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TITLE: microRNA Replacement Therapy for ALS Treatment

PRINCIPAL INVESTIGATOR: Samuel Pfaff, Ph.D.

CONTRACTING ORGANIZATION: The Salk Institute for Biological Studies, La Jolla, CA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall goal of this grant is to determine whether ALS affects the expression and/or function of a critical microRNA-218 (miR-218) using mouse genetics. We have generated a novel mouse line that detects miR-218 activity (tg-miR-218-rep) and used it to establish that we can induce deletion of miR-218 in adult motor neurons using a novel intercross of the following animals: miR-218-1 -/- ; miR-218-2 CreER/fl; tg-miR-218-rep +/-. This inducible system for eliminating miR-218 will help to establish for the first time whether both alleles of miR-218 are required in mature motor neurons. We have determined that our reporter detects levels of miR-218 in SOD1(G93A) and PFN1(C71G) mice at end-stage, as well as in advanced disease TDP43(Q331K) and aging, asymptomatic BAC500 mice. The sensitivity of our reporter gene suggests that motor neurons maintain detectable levels of miR-218 at late stages of disease. We have hypothesized that elevated miR-218 may be protective against some ALS-causing mutations. To test this hypothesis, we have generated a new self-complementing retroAAV virus carrying miR-218 that will be injected into ALS mouse models. Finally, we have tested and identified a miR218 mimic molecule using cell culture screens. This modified oligonucleotide can be used as an alternative approach to increase miR-218 in ALS.

15. SUBJECT TERMS

ALS, microRNA, miR-218, motor neuron, neurodegeneration, PFN1(C71G), reporter, retroAAV, SOD1(G93A), TDP43(Q331K).

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1. INTRODUCTION

Motor neuron death drives ALS progression either due to both cell-autonomous and non-cell-autonomous effects. Mutations in more than 40 genes have been linked to the development of ALS. Of these genes, C9orf72, TDP43, FUS/TLS and SOD1 account for most of the known familial cases. C9orf72, TDP43 and FUS/TLS are genes that interact with RNAs in control their processing and transport. In particular, TDP43 and FUS/TLS interact with the nucleases involved in microRNA generation from longer precursor RNAs. We previously characterized miR-218 as the most highly enriched microRNA in motor neurons, observing neurodegenerative-like defects in mice embryos lacking miR-218. MiR-218 controls the expression of ~300 genes, many of them involved in cell homeostasis. In this grant we hypothesize that reduced levels of functional miR-218 contribute to the progression of ALS. Consequently, we predict that genetically-raising miR218 levels will slow ALS progression, and if our hypothesis is correct we logically predict that genetically-reducing the levels of miR218 will accelerate the disease. Our proposal takes advantage of mouse genetics to determine the natural history of miR-218 and its regulatory network in adult mice in the context of ALS. Our studies will also examine whether the genes regulated by miR-218 themselves have therapeutic potential.

2. KEYWORDS

ALS, microRNA, miR-218, motor neuron, neurodegeneration, PFN1(C71G), reporter, retroAAV, SOD1(G93A), TDP43(Q331K).

3. ACCOMLIPSHMENTS

3a. What were the major goals of the project?

The major goals of our project are to:

1. Create a detailed survey of miR-218 function in the spinal cord
2. Perform miR-218-associated candidate therapeutic approach for ALS
3. Probe miR-218 mimics as ALS therapeutic agents

3b. What was accomplished under these goals?

Progress on each task is described below the statement of work for each set of experiments.

Experiment	Timeline (Months)	Site Personnel (#mice)
Aim 1 Detailed survey of miR-218 function in the spinal cord.		
Task 1: IACUC approval currently in place (protocol 11-00020)	0	Dr. Sam Pfaff
Milestone: ACURO Approval	3	

Aim 1, Task 1. As described in the Year 1 report, our project received ACURO approval in January 2019. All of our aims involve work in mice and therefore animal crosses for the project began at this stage. We have obtained ALS mouse models animals for SOD1(G93A), TDP-43(Q331K) and PFN1(C71G). These animals have been intercrossed with miR-218 knockout mutants and with a novel miR-218 mouse reporter line (tg-miR-218-rep; described below in task 4) to generate the genetic combinations needed for experimentation. Initial

characterization of the off spring has begun. Overall, these genetic experiments are underway as described in the grant.

Task 2: LCM capture of motor neuron for SmartSeq2 and Taqman analysis, together with histology analysis in WT mice.	0-4	Dr. Giancarlo Costaguta Shawn Driscoll Dr. Sam Pfaff (12 WT mice)
Task 3: LCM capture of motor neuron for SmartSeq2 and Taqman analysis, together with histology analysis in ALS mouse models and tamoxifen-inducible 218DKO mice.	4-12	Dr. Giancarlo Costaguta Shawn Driscoll Dr. Sam Pfaff (12 mice of each SOD1(G93A), PFN1(C71G), Tam-218DKO)

Aim1, Tasks 2 and 3. Our expectation was to perform next generation sequencing from nuclei isolated from mice cohorts bearing miR-218-1 -/- miR-218-2 CreER/fl tg-miR-218-rep +/- and a control cohort miR-218-1 -/- miR-218-2 +/fl tg-miR-218-rep +/- that have been injected with Tamoxifen to induce Cre-dependent expression and deletion of miR-218 from adult motor neurons. In our Year 2 progress report we found that our GFP-reporter still detected miR-218 3-months after Cre-dependent ablation of miR-218 in motor neuron. As well, we had observed that conditional deletion of miR-218 in adults produced partial neuromuscular junction (NMJ) loss ~12-15 month after Tamoxifen injection. For the current period, we set to wait at least 12 months after miR-218 Cre-dependent ablation to perform next generation sequencing. In preparation for the experiment, we sampled mice at ~15 months post-miR-218 ablation and noticed that our reporter still detected miR-218 (data not shown). This result suggests that (1) Cre activity could have been lower in the context of the miR-218 CreER and fl alleles (in previous experiment we observed robust miR-218^{CreER} expression in motor neurons bearing a TdTomato-based Cre reporter gene) and/or that (2) levels of miR-218 at the time of miR-218 gene-ablation are elevated enough and turnover is slow enough that a significant amount of miR-218 is retained in motor neurons to last through life. We quantified a cohort of ~ 18 months old miR-218-1-/- miR-218-2 CreER/fl mice using antibodies against VAChT and miRNAscope to detect miR-218 in consecutive slides to determine what percentage of neurons have detectable levels of miR218 at ~15 month post miR-218-2 fl conditional ablation.

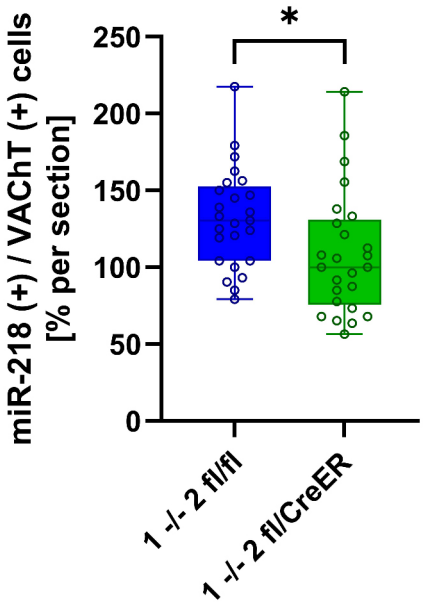

Figure 1. **Lower number of miR-218 (+) cells upon miR-218 ablation**. Animals were injected with Tamoxifen at ~ 3 m.o. and spinal cords from 4 animals at ~ 18 m.o. from each genotype were fixed and processed to perform microRNAscope (miR-218) or immunohistochemistry (VAChT) in paired consecutive 12 µm deep lumbar sections. For each animal, 8 different section pairs > 200 µm apart were processed and only those pairs with intact ventral spinal cord sections were included in the final quantitation. A miR-218 positive cell was determined as aggregation of ruby red at or near nuclei, while VAChT(+) cells were those that combined a positive signal with the presence of Z-buttons and colocalize with nuclear staining (Hoechst). As a result, miR-218(+) events regularly outnumbered VAChT(+), explaining > 100% values. Treatment required for miRNAscope staining likely changes the dimension of the spinal cord section, complicating a 1:1 correlation between miRNAscope and IHC datasets. Such detailed analysis is ongoing. miR-218-1 -/- miR-218-2 fl/fl (blue, n = 25, mean = 132 % ± 6); miR-218-1 -/- miR-218-2 fl/CreER (green, n = 25, mean = 108 % ± 8). Two -tailed T-test, p = 0.0279.

Ablation of miR-218 causes a small significant reduction in the number of cells with detectable levels of miR-218 (Figure 1). At first glance, such small reduction could be attributed to inefficient Cre expression and/or inaccessibility to the miR-218 fl allele. However, closer examination of the miRNAscope staining suggests that miR-218 stability or basal residual expression may explain its presence long after Cre-dependent allele ablation is triggered (Figure 2). To this end, we observe a clear difference between control and experimental samples: while control samples show robust expression of miR-218 as denoted by the strong ruby-red labelling (Figure 2A, arrowheads), experimental samples present much weaker miR-218 staining (Figure 2C, arrowheads). MicroRNAs half-lives could go up to days, with one group estimating miR-218 half-life in contact-inhibited MEF cells between 15.6 h. and 158.7 h. (Kingston and Bartel (2019) Genome Res., DOI: 10.1101/gr.251421.119). Motor neurons express high levels of miR-218 (~20% of total microRNAs at e12.5), therefore it is possible that detectable levels of miR-218 remain after ablation if its half-life is in the order of days. Alternatively, the miR-218 CreER allele lies directly downstream the miR-218 promoter but upstream of the miR-218 locus, and although it functionally phenocopies miR-218-2 KO (no viable miR-218-1 -/- miR-218-2 -/CreER neonates are observed, Costaguta and Pfaff it is possible that miR-218-2

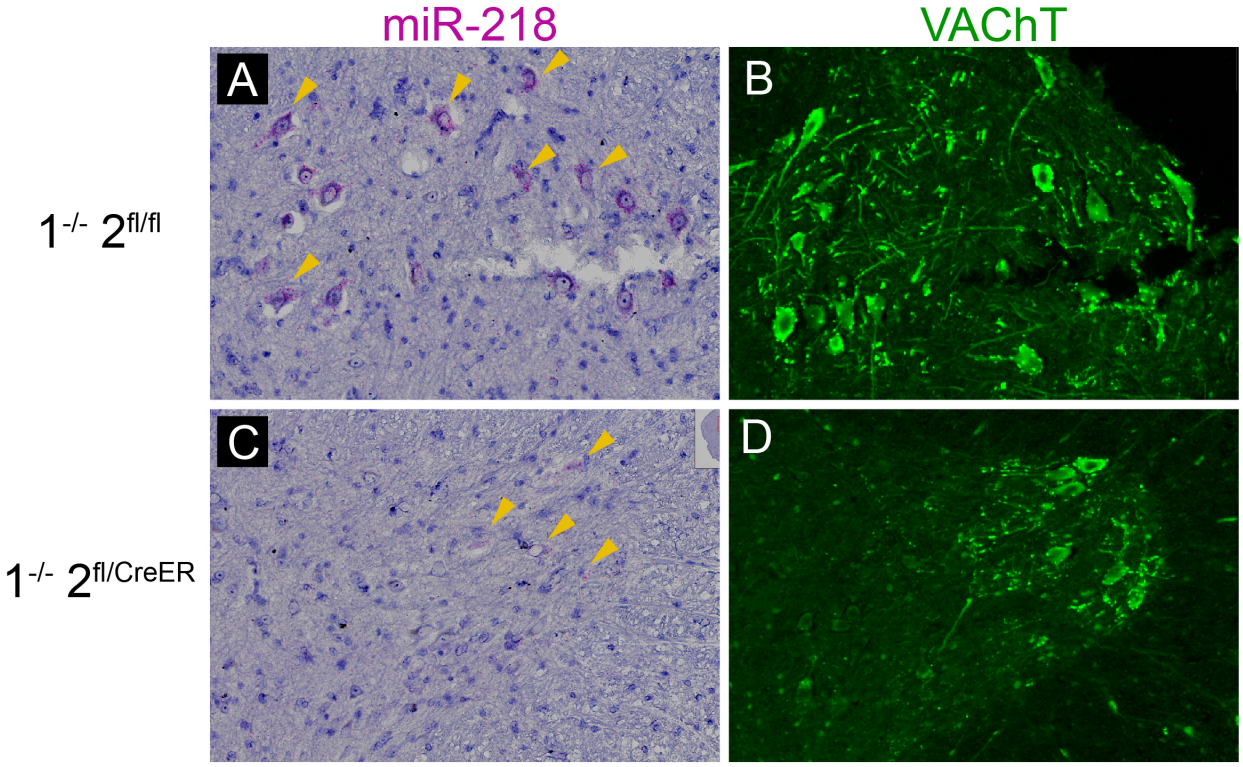

Figure 2. Ablation of miR-218 sharply decreases miR-218 levels. Selected images from the experiment described in Figure 1. **(A), (C)** miRNAscope staining of miR-218; **(B), (D)** immunohistochemistry for VAChT. Consecutive 12 µm sections are shown for each genotype. Signal for miR-218 (ruby red) is apparent on control animals (A, arrowheads) but much weaker upon miR-218 ablation (C, arrowheads). Control (A, B) and experimental animals (C, D) show internally consistent numbers of miR-218(+) and VAChT(+) cells. Therefore, similar cell counts in control and experimental animals hide the dramatic decrease in miR-218 levels between both groups.

218 expression. This complex result underscore the challenges of performing genetic-based experiments in mice. We are proceeding with an alternate strategy for future experiments based on the use of AAV-retro vectors as explained further in Aim 2.

Task 4: Histological analysis of motor neurons from mice bearing a miR-218 reporter in WT and ALS mouse models.	6-12	Dr. Giancarlo Costaguta Dr. Sam Pfaff (12 mice of each WT, SOD1(G93A), PFN1(C71G), tam-218DKO, all crossed to tg-miR-218rep)

Aim 1, Task 4. As part of reports for Year 1 and Year 2, using a miR-218 reporter line (tg-miR-218-rep), we detected miR-218 in motor neurons of SOD1(G93A), PFN1(C71G) and TDP-43(Q133K) mice at all diseases stages. Our BAC500 mice did not show ALS-like phenotypes and we were able to detect miR-218 in motor neurons of female animals up to 1 year of age.

Milestone: Expression and miR-218 activity map in WT and ALS mouse models.	12	

Aim 1, Milestone. We have detected miR-218 in motor neurons at end-stage of SOD1(G93A) and PFN1(C71G) mice, advanced disease stage for TDP-43(Q331K), and aging BAC500 mice. The presence of miR-218 at advance disease stage for the TDP-43(Q331K) and end stage for SOD1(G93A) and PFN1(C71G) models suggests that detectable levels of miR-218 may be present near or at the time of motor neuron death. A single allele of miR-218-1 can partially suppress expression of GFP in our reporter. Published data from our laboratory places miR-218-1 +/+ as contributing to up to ~36% of total miR-218 in embryonic motor neurons (Amin et al., Neuron (2021) DOI: 10.1016/j.neuron.2021.07.028). These data suggest that motor neurons at terminal disease stage in our ALS models contain miR-218 at levels of at most 36% as observed in healthy motor neurons. The continued detection of miR-218 after 12-15 months of Cre-dependent ablation of the miR-218-2 fl allele prevented next generation sequencing analysis under our planned format. We will adopt a viral strategy for the conditional deletion of miR-218-2 fl followed by next generation sequencing in experiments that fall beyond the scope of this grant.

Aim 2 MiR-218-associated candidate therapeutic approach for ALS.		
Task 1: Conditions for quantitative scAAV9 delivery to motor neurons in WT mice.	0-4	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff 6 WT mice

Aim 2, Task 1. In our Year 1 report we explained the switch from using scAAV9 virus to retroAAV, and had cloned the retroAAV virus for overexpressing miR-218 constitutively or conditionally in Cre-expressing cells.

Task 2: Delivery of scAAV9 bearing miR-218 or mock to ALS mouse models with behavioral and histological analysis.	4-12	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff
		(48 mice of each SOD1(G93A), PFN1(C71G) and 60 FUS(R521C) mice)

Aim 2, Task 2. In our Year 2 report we generated breeding mouse pairs SOD1(G93A)$^{+/-}$ x ChAT:Cre$^{+/+}$ for targeted expression of miR-218 in motor neurons using our conditional miR-218 virus (retroAAV-LSL-miR-218). We injected 5 newborn mice (p0-p3) with virus in the hindlimb. The expression of the viral constitutive mCardinal reporter showed widespread distribution along the ventral spinal cord at p70, regardless of mouse genotype (n=1 for each genotype), estimating a total of ~4500 cells were infected per spinal cord (Figure 3). The ventral location of the virus(+) cells and its good colocalization with motor neurons indicated that constitutive expression of miR-218 would suffice to target motor neurons using AAV-retro.

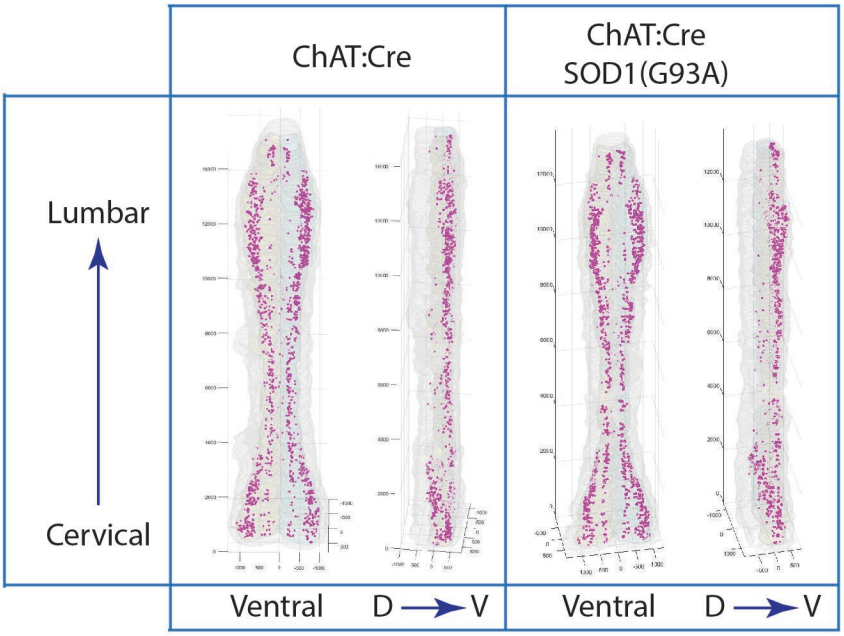

Figure 3. Infection by retroAAV-LSL-miR-218 covers the full spinal cord. Reconstruction using Build-a-Brain (Jeff Moore) of spinal cords from p70 mice injected in the hindlimb at p0-p3 with retroAAV-LSL-miR218. Serial sections were collected on a slidescanner microscope (Olympus). Magenta dots represent cell nuclei positive for mCardinal fluorescence, a constitutive reporter part of retroAAV-LSL-miR218. D = dorsal, V = Ventral.

We injected WT (n=8) or SOD1(G93A)$^{+/-}$ (n=10) mice with retroAAV-miR-218 at p0-p4, which drives constitutive expression of miR-218 and an mCardinal reporter. Disease onset in SOD1(G93A)$^{+/-}$ mice occurs at ~p90, and by ~p120 hindlimb tremors and loss of grip strength are evident, with death occurring between p130-p140. Viral injections did not alter the average survival of the SOD1(G93A) population (Figure 4).

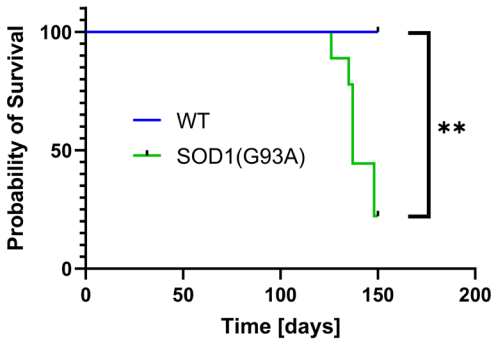

Figure 4. Viral injection of miR-218 into SOD1(G93A) does not increase survival. Survival curve for WT (blue, n=8) or SOD1(G93A) (green, n=9) mice injected with retroAAV-miR218 between p0-p4. Median survival for SOD1(G93A) mice is p137 and Chi Square p value is 0.0023 when compared to WT. Two mice from the SOD1(G93A) cohort survived past p150, showing different levels of hindlimb paralysis and were used for histological analysis.

We measured hindlimb grip strength on our cohort at different time points starting at p87 (Figure 5). While we observed a significant decline in the SOD1(G93A) population's grip strength at p124, we noticed a wide variance within this population, where at least two mice showed values associated with normal grip strength until euthanasia, when hindlimb paralysis was advanced. The lack of a control population for SOD1(G93A) mice with a mock viral injection precludes any conclusions from this observation. However, histological analysis of 2 surviving mice from the SOD1(G93A) + retroAAV-mir218 cohort suggests that miR-218 may have an effect during disease progression.

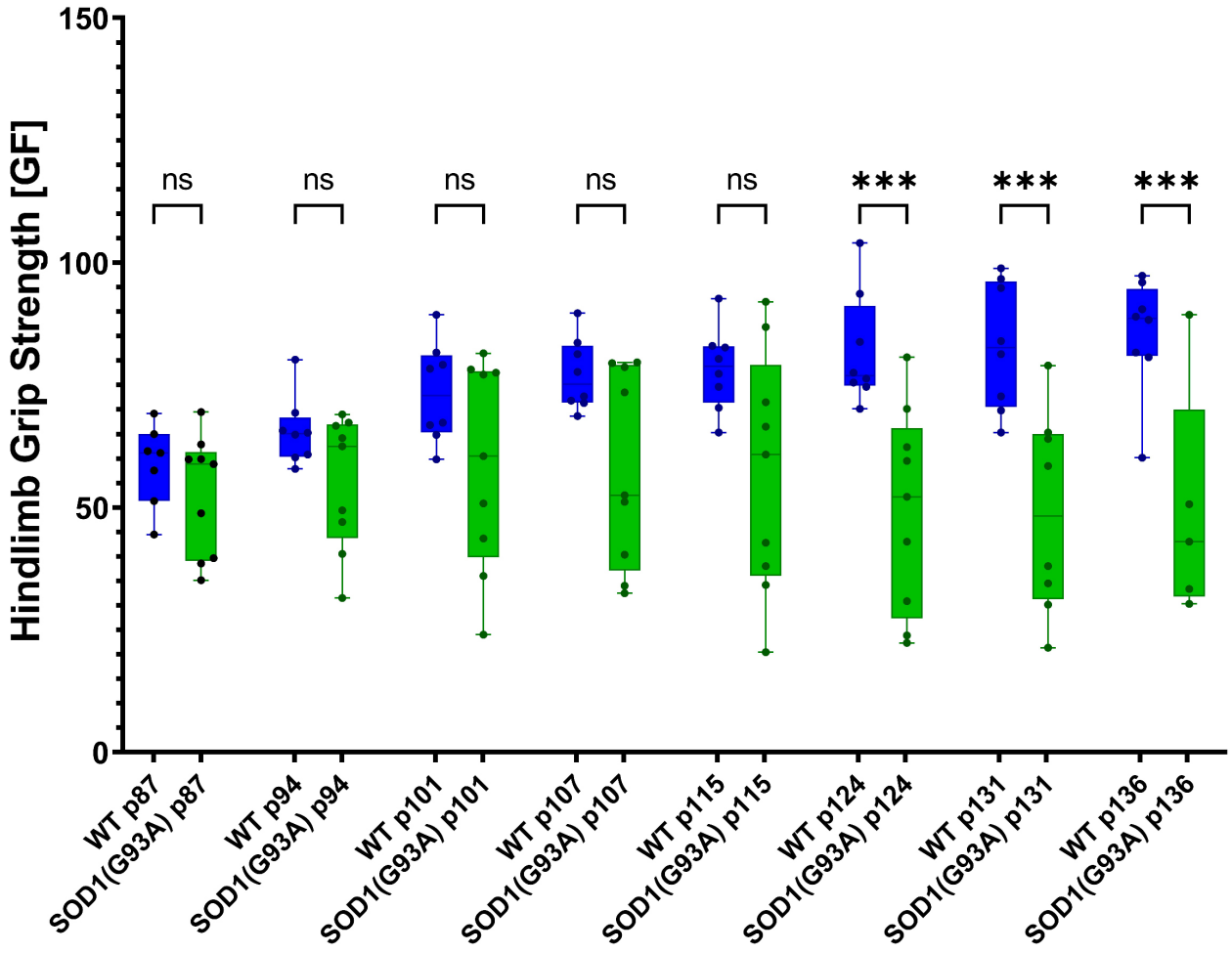

Figure 5. Hindlimb grip strength decline in SOD1(G93A) mice injected with retroAAV-miR218. WT (blue, n=8) or SOD1(G93A) (green, n=9) mice were injected with retroAAV-miR218 at p0-p4 and grip strength was measured starting at p87. While the WT cohort shows cohesive, normal values for grip strength, SOD1(G93A) mice show grip strength decline with time and large variance in their behavior.

In order to contextualize how disease progression and behavior related to ectopic miR-218 expression we performed a histological analysis of surviving mice near their clinical endpoint (Figure 6). RetroAAV-miR218 contains nuclear mCardinal as a reporter, and together with VAChT staining we assessed the infection coverage of our viral vector (Figure 6A). Analysis from 4 WT and 2 SOD1(G93A) mice at ~p150 showed that about 37% of VAChT(+) lumbar cells express mCardinal in WT mice, number that increases to

66% in SOD1(G93A) mice. This result has two implications: (1) The increased ratio of infected cells in SOD1(G93A) at disease end-stage suggests that ectopic expression on miR-218 provides a survival advantage to VAChT(+) cells, and that (2) in the context of ALS mouse models, where 50% or more motor neuron death is required to observe a disease phenotype, the coverage given by a rate of 37% infection is likely below the critical mass necessary to observe increased survival and/or improved behavioral outcomes. It is possible that the variance observed for grip strength in the SOD1(G93A) cohort is due in part to better survival of motor neurons ectopically expressing miR-218. Although beyond the scope of this grant, we are currently performing tests to see whether an increase of infection rate will translate in improved clinical outcomes.

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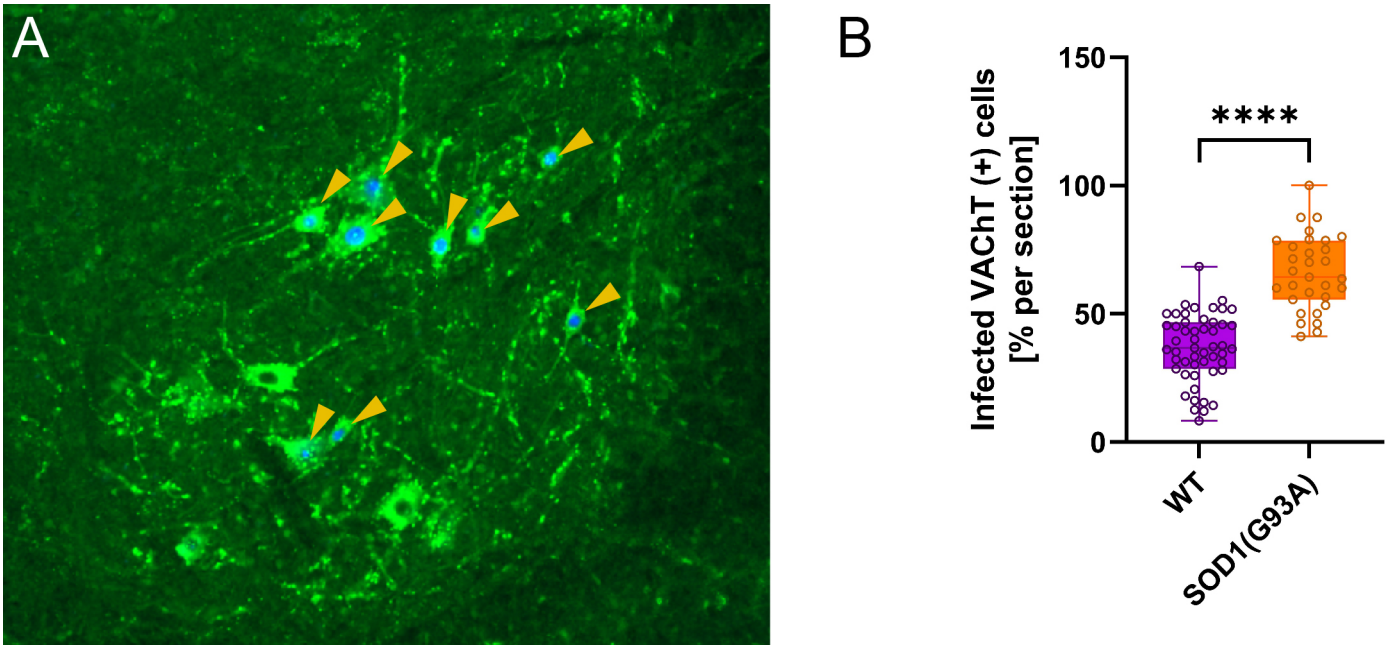

Figure 6. Enrichment of retroAAV-miR-218 in VAChT(+) cells at disease end-stage of SOD1(G93A) mice. Deep lumbar spinal cord sections from 4 WT (51 sections) and 2 SOD1(G93A) (31 sections) ~p150 mice, all infected with retroAAV-miR-218, were quantified for **(A)** VAChT (green) and mCardinal (blue) colocalization. Cytosolic staining of VAChT colocalizes with nuclear mCardinal staining (orange arrowheads). The data is represented as **(B)** percentage of VAChT(+) co-expressing mCardinal in either genotype. An average of 37% VAChT(+) show co-expression of mCardinal, on a given WT spinal cord section, while the average increases to 66% in samples from disease end-stage SOD1(G93A) mice. Two tailed T-test $p < 0.0001$.

Task 3: Delivery of scAAV9 bearing Relay218 genes to ALS mouse models with behavioral and histological analysis.	9-21	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff (48 mice of each SOD1(G93A) and PFN1(C71G))

Aim 2, Task 3. We decided to focus our efforts on Aim2, Task 1 due to our intriguing data from the constitutive miR-218 viral infection experiments. We will address the experiments detailed in Task 3 in future work after we successfully increase the infectivity of retroAAV-miR-218 on SOD1(G93A) $^{+/-}$ mice.

Milestone: Disease modification by miR-218 overexpression.	21	

Aim 2, Milestone. We have identified a highly efficient transgenic AAV system that can be delivered by simple intramuscular injection at or near birth. Experiments using a scAAV-retro virus with constitutive expression of miR-218 (retroAAV-miR-218) in SOD1(G93A)$^{+/-}$ mice show interesting preliminary results with respect to cell survival, apparently increasing the survival or VAChT(+) cells under disease conditions.

Aim 3 MiR-218 mimics as ALS therapeutic agents.		
Task 1: Intrathecal delivery conditions for [miR-218].	3-6	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff (20 Tam-218DKO mice)
Task 2: LD50 determination for [miR-218].	6-9	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff (30 WT mice)
Task 3: Delivery of [miR-218] to mouse models of ALS.	9-24	Miriam Gullo Dr. Giancarlo Costaguta Dr. Sam Pfaff (120 mice of each WT and SOD1(G93A), 144 PFN1(C71G) mice)

Aim 3, Tasks 1 through 3. On the progress report for Year 2 we conditioned analyzing effects of miR-218 mimic molecules on having a positive outcome with our retroAAV strategy as stated on Aim2. The promising results described on Aim2 will prompt future work on the efficacy of miR-218 mimics we identified on our Year 1 report.

Milestone: Disease modification by intrathecal delivery of [miR-218].	24	

Aim 3, Milestone. The data described on Aim2 will drive the future design of experiments that will test the effects of miR-218 mimic in ALS mouse models as an alternative to the use of viral vectors.

3c. What opportunities for training and professional development has the project provided?

The support provided by this grant has been of great value in the professional development of Dr. Costaguta, allowing him to attend and present our work in meetings relevant for ALS and related motor neuron diseases:

i) 2021 Virtual SMA Research & Clinical Care Meeting

ii) The 23rd International SMA Researcher Meeting. June 2019. Poster presentation.

iii) Gordon Research Conference - Amyotrophic Lateral Sclerosis (ALS) and Related Motor Neuron Diseases. Mechanisms of Motor Neuron Degeneration and Therapeutic Intervention. July 2019. Poster presentation.

iv) SBP's 40th Annual Symposium - Regulation of Neural Function in Health and Disease. November 2019. Poster presentation.

3d. How were results disseminated to communities of interest?

We have presented posters with our results in conferences with participation of the ALS as well as SMA and neurodegenerative diseases scientific communities.

3e. What do you plan to do during the next reporting period to accomplish the goals?

Not Applicable.

4. IMPACT

4a. What was the impact on the development of the principal discipline(s) of the project?

We are investigating how miR-218, a molecule with the ability to regulate the expression of about 300 genes in motor neurons, changes through life and in ALS in order to gain insights on the pathways that maintain proper motor neuron function. We hypothesize that elevated expression of miR-218 in motor neurons with insufficient levels of this microRNA could slow-down progression or delay onset of ALS. This approach would represent an entirely new direction for targeting ALS disease.

4b. What was the impact on other disciplines?

The tools generated in this study have helped to inform neuroscientists about how gene networks are regulated, and provided mouse genetic tools for studying the spinal cord.

4c. What was the impact on technology transfer?

If miR-218 holds therapeutic potential for ALS, it would be an attractive molecule for industry to adopt in their development for new ALS interventions.

4d. What was the impact on society beyond science and technology?

The laboratory has had students and lay visitors. We have collaborated with an artist to develop art work based on our scientific images.

5. CHANGES/PROBLEMS

5a. Changes in approach and reasons for change.

There has not been a change in approach for the reported period.

5b. Actual or anticipated problems or delays and actions or plans to resolve them.

None

5c. Changes that had a significant impact on expenditures.

None

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards and/or select agents.

No significant changes. All procedures on mice are currently approved as in our IACUC protocol 11-00020.

5e. Significant changes in use or care of human subjects.

No human subjects

5f. Significant changes in use or care of vertebrate animals.

None

5f. Significant changes in use or care of biohazards and/or select agents.

Nothing to report.

6. PRODUCTS

6a. Publications, conference papers, and presentations.

i) Amin ND, Senturk G, Costaguta G, Driscoll S, O'Leary B, Bonanomi D, Pfaff SL. A hidden threshold in motor neuron gene networks revealed by modulation of miR-218 dose. Neuron. 2021 Aug 25:S0896-6273(21)00572-9. doi: 10.1016/j.neuron.2021.07.028. Epub ahead of print. PMID: 34450025.

ii) The 23rd International SMA Researcher Meeting. June 2019. Poster presentation.

iii) Gordon Research Conference - Amyotrophic Lateral Sclerosis (ALS) and Related Motor Neuron Diseases. Mechanisms of Motor Neuron Degeneration and Therapeutic Intervention. July 2019. Poster presentation.

iv) SBP's 40th Annual Symposium - Regulation of Neural Function in Health and Disease. November 2019. Poster presentation.

6b. Website(s) or other internet site(s).

Nothing to report.

6c. Technologies or techniques.

All technologies developed will be shared according to the scientific community guidelines.

i) **Mouse reporter lines for miR-218**. Allows for the detection of low levels of miR-218 by the observation of GFP expression in motor neurons.
ii) **Mouse line with conditional expression of miR-218 reporter**. The reporter is activated by the presence of Cre recombinase, allowing the analysis of miR-218 levels in different cell types and tissues. Currently under characterization.
iii) **retroAAV-miR-218**. It delivers ectopic expression of miR-218. Currently under characterization.
iv) **retroAAV-LSL-miR-218**. It delivers conditional expression of miR-218 to cells or tissues expressing the Cre recombinase. Currently under characterization.

6d. Inventions, patent applications, and/or licenses.

Nothing to report.

6e. Other products.

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. What individuals have worked on the project?

Name:	Samuel L. Pfaff
Project Role:	Principal Investigator
Researcher Identifier	
Nearest person month worked:	1.8
Contribution to project	Plan and interpret experiments, ensure that regulatory and reporting requirements are met, prepare manuscripts and coordinate the sharing of reagents and communication of results.
Funding Support:	NIH

Name:	Giancarlo Costaguta
Project Role:	Senior Research Assistant
Researcher Identifier	
Nearest person month worked:	12
Contribution to project	Performs experiments in Aims 1-3. He is an experience molecular-geneticist with a background in the cellular pathways associated with neurological disease.
Funding Support:	DOD

Name:	Shawn Driscoll
Project Role:	Bioinformatics Specialist

Researcher Identifier	
Nearest person month worked:	1.2
Contribution to project	Performs statistical analysis and bioinformatics characterization of the gene expression data.
Funding Support:	NIH

Name:	Miriam Gullo
Project Role:	Research Technician
Researcher Identifier	
Nearest person month worked:	12
Contribution to project	Assist with all mouse experiments. She is a highly experienced technician who helps to breed, genotype, and process tissue from the mice used in Aims 1-3.
Funding Support:	DOD

7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

ACTIVE	NIH/NINDS 5 U19 NS112959-03	Spinal Circuits for the Control of Dextrous Movement (Goulding, PI)	09/15/2019-06/30/2024	annual direct
ACTIVE	NIH/NINDS 1 R03 NS121480-01	Tools for regulated expression control of miR-218 (Pfaff, PI)	05/15/2021-10/31/2022	annual direct
ACTIVE	NIH/NINDS 1 R21 NS121846-01	MiR-218 regulatory networks in adult mice and its relationship to ALS (Pfaff, PI)	05/15/2021-10/31/2022	annual direct
ACTIVE	NIH/NINDS 1 R01 NS123160-01	Characterization of spinal circuits underlying motor synergy function (Pfaff, PI)	09/01/2021-08/31/2026	annual direct
ACTIVE	Department of Defense W81XWH-20-1-0423	Genetic Therapy Solution for Duchenne Muscular Dystrophy (Pfaff, PI)	09/01/2020-08/31/2022	annual direct

7c. What other organizations were involved as partners?

None.

8. SPECIAL REPORTING REQUIREMENTS

8a. Collaborative awards.

Nothing to report.

8b. QUAD charts

9. APPENDICES