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TITLE: Integrating Environmental, Genomic, and Functional Data to Characterize Individual Risk for Parkinson's Disease

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CONTRACTING ORGANIZATION: Northern California Institute for Research (NCIRE)
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14. ABSTRACT

The overall purpose of this work is to identify methods for characterizing which individuals may be particularly susceptible to specific toxic insults, and the underlying mechanisms. We have three Aims: 1) use iPSC-derived neuronal cell lines to validate and extend our prior work that found an 11-fold increased risk of Parkinson's disease (PD) in persons exposed to the herbicide paraquat who lacked functional glutathione-S-transferase T1 (GSTT1), a key metabolic enzyme with anti-oxidant function; 2) perform whole genome sequencing of existing DNA specimens from a PD study with highly characterized pesticide exposures, using bioinformatic tools to identify likely functional gene-environment interactions; and 3) test our predictions using iPSC-derived neuronal cell lines that model these genetic variations, and exposing cellular lines to these same environmental agents. This is a partnering PI project with W81XWH-20-1-0709. The scope of the -0710 project is to accomplish Aims 1&3.

To date, for Aims &3, we have established 3 clones of iPSC-derived dopamineergic neurons and have down-regulated GSTT1 in one of these lines so far. We have also established markers of oxidative stress and cell death, and used these markers to established dose-response curves for the 6 toxicants pertinent to this study. For Aim 2, we have completed sequencing of genomic DNA of 270 individuals, 5 more than specified in the SOW. In combination with existing sequencing data for this population we now have sequencing data for 340 study participants. Using a range of curation approaches and imputation of GWAS data we have and continue to identify compelling gene-pesticide interactions. In the next year of the project we will incorporate sequencing data to identify likely causal variants which will be validated as Aim 3 .

15. SUBJECT TERMS

Parkinson's disease; genetics; environment; pesticides; toxicants; gene-environment interaction

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I. INTRODUCTION

Parkinson's disease (PD) is a growing public health problem. As many as 1.5 million people in the U.S. currently suffer from PD, with an estimated 70,000 newly diagnosed cases per year. Veterans may be at increased risk, and more than 100,000 veterans with PD are currently treated in the Veterans Health Administration (VHA). Highly penetrant genetic causes of PD are uncommon. Numerous lines of research implicate a role for environmental causes, yet disease clusters are rarely identified. The vast majority of PD is therefore likely due to the deleterious effects of environmental factors on a background of genetic susceptibility, so-called gene-environment interaction (G*E). G*E is measured epidemiologically by the statistical deviation of the joint effects from independence. This statistical deviation is assumed to reflect an underlying biological interaction, but this assumption requires verification in valid biological models. The current project explores G*pesticide interaction in a highly exposed well-characterized cohort of professional pesticide applicators. Our aims are to 1) validate the epidemiologically observed G*E interaction in a cellular model; 2) identify novel, likely causal G*E interactions of particular relevance for service members; and 3) validate these novel interactions in cellular models.

II. KEYWORDS

Parkinson's disease; genetics; environment; pesticides; toxins; gene-environment interaction

III. ACCOMPLISHMENTS

This is a report of a collaborative (dual PI) project. The following tasks/milestones/accomplishments pertain to both projects, with the responsible PI noted in brackets to the right of each major task. The PI for project W81XWH-20-1-0709 is Samuel Goldman, MPH, MD; and the PI for project W81XWH-20-1-0710 is Raymond Swanson, MD.

A. What were the major goals of the project?

Major Task 1: Implement procedures and methods for genetic data analysis and cell culture models [Swanson & Goldman]

Subtask 1.1: Establish Study Team Processes, regular meetings, train all study staff (months 1-3).
Subtask 1.2: Develop study databases, data security, quality assurance methods (months 3-5).
Subtask 1.3: Use the WTC11 iPSC line to model GSTT1 deficiency (months 6-12).

Major Task 2: Determine the consequences of GSTT1 loss of function for susceptibility to paraquat injury [Swanson]

Subtask 2.1: Differentiate at least 9 iPSC lines (3 each) with GSTT1 intact, down regulated, or deleted into dopaminergic (DA) neurons. (months 12-18)
Subtask 2.2: Treat the 9 lines of differentiated DA neurons with paraquat and other putative PD-related toxicants likely to be encountered in military settings (permethrin, 2,4-D, trichloroethylene) or left untreated. (months 15-20)

Subtask 2.3: In the lines of differentially expressing GSTT1 DA neurons, assess reactive oxygen species (ROS) generation, ROS sensitivity, a-synuclein levels and aggregation, neurite retraction and neurodegeneration. (months 18-24)

Milestone #1: *Development and characterization of GSTT1- deficient cell line (months 12-24)*

Major Task 3: Determine if GSTT1 overexpression can rescue neurodegeneration. (months 18-24) [Swanson]

Subtask 3.1: Using similar approaches as above, determine whether ectopic expression of GSTT1 slows or blocks toxicant-induced or synuclein-dependent neurodegeneration in the context of GSTT1 deletion and reverses ROS and aggregation phenotypes. This will be accomplished using transfection of the 9 previously generated cell lines

Subtask 3.2: Using similar approaches as above, in 9 lines determine whether treatment with antioxidants slows or blocks toxicant-induced or synuclein-dependent neurodegeneration in the context of GSTT1 deletion and reverses ROS and aggregation phenotypes.

Major Task 4: Whole-genome sequencing of 265 FAME study subject specimens [Goldman]

Subtask 4.1: prepare high quality aliquots of banked DNA from 265 study subjects to specified concentrations (months 4-6).

Subtask 4.2: DNA specimens from 265 subjects will be sequenced, and sequence data stored on multiple high capacity drives, and data uploaded to UCSF/Gladstone secure servers (months 7-12).

Milestone #2: *FAME sequencing completed (months 6-12)*

Major Task 5: Quantification of pesticide and other toxicant exposures within the FAME study database comprised of 498 study subjects in total (115 case and 383 controls) [Goldman]

Subtask 5.1: classify historical exposures for 498 FAME subjects, derive cumulative exposure estimates by duration and intensity for each agent (months 9-15)

Subtask 5.2: classify exposures for 498 FAME subjects according to mechanistic classes (e.g., mitochondrial Complex I inhibitors; redox-cycling/oxidative stressors; a-synuclein pro-aggregants) (months 12-16).

Subtask 5.3: classify serum toxicant exposures 498 FAME subjects (months 14-18).

Major Task 6: perform rare burden analysis and machine learning to identify genes likely to increase susceptibility to specific toxicants in FAME, pooling data from the 265 subjects sequenced in the current project with existing sequencing data for 70 subjects, for a total of 335 subjects [Goldman]

Subtask 6.1: Annotation of sequencing data for 335 FAME subjects (months 14-18)

Subtask 6.2 rare-burden analysis of variants for 335 FAME subjects (months 18-30)

Subtask 6.3 conduct exposed-only analyses of gene*environment interaction for 335 FAME subjects

(months 20-30)

Subtask 6.4: use Machine Learning approaches for 335 FAME subjects to identify genes that may contribute to neurodegeneration in Parkinson's disease (months 24-30)

Milestone #3: *identification of combinations of variants likely to increase susceptibility to specific toxicants (months 24-30)*

Major Task 7: Validate novel G*E interactions relevant to PD pathogenesis [Swanson]

Subtask 7.1: lower the expression of the genes in human DA neurons and determine whether that confers sensitivity to specific toxicants, using the same paradigm and 9 cell lines as for Major Tasks 1 & 2 above (months 20-30)

Subtask 7.2: Determine the robustness of validated G*E interactions using additional cell lines. We will generate > 3 lines for each gene of interest. (months 24 -32)

Subtask 7.3: Using the lines above, demonstrate that specific genetic variants identified in FAME are sufficient to mediate G*E interaction. (months 32-36)

Milestone #4: *Manuscript on use of the identification and validation of specific genetic susceptibility to specific toxicants (months 32-36).*

B. What was accomplished under these goals?

Subtask 1.1: Establish Study Team Processes, regular meetings, train all study staff (months 1-3). [Swanson & Goldman]

Accomplishments: This Subtask is completed. We established regular meetings within the Goldman group, and between the Goldman, Swanson & Gladstone (subcontract) groups. These meetings have been held biweekly since August, 2020. All study staff are trained in study procedures and human subjects requirements of each of the associated institutions, with regular training updates as required.

Subtask 1.2: Develop study databases, data security and quality assurance methods (months 3-5). [Swanson & Goldman]

Accomplishments: This Subtask is completed. Study databases have been developed, and essential de-identified datasets are accessible to appropriate study staff. All databases are regularly backed up. Genomic sequencing data reside on redundant devices to ensure integrity and protect from any unlikely data loss. Intensive quality assurance procedures were implemented during DNA processing, prior to and during sequencing, and throughout the annotation pipeline.

Subtask 1.3: Use the WTC11 iPSC line to model GSTT1 deficiency (months 6-12). [Swanson]

Accomplishments: This Subtask is partially completed. The process of differentiating the WTC11 iPSC line into dopaminergic neurons has been optimized and GSTT1 genotype of this cell line has been established. However, stable CRISPR-mediated downregulation of GSTT1 has not yet been accomplished.

Subtask 4.1: prepare high quality aliquots of banked DNA from 265 study subjects to specified concentrations (months 4-6). [Goldman]

Accomplishments: This Subtask is completed. Banked DNA for 285 study subjects was aliquoted after assessment for quality and concentration, and prepped according to the sequencing contractor's specifications. Additional aliquots were prepped to account for instances of possible low DNA quality or other potential QA failures.

Subtask 4.2: DNA specimens from 265 subjects will be sequenced, and sequence data stored on multiple high capacity drives, and data uploaded to UCSF/Gladstone secure servers (months 7-12). [Goldman]

Accomplishments: This Subtask is completed. Genomic DNA was successfully sequenced for 270 subjects, 5 more than our target goal. Sequencing data quality was reviewed, and data uploaded to secure servers at UCSF/Gladstone.

Milestone #2: FAME sequencing completed (months 6-12)
Accomplishments: Milestone #2 was fully completed on schedule.

Subtask 5.1: classify historical exposures for 498 FAME subjects, derive cumulative exposure estimates by duration and intensity for each agent (months 9-15) [Goldman]

Accomplishments: Work is ongoing. We anticipate full completion in accord with the specified timeline.

Subtask 5.2: classify exposures for 498 FAME subjects according to mechanistic classes (e.g., mitochondrial Complex I inhibitors; redox-cycling/oxidative stressors; a-synuclein pro-aggregants) (months 12-16). [Goldman]

Accomplishments: This Subtask is largely completed, and will be fully completed in accord with the specified timeline.

Subtask 5.3: classify serum toxicant exposures 498 FAME subjects (months 14-18). [Goldman]

Accomplishments: This Subtask is initiated. We are using lipid-adjusted values of persistent pollutants including polychlorinated biphenyl compounds and organochlorine pesticides. A beta-substitution method is being used to impute values below the limits of detection.

Subtask 6.1: Annotation of sequencing data for 335 FAME subjects (months 14-18) [Goldman]

Accomplishments: We have begun the annotation of sequencing data for 340 study subjects. In addition, we have taken advantage of existing GWAS SNP data in this population to help inform next steps for analysis of sequencing data. Specifically, we performed imputation on approximately 750,000 SNPs typed on the Affymetrix UKBrainbank array. We have identified all SNP*pesticide-use interactions that are nominally statistically significant in either additive or binary models. Because study Aim 3 can only validate epidemiological associations in achievable cellular models, we have restricted analyses to SNPs that are in or near coding regions, or that are eQTL or sQTLs. Initially we are exploring subsets of interest: 1) PARK genes, 2) PD-associated SNPs, 3) genes involved in pesticide metabolism and transport, and that are expressed in brain.

C. What opportunities for training and professional development has the project provided?

Nothing to Report.

D. How were the results disseminated to communities of interest?

Nothing to report.

E. What do you plan to do during the next reporting period to accomplish the goals?

We plan to complete the in-process Subtasks noted in Section B above. In addition, we plan to initiate work on the subtasks below.

Subtask 2.1: Differentiate at least 9 iPSC lines (3 each) with GSTT1 intact, down regulated, or deleted into dopaminergic (DA) neurons. (months 12-18) [Swanson]

Subtask 2.2: Treat the 9 lines of differentiated DA neurons with paraquat and other putative PD-related toxicants likely to be encountered in military settings (permethrin, 2,4-D, trichloroethylene) or left untreated. (months 15-20) [Swanson]

Subtask 2.3: In the lines of differentially expressing GSTT1 DA neurons, assess reactive oxygen species (ROS) generation, ROS sensitivity, a-synuclein levels and aggregation, and neurite outgrowth (months 15-20) [Swanson]

Subtask 3.1: Using similar approaches as above, determine whether ectopic expression of GSTT1 slows or blocks toxicant-induced or synuclein-dependent neurodegeneration in the context of GSTT1 deletion and reverses ROS and aggregation phenotypes. This will be accomplished using transfection of the 9 previously generated cell lines (months 18-24) [Swanson]

Subtask 3.2: Using similar approaches as above, in 9 lines determine whether treatment with antioxidants slows or blocks toxicant-induced or synuclein-dependent neurodegeneration in the context of GSTT1 deletion and reverses ROS and aggregation phenotypes. (months 18-24) [Swanson]

Subtask 6.2 rare-burden analysis of variants for 335 FAME subjects (months 18-30) [Goldman]

Subtask 6.3: conduct exposed-only analyses of gene*environment interaction for 335 FAME subjects (months 20-30) [Goldman]

Subtask 7.1: lower the expression of the genes in human DA neurons and determine whether that confers sensitivity to specific toxicants, using the same paradigm and 9 cell lines as for Major Tasks 1 & 2 above (months 20-30) [Swanson]

IV. IMPACT

A. What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

B. What was the impact on other disciplines?

Nothing to Report

C. What was the impact on technology transfer?

Nothing to Report

D. What was the impact on society beyond science and technology?

Nothing to Report

V. CHANGES/PROBLEMS

A. Changes in approach and reasons for change

Nothing to Report

B. Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

C. Changes that had a significant impact on expenditures

Nothing to Report

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

Nothing to Report

E. Significant changes in use or care of human subjects

Nothing to Report

F. Significant changes in use or care of vertebrate animals

Nothing to Report

G. Significant changes in use of biohazards and/or select agents

Nothing to Report

VI. PRODUCTS

A. Publications, conference papers, and presentations

Nothing to Report

a. **Journal publications**

Nothing to Report

b. **Books or other non-periodical, one-time publications.**

Nothing to Report

c. **Other publications, conference papers, and presentations.**

Nothing to Report

B. Website(s) or other Internet site(s)

Nothing to Report

C. Technologies or techniques

Nothing to Report

D. Inventions, patent applications, and/or licenses

Nothing to Report

E. Other Products

Nothing to Report

VII. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

A. What individuals have worked on the project?

Name	Role	Researcher Identifier	Nearest person-months worked	Contribution to Project	Funding support
Samuel Goldman, MD, MPH	PI	0000-0002-3039-9927	2	Project Oversight; FAME study exposure and genotyping lead	W81XWH-20-1-0709
Kathleen Comyns, MPH	Project Coordinator	None	3	Project coordination	W81XWH-20-1-0709
Raymond Swanson, MD	PI	000-0002-3664-5359	2	Project oversight; cell culture studies; data analysis	W81XWH-20-1-0710
Jiejie Wang, PhD	Post-doctoral	None	6	iPSC preparation	W81XWH-20-1-0710
Rebecca Fong, BS	Technician	None	5	Immunostaining and data analysis	W81XWH-20-1-0710

Julia Kaye	Scientific Program Leader II	0000-0001-7442-0882	1	Project oversight and management	W81XWH-20-1-0710 (subcontract)
Steven Finkbeiner	Center Director	0000-0002-3480-394X	0.25	Project oversight	W81XWH-20-1-0710 (subcontract)
Leandro De Araujo Lima	bioinformatician	None	2	Bioinformatics work	W81XWH-20-1-0710 (subcontract)
Mariya Barch	Staff Scientist	None	0.25	Microscope and Image analysis Pipeline support	W81XWH-20-1-0710 (subcontract)
Roma Patterson	Robotic Engineer	None	0.25	Microscope support	W81XWH-20-1-0710 (subcontract)
Shiron Drusinsky	Research Engineer	None	4	Bioinformatics work	W81XWH-20-1-0710 (subcontract)
Sina Dabiri	Software Engineer	None	0.25	Microscope and Image analysis Pipeline support	W81XWH-20-1-0710 (subcontract)
Stephanie Lam	Software Engineer	None	0.25	Microscope and Image analysis Pipeline support	W81XWH-20-1-0710 (subcontract)
Zohreh Faghihmonzavi	Research Associate	None	4	iPSC cell culture differentiation, and robotic imaging	W81XWH-20-1-0710 (subcontract)

B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Samuel Goldman:

Previously active projects that have closed:

Michael J. Fox Foundation
A131409 (PI: Goldman)
08/28/2018 – 08/27/2020
(0.96 calendar months)
Mitochondrial DNA damage in PD and control in a pesticide-exposed cohort

UCSF Academic Senate
A7501268 (PI: Goldman)
2/1/2019-7/31/2020
(0.36 calendar months)
The colonic microbiome preceding Parkinson's disease: a pilot study

Newly active projects:

Michael J. Fox Foundation
A135062 (PI: Tanner)
8/1/2020-7/31/2022
384,642 (1.2 calendar months)
Bipolar Disease and PD

Michael J. Fox Foundation
A136995 (PI: Goldman)
5/1/2021-4/30/2023
(1.2 calendar months)
Collaborative Study of Environmental Risk Factors for Parkinson's Disease in a Large Prospective Veteran Cohort (MILCO-PD)

Raymond Swanson: no changes

Julia Kaye

Previously active projects that have closed:
None.

Newly active projects:
Livermore Lab Foundation
(Finkbeiner PI)
01/01/21 – 12/31/21
(0.12 calendar months)
Examination of TDP43 Dynamics in ALS Patient-derived Neurons

Steven Finkbeiner

Previously active projects that have closed:
NIH/ NIA
RF1 AG058447-01 (PI: Finkbeiner)
06/01/18–05/31/21
(1.4 cal months)
Discovery of Novel Drugs that Increase Tau Clearance to Treat Alzheimer's Disease

UCI/ NIH
U54 NS091046 (PI: Thompson)
09/30/14–06/30/20
(0.12 cal months)
Neuron and Glial Cellular Signatures from Normal and Diseased iPS Cells

Michael J. Fox Foundation
(PI: Finkbeiner)
01/01/19–12/31/20
196,539 (0.12 cal months)
Applications of Deep Learning to Assess PD Pathology

Newly active projects:
Livermore Lab Foundation
(Finkbeiner PI)
01/01/21 – 12/31/21
(0.12 calendar months)
Examination of TDP43 Dynamics in ALS Patient-derived Neurons

NIH-R01
R01AG064579 (PI: Finkbeiner)
04/15/20 – 03/31/25
(0.91 calendar months)
Cell and Network Disruptions and Associated Pathogenesis in Tauopathy and Down Syndrome

C. What other organizations were involved as partners?

Nothing to report

VIII. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

This is a collaborative award: W81XWH-20-1-0709 (PI: Goldman), W81XWH-20-1-0710 (PI: Swanson). A duplicative report is being submitted for each award, with tasks clearly marked with the responsible PI. Both PIs are affiliated with the Northern California Institute for Research and Education (NCIRE).

QUAD CHARTS: The Quad Chart is submitted as a separate attachment.

IX. APPENDICES

None