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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.

In work performed for Aim 1 during this reporting period, we established 3 clones of iPSC-derived dopaminergic 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 superoxide formation, DNA damage, and cell death. We have also established long-term iPSC cultures for assessing α-synuclein aggregate formation, induced by viral-mediated α-syn overexpression and exposure to pre-formed α-syn fibrils. Work ongoing is evaluating the effect of CRISPRi – mediated GTT1 downregulation on these dose-response curves, in cells with and without α-synuclein aggregates. In the next reporting period, selected genes identified in the Aim 2 work (W81XWH-20-1-0709) will be downregulated using this same CRISPRi method to assess for biological importance.

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; toxicants; 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?

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). [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 at Gladstone, and essential de-identified datasets are accessible to appropriate study staff. All databases are regularly backed up at Gladstone and stored on a secured server. 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. The genomic data is stored at Gladstone is fully de- identified and maintained separately from the patient sample data at UCSF.

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

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. To evaluate the effects of GSTT1 knockdown, we established the CRISPRi platform[1-9] at Gladstone. We cloned 3 gRNAs against GSTT1 into the pMK1334 vector, which expresses gRNAs in a robust fashion[9]. We assessed the knockdown efficacy of each gRNA by transfecting them into the WTC11 iPSC line, which harbors dCas9-KRAB at the CYBL safe-harbor locus[10]. After selecting for cells that harbor the pMK1334 vector with puromycin for ~4 days, we harvested the cells and performed RT-PCR to confirm the knockdown. We observed that GSTT1-gRNA3 achieved significant reduction in GSTT1 expression compared to the control (**Figure 1**). We did not achieve complete knockdown of GSTT1, but this is likely due to the transient transfection approach, as previous studies from our lab indicate that viral expression is required for robust knockdown. Therefore, we sent the pMK1334 vector that harbors the GStTT1-3 gRNA for viral packaging, which we will assess in the next experiments

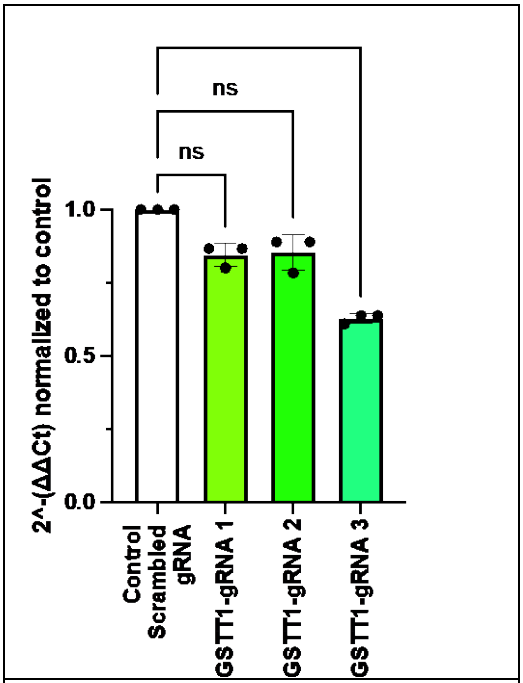


Figure 1. Transient transfection of the GSTT1-3 gRNA in WTC11 iPSCs results in significant reduction in GSTT-1 expression. Three gRNAs targeting GSTT1 were tested. RT-qPCR was performed using primers against GSTT1 and the housekeeping gene GAPDH. n=3 experiments. Simple linear regression using a Kruskal-Wallis test, p= 0.059 comparing control scrambled gRNA to GSTT1-3 gRNA.

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)*

We differentiated a BJ5 cell line that harbors a tDTomato: fluorescent reporter under the tyrosine hydroxylase (TH) gene, which allows selective visualization of DA neurons[11]. Our DA differentiation protocol yielded many TH-positive DA neurons (Figure 2), and we confirmed this by staining using a TH antibody (data not shown). We subjected the BJ5 line to dosing of 25 uM paraquat and then imaged every 24 hours using Robotic Microscopy (RM)[12-14]. We observed a robust decrease of DA neurons over time (**Figure 2**) compared to neurons not dosed (Data not shown). To better evaluate the effects of paraquat and another pesticide, rotenone, we differentiated the BJ5 line

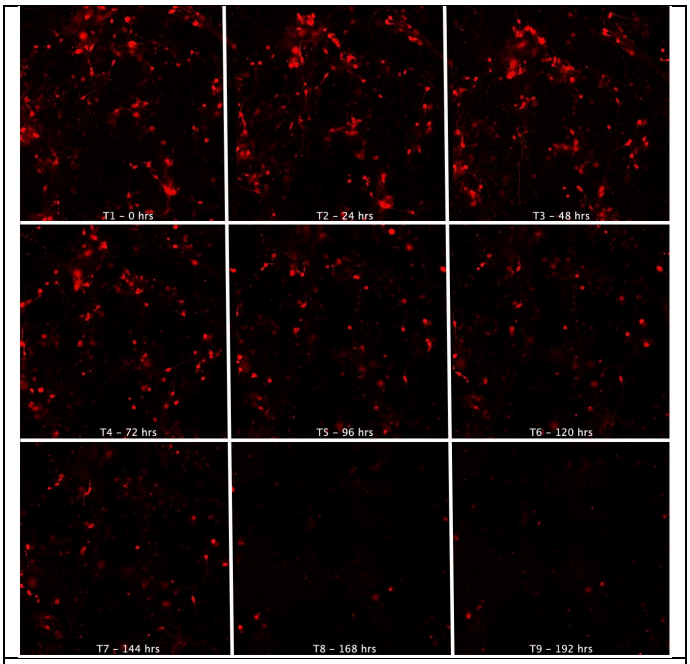


Figure 2. DA neurons die after pesticide exposure. The BJ5 line harbors the tDTomato fluorescent reporter tagged to the tyrosine hydroxylase (TH) promoter was differentiated into DA neurons[11] and dosed with 25 uM paraquat at day ~33, and then imaged every 24 hours using RM. The loss of fluorescence is readily detected by 72 hours (fourth panel). Each panel is ~600 um wide.

and two additional control iPSC lines (WTC11 and 4337) into DA neurons, dosed them with varying concentrations of rotenone and paraquat, and subjected them to daily imaging for 9 days. We subjected the images to our custom-built cell tracking algorithm to count the number of cells over time. We normalized the change in cell number over time compared to the initial timepoint, calculating the ratio of the number of cells detected at each time point to the initial number (Figure 3). We then applied a simple linear regression model to estimate the slopes of the change of the number of cells over time. We found that treatment with either rotenone or paraquat reduced the number of DA neurons over time (Figure 3). The effect appears to be somewhat dose-dependent (**Figure 3,** for example compare 25uM to 5 uM).

While monitoring cell count over time can approximate the rate of cell death, it is not an ideal method to evaluate the probability of death. To more accurately identify cellular mechanisms of neurodegeneration, we recently developed a novel biosensor that we call genetically encoded fluorescent cell-death indicator, or GEDI[10, 15, 16]. GEDI contains a red fluorescent sensor (RGEDI) that specifically detects intracellular Ca^{2+} levels only achieved when cells are irreversibly committed to die. It is the most specific and earliest live marker of a commitment to degeneration we have found. Linked to the RGEDI sensor is a green fluorescent marker of cell morphology (EGFP). Calculating the ratio of RGEDI to EGFP fluorescence, which we refer to as the GEDI ratio (%GEDI), provides a reliable prediction of whether a neuron is destined to live or die[10, 15, 16]. Cells that are dying can be visualized by a yellow fluorescent signal apparent by overlaying the red and green channels (Figure 4). Our preliminary results show that exposure to 25 uM rotenone induces a high level of cell death (**Figure 4**). This effect can be quantified by a linear mixed model we developed in-house (data not shown). This approach will allow us to assess the effects of knocking down both GSTT1 and other genes we find from milestone 2 of this project in a high throughput way. We will report the use of GEDI for our pesticide-based human model in the next progress report.

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

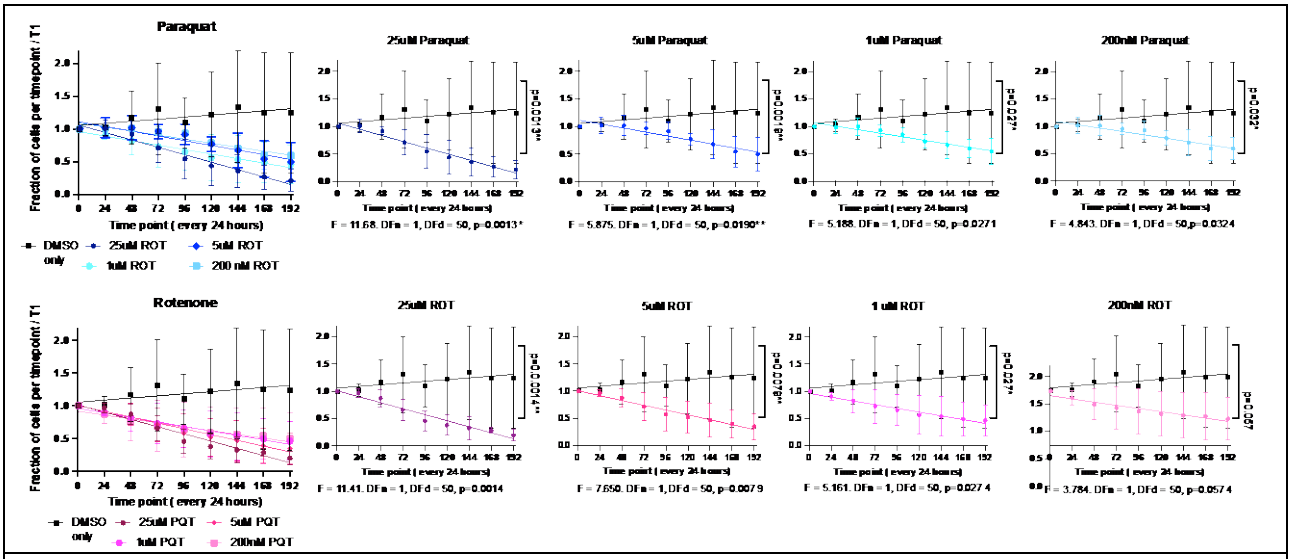


Figure 3. Dosing DA neurons with paraquat and rotenone decreases cell viability over time. 2 control iPSC lines (WTC11 and 4337) were differentiated into DA neurons and transduced with a synapsin: EGFP biosensor. Another line, BJ5, harbors a tDTomato fluorescent reporter integrated under the tyrosine hydrolase promoter to visualize TH positive neurons[11]. Dosing began at day ~33 of differentiation and cells were imaged every 24 hours. Cells were tracked and counted at each time point. The number of cells per timepoint was normalized by the total number of cells at the very first time point (Day 0) and plotted. The dose of either paraquat (top) or rotenone (bottom) is listed for each graph. Linear regression was used to evaluate if the change in cell count was different across the different groups. n = experiments (~ 100,000 neurons).

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.

The work for these subtasks is ongoing.

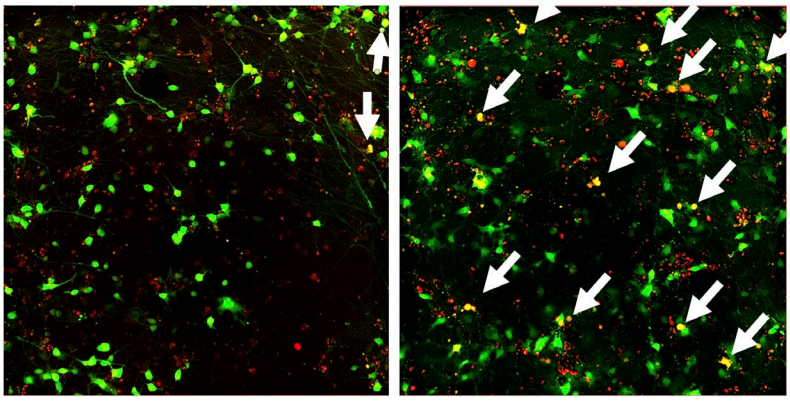


Figure 4. Implementing the GEDI biosensor in DA i-neurons. GMK2 DA-neurons were transduced with GEDI[10, 15, 16] and imaged after 5 days in the RFP and GFP channels, and merged for visualization. DA neurons were dosed with vehicle alone (left) or 25 uM rotenone (right). Live cells appear green (low GEDI ratio) and dead cells appear yellow (high GEDI ratio). Arrows indicate dead neurons.

Major Task 4: Conduct 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). [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.

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)

Accomplishments: Cumulative exposure estimates are derived. We anticipate that due to limited sample size of the exposed sub-cohorts, gene-environment analyses of cumulative exposures will be limited predominantly to sensitivity analyses of interaction among those with high-level cumulative exposures to a given agent.

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).

Accomplishments: This Subtask is completed, though we continue to regularly search the scientific literature for additional insights regarding pesticide toxicologic mechanisms and potential mechanisms of specific relevance to PD etiology.

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

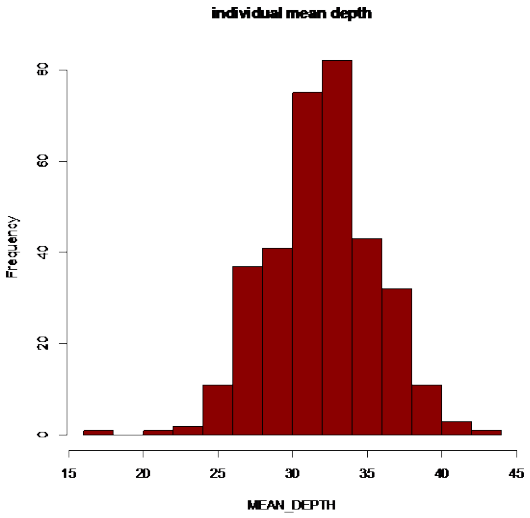
Accomplishments: We have derived lipid-adjusted values of previously measured persistent organic toxicants including polychlorinated biphenyl compounds and organochlorine pesticides. We used a beta-substitution method to impute values below the limits of detection.

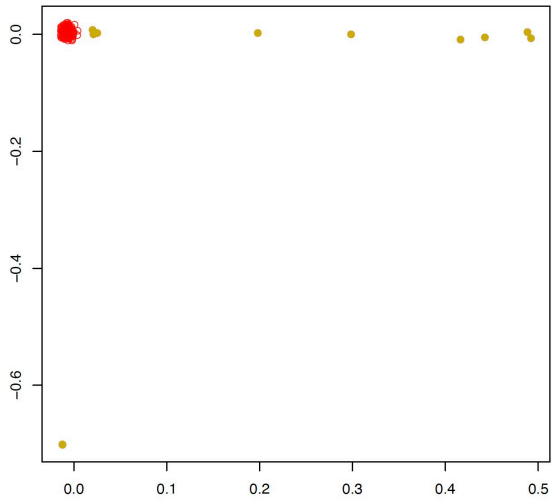
Major Task 6: perform rare burden analysis and machine learning to identify genes likely to increase susceptibility to specific toxicants in FAME, pooling data from the 265 subjects sequenced in the current project with existing sequencing data for 70 subjects, for a total of 335 subjects [Goldman]

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

Accomplishments: We have completed the QA and annotation of sequencing data for 340 FAME study subjects. Median sequencing depth per individual was 32 (see Figure below). Only a single individual with depth below 20 was excluded from analyses. Principal components analysis (see Figure below)

identified a handful of (already self-identified) non-white individuals in this predominantly white European-descent study cohort, as well as a highly-related pair that necessitated the removal of one subject from analyses. Variants have been annotated as high impact (predicted loss of function, missense, n=1,233), low impact (n=182,133) or modifier (22,241,663). Common variants (minor allele frequency MAF 1-50%) (n=7,924,634) have been extracted.





Subtask 6.2: rare-burden analysis of variants for 335 FAME subjects (months 18-30)
Accomplishments: We have implemented rare-burden analyses (minor allele frequency < 1%). Initial focus is on high impact variants (predicted loss of function, missense). Subsequent pooled analyses will additionally consider common variants. The tables below provide preliminary examples of associations of PD with candidate genes, and among all genes.

Candidate Genes	RANGE	NumVar	Pvalue
TKT	3:53259652-53290130;3:53258722-53290130	11	0.0074
POR	7:75544419-75616173	14	0.0150
GPX2	14:65405871-65409531	7	0.0552

All Genes	RANGE	NumVar	Pvalue
MIR548N	2:179246804-179541009	228	3.52 E-09
TTN	2:179610046-179672150;2:179390717-179672150;2:179390717-179672150;2:179390717-179672150	364	1.42 E-08
LAMA5	20:60884120-60942368	87	4.36 E-08
HSPG2	1:22148736-22263750	78	2.67 E-06
OBSCN	1:228395860-228548951;1:228395860-228566575	95	7.85 E-06
MUC16	19:8959519-9092018	150	8.44 E-06
MACF1	1:39796809-39952810;1:39547088-39952810	85	8.19 E-05

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

Accomplishments: We have conducted association analyses in pesticide exposed-only sub-cohorts for applicators of 1) rotenone, 2) paraquat, 3) permethrin, 4) benomyl, 5) dieldrin, 6) paraquat or diquat, 7) any pyrethroid, 8) dieldrin or aldrin, 9) any benzimidazole. The tables below provide preliminary examples of rare burden interactions among a) a priori candidate genes involved in xenobiotic metabolism, transport, and glutathione metabolism, and b) among all genes.

Candiate Genes	Pesticide	Exposed n	NumVar	P-value
ABCC3	Rotenone	35	29	0.041
AHRR	Pyrethroid	54	5	0.005
	Permethrin	52	5	0.011
	Benzimidazole	36	5	0.034
RDH5	Benzimidazole	36	6	3.36 E-07
	Dieldrin	38	6	0.004
CAT	Paraquat	72	9	0.043
CPA6	Paraquat	72	5	0.044
CYP1B1	Pyrethroid	54	11	0.010
	Permethrin	52	11	0.012
CYP2C18	Permethrin	52	5	0.002
	Pyrethroid	54	5	0.007
CYP2E1	Paraquat	72	11	0.043
	Dieldrinlike	120	11	0.062
DHRS9	Paraquat	72	8	0.011
	Paraquatlike	75	8	0.015
GSR	Dieldrinlike	120	9	0.028
	Pyrethroid	54	9	0.042
	Permethrin	52	9	0.046
GSTM4	Benzimidazole	36	4	0.030
	Dieldrinlike	120	4	0.036
	Paraquat	72	4	0.041
	Paraquatlike	75	4	0.042
POR	Dieldrinlike	120	14	0.002
	Paraquat	72	14	0.012
SLC1A1	Permethrin	52	4	0.003
SLC1A2	Rotenone	35	15	0.019
SLC1A3	Paraquat	72	5	0.015
	Paraquatlike	75	5	0.031
SLC25A22	Dieldrin	38	6	0.004
	Paraquatlike	75	6	0.049
SLC7A11	Benomyl	22	9	-8.88E-16
	Dieldrin	38	9	0.004

SLC7A9	Rotenone	35	7	0.017
NT5C1B-RDH14	Dieldrinlike	120	11	0.004
UGT1A7	Dieldrinlike	120	29	0.013

All Genes	Pesticide	Exposed n	NumVar	P-value	Name	Function
IPO5	Benomyl	22	11	7.78 E-150	Importin 5	Functions in nuclear protein import as nuclear transport receptor. Serves as receptor for nuclear localization signals (NLS) in cargo substrates. Mediates nuclear accumulation of synuclein
WNK1	Benomyl	22	27	8.95 E-11	WNK Lysine Deficient Protein Kinase 1	Serine/threonine kinase which plays an important role in the regulation of electrolyte homeostasis, cell signaling, survival, and proliferation. Autophagy inhibitor, regulates NLRP3 inflammasome activation
PLXNB2	Benomyl	22	36	1.45 E-09	Plexin B2	transmembrane receptors that participate in axon guidance and cell migration in response to semaphorins.
PRICKLE1	Benzimidizole	36	10	3.36 E-07		Rs148294058 (LINC02451) associated with PD
GTF2B	pyrethroid	54	1	1.91 E-05	General Transcription Factor IIB	required for transcription initiation by RNA polymerase II.

In addition, we have taken advantage of existing GWAS SNP data in this population to help inform next steps for in vitro modeling. 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. In addition to "hypothesis-free" analyses, we have also explored pesticide interactions with genes and variants of *a priori* interest: 1) PARK genes, 2) PD GWAS-associated SNPs, and 3) genes involved in pesticide metabolism and transport. Selected preliminary results are reported in the tables below.

PARK Gene	Pesticide	dbSNPRSID	Location	OR_GE	OR_E_noG	OR_G_noE	OR_interaction	Additive p-val
PINK1	benzimidazole	rs148871409	**Nonsynon**	7.93	0.99	1.08	7.40	0.038
ATP13A2	fungicide	rs3738815	Synonymous	2.98	0.86	0.84	4.12	0.027
PARK7	Dieldrin	rs4908488	Intron	5.37	1.52	0.95	3.70	0.047
GIGYF2	permethrin		Intron	16.44	1.59	1.78	5.80	0.037
SNCA	rotenone	rs11097234	Intron	6.11	2.26	0.83	3.26	0.038
SNCA	rotenone	rs12502363	Intron	5.98	2.21	0.81	3.33	0.035
LRRK2	Blazer	rs7308720	**Nonsynon**	3.31	0.91	0.97	3.77	0.045
LRRK2	Blazer	rs10878372	Intron	2.60	0.83	1.03	3.06	0.043
LRRK2	rotenone	rs17466339	Intron	9.66	2.53	0.82	4.67	0.035
PARK2	Benomyl	rs9355360	Intron	4.83	0.89	1.05	5.12	0.049
PARK2	fungicide	rs58468575	Intron	2.68	0.92	0.85	3.39	0.047
PARK2	Cyanides	rs78224461	Intron	12.12	1.03	1.09	10.72	0.044
PARK2	Blazer	rs9458273	Intron	2.27	0.84	0.80	3.36	0.043
PARK2	Dieldrin	rs9456751	Intron	2.82	1.06	0.82	3.24	0.037
PARK2	Ferbam	rs3019443	Intron	3.17	0.86	0.87	4.22	0.032
PARK2	rotenone	rs9346879	Intron	4.84	1.35	0.88	4.07	0.030
PARK2	fungicide	rs11966738	Intron	2.83	0.97	0.86	3.36	0.030

PARK2	Benomyl	rs112913800	Intron	16.11	1.04	1.18	13.15	0.020

A priori Gene	Pesticide	dbSNPRSID	Location	OR_GE	OR_E_noG	OR_G_noE	OR_interaction	Additive p-val
ABCB9	benzimidazole	rs116887147	Intron	15.62	0.96	1.20	13.55	0.031
ABCC1	pyrethroid	rs188352772	Intron	6.43	0.99	1.21	5.36	0.031
ABCC1	pyrethroid	rs79686715	Intron	12.62	1.30	1.05	9.22	0.040
ABCC1	benzimidazole	rs17287570	Intron	3.10	0.93	0.83	4.01	0.041
ABCC4	Dieldrin	rs4148493	Intron	3.58	1.11	0.81	3.99	0.015
ABCC4	rotenone	rs3742106	Utr3	6.50	1.91	0.95	3.56	0.031
ABCC4	benzimidazole	rs7324065	Intron	11.73	1.03	0.84	13.60	0.033
ABCC4	Blazer	rs78302301	Intron	11.49	0.97	0.87	13.64	0.036
ABCC4	Cyanides	rs9561784	Intron	5.03	0.86	1.11	5.29	0.038
ABCC4	rotenone	rs4148551	Utr3	6.70	2.10	1.02	3.13	0.050
ABCC6	permethrin	rs16967441	Intron	6.25	1.25	0.90	5.57	0.016
ABCC6	Ferbam	rs12598559	Intron	5.10	1.00	1.10	4.63	0.047
ABCC8	Dieldrin	rs4148618	Intron	4.70	1.21	0.90	4.32	0.020
GCLC	Blazer	rs16883912	Intron	4.36	0.81	0.88	6.17	0.016
GCLC	Benomyl	rs16883912	Intron	19.91	0.90	1.00	22.24	0.022
GCLC	pyrethroid		Intron	22.20	1.29	1.57	10.96	0.040
GLRX	rotenone	rs7700814	Intron	16.10	2.25	1.15	6.24	0.023
GLRX	rotenone	rs885303	Intron	6.48	1.77	0.85	4.28	0.025
GLRX	rotenone	rs74587548	Intron	35.48	2.48	1.25	11.49	0.035
GLRX	Ferbam	rs74587548	Intron	8.60	1.11	1.41	5.47	0.049
GSTM1	Aldrin	rs1056806	Synonymous	4.26	1.04	0.81	5.06	0.033
MGST1	Ferbam	rs73064116	Utr3	6.05	0.97	0.98	6.36	0.011
MGST1	benzimidazole	rs9332929	Intron	3.40	0.85	1.00	3.99	0.038
MGST1	Ferbam	rs10846355	Intron	4.14	0.99	1.14	3.67	0.039
NQO1	benzimidazole	rs1800566	**Nonsynon**	4.64	0.84	1.14	4.82	0.028
NXN	Ferbam	rs7223906	Intron	17.15	1.13	1.31	11.52	0.027
NXN	Blazer	rs17693812	Intron	2.86	0.91	0.92	3.43	0.048
SLC1A1	paraquat	rs10974575	Intron	7.01	1.67	0.86	4.90	0.023
SLC1A4	Benomyl	rs2268483	Intron	15.48	1.00	0.88	17.52	0.011
SLC1A4	Ferbam	rs2268483	Intron	5.26	1.04	0.85	5.94	0.032
SLC7A11	Dieldrin	rs4863768	Intron	3.18	1.02	0.85	3.67	0.022
SLC7A11	permethrin	rs72712336	Intron	7.91	1.30	1.51	4.04	0.037
TXNDC8	pyrethroid	rs7041938	**Nonsynon**	4.83	1.29	1.06	3.56	0.028
TXNRD1	Dieldrin	rs11111945	Intron	5.96	1.37	0.82	5.35	0.030
TXNRD2	Benomyl	rs7410379	Intron	5.83	0.83	0.93	7.52	0.026

Milestone #3: identification of combinations of variants likely to increase susceptibility to specific toxicants

Accomplishments: Good progress is being made toward this Milestone.

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 for the current funding interval. An abstract has been accepted for presentation at the 2022 Society for Neuroscience meeting in November 2022.

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 complete 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.

Subtask 2.2: Treat the 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.

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

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.

Subtask 6.2 rare-burden analysis of variants for 335 FAME subjects (months 18-30) [Goldman]. We will continue to explore rare-variation, including variation in non-coding regions likely to be eQTL or sQTL sites, or to otherwise influence gene expression.

Subtask 6.3: conduct exposed-only analyses of gene*environment interaction for 335 FAME subjects (months 20-30) [Goldman]. We will expand exposed-only analyses to identify meaningful gene*environment interactions that are likely to be causal by incorporating rare and common variation in coding regions, and in eQTL and sQTLs, and by using WGS data to explore strong GWAS associations.

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). We will accomplish this Subtask. We additionally will explore multigenic models for assigning individual risk scores for pesticide-exposed individuals.

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.

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.

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

Milestone #4: Manuscript on use of the identification and validation of specific genetic susceptibility to specific toxicants. We will prepare manuscript(s), and will present findings at scientific meetings.

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

There was a 2-month interval in which dopaminergic differentiation of the iPSC cells was unsuccessful, and a separate 4-month interval in which viability of the iPSC cells was insufficient for long-term cultivation. These technical issues are now resolved, but they significantly slowed progress on subtasks 3.1 and 3.2.

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	3	Project Oversight; FAME study exposure and genotyping lead	W81XWH-20-1-0709
Kathleen Comyns, MPH	Project Coordinator	None	2.2	Project coordination	W81XWH-20-1-0709
Raymond Swanson, MD	PI	000-0002-3664-5359	1.8	Project oversight; cell culture studies; data analysis	W81XWH-20-1-0710
Rebecca Fong, BS	Technician	None	7	Immunostaining and data analysis	W81XWH-20-1-0710
Julia Kaye	Scientific Program Leader II	0000-0001-7442-0882	1.7	Project oversight and management	W81XWH-20-1-0710 (subcontract)
Leandro De Araujo Lima	Bioinformatician	None	1.9	Bioinformatics work	W81XWH-20-1-0710 (subcontract)
Eric Mockler	Research Engineer	None	2.4	Microscope and Image analysis Pipeline support	W81XWH-20-1-0710 (subcontract)
Zohreh Faghihmonzavi	Research Associate	None	5.3	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

P0543866 (PI: Akbilgic)
9/1/2019-8/31/2021
(1.2 calendar months)
Applying Artificial Intelligence for Early Identification of Parkinson's Disease

Newly active projects:

None.

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
$231,917 (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

References Cited:

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Integrating Environmental, Genomic, and Functional Data to Characterize Individual Risk for PD

ERMS/Log Number PD190037 & PD190037P1 Annual Report Year 2

Award Numbers W81XWH-20-1-0709 and W81XWH-20-1-0710

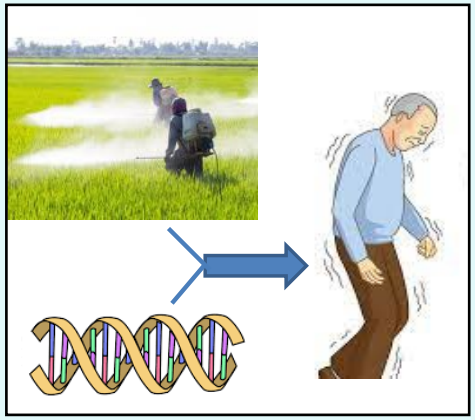
PIs: Samuel Goldman and Raymond Swanson **Org:** Northern California Institute for Research and Education **Total Award :** $2,397,928

Study Aims and Approach

• Identify genetic variants in the FAME cohort that confer susceptibility to pesticide-induced Parkinson's disease.
  - DNA samples from the FAME cohort will undergo SNP analysis and whole genome sequencing. Variants associated with increased risk of PD will be identified using advanced bioinformatic methods.

• Establish a cell culture model using human iPSC-derived dopaminergic neurons to evaluate susceptibility to pesticides and related compounds.
  - The cultures will be evaluated for oxidative stress, α-synuclein aggregation, neurite retraction, and survival

• Use the cell culture model to biologically validate variants identified in the FAME cohort
  - Genetic loci of interest will be downregulated or introduced using CRISPR/cas-9 to evaluate effect on cellular response to pesticides and related compounds of military relevance.



Environmental and genetic factors interact in poorly understood ways to cause Parkinson's disease (PD). This project aims to identify and biologically validate genetic variants that modulate individual susceptibility to toxicant-induced PD.

Accomplishment: The FAME database has been evaluated to identify SNPs associated with increased susceptibility to certain pesticides. An iPSC-derived dopaminergic neuron culture system has been established for evaluating pesticide – gene interactions on oxidative stress and cell survival.

Timeline and Cost

Milestones / CY	20	21	22	23
Development and characterization of GSTT1- deficient cell lines.				
FAME SNP and WGS data analysis				
Use the cell lines to evaluate GSST1 effects on pesticide toxicity				
Evaluate additional gene variants and manuscript submission				
Estimated Budget ($2,398K)	**$440K**	**$894K**	**$671K**	**$393K**

Updated: 8/21/22

Goals/Milestones

CY20 Goals – Procedure implementation
☑ Implement team organization, genomic analysis methods, and cell culture system.

CY21 Goals – FAME database analysis / genetic manipulation of cells ☑ Complete FAME sequencing and SNP analysis
☑ Establish GSTT1-modulated iPSC neuronal lines.

CY22 Goals – WGS analysis, complete GSTT1 studies
☑ Analysis of whole genome sequence data
☐ Evaluate GSTT1 up/down regulation on cell response to pesticides.

CY23 Goals – Validate additional gene-pesticide interactions, reporting
☐ Evaluate additional gene variants in the iPSC model
☐ Manuscript preparation

Comments/Challenges/Issues/Concerns - 2-month delay in dopaminergic differentiation of iPSC cells; 4-month delay in long-term cultivation of viable iPSCs. These technical issues are now resolved.

Budget Expenditure to Date

Projected Expenditure: $723,434 (-0709); $1,674,494 (-0710)
Actual Expenditure: $493,998 (-0709); $936,452 (-0710)