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TITLE: Functional Genetics for Predisposition to Development of Type 2 Diabetes in Obese Individuals

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

T2D frequently occurs together with obesity. In fact, the vast majorities of T2D individuals from both civilian and Veteran populations are or have been overweight or obese. Surprisingly, the majority of the obese subjects, both Veterans and non-veterans, will never develop T2D and their metabolic tissues will never lose the ability to respond to insulin. This fact indicates the critical role of the genetic predisposition to T2D development in obesity. The proposal assesses for the first time if pathogenic single nucleotide variations (SNVs) within three genes, i.e., *PIKFYVE*, *FIG4* and/or *VAC14*, found previously to be involved in glucose homeostasis, are linked with T2D development in obese individuals. The expected changes will be related to functional differences in the performance of the PIKfyve enzymatic activity. A total of 96 obese patients with or without T2D (DM and non-DM), 48 per group, with similar average BMI and age have been selected and included in the analyses. Fat tissue for analyses has been collected and the DNA was isolated. Samples were subjected to next-generation exome sequencing. Several promising SNVs in the three genes, with $p < 0.05$, associated specifically with T2D in the obese individuals have been already identified. Determining their position within the gene and the potential functional outcome is currently in progress.

15. SUBJECT TERMS

type 2 diabetes mellitus; morbid obesity; PIKFYVE, FIG4 and VAC14 genes; single nucleotide variations

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1. **INTRODUCTION:** Patients suffering from Type 2 Diabetes mellitus (T2D) are usually overweight or obese. However, only a small fraction of even morbidly obese individuals develops T2D. Since T2D is a familial disease, a genetic predisposition for the development of T2D is expected to play a major role in obese individuals. Basic studies have shown that manipulating the levels of 3 cellular proteins -PIKfyve (encoded by *PIKFYVE* gene), Sac3 (encoded by *FIG4* gene) and ArPIKfyve (encoded by *VAC14* gene) – which associate in a functional complex, markedly affects insulin sensitivity of fat cells. Furthermore, knockout of mouse *pikfyve* selectively in muscle or fat cells causes whole body insulin resistance in transgenic mice. Therefore, it is plausible that mutations in the three genes of interest could cause tissue insulin resistance and thereby predispose the obese individuals to develop T2D.

2. **KEYWORDS:** T2D (type 2 diabetes mellitus); morbid obesity; PIKFYVE, FIG4 and VAC14 genes; single nucleotide variations

3. **ACCOMPLISHMENTS:** It should be mentioned that there was misunderstanding related to the start day of this award. Whereas our University official and myself were informed by DoD that the start day will be June 15, 2017 as we requested, in reality the start day given by DoD was April 15, 2017. The later start day was requested based on the delayed decision by the our University IRB committee. However, DoD assured us that I will be allowed to receive 1-year no-cost extension. Therefore, our actual work on the project has begun 2 months after the official day of the award due to administrative miscommunication.

The proposed goals of the project for the first year are completed according to our SOW. Namely the goals for the first year as appeared in our SOW (Table 1) were:

Table 1: Statement of work actual start date: **June 15/2017**

Specific Aim 1: Identify non-synonymous single nucleotide variations (SNVs) within *PIKFYVE*, *FIG4* or *VAC14* genes specifically associated with T2D in obesity by exome sequencing:	**Timeline Months (-)1-7**	Accomplished
Major Task 1 Request for approval for research with Human Anatomical Substances and assembly of the two groups		
Subtask 1: U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO) approval prior to research implementation for the entire study	(-)1-2	Accomplished
Subtask 2: Local IRB/EC Approval for the entire study	(-)1-2	Accomplished
Subtask 3: Evaluate and Divide participants to two groups: Obese with	3	Accomplished

T2D and Obese without T2D		
Major Task 2 **Isolate genomic DNA and perform whole exome sequencing**		
Subtask 1: Isolate genomic DNA	4-5	Accomplished
Subtask 2: Exome sequencing on fee-for-service basis by next generation sequencing technology at the Wayne State University Core for Applied Genomics Technology Center (AGTC). I have already consulted with Dr. Susan Land, Director of the AGTC Core, who has agreed to accept and prioritize our project.	6	Accomplished
Subtask 3: Perform bioinformatics consulting with bioinformatician about the computational predictions from all available web servers.	7	Accomplished
Milestone(s) Achieved: Identified are coding SNVs within the three genes associated with T2D in obesity	7	Accomplished
Specific Aim 2: Establish T2D-associated coding SNVs in *PIKFYVE*, *FIG4* and/or *VAC14* in obesity, leading to quantitative differences in synthesis of PI(3,5)P2 and/or PI5P	Months 8-18	
Major Task 3 **Prioritize SNVs for analyses**		
Subtask 1: Analyze the coding SNVs relative to its localization in the protein itself and in the triple protein complex using the interaction map. Prioritize SNVs	8	In process
Subtask 2: Select fat tissue with the prioritized SNVs	9	In process
Milestone(s) Achieved: Selected are ~50 priority T2D-causing SNVs in the three proteins	9	In process
Subtask 3: Perform functional assays of the lipid kinase activity and analyze proteins levels by WB simultaneously in order to thaw tissue only once.	9-13	In process
Subtask 4: Mutagenesis to introduce the variations in the cDNA and cell transfections	14-16	In process
Subtask 5: Relate changes in protein levels with the data from the functional assay	17	In process
Milestone(s) Achieved: Design a map with T2D-causing SNVs in the three proteins that alter synthesis of PI(3,5)P2 and PI5P	18	In process
Subtask 7: Deposit the identified T2D-causing SNVs in the three proteins that alter synthesis of PI(3,5)P2 and PI5P in the related webservers	18	In process

- ***What was accomplished under the above goals:***

1) As proposed, ninety six (96) morbidly obese individuals with or without T2D were selected and were matched by gender and race [AA (African American), W (White)] as shown in the Table 2 below:

Female				Male			
Obese non-Diabetic		Obese Diabetic		Obese non-Diabetic		Obese Diabetic	
32		32		16		16	
AA	W	AA	W	AA	W	AA	W
21	11	21	11	10	6	10	6

Table 2: Selected individuals for the study

The age between the obese diabetic group (a total of 48) and obese non-diabetic group (a total of 48) were not statistically different (Table 3)

	Obese non-Diabetic	Obese Diabetic
Total	48	48
Age	mean = 42.0 ± 2.85	mean = 47.0 ± 1.5
BMI	mean = 49.4 ± 1.06	mean = 47.9 ± 1.01

Table 3: Age and BMI of the groups

2) As proposed, the frozen samples of abdominal fat from these individuals, collected during laparoscopic bariatric surgery of morbidly obese individuals were successfully used for genomic DNA extraction and next generation exome sequencing of the genes of interest. As proposed, we used fee-for-service Illumina next-generation sequencing housed by the AGTC/Karmanos Genomics Core for the Cancer center located in our Institution.

3) The result of the exome sequences came back to us about 2 months ago. We focused on the sequences of the 3 genes of interest, namely *PIKFYVE, VAC14* and *FIG4* as proposed. In those three, we identified numerous (>400) SNPs.

4) We subjected the SNPs to analysis for the association between polymorphisms and T2D using a logistic regression model, by adjusting age, gender and BMI. Associations were examined in each of the two populations separately.

5) So far, our bioinformatics analysis indicated that in the African American (AA) population, a total of 13 SNPs identified in the three gene sequences were significantly ($p<0.05$) or marginally ($p<0.1$) associated with T2D. In the Caucasian population, as many as 30 polymorphisms were identified in the exome sequencing with statistical ($p<0.05$) or marginal ($p<0.1$) significance to be associated T2D.

6) In summary, all planned activities specified in Specific aim 1 of the SOW are successfully completed and we are beginning the detailed analysis of the SNVs associated with T2D in the morbidly obese patients.

- ***What do you plan to do during the next reporting period and beyond***

We will continue with our tasks as specified in the Table of Statement of work. Specifically:

1 - We will perform a quality control with a HWE (Hardy-Weinberg equilibrium) test;

2 - We will further validated the established polymorphisms by using independent genotyping techniques;

3 - We will progress with the in vitro analyses of the PIKfyve product PtdIns(3,5)P_2 and PtdIns5P of the samples that indicate statistically significant SNP. This will allow us to make an association between polymorphisms and enzymatic activities.

4. **IMPACT:** The preliminary analysis supports the hypothesis that single nucleotide variations in the genes of interest are associated with T2D in certain morbidly obese patients. At this point it is early to determine the impact on other disciplines, on technology transfer and on society.

5. **CHANGES/PROBLEMS:** Besides the shifted start day (from June 15 to April 15) there are no problems to report so far. We expect to request and to be granted a one year no-cost extension to complete the proposed project

6. **PRODUCTS**: An Abstract has been submitted in the 2018 Annual Meeting of the American Diabetes Association.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS: The following individuals**

Name, ID	Assia Shisheva
Role	Principal Investigator
Contributions	Managing all activities
Nearest Month worked	1.7
Funding support	none

Name, ID	Berhane Seyom
Role	Co-Investigator
Contributions	Provided all specimens, helped organizing matched subgroups for the study
Nearest Month worked	0.6
Funding support	None

Name	Diego Sbrissa
Role	Researcher
Contributions	Worked on organizing all samples and details of DNA extraction and quality for next generation sequencing
Nearest Month worked	12
Funding support	None

Name	Ognian Ikonomov
Role	Part-time (hourly) technician
Contributions	Helped with IRB protocol preparation and initial analyses and bioinformatics
Nearest Month worked	Hourly based
Funding support	None

8. **SPECIAL REPORTING REQUIREMENTS**: As indicated above, we started later than the indicated date, April 15, 2017 as our IRB required longer than anticipated time to be approved by our University IRB committee and that by the DoD. Due to these early start date and the delay of the IRBs, and the PI will require one additional year to complete the project

9. **APPENDICES:** None