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TITLE: Rare Variants in Systemic Sclerosis (SSc, Scleroderma)

PRINCIPAL INVESTIGATOR: Maureen D. Mayes, MD, MPH

CONTRACTING ORGANIZATION: University of Texas Health Science Center

Houston, Texas 77030-5400

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14. ABSTRACT

The subject/topic area of this research is Systemic Sclerosis (SSc, Scleroderma). The purpose of the research is to identify genetic variants that contribute to SSc disease susceptibility and influence outcome. The approach involves whole genome sequencing of 100 trios (300 individuals including affected case and both parents). Previous Genome-Wide-Association-Studies (GWAS) have identified gene regions that are associated with disease but the majority of these are in non-coding areas so the impact of these variants is unclear. This study will identify rare variants (both inherited and de novo mutations) and will analyze these mutations according to the role they likely plan in disease pathogenesis. The immediate outcome of this project will be identification of the causal variants in multiple pathways associated with SSc susceptibility with the long-range impact will be the identification of the role these variants plan in disease causation and severity/outcome.

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1. **INTRODUCTION:** The subject/topic area of this research is Systemic Sclerosis (SSc, Scleroderma). The purpose of the research is to identify rare genetic variants that contribute to SSc disease susceptibility and influence outcome. The approach involves whole genome sequencing of 100 trios (total of 300 individuals including affected case and both parents). Previous Genome-Wide-Association-Studies (GWAS) have identified gene regions that are associated with disease but the majority of these are in non-coding areas so the impact of these variants is unclear. This study will identify rare variants (both inherited and de novo mutations) and will analyze these mutations according to the role they likely plan in disease pathogenesis. The immediate outcome of this project will be identification of the causal variants in multiple pathways associated with SSc susceptibility with the long-range impact being the identification of the role these variants plan in disease causation and severity/outcome.
2. **KEYWORDS:** Systemic Sclerosis, Scleroderma, Whole Genome Sequencing (WGS), genetic variants, rare variants.
3. **OVERALL PROJECT SUMMARY:** This study is on track for completion of the largest whole genome sequencing (WGS) project in scleroderma. The samples have been successfully sequenced and quality control procedures are being applied. The "raw" sequencing data have been uploaded to the DRAGEN (DRAGEN = **D**ynamic **R**ead **A**nalysis for **Gen**omics) platform at Baylor College of Medicine (see separate Progress Report by Co-PI Dr. Brendan Lee) for mapping, alignment, sorting, duplicate marking and variant calling. The analysis has just started for data interpretation, variant calling and identification of rare variants and de novo variants in individual cases and those in common among scleroderma cases. With these analyses, interpretation and genotype/phenotype correlations will be done.

   This will represent the largest, most complete and detailed whole genome sequencing (WGS) project done to date in scleroderma. It is anticipated that the data generated will lead to multiple publications as well as additional avenues of investigation and, finally, insight regarding susceptibility and pathogenesis.

4. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**
     - Task 1: Institutional Review Board (IRB) and DOD Human Research Protection Office (HRPO) approval. Timeline months 1-6 (15 Aug 2018 – 14 Jan 2019); actually HRPO approval was received later than expected on 11 Jul 2019 due to a miscommunication– but this has been resolved..
       - UT Houston IRB approval was obtained previously under the general approval for the genetics studies in SSc; a notice was sent to the IRB and accepted as a defined sub-study with the most recent annual reviews up-to-date as of 22 Jan 2019; so **completed**.
       - (Note Baylor College of Medicine – BCM - IRB approval is handled by the Collaborating Investigator, Dr. Brendan Lee, in his report); **completed**.
       - HRPO approval was received 11 Jul 2019 (HRPO Log Number E00551.1a) so **completed**.
     - Task 2: Prioritization, preparation and distribution of samples to be sent to BCM for genotyping: Timeline months 7- 8 (15 Feb 2019 – 14 Apr 2019)

        - Samples were identified on the basis of protocol-defined criteria and prepared for shipping during this time and were subsequently sent to BCM. So **completed.**

    - Task 3: Sequencing of 300 samples with appropriate quality control measures: Timeline months 9-18 (15 Apr 2019 – 14 Feb 2020)

        - All samples were sent to BCM for whole genome sequencing; of the samples sent 2 failed quality control measures and new replacement aliquots were prepared and sent to BCM on 19 Aug 2019. So **completed.**

    - Task 4: Processing, including imputation using the generated sequence data. Timeline months 19-21 (15 Feb 2020 – 14 May 2020). **The sequencing data have been generated and uploaded to the DRAGEN platform for alignment and preparation for variant calling – completed.**

    - Task 5: Data analysis and prioritization of candidates for validation in other, future cohorts. Timeline months 22-36 (15 May 2020 – 14 Aug 2021). **This is now in process.**

    - Task 6: Association analysis of the most likely identified variants with clinical disease features. Timeline months 34-36 (15 May 2021 – 14 Aug 2012). **Awaiting data from Task 5**

    - Task 7: Preparation of manuscripts for publication. Timeline months 34-36 (15 May 2021– 14 Aug 2021). **Awaiting data from Tasks 5 and 6**.

    - Task 8: Quarterly meetings between the UT-H and BCM teams to coordinate all aspects of the project and review and interpret data as it becomes available. Timeline months 3-36 (15 Nov 2019 – 14 Aug 2021). **Ongoing.**

        - We are now **meeting monthly** in virtual capacity (via Zoom) due to the pandemic. Monthly meetings are considered necessary at this stage due to the volume of data and complexity of the analysis. We are up-to-date on these and plan to continue with monthly meetings in this exciting phase of the project.

- **What was accomplished under these goals?**

    - Tasks 1, 2, 3 and 4 have been completed; Task 5 is well underway with analysis of sequencing data and variant calling expected to be completed in the next few months. Tasks 6 and 7 should be accomplished in the next and final year of the project. Task 8 (quarterly meetings – actually now monthly meetings of the UT-H and BCM teams) is ongoing as scheduled.

- **What opportunities for training and professional development has the project provided?**

    - Nothing to report**.**

- **How were the results disseminated to communities of interest?**

    - Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

        - Now that the sequencing data have been generated and uloaded to the DRAGEN platform for variant calling, we fully anticipate that the analysis of rare and de novo variants will be completed on time in the final year fof the project with genotype-phenotype correlations. Validation in a larger cohort should be done as well.

5. **IMPACT:** *Describe 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?**
        - Nothing to Report.
        - Nothing to Report at this time.
    - **What was the impact on other disciplines?**
        - Nothing to Report.
    - **What was the impact on technology transfer?**
        - Nothing to Report.
    - **What was the impact on society beyond science and technology?**
        - Nothing to Report.
6. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:*
    - **Changes in approach and reasons for change**
        - There have been no changes in approach.
    - **Actual or anticipated problems or delays and actions or plans to resolve them**
        - There was a delay in HRPO approval due to a miscommunication, but this was resolved and the project was able to move forward in a timely fashion.
    - **Changes that had a significant impact on expenditures**
        - There were no changes that had a significant impact on expenditures.
    - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
        - None
    - **Significant changes in use or care of human subjects - None**
    - **Significant changes in use or care of vertebrate animals. None**
    - **Significant changes in use of biohazards and/or select agents None**
7. **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**

  - **Journal publications.** None to date.
  - **Books or other non-periodical, one-time publications.** None to date.
  - **Other publications, conference papers, and presentations.** None to date.

- **Website(s) or other Internet site(s)**
  None to date.
- **Technologies or techniques**
  None to date.
- **Inventions, patent applications, and/or licenses**
  None to date.
- **Other Products**
  None to date.

8. **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."*

Name:	Maureen D. Mayes, MD, MPH (No change from submission)
Project Role:	Principal Investigator; Initiating PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID = 0000-0001-5070-2535
Nearest person month worked:	3.0 calendar months
Contribution to Project:	Dr. Mayes is responsible for the overall conduct of the study and for the timely completion of all aspects (Tasks 1 through 8); she supervises UT project personnel and organizes the weekly UT meetings, the now monthly UT-BCM team meetings to review progress, potential problems, data collection and results.
Funding Support:	**New:** No new sources of funding support since last annual progress report (9/14/2019) Other projects, listed previously, are ongoing.

Name:	Dianna Milewicz, MD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6 calendar months
Contribution to Project:	Dr. Milewicz has a strong background in the genetic basis of vascular diseases. Her role to date is that she has provided guidance on study design thus far and in the upcoming years she will advise on the analysis and interpretation of the genetic data. She has served as an advisor on Dr. Mayes' previous scleroderma genetic studies and has worked with the Baylor College of Medicine Genetics group on multiple projects
Funding Support:	**New since last report:** NIH/NHLBI: Genetic predisposition to thoracic aortic aneurysms/dissections. John Ritter Foundation: John Ritter research program in aortic and vascular diseases NIH/NCATS: Center for clinical and translational sciences Genetic Aortic Disorders Association Canada: MAC maintenance for future expansion to MSC2-HTAD NIH/NHLBI: Genetic basis of early onset bicuspid aortic valve disease

Name:	Claudia Pedroza, PhD (no change from submission)
Project Role:	Co-Investigator, statistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6 calendar months
Contribution to Project:	Dr. Pedroza has been involved in the planning and implementation of the project to date to ensure that data will be interpreted in light of available clinical outcomes.
Funding Support:	No changes

Name:	Patricia Gonzales, LVN (no change from submission)
Project Role:	Project Coordinator
Researcher Identifier (e.g. ORCID ID):	Not Applicable
Nearest person month worked:	3.6 calendar months
Contribution to Project:	Ms Gonzales is responsible for the day-to-day operations of the study, overseeing database queries and reporting to the investigators regarding progress, time lines and review of expenditures.
Funding Support:	

Name:	Julio Charles (no change from submission)
Project Role:	Laboratory Manager
Researcher Identifier (e.g. ORCID ID):	Not applicable
Nearest person month worked:	3.0 calendar months
Contribution to Project:	Mr Charles is responsible for overseeing the selection, aliquoting and distribution of samples for genotyping and sequencing studies. He coordinates delivery to the Baylor research lab; he attends the weekly lab meetings as well as the project-specific quarterly meetings between the UT and Baylor research groups.

Funding Support:	

Name:	Hau Pham (no change from submission)
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	Not applicable
Nearest person month worked:	3.0 calendar months
Contribution to Project:	Ms. Pham is responsible for the day-to-day work in the Rheumatology Research Lab, to implement sample selection, DNA quantification, DNA measurement and record keeping of samples distributed to Baylor as well as DNA extraction on new samples as needed, and autoantibody determination on these new samples.
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - None
- **What other organizations were involved as partners?**
  - Note: Dr. Brendan Lee at Baylor College of Medicine was included in the original application as Collaborating/Partnering PI. and no changes have been made to this relationship; he continues as Collaborating/Partnering PI. As collaborating PI he will submit his own annual report.

9. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** The Collaborating PI (Dr. Brendan Lee, Baylor College of Medicine) will submit a separate report as required.
   - **QUAD CHARTS:** The Quad chart will be attached and uploaded on the website.
10. **APPENDICES:** Not applicable.