AWARD NUMBER: W81XWH-18-1-0173
GRANT12462693

TITLE: Proteomic-Based Biomarkers for Risk of Progression in Early Prostate Cancer

PRINCIPAL INVESTIGATOR: Justin R. Gregg, MD

CONTRACTING ORGANIZATION: University of Texas MD Anderson Cancer Center Houston, TX 77030-4009

REPORT DATE: AUGUST 2019

TYPE OF REPORT: Annual report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
AUGUST 2019	Annual	7/15/2018 – 07/14/19

4. TITLE AND SUBTITLE

Proteomic-Based Biomarkers for Risk of Progression in Early Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-18-1-0173

5b. GRANT NUMBER
GRANT12462693

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas MD Anderson Cancer Center
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Houston, TX 77030-4009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Active surveillance is an increasingly utilized strategy for the management of newly diagnosed localized prostate cancer, ideally limiting morbidity associated with local treatment while safely treating men with aggressive disease who are at risk of progression. Identification of non-invasive biomarkers of disease progression would help improve the care of men by determining who can safely be watched on surveillance (and avoid life-altering radical treatment). I performed a comparative analysis using untargeted proteomic data from mass spectrometry performed on the plasma of 16 active surveillance patients with early progression and 16 with indolent disease, obtaining candidate circulating proteins for association with disease aggression. This report details my work verifying candidate markers using ELISA on baseline prostate cancer patient plasma, and expanding the work to include more patients. It also discusses the career development and training that I was able to complete thanks to this grant, including online courses and grantsmanship training.

15. SUBJECT TERMS
Prostate cancer, proteomics, biomarker, active surveillance

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	20	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Active surveillance is an increasingly utilized strategy for the management of newly diagnosed localized prostate cancer, ideally limiting morbidity associated with local treatment while safely treating men with aggressive disease who are at risk of progression. Identification of non-invasive biomarkers of disease progression would help improve the care of men by determining who can safely be watched on surveillance (and avoid life-altering radical treatment). I performed a comparative analysis using untargeted proteomic data from mass spectrometry performed on the plasma of 16 active surveillance patients with early progression and 16 with indolent disease, obtaining candidate circulating proteins for association with disease aggression. This ongoing study is working to verify and expand this analysis, seeking to identify circulating proteomic-based markers of disease aggression.

2. KEYWORDS:

Proteomic; prostate cancer; active surveillance; biomarkers

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major task 1: training and education development
Subtask 1: audit courses on courera.org. **STATUS: complete** (certificates in appendix)
Subtask 2: present at department meeting. **STATUS: complete**. Ongoing presented at Division of Surgery grand rounds 5/8/19
Subtask 3: attend workshops related to grantsmanship. **STATUS: complete**. Attended full day RO1 grant writing workshop
Subtask 4: attend international scientific meeting. **STATUS: incomplete**
Major task 2: Candidate protein marker confirmation
Subtask 1: obtain, run and optimize confirmatory ELISA. **STATUS: 80% complete**.
Subtask 2: Analyze protein expression data. **STATUS: 80% complete**
Major task 3: Evaluate candidates as markers of progression
Subtask 1: Identify cases in the surveillance cohort for expanded ELISA use. **STATUS: complete**
Subtask 2: Determine if additional samples needed. **STATUS: complete**
Subtask 3: Quantify candidate markers of protein expression. STATUS: incomplete
Subtask 4: Analyze data. **STATUS: incomplete**

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and

negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The major activities for this reporting period surrounded training and education and deep investigation of biomarkers using ELISA. For each ELISA, a commercial kit was purchased and strips were verified with stock human plasma to ensure an appropriate dilution. Then, the dilution was performed using human samples of matched aggressive (early progression) and indolent (no progression over a number of years) baseline men from patients enrolled on active surveillance. ELISA was then performed as outlined in manufacturer instructions, and optical density measured in the laboratory. Results of ELISAs are found in the appendix. In short, while some suggestive trends exist, we have not yet confirmed a marker of interest to be associated with aggressive disease using the ELISA assay. Promising markers were expanded into a larger cohort to improve sample size, and results are included, as well. At present, no marker is clearly associated with aggressive disease, though this phase of study is ongoing.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Through this award I have been able to successfully complete a number of training and development activities. As listed in the above "accomplishments" section, I successfully completed two online courses related to statistics and genomics. I was able to broaden my understanding regarding the breadth of genomic studies that are possible, and also gained knowledge about the use and deployment of statistics, particularly as they pertain to biomarker analysis. I was also able to attend a professional development activity related to competition for RO1 awards. Here, I learned about the different categories of applications and also individual applicants. I also received tips regarding the format, composition, and narrative approach of RO1 submission. These experiences will prove invaluable in my future role as an independent investigator.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report at this time

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Professional and development goals as stated in this grant are met other than travel to an international meeting. I plan to attend the next meeting of the EAU, at which I will present findings obtained through this grant. I will use this opportunity to learn more about the field of prostate cancer and related biomarkers, and also to network with world renowned researchers, including discussions about future collaborations (if possible).

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

This work will likely lead to the use of ELISA technology to improve risk stratification of men with localized prostate cancer. While further work will need to be completed at the conclusion of the grant, there is a high likelihood that circulating markers of disease aggression will contribute to clinical management.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Note that prior to initiation of ELISA analyses, an error was noted in initial proteomic data extraction and normalization. Therefore, the analysis and normalization were repeated, leading to different targets of interest being investigated for verification using ELISA. Interestingly, multiple targets were mechanistically related to ongoing pathway analyses being completed by the research team through metabolomics-related investgations.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

In some cases, detection of ELISA optical density was not uniform. Some experiments were repeated, and after discussions with ELISA experts from Dr. Hanash's laboratory, we decided to initiate use of a DDM detergent to improve detection.

Additionally, thus far no target of interest has been confirmed to be clearly associated with progression. While we are still working on targets of interest, we have not pursued broad expansion to over 400 surveillance patients. We have expanded the verification cohort using additional matched aggressive and indolent cases, are utilizing additional proteomic data to identify new targets, and are considering pursuing alternative related pathways to offer further mechanistic insights. We have obtained a no-cost extension in order to complete this work during this academic year.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Material related to this grant was presented at the University of Texas MD Anderson Division of Surgery grand rounds on localized prostate cancer on 5/8/19.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*

- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

No change, the only funded individual is the PI, Justin R. Gregg. Core activities in Dr. Thompson's laboratory were funded as part of this grant as outlined in the budget and were performed by multiple individuals.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix 1: Certificates of completion of Coursera courses
Appendix 2: Results from ELISA assays

JOHNS HOPKINS
UNIVERSITY

COURSE CERTIFICATE

05/19/2018

Justin Gregg

has successfully completed

Introduction to Genomic Technologies

an online non-credit course authorized by Johns Hopkins University and offered through Coursera

EDUCATION FOR EVERYONE · coursera · COURSE CERTIFICATE

Steven L. Salzberg, PhD
McKusick-Nathans Institute of Genetic Medicine
Johns Hopkins University

Jeffrey Leek, PhD
Department of Biostatistics
Johns Hopkins Bloomberg School of Public Health

Verify at coursera.org/verify/H28WJ4SB5ZLA

Coursera has confirmed the identity of this individual and their participation in the course.

JOHNS HOPKINS
UNIVERSITY

COURSE CERTIFICATE

04/06/2018

Justin Gregg

has successfully completed

Statistics for Genomic Data Science

an online non-credit course authorized by Johns Hopkins University and offered through Coursera

Jeffrey Leek, PhD
Department of Biostatistics
Johns Hopkins Bloomberg School of Public Health

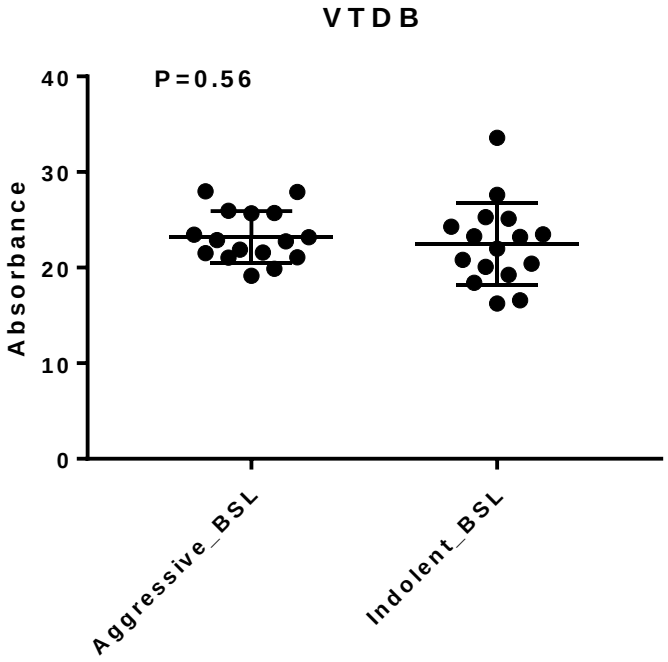
Verify at coursera.org/verify/24LC63ZJUC67

Coursera has confirmed the identity of this individual and their participation in the course.

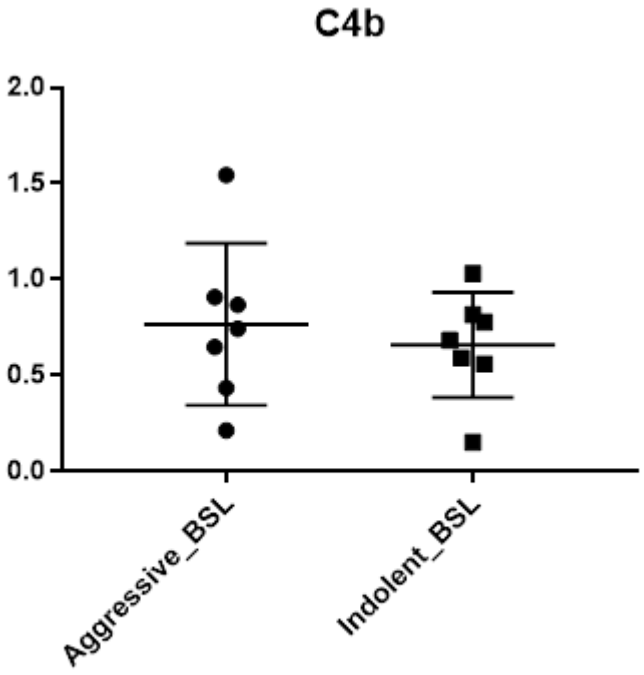
APPENDIX 2: Results of ELISA verification quantifying proteins of interest in baseline active surveillance patient plasma

ELISA performed in initial group of 16 cases and 16 controls:

1. Vitamin D Binding Protein



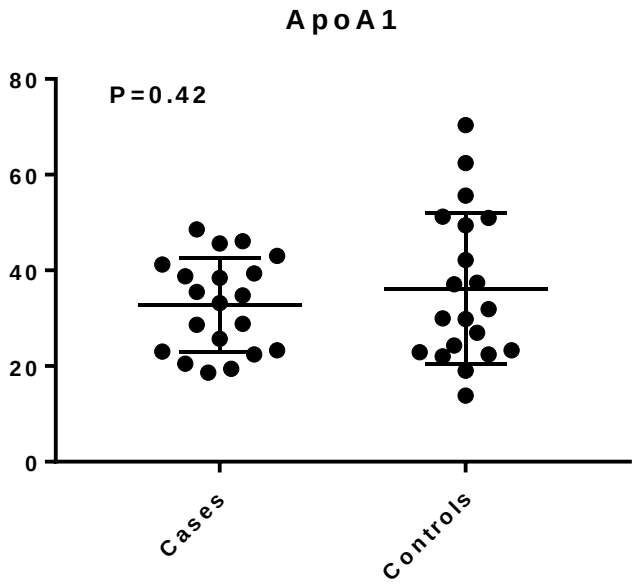
2. Complement factor C4b



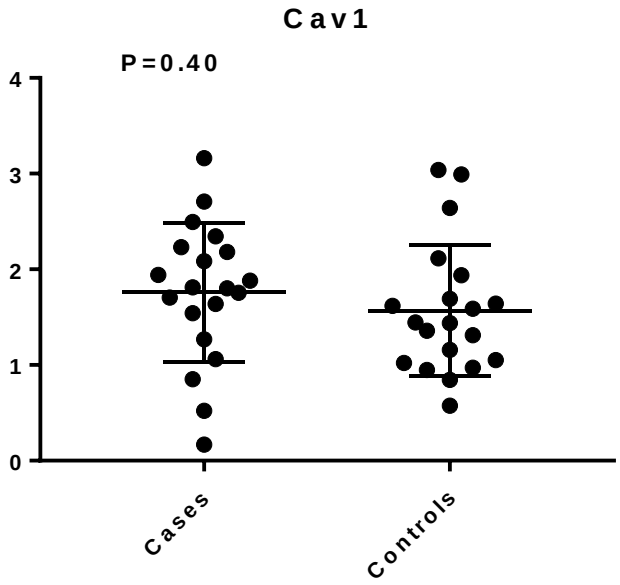
Note: These results may be limited, as dilution likely limited detection (many samples did not reach threshold for detection)

ELISA below performed in larger cohort 20 cases and 20 controls:

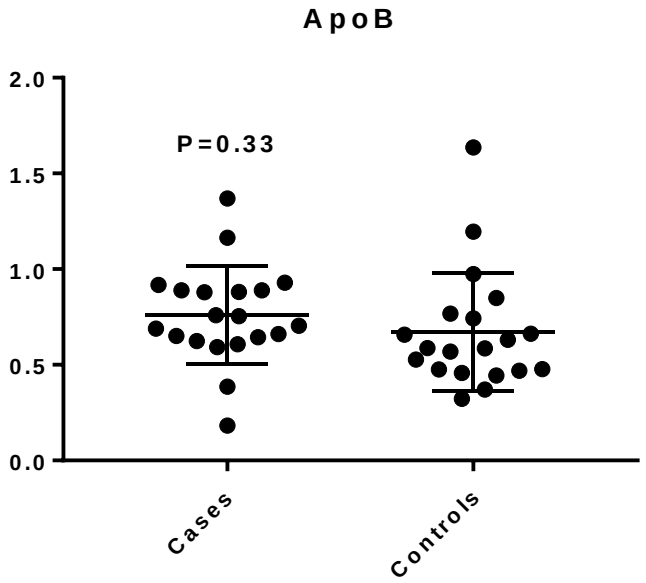
3. Apolipoprotein A1



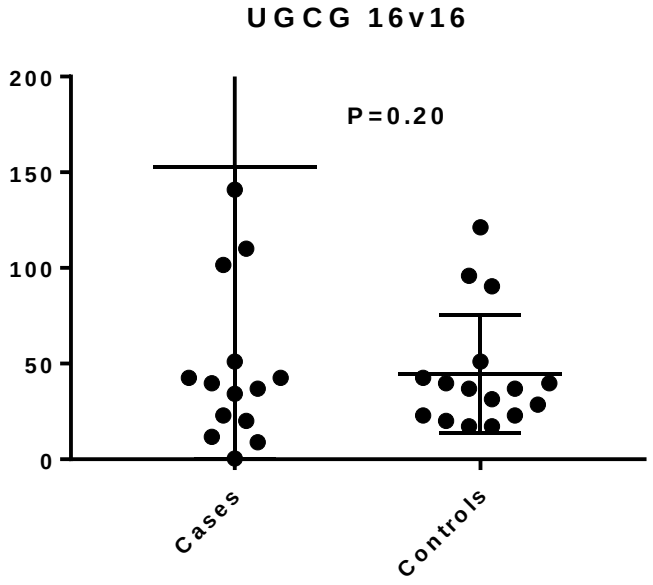
4. Caveolin-1



5. Apolipoprotein B

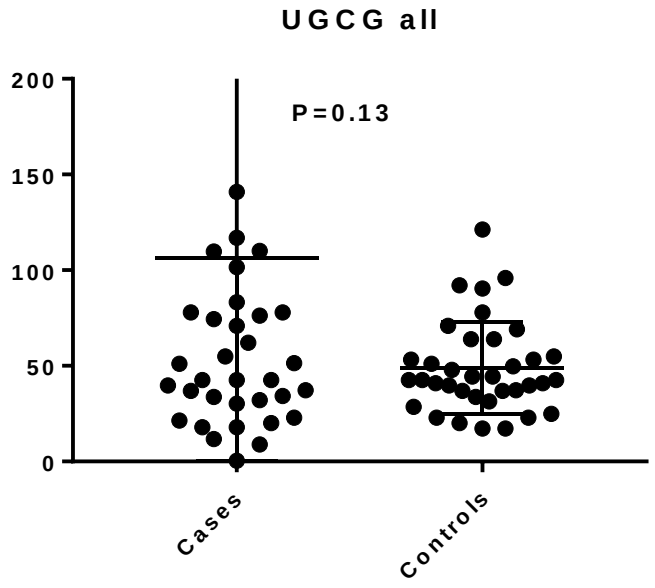


6. UDP-glucose ceramide glucosyltransferase



Please note that results appeared promising and therefore were expanded to a larger cohort.

36 cases and 36 controls:



Note here that many cases were at the low end of detection. Therefore, after discussion with members of Dr. Hanash's team, a detergent DDM was used to hopefully improve detection of UGCG presence.

Results using the detergent are shown below: