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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Frontotemporal dementia (FTD) is one of the major causes of dementia in adults under the age of 65. To better understand this disease, we focus our studies on one of the most prevalent subsets of FTD patients, those carrying a mutation in the *C9orf72* gene (C9 FTD). To gain more knowledge about how mutations in C9orf72 lead to FTD, we are generating a genetic signature profile of postmortem patient tissues. Our studies provide first insights into C9-mediated FTD disease pathogenesis, with the goal of identifying novel therapeutic targets for future therapeutic interventions. To accomplish this goal, we generated cell type specific gene expression profiles from C9 FTD patient frontal cortex postmortem autopsy tissue samples using the novel technology of single nuclei RNA sequencing (snRNA seq). This transcriptome analysis will now be compared and validated to a genotypic and phenotypic analysis of C9 FTD patient derived induced pluripotent stem cells (iPSCs) differentiated into cortical neurons. This cell model is currently being established after collecting and reprogramming the patient subgroup-specific iPSC lines. We will combine genetic signature profiles from these two disease models, which will then allow us to select candidate genes that we hypothesize play a significant role in C9 FTD disease pathogenesis. We have also early data on the establishment of FTD-relevant behavioral in vivo assays using Drosophila models of C9orf72. Eventually, we will test whether the aberrantly expressed genes identified from patient tissue and cells will affect the behavioral cognitive phenotypes of C9 FTD flies.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

1. Introduction

Frontotemporal dementia (FTD) is one of the major causes of dementia in adults under the age of 65, affecting neurons in the frontal and temporal cortices of patients, which leads to clinical abnormalities in behavior, language and personality. FTD still remains a poorly understood disease, especially in regards to its underlying cellular and molecular mechanisms. The genetic, pathological and clinical heterogeneity exacerbates this lack of knowledge and the resulting absence of specific therapeutic interventions that could ameliorate or even prevent disease symptoms. We therefore proposed to study molecular and cellular alterations in affected cortical neurons using single cell type specific approaches, followed by aberrant gene candidate validations in human cell models and animal models of disease. The focus of this project is on the most common genetic form of FTD, the hexanucleotide repeat expansion in the *C9orf72* gene (C9). We hypothesize that **the cell-type specific C9 – FTD genetic profiles and functional phenotypes are related to FTD-specific disease pathogenesis.** Our studies will provide first insights into C9-mediated FTD disease pathogenesis, with the goal of identifying novel therapeutic targets for future therapeutic interventions. To test this hypothesis, we will obtain several cell type specific gene expression profiles from C9 FTD patient frontal cortex postmortem autopsy tissue samples using the novel technology of single nuclei RNA sequencing (snRNA seq; Aim 1). This transcriptome analysis will be compared to, and validated against genotypic and phenotypic analyses of C9 FTD patient derived induced pluripotent stem cells (iPSCs) differentiated into cortical neurons (Aim 2). Combining the genetic signature profiles from the two single cell specific models, we will select candidate genes that we hypothesize play a significant role in C9 FTD disease pathogenesis. To test whether these genes will affect the behavioral cognitive phenotypes of C9 FTD patients, we will use a Drosophila (fruit fly) model of C9 FTD and perform genetic interaction studies of these candidate genes in this novel, in vivo dementia model (Aim 3).

2. Keywords

Frontotemporal dementia, FTD, C9orf72, transcriptome, single nuclei RNA sequencing, iPSC

3. Accomplishments

Due to the worldwide pandemic caused by Covid19 and temporary shutdown of all laboratories at all three sites, we have not been able to meet all of the goals and milestones proposed in our application, but have nevertheless made significant progress. Below, we describe in detail the progress on the individual aims and sub-aims for the first year of our project funding.
The Highlights of our accomplishments are:

- Collection of all required postmortem brain tissue samples from varying brain banks (nationally and internationally)
- Optimization of nuclei isolation for 10x genome analysis
- Nuclei isolation of all proposed brain tissue samples
- 10x genome preparation of all isolated nuclei samples
- Collection of C9orf72 FTD patient fibroblasts and successful reprogramming into iPSCs
- Successful breeding of mutant C9orf72 fly strain and initial characterization of MBs
- Successful breeding of mutant C9orf72 fly strain and initial characterization of MBs
- Establishment of behavioral assay to measure FTD-like behavioral defects

a. What were the major goals of the project?

	Timeline	Site 1 Dr. Sattler	Site 2 Dr. Jensen	Site 3 Dr. Zarnescu	Completion date/ percentage of completion
Specific Aim 1: Single cell RNA sequencing of C9 FTD patient frontal cortex					
Major Task 1: Development of a single nuclei RNA sequencing approach for frozen postmortem FTD patient tissue					
Subtask 1: Development of successful tissue dissociation of fresh frozen postmortem tissue					
Dr. Sattler's laboratory will perform the tissue dissociation process and the isolation of single nuclei	1 to 24	X	X		100%
Dr. Van Keuren-Jensen's laboratory will provide resources for assessing quality of dissociated single nuclei by staining and RNA integrity measurements	1 to 24		X		100%
Subtask 2: single nuclei RNA sequencing using 10x technology					
We will use the 10x single cell platform to generate transcriptomic data	6 to 30		X		50%
Milestone #1: Successful sc RNA sequencing with acceptable quality control criteria	6 to 12				50%
Major Task 2: Data analysis of sc RNA sequencing					
Subtask 1: RNA sequencing pathway analysis for each individual cell type					
We will use SMART-Seq2 data to define expression profiles for each cell type and identify significant differences and associations between the cells.	6 to 30		X		not initiated
Pathway analysis will be applied to identify aberrant disease- and brain-region specific pathways	6 to 30		X		not initiated
Specific Aim 2: Genotypic and phenotypic analysis of C9 FTD hiPSC cortical neurons					
Major Task 3: Generation of patient-derived iPSC cortical neurons (CNs)					
Subtask 1: iPSC differentiation into cortical neurons.					
Existing validated protocols will be used to differentiate iPSCs into cortical forebrain neurons	1 to 36	X			25%
Subtask 2: RNA seq analysis of iPSC cortical neurons					
Dr. Sattler laboratory will isolate RNA from iPSC CNs and processed for RNA sequencing analysis	6 to 20	X			not initiated
Dr. Van Keuren-Jensen's laboratory will perform standard RNA seq analyses on isolated RNA	6 to 20		X		not initiated
Subtask 4: Morphological phenotypic characterization of iPSC cortical neurons					
iPSC CNs will be examined for dendritic arborization and spine density	6 to 30	X			not initiated
Subtask 5: Functional phenotypic characterization of iPSC cortical neurons					
iPSC CNs will be examined for functional synapse numbers and electrical firing activities using MEA	6 to 30	X			not initiated
iPSC CNs will be treated with glutamate to test for increased susceptibility to cell stressors	6 to 30	X			not initiated
Specific Aim 3: Validation of FTD disease-relevant pathways/genes using a C9 FTD fly model					
Major Task 4:					
Subtask 1: Development and validation of C9 FTD fly behavioral dementia assay					
Identify morphological and behavioral (memory) phenotypes in flies expressing C9 expansions in MB neurons	1 to 12			X	25%

b. What was accomplished under these goals?

Major activities

The major activity for this first year support was the collection of patient postmortem autopsy brain tissue samples with strong clinical demographic documentation to ensure the selection of the proposed C9orf72 patient subgroups: ALS, ALS/FTD and FTD. These tissues were obtained from three different brain banks: Body and Brain Donation Program at Banner Sun Health Research Institute (Dr. Tom Beach); Target ALS Human postmortem tissue core (Dr. Lyle Ostrow); Queen Square Brain Bank, UCL Queen Square Institute of Neurology (Dr. Jon Rohrer). Table 1 below lists all tissue samples and the corresponding clinical information.

Meanwhile, using existing control patient tissue samples, we optimized the process of isolating intact cellular nuclei from frozen tissue samples, which is required for the proposed 10x genomics analyses of single nuclei. The optimized isolation protocol is described below.

Finally, albeit delayed due to the Covid19-induced pandemic and laboratory shut downs, we finalized nuclei isolation and 10x genomics preparations of all of our proposed tissue samples (see Table 1 below).

In addition, we obtained 5 C9orf72 FTD patient fibroblast lines through our collaborations with UCL (Drs. Rohrer and Wray). After internal cell propagation, three patient lines were submitted to the Baylor College of Medicine Stem Cell Core Facility for reprogramming into iPSCs. A characterization of the successful generation of these iPSC lines is provided below.

Finally, we cross bred mutant C9orf72 fly models to a genetic background which is more suited for behavioral studies, and initiated first behavioral assay optimizations and validations.

Specific objectives

One objective for this funding period was the generation, validation and use of an optimized single nuclei isolation protocol to perform single nuclei transcriptomics analyses on frontal cortex (disease-affected brain region) and occipital cortex (disease un-affected brain region) postmortem brain tissue samples obtained from clinically confirmed C9orf72 patient subgroups: FTD, FTD/ALS, ALS; in addition to healthy control subjects.
An additional objective was the collection and/or generation of C9orf72 patient iPSC lines from the three C9orf72 patient subgroups: FTD, FTD/ALS, ALS; in addition to healthy control subjects.
Finally, the last objective for this funding period was the establishment and validation of FTD-relevant behavioral assays in a C9orf72 fly model.

Significant results

Successful collection of C9orf72 patient brain tissue samples

We have been able to obtain de-identified C9orf72 patient and healthy subject control postmortem brain tissue samples (frontal cortex and occipital cortex) from three different brain banks: Body and Brain Donation Program at Banner Sun Health Research Institute (Dr. Tom Beach); Target ALS Human postmortem tissue core (Dr. Lyle Ostrow); Queen Square Brain Bank, UCL Queen Square Institute of Neurology (Dr. Jon Rohrer). The collaborative connection to the UCL brain bank was established after we were received funding for this project and has greatly increased our number of patient and control samples for these studies. The demographics of all samples is listed below in Table 1. All tissues are divided into 30-50mg pieces and stored at -80C at the Barrow Neurological Institute.

Table 1. Patient demographics of postmortem brain tissue samples

Tissue Identifier	Source	Disease Group	Pathological Diagnosis	Sex	Race	Mutation
JHU 22	TargetALS - John Hopkins	C9+ ALS		M	B	*C9ORF72*
JHU52	TargetALS - John Hopkins	C9+ ALS		M	W	*C9ORF72*
JHU54	TargetALS - John Hopkins	C9+ ALS		F	W	*C9ORF72*
JHU67	TargetALS - John Hopkins	C9+ ALS		M	W	*C9ORF72*
JHU92	TargetALS - John Hopkins	C9+ ALS		M	W	*C9ORF72*
JHU120	TargetALS - John Hopkins	C9+ ALS	ALS with TDP	F	W	*C9ORF72*
CU-16	TargetALS - Columbia	C9+ ALS		M	W	*C9ORF72*
BH 19-28	Tom Beach, Banner Health	C9+ ALS	ALS with TDP	M		*C9ORF72*
JHU119	TargetALS - John Hopkins	C9+ FTLD-ALS		F	W	*C9ORF72*
CU-25	TargetALS - Columbia	C9+ FTLD-ALS	FTLD-ALS	M	W	*C9ORF72*
CU-34	TargetALS - Columbia	C9+ FTLD-ALS	FTLD-ALS Type C?	M	W	*C9ORF72*
CU-50	TargetALS - Columbia	C9+ FTLD-ALS	FTLD-ALS Type C	M	W	*C9ORF72*
CU-60	TargetALS - Columbia	C9+ FTLD-ALS	FTLD-ALS Type B	F	W	*C9ORF72*
CU-62	TargetALS - Columbia	C9+ FTLD-ALS	FTLD-ALS Type B	F	W	*C9ORF72*
BH 03-23	Tom Beach, Banner Health	C9+ FTD	FTLD-TDP	F		*C9ORF72*
BH 04-53	Tom Beach, Banner Health	C9+ FTD	FTLD-TDP	F		*C9ORF72*
BH 11-34	Tom Beach, Banner Health	C9+ FTD	FTLD-TDP	F		*C9ORF72*
BH 19-13	Tom Beach, Banner Health	C9+ FTD	FTLD-TDP and AD	M		*C9ORF72*
QSBB P65/08	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
QSBB P16/09	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
QSBB P70/10	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
QSBB P56/13	Queen Square, UCL	C9+ FTD	FTLD-TDPA	F		*C9ORF72*
QSBB P23/15	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
QSBB P17/17	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
QSBB P99/17	Queen Square, UCL	C9+ FTD	FTLD-TDPA	M		*C9ORF72*
JHU95	TargetALS - John Hopkins	Non-neuro controls	Control	M	W	N/A
JHU96	TargetALS - John Hopkins	Non-neuro controls	Control	F	B	N/A
JHU101	TargetALS - John Hopkins	Non-neuro controls	Control	F		N/A
JHU110	TargetALS - John Hopkins	Non-neuro controls	Control	M	B	N/A
JHU123	TargetALS - John Hopkins	Non-neuro controls	Control	M	W	N/A
GBB 17-21	TargetALS- Georgetown	Non-neuro controls	Control	M	B	N/A
GBB 18-13	TargetALS- Georgetown	Non-neuro controls	Control	F	B	N/A
QSBB P29/12	Queen Square, UCL	Non-neuro controls	Control	F		N/A
QSBB P77/12	Queen Square, UCL	Non-neuro controls	Control	M		N/A
QSBB P22/13	Queen Square, UCL	Non-neuro controls	Control	F		N/A
QSBB P43/13	Queen Square, UCL	Non-neuro controls	Control	M		N/A
QSBB P48/15	Queen Square, UCL	Non-neuro controls	Control	F		N/A
QSBB P17/16	Queen Square, UCL	Non-neuro controls	Control	F		N/A
QSBB P24/17	Queen Square, UCL	Non-neuro controls	Control	F		N/A
BH 09-50	Tom Beach, Banner Health	Non-neuro controls	Control	M		N/A
BH 17 -19	Tom Beach, Banner Health	Non-neuro controls	Control	F		N/A
BH 99 -44	Tom Beach, Banner Health	Non-neuro controls	Control	M		N/A
BH 18 -78	Tom Beach, Banner Health	Non-neuro controls	Control	M		N/A
BH 18 -64	Tom Beach, Banner Health	Non-neuro controls	Control	M		N/A

Optimization of single nuclei isolation from frozen brain tissue samples

Several published protocol for frozen brain tissue single nuclei isolation were trialed and optimized for generation of a final protocol, which then was validated for reproducibility using healthy control brain tissue:

Single nuclei isolation protocol

1. Pre-cool dounce homogenizers and buffers on ice, set centrifuge to 4˚C.
2. Add 500 ul of chilled Nuclei EZ prep lysis buffer (complete stock/Nuclei EZ Prep buffer/RNasin Plus) to dounce homogenizer on ice, place 30-50 mg piece of tissue in buffer, let sit for 2.5-5 mins.
3. Dounce tissue 10-15 times with loose pestle and then 10-20 times with tight pestle dependent on observation of homogenate.

4. Add 500 ul of chilled Nuclei EZ prep lysis buffer to 1.5 ml tube with 70 um filter, transfer homogenate to filter. Pipette mix homogenate a few times as homogenate sits in lysis buffer for 2.5-5 mins.
5. Centrifuge homogenate at 500g for 5 min at 4˚C and remove as much supernatant as possible. Resuspend in 1 ml of Nuclei EZ prep lysis buffer and let sit on ice for 5 mins.
6. Centrifuge the nuclei at 500g for 5 min at 4˚Cm again remove as much supernatant as possible. Add 500 ul of chilled wash buffer (PBS/BSA/RNasin Plus) and incubate for 5 mins to allow buffer interchange, after incubation add 500 ul of wash buffer and mix thoroughly.
7. Centrifuge the nuclei at 500g for 5 min at 4˚C, remove as much supernatant as possible and resuspend in 250 ul - 1 ml of wash buffer (dependent on pellet size)
8. Incubate sample with NucBlue or NucGreen and take 10 ul aliquot for Countess nuclei count.
9. Proceed directly to FACS sorting of NucBlue or NucGreen positive nuclei at 15,000 events into 10x RT mix minus RT enzyme.

Generation of single nuclei from postmortem tissue, followed by FACS purification

Using the optimized protocol above, we isolated single nuclei from a total of 48 brain tissue samples: 3xC9orf72 patient subgroups (FTD, ALS, ALS/FTD) and healthy controls (6 individuals per group), 2 brain regions were collected from each individual (Frontal cortex and Occipital cortex). Additionally, we included 12 10x chip controls using the frontal cortex of the healthy control to determine any batch effects. Immediately following the tissue homogenization and nuclei isolation, nuclei were sorted via Fluorescent activated cell sorting (FACS) using a 100 um sorting chip on a Sony SH800S cell sorter gated based on GFP/NucGreen signal. We used the single cell purification setting and sorting time varied depending on sample. Figure 1 shows a representative image of a sample after FACS of NucGreen positive nuclei, which were sorted directly into 10x 3' v3 RT reaction buffer for 10x genomics GEM (Gel Beads-in-emulsion) formation.

10x 3' v3 sample cDNA were cleaned up and amplified based on manufacturer's recommendations. cDNA was processed through library prep based on 10x recommendations with changes only at the sample index PCR based on manufacturer's recommendations. Libraries were pooled and sequenced on an iSeq 100 v2 flowcell and reads were normalized prior to sequencing on a NovaSeq S2 at a minimum of 50,000 read pairs per cell using run parameters of 28 cycles for Read 1, 8 cycles for i7 index, 0 cycles for i5 index, and 91 cycles for read 2 index.

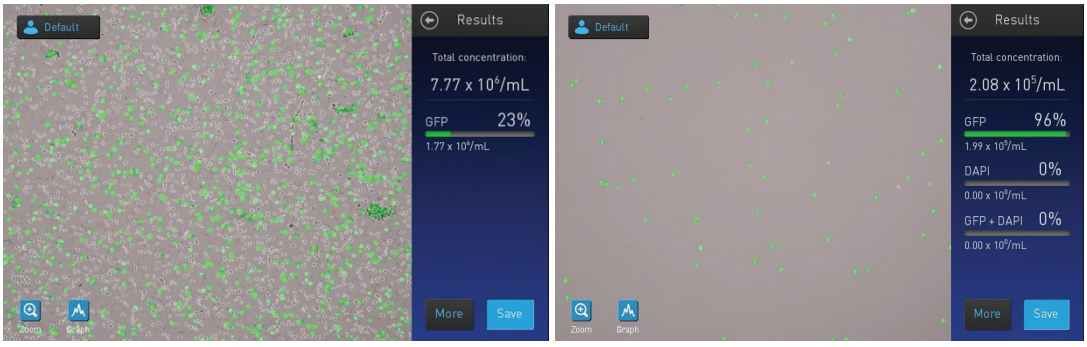

Figure 1. Optimization of nuclei preparations. The figure on the left depicts data from the Countess after cell dissociation, centrifugation and resuspension. The figure on the right displays nuclei after FACS. We have optimized the prep to reduce debris and capture intact nuclei.

Successful generation of 10x genomics single nuclei transcriptome profile of C9orf72 patient subgroup brain tissue

We have preliminary data supporting the successful sequencing and clustering of single nuclei data using control tissue (n=7 samples). With our optimized protocol, we have 4,800 cells per sample sequenced, which exceeds our original goal of 2,500 nuclei/sample. We currently get an average of 43,580 post-processing reads per cell. Our goal is 50,000 reads per cell and we will increase our read depth accordingly. Figure 2 displays the clustering using the nuclei captured with our control tissue. We can increase the specificity of the clusters in future iterations of analysis, but for now we are showing the clusters with minimal clean up and processing.

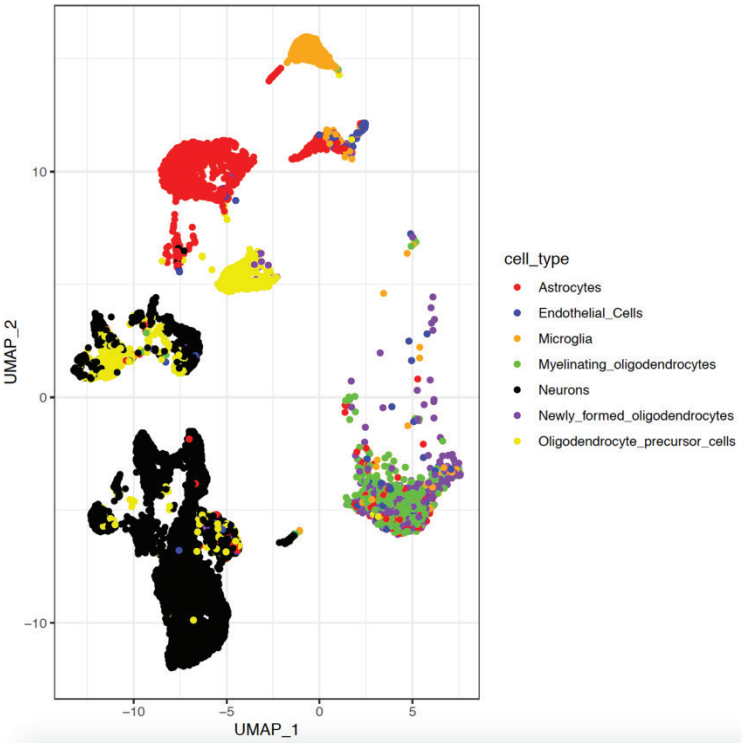

Figure 2. Cell-type clustering after snRNA seq. Clustering analysis of 4800 nuclei of healthy control tissue from frontal cortex

Collection of C9orf72 patient subgroup iPSC, including the generation of new iPSCs from C9orf72 FTD patient fibroblasts

We have successfully collected all of the patient lines required for our studies (Table 2). In addition to existing iPSCs obtained from varying sources, we also contracted out the reprogramming of 3 C9-FTD patient fibroblasts into iPSCs. Albeit with delay due to Covid-19, these cell lines were just received from the Baylor Stem Cell Core facility, with all required quality control assessments for pluripotency. The iPSCs are currently being expanded in the Sattler laboratory and prepared for cortical neuron differentiations.

Table 2. List of patient iPSC lines and demographics

Cell Line	Cell Line Source	Diagnosis	Mutation	Age at Onset	Age at Sample Collection	Sex	Baseline CBS	FTLD-CDR-SOB	MMSE	Baseline ALS-FRS
YELLOW-IoN 326	UCL	C9-FTD	C9ORF72	55	57	Male		10	27	
YELLOW-IoN 357	UCL	C9-FTD	C9ORF72	40	57	Male		11.5	24	
YELLOW-IoN 457	UCL	C9-FTD	C9ORF72	65	68	Female		18	14	
CS0LPKiALS-n1 *	AnswerALS / Cedar Sinai	C9-ALS with cognitive symptoms	C9ORF72	65	67	Male	7			36
CS2DDGiALS-n1 *	AnswerALS / Cedar Sinai	C9-ALS with cognitive symptoms	C9ORF72	55	57	Female	12			37
CS3EGMiALS-n5 *	AnswerALS / Cedar Sinai	C9-ALS with cognitive symptoms	C9ORF72	64	66	Male	12			43
CS0BUUiALS-n3	AnswerALS / Cedar Sinai	C9-ALS	C9ORF72	60	63	Female	Not Collected			Not Collected
CS1HKRiALS-n3	AnswerALS / Cedar Sinai	C9-ALS	C9ORF72	52	58	Male	Not Collected			45
CS5MNZiALS-n2	AnswerALS / Cedar Sinai	C9-ALS	C9ORF72	50	51	Female	Not Collected			31
CS25iCTR-18nxx	Cedar Sinai	Control	N/A	N/A	76	Male	N/A			N/A
CS83iCTR-33nxx	Cedar Sinai	Control	N/A	N/A	21	Female	N/A			N/A
CS14iCTR-nxx	Coriell Cell Repositories	Control	N/A	N/A	Unknown	Female	N/A			N/A

Generation of C9orf72 repeat expansion Drosophila fly strains

We have introduced the C9 constructs (G4C2 3X, G4C2 RNA only 108X and polyGR 100X) as well as the w; attP40 genetic background control in a “true” wild-type background called OR-R (Oregon-R). We found this to be a necessary step to reduce variability, a standard procedure in the Drosophila behavior field. We now have all these stock in hand, ready for the behavioral experiments proposed in the application. Due to COVID-19 we were unable to repeat the learning and memory assays based on wasp exposure as proposed but plan to do so in the coming weeks. We have begun characterizing the morphological phenotypes of C9 expressing flies by crossing a Kenyon cell specific GAL4 driver (SS01276) with various C9 lines and controls. Preliminary results show that young flies (Day 1) expressing 108G4C2 RO (RNA only) repeats reveal a possible midline crossing phenotype while those expressing 100 polyGR repeats appear to exhibit abnormal axonal projections (Figure 3).

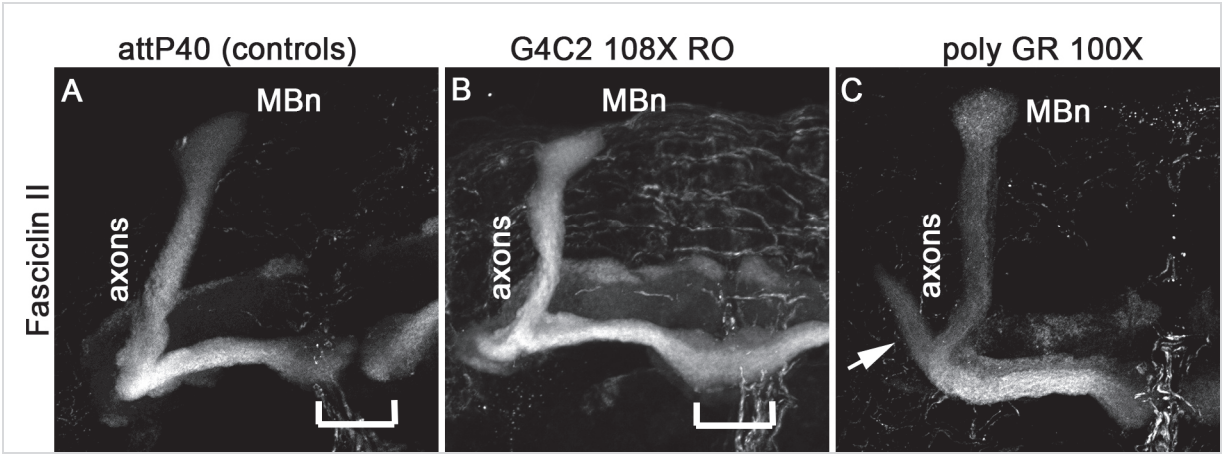

Figure 3. Expression of C9 transgenes affects mushroom body neuron (MBn) morphology. MBns were labeled with the cell membrane markers Fasciclin II. Genotypes as indicated. Brackets in A and B indicate the midline and arrow in C indicate abnormal axonal projections. Images were acquired from dissected brains of 1day old adult flies.

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

Not applicable

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 aim to address the following aspects of our proposal during the second year of our award period:

- Complete the snRNA sequencing of isolated nuclei and immediately move on to bioinformatics analyses of these data sets
- Initiate differentiations of C9 FTD iPSCs and those from the other experimental groups into cortical neurons and start molecular and cellular analyses, unbiased and also guided with the bioinformatics analyses from snRNA seq data.
- We will establish the wasp exposure based learning and memory assays and complete the morphological analyses of C9 transgenics in a behaviorally appropriate OR-R background. In addition, we will perform memory assays based on Y maze performance, sleep/activity and lifespan assays. As preliminary data is generated from the bioinformatics analyses of snRNA seq data we will begin genetic interactions with top candidate genes.

4. Impact

a. What was the impact on the development of the principal disciplines of the project?

Even the early stages of this project will provide the scientific community with technical advice and validation steps that might improve the difficult approach of isolation single nuclei from frozen autopsy patient brain tissues. In addition, the establishment of FTD-relevant behavioral fly assays will be of great value to scientists working in the field of cognitive impairment and diseases characterized by these deficits.

b. What was the impact on other disciplines?

The optimization of single nuclei isolation could easily be transferred to any other disease studies, either other CNS diseases, but also diseases affecting peripheral tissues and organs.

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

5. Changes/Problems
a. Changes in approach and reasons for change
There have been no changes in our approach or objectives for this project.

b. Actual or anticipated problems or delays and actions or plans to resolve them
Due to the Covid-19 pandemic and the temporary shutdown of all participating laboratories, we are slightly delayed with our anticipated milestones. However, we are expecting to be able to catch up with these milestones throughout the second funding period.

c. Changes that had a significant impact on expenditures
None noted

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None noted

e. Significant changes in use or care of human subjects
None noted

f. Significant changes in use or care of vertebrate animals
None noted

g. Significant changes in use of biohazards and/or select agents
None noted

6. Products
a. 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
- We will make all of our snRNA seq data sets publicly available to other researchers
- The newly reprogrammed C9 FTD iPCS cell lines will also be made available to researchers in the field
- The protocols and assays developed for FTD-relevant Drosophila behavior will be shared with other researchers

7. Participants & Other Collaborating Organizations
a. What individuals have worked on the project

Sattler Lab:

Name	Rita Sattler	Lauren Gittings	Mo Roberts
Project Role	PI	Postdoctoral Fellow	Research Technician
Research Identifier (e.g. ORCID ID)			
Nearest person month worked:	2	6	12
Contribution to Project	Project design and supervision	Dr. Gittings has collected all of the brain tissue samples, and has particpated in single nuclei isolation process. She has further collected all patient iPSC and fibroblast lines, and oversaw the reprogramming of fibroblasts into iPSCs	Ms.Roberts has been aiding in the single nuclei protocol optimization and the actual isolation process
Funding support		Fein Foundation	

Van Keuren-Jensen Lab:

Name	Kendal Van Keuren- Jensen	Jeryy Antone	Elizabeth Hutchins	Bessie Meechoovet	Amber Logemann
Project Role	PI	Computational Scientist	Computational Scientist	Research Associate	Research Associate
Calendar month worked	0.6	9	3	1.68	6.96
% effort worked	5%	75%	25%	14%	58%
Contribution to project	Project design and Supervision	Dr. Antone has been working closely with Dr. Van Keuren-Jensen providing statistical analysis and informatics support.	Dr. Hutchins has been working closely with Dr. Van Keuren-Jensen providing statistical analysis and informatics support.	Ms. Meechoovet is working on the single nuclei protocol optimization and the isolation process in addition to the 10X genomics sample preparation and RNA sequencing process.	Ms. Logemann is working on the single nuclei protocol optimization and the isolation process in addition to the 10X genomics sample preparation and RNA sequencing process.

Zarnescu Lab:

Name	Daniela Zarnescu	Rebekah Keating Godfrey	Haley McMullen
Project Role	Co-I	post-doc	MS student
Research Identifier (e.g. ORCID ID)			
Nearest person month worked:	0.5	1 month	250 hours
Contribution to Project	Project design and supervision	Imaging MB neuron phenotypes in C9 ALS flies	Generated C9 ALS stocks in a OR-R genetic background; trained in and performed behavioral assays

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

Updated support for:
Dr. Rita Sattler, PI

11/01/20 – 08/31/23 Mechanisms underlying the protective role of glycolysis
R21 NS115514-01A1
NIH NINDS
$150,00/$40,000
PI: Zarnescu D
Role: Co-I, 0.6 cal mos
Major Goal: To investigate the role of glycolysis in motor neuron degeneration

08/01/20 – 07/31/21 Role of microglia in C9orf72 ALS/FTD
Robert Packard Center for ALS Research
$50,000/$25,000
Multi-PI: Sattler R; Ichida J
Major goals: To determine how neuron-immune response contributes to disease pathogenesis in C9orf72 ALS/FTD

07/01/20 – 06/30/21 Postdoctoral Fellowship
Barrow Neurological Foundation
PI: Gittings
$85,000
Role: Mentor
Major goals: Identifying the mechanisms of disease pathogenesis in the C9orf72 ALS/FTD spectrum using snRNA sequencing in postmortem autopsy brain tissues and patient-derived iPSC cortical neurons.

07/01/20 – 06/30/22 Interplay between C9orf72 ALS and neurotropic viruses: Impact on viral and ALS disease pathogenesis
Valley Research Partnership Program Award (VRP)
$49,640/$24,480
Multi-PI: Sattler R; Gustin, K
Major Goal: to test the hypothesis that C9orf72 repeat expansions alter the host antiviral response and that viral infection will exacerbate C9of72-mediated ALS disease pathogenesis.

07/01/20 – 06/30/21 Synapse loss in frontotemporal dementia (FTD): Validation by immunohistochemistry in FTD/ALS patient post-mortem tissues and PET imaging in a FTD mouse model.
Arizona Alzheimer's Research Consortium/BNF
$54,850
PI: Sattler R, 1.2 cal mos
Major Goal: To validate the use of 18[F]-FDG PET tracer as a marker for synapse damage or loss in a progranulin knock out mouse model for FTD.

11/01/19 – 08/31/21 RNA Dysregulation in neurodegeneration
RO1 Supplement award (NCE)
NIH NINDS
$377,974/$91,000
PI: Zarnescu D
Role: Co-PI, 1 cal mos
Major Goal: To investigate the role of TDP43 in cognitive impairment in FTD using Drosophila models of disease as well as patient-derived iPSC cortical neurons.

09/15/19 – 12/31/20 Matrix Metalloproteinase 2/9 inhibitor for the treatment of ALS
SBIR (NCE)
NIH NINDS
$457,639/$25,000
PI: Sucholeiki (Aquilus Pharma)
Role: Co-I, 0.6 cal mos

01/01/19 – 12/31/20 Studies of disease mechanisms and biomarker development for FTD
Fein Foundation
$500,000/$150,000
Multi-PI: Sattler/Bowser/Mufson/Shefner
Major goals: To study mechanism of FTD disease pathogenesis using patient-derived iPSC cortical neurons and FTD patient postmortem autopsy tissue.

Dr. Kendal Van Keuren-Jensen, PI

03/01/20 – 03/31/21 Extracellular Vesicles
TGen (Dorrance) Foundation
$843,810
Multi PI: Jensen/Von Hoff
The goal of the multiple organ and sample study is to find out more about extracellular vesicles.

06/01/19 – 05/31/20 3R0NS091299-05S1 : RNA dysregulation in neurodegeneration
NIH (University of Arizona)
$30,000
Co-Investigator
The goal is to fill a critical gap in our understanding of the molecular mechanisms underlying the ALS/FTD spectrum disorder.

Dr. Daniela Zarnescu, Co-I

11/01/20 – 08/31/23 Mechanisms underlying the protective role of glycolysis
R21 NS115514-01A1
NIH NINDS
$150,000
PI: Zarnescu D
Major Goal: To investigate the role of glycolysis in motor neuron degeneration

11/01/19 – 08/31/21 RNA Dysregulation in neurodegeneration
RO1 Supplement award (NCE)
NIH NINDS
$377,974/$91,000
PI: Zarnescu D
Major Goal: To investigate the role of TDP43 in cognitive impairment in FTD using Drosophila models of disease as well as patient-derived iPSC cortical neurons.

c. What other organizations were involved as partners?

We reached out to our collaborators from the Queen Square Brain Bank and the Institute of Neurology at UCL, London, UK to supplement both our postmortem brain tissue collection, but also our C9 patient iPSC collection.

8. Special Reporting Requirements

a. Collaborative Awards

b. Quad Charts

9. Appendices- None