleotide Change	Amino Acid Position	Amino Acid Change	Is Change Assymetric?
1	261	CGC to CTC	38	Arginine to leucine	Y
1	174	T-TC to TCTC	9	Frameshift; phenylalanine to serine	Y
1	310	GCC to GCT	54	Alanine to alanine	N
1	222	TCC to TTC	25	Serine to phenylalanine	Y
1	304	TTC to TTT	52	Phenylalanine to phenylalanine	N
1	269	GAC to TAC	41	Aspartic Acid to Tyrosine	Y
6	819	GAG to GTG	224	Glutamic Acid to Valine	Y
6	793	TTT to TTG	215	Phenylalanine to Leucine	Y

Table 2. Primer Sequences for Exons 1 and 6

Exon	Expected PCR Product Size	Forward Primer Sequence	Reverse Primer Sequence
I	926	5' – CTC CTC CAT TCT CGC TTC TG – 3'	5' – AGG AAG TGC CAC AAG AGC AC – 3'
VI	220	5' – TTA AGG GGT ATC ATC TTA TTT CGT C – 3'	5' – TTC TAA CAC CCA CAC ATT ACT GC – 3'

Results

PCR Amplification and Sequencing

Test DNA amplified with primers for exons 1 (Figure 1) and 6 (Figure 2) produced single bands of the expected sizes.

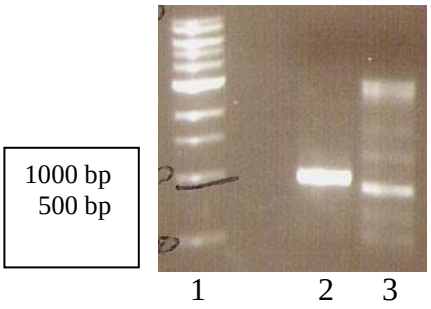


Figure 1. PCR amplification of exon 1 with DMSO (lane 2) and without DMSO (lane 3). DMSO is required to produce a single band.

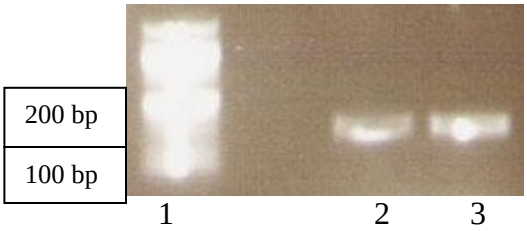


Figure 2. PCR amplification of exon 6 with DMSO (lane 2) and without DMSO (lane 3). Both conditions produced a single band of the expected size.

Sequencing

Exon 1: Chromatograms and interpreted sequences from the chromatograms indicated that there were multiple gene products produced through PCR (data not shown). Nested PCR in which smaller portions of amplified products are re-amplified with different primers failed to separate the multiple products. No further sequence analyses of this exon are being planned.

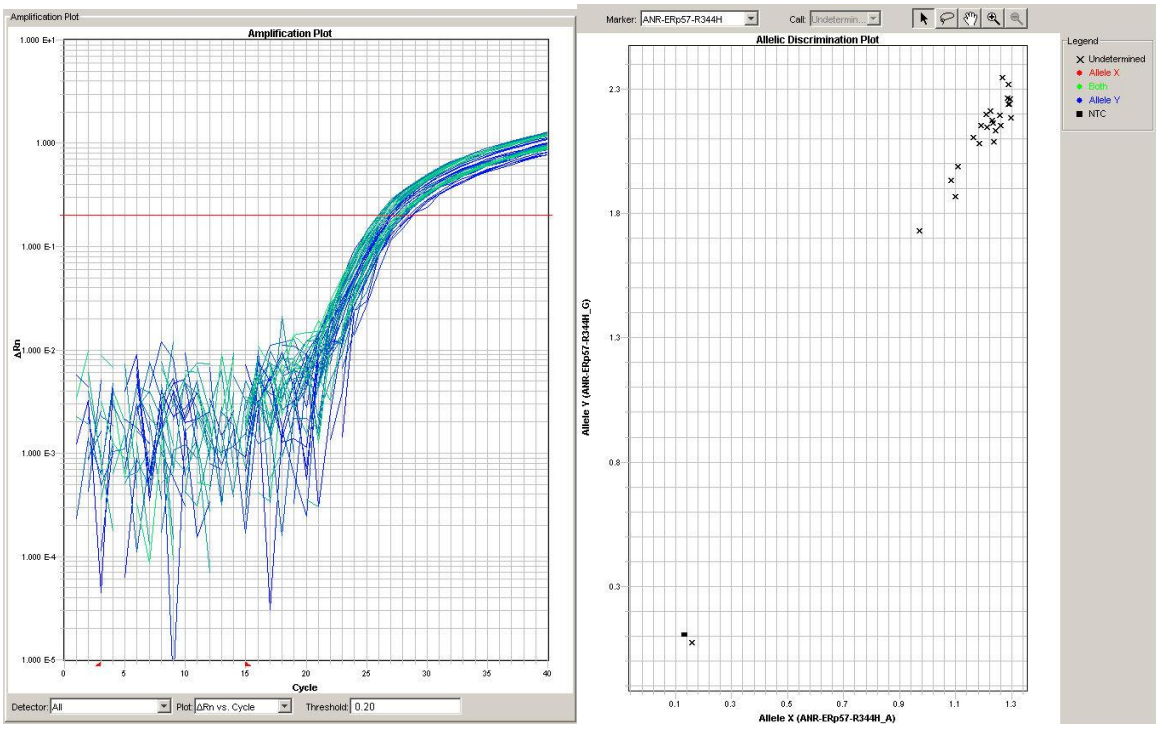
Exon 6: Chromatograms and interpreted sequences from the chromatograms show a single, identical sequence for the control and 25 patient DNA templates with no polymorphisms (data not shown).

Taqman Assays

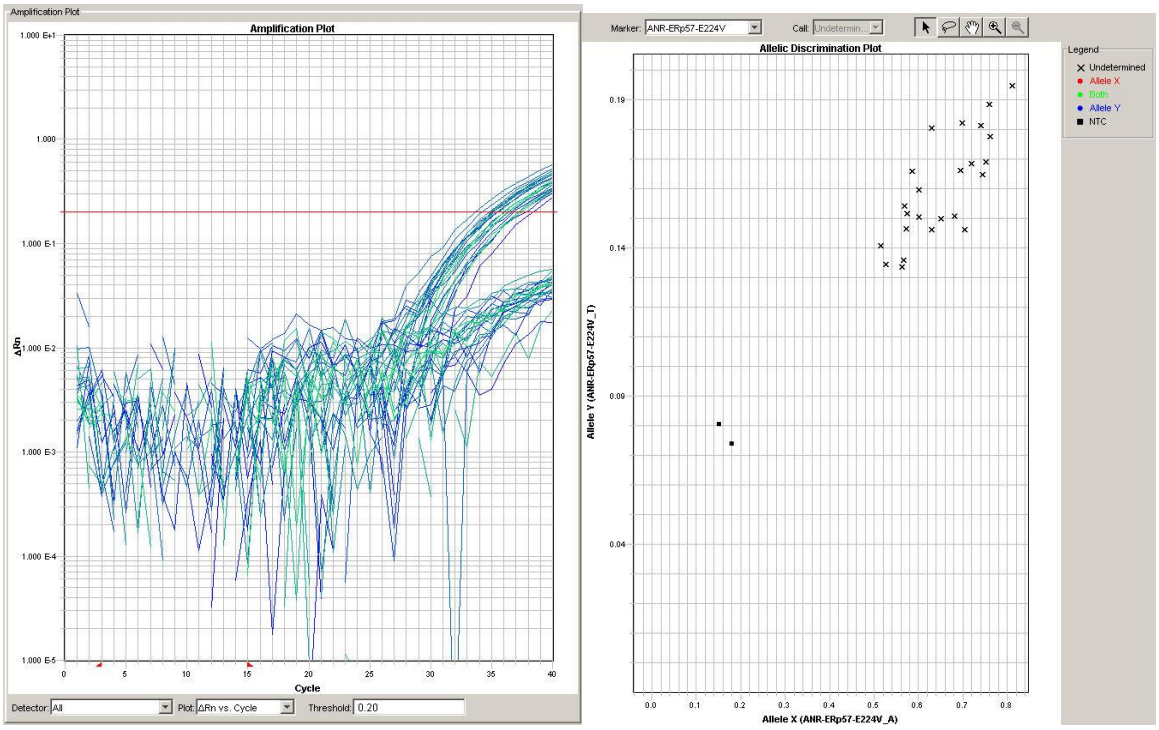
Neither the single previously available assay for exon 1 nor any of the custom designed assays produced any results. The probes either failed to amplify either allele or they amplified both in all samples (Figure 3). The control (MATP, last panel) worked as expected.

Figure 3. Taqman Genotype Assay Results for Exon 1.

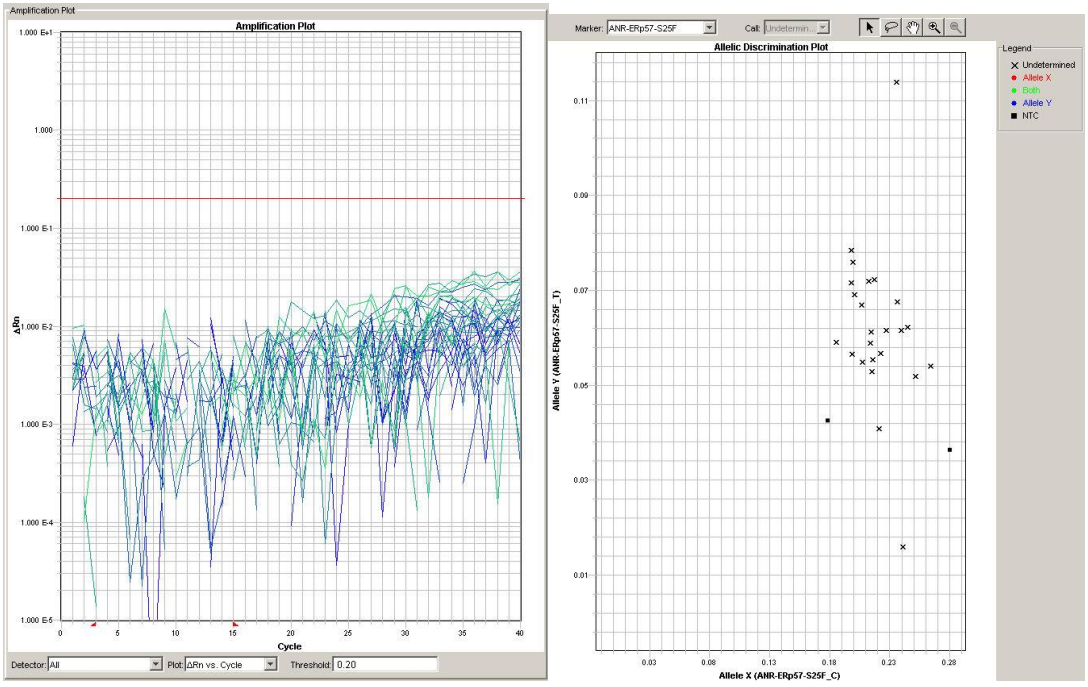
ERp57-R344H Failed (both probes amplified in all samples)



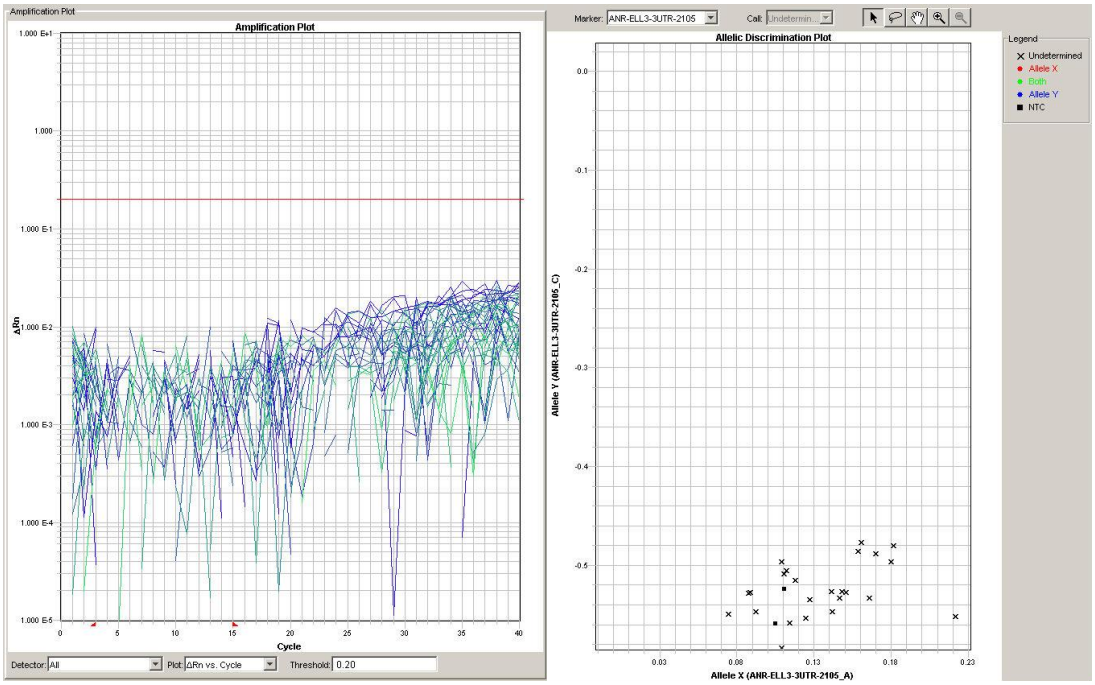
ERp57-E224V Failed (the one probe did not amplify in any samples)



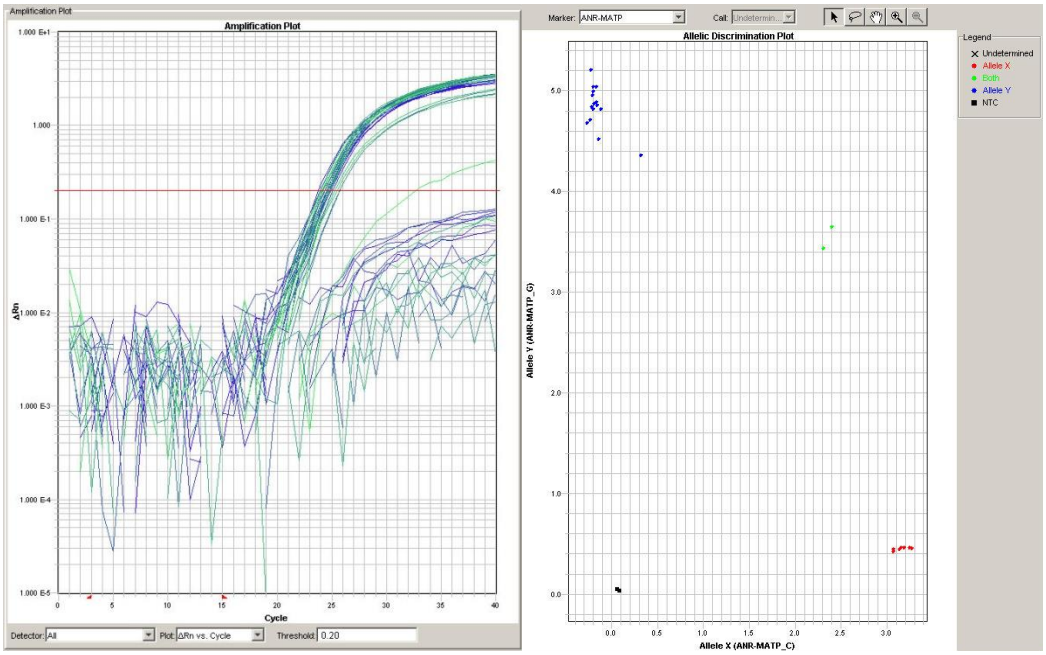
ERp57-S35F Failed (No amplification)



ELL3-3UTR-2105 Failed (no amplification)



MATP Worked



Conclusions

The DNA gels of PCR products that showed a single band of the expected size indicate that the proper PCR conditions and appropriate primers were used. The sequence results for exon 1 for which single product sequences could not be obtained combined with information from the

literature that multiple genes exist for this sequence provide strong evidence that reliable sequence results are not readily obtainable for this exon. The decision was made to not pursue this line of inquiry under the present funding at this time. The sequence results for exon 6 indicate that no polymorphisms were found for 25 subjects (50 DNA strands). This is a sufficiently large sample size to indicate that any polymorphism that may exist is present in this population at too low a frequency to provide significant information. Similar results from concurrent research projects showing a lack of polymorphic variants in other exons provide strong evidence that functional polymorphisms are not present in this gene.

The second specific aim was not pursued as the focus was to investigate the effects of polymorphisms in $1,25D_3$-MARRS on its interactions with two polymorphic variants of nVDR. As there are no polymorphic variants of $1,25D_3$-MARRS this specific aim could not be addressed. Interactions with wild-type $1,25D_3$-MARRS and the two polymorphisms of nVDR can be pursued, but the focus will then be on nVDR and not the novel receptor, $1,25D_3$-MARRS. Overall, these results suggest that any observed differences in anti-cancer effects of $1,25(OH)_2D_3$ that are mediated by 1,25D3-MARRS are probably due more to intracellular protein:protein interactions and transcriptional pathways than to genetic differences.

Literature Cited

Bai, Y., Yu, Y. Yu, B., Ge, J., Ji, J., Lu, H., Wei, J., Weng, Z., Tao, Z., and Lu, J. 2009. Association of vitamin D receptor polymorphisms with the risk of prostate cancer in the Han population of Southern China. BMC Med Genet. Dec. 4 (10): 125.

Costa, J.L., Eijk, P.P., van de Wiel, M.A., ten Berge, D., Schmitt, F., Narvaez, C.J. Welsh, J., Ylstra, B. Anti-proliferative action of vitamin D in MCF7 is still active after siRNA-VDR knock-down. BMC Genomics 10: 499.

Holick, C.N., Stanford, J.L., Kwon E.M., Ostrander, E.A., Nejentsev S., and Peters, U. 2007. Comprehensive association analysis of the vitamin D pathway genes, VDR, CYP27B1, and CYP24A1, in prostate cancer. Cancer Epidemiol Biomarkers Prevention 16(10):1990-9.

Roff, A. and R.T. Wilson. 2008. A novel SNP in a vitamin D response element of the CYP24A1 promoter reduces binding, transactivation , and gene expression. J Steroid Biochem Mol Biol (112): 47-54.

Tamez, S., Norizue, C., Ochiai, K., Takahashi, D., Shimojima, A. Tsutsumi, Y., Yanaihara, N., Tanaka, T., Okamoto, A., and Urashima, M. 2009. Vitamin D receptor polymorphisms and prognosis of patients with epithelial ovarian cancer. Br J Cancer 101(12):1957-60.

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer

Project 3 – Zhang – Publication 2 – Lung Cancer

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
2.				☐Submitted ☐Accepted ☐Published
3.				☐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: The results obtained were all negative. These are important results, but difficult to publish. These results will be published in Lincoln University's Science Journal, IHE within the next 6 months.

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.

None

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Susan E. Safford, Ph.D.
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Phone: (484) 365-7512 • Email: safford@lincoln.edu

(a) Education and Training

University of Southern Mississippi	Hattiesburg, MS	B.S. Biology	1977
University of Massachusetts at Amherst	Amherst, MA	M.S. Fishery Biology	1985
The University of Texas at Austin	Austin, TX	Ph.D. Zoology	1992
University of Kentucky	Lexington, KY	Post-doctoral Fellow	1992-1993

(b) Appointments

2008-present Professor, Dept. of Biology Lincoln University, Lincoln University, PA
2001-2008 Associate Professor, Dept. of Biology Lincoln University, Lincoln University, PA
1993-2001 Assistant Professor, Dept. of BiologyLincoln University, Lincoln University, PA
2007-Present Adjunct Professor, Dept. Biol. Science University of Delaware, Newark, DE
1994-2001 Part-time Instructor, Dept. Biol. Science University of Delaware, Newark, DE
Course and curriculum development: developed, implemented, and taught an Endocrinology class as an upper elective for Biology majors; it is currently being offered for the second time (Fall 2011)
Began the process of revamping the laboratory that accompanies Vertebrate Physiology, an upper level elective, through the purchase and use of iWORX physiographs, Fall 2010

(c) Publications

Brian J. Grindel, Rohe, B**., Safford, S.E.,** Bennett, J.J., and Farach-Carson, M.C. 2011. Tumor necrosis factor α treatment of HepG2 cells mobilizes a cytoplasmic pool of ERp57/1,25D_3-MARRS to the nucleus. *J Cell Biochem*. 112: 2606–2615.

Safford, S.E., Fawehinmi, M., Oyekanmi, O., Rohe, B., Nemere, I., and Farach-Carson, MC. 2008. Preparation of a stably transfected cell line with an expression vector for the 1,25D3-MARRS receptor. *IHE* 1(1): 41-48.

Rohe, B., **S.E. Safford**, I. Nemere, M.C. Farach-Carson. 2007. Regulation of expression of 1,25D_3-MARRS/ERp57/PDIA3 in rat IEC-6 cells by TGF□ and 1,25$(OH)_2D_3$. Steroids 72(7): 144-150.

Rohe, B., **S.E. Safford**, I. Nemere, and M.C. Farach-Carson. 2005. Identification and characterization of 1,25D_3-membrane-associated rapid response, steroid (1,25D_3-MARRS)-binding protein in rat IEC-6 cells. Steroids 70:458-463.

Nemere, I., M.C. Farach-Carson, B. Rohe, T.M. Sterling, A.W. Norman, B.D. Boyan, and **S.E. Safford**. 2004a. Ribozyme knockdown functionally links a 1,25(OH)2D_3 membrane binding protein (1,25D_3-MARRS) and phosphate uptake in intestinal cells. PNAS 101(19): 7392-7397.

Nemere, I., **S.E. Safford**, B. Rohe, M.M. DeSouza, M.C. Farach-Carson. 2004b. Identification and characterization of 1,25D_3-membrane associated rapid response, steroid (1,25D_3-MARRS) binding protein. J Steroid Biochem Mol Biol 89-90: 281-285.

Safford, S.E., Oberley, T.D., Urano, M. and St. Clair, D.K. 1994. Suppression of Fibrosarcoma Metastasis by Elevated Expression of Manganese Superoxide Dismutase. Cancer

Research (54): 4261-4265.

Safford, S.E. and H. Booke. 1992. Lack of Biochemical Genetic and Morphometric Evidence for Discrete Stocks of Northwest Atlantic Herring, *Clupea harengus harengus*. Fish. Bull. 90: 203-210.

Safford, S.E. 1991. A New Record of *Paulinella ovalis*: Filosea: Euglyphina. Contributions in Marine Science (32): 21-26.

(d) Current Funded Research Activity

September, 2010 – June 2012 **RIMI –** "Identification of Polymorphisms and Functional Mutations in 1,25D_3-*M*embrane *A*ssociated *R*apid *R*esponse *S*teroid-Binding (1,25D_3-MARRS) Protein". This grant provides $25,000 to identify additional polymorphisms in several more exons of the ERp57 gene and to explore the effect specific mutations in exon 1 have on calcitriol binding of ERp57.

September, 2008 – June, 2012 **NIH: SCORE 1** – "Investigations into the signaling pathways of 1,25D3-MARRS". This grant provides $175,000 a year for each of four years. The goals of this proposal are to identify proteins that interact with 1,25D3-MARRS in each of the main cell compartments through immunoprecipitation, cell compartmentalization, isoelectric focusing, and mass spectrophotometry.

September, 2009 – December, 2010 **NIH: SCORE 1 Administrative Supplement in Collaborative Science** – "Investigations into the signaling pathways of 1,25D3-MARRS". This grant provides $75,000. The goals of this proposal are to form a new collaboration (formed with Dr. Robin Wilson of Penn State Medical Center).

January 1, 2010 – June 30, 2012 – **CURE** – "Polymorphisms in the 1,25D_3-MARRS Receptor: Characterization and Association with serum vitamin D levels". This grant provides $28,000 for the entire period. The goal of this grant is investigate existing tissue and blood samples to determine the prevalence of polymorphisms in the 1, 25D_3-MARRS receptor.

(e) Synergistic Activities

IRB Member, Lincoln University
Research mentor to students, including underrepresented groups, in protein science and endocrinology research through an NSF LEAPS grant held by Lincoln's Science Dean
Recipient of the first Hildrus A. Poindexter Distinguished Research Award, Lincoln University Board of Trustees, 2006
Panel reviewer for NIH SCORE grant proposals, 2010

(f) Collaborators

Robin Taylor-Wilson, Penn State College of Medicine
David Usher and Robert Sikes, The University of Delaware