AWARD NUMBER: W81XWH-19-1-0209, AL180020

TITLE: Modulation of Nuclear Transport and the Nuclear Pore Complex in Sporadic ALS

PRINCIPAL INVESTIGATOR: Jeffrey D. Rothstein MD, PhD

CONTRACTING ORGANIZATION: Johns Hopkins University

REPORT DATE: OCTOBER 2021

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2021	FINAL	7/1/19-6/30/21

4. TITLE AND SUBTITLE

Modulation of Nuclear Transport and the Nuclear Pore Complex in Sporadic ALS

5a. CONTRACT NUMBER
W81XWH-19-1-0209

5b. GRANT NUMBER
W81XWH-19-1-0209

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University,
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

Growing evidence points to disruption of nuclear transport in neurodegenerative diseases, (recently reviewed3). Recently, our laboratory and others identified defects in nucleocytoplasmic transport in models of familial ALS due to a hexanucleotide repeat expansion in C9orf72 (C9), across multiple model systems, patient induced pluripotent stem cell (iPSC)-derived neurons, and postmortem tissue.4-7 Working with collaborators in academics and industry, we have now identified multiple candidate therapies for reversing C9-mediated nuclear transport deficits. We have also begun to screen for nuclear transport deficits in sporadic ALS (sALS) iPSC-derived motor neurons (iPSN). Although more heterogeneous than C9-ALS, we find evidence of both nuclear transport and nuclear pore complex disruption in sALS iPSN. These data suggest that sALS, like C9-ALS, involves a defect in nuclear transport that is a promising and druggable target. In the proposed aims, we will further define the nuclear transport deficits in sALS iPSN, and test candidate drugs for reversing these deficits. This study will identify lead agents for reversing nuclear transport defects in sALS and allow us to correlate specific "nuclear transport signatures" at the level of iPSN with response to therapy.

15. SUBJECT TERMS
ALS, nuclear pore, sporadic, therapy, nucleoporins,

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Growing evidence points to disruption of nuclear transport in neurodegenerative diseases. We have now identified candidate therapies for reversing C9-ALS mediated nuclear transport deficits. We have also begun to screen for nuclear transport deficits in sporadic ALS (sALS) iPSC-derived motor neurons (iPSN) and find nuclear pore complex disruption in sALS iPSN. We have identified the underlying pathway and developed novel antisense therapy as well

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

ALS, C9orf72, nuclear pore, nup, Pom121, nuclear transport, SIM, confocal

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The original specific AIMS:

Aim 1. Define the structural and functional nuclear transport defects in sALS. *We hypothesize that sALS iPSN will show structural deficits in the NPC and functional impairment of nuclear transport dynamics.*

Aim 2. Investigate the ability of candidate nuclear transport drugs to reverse nuclear transport defects in sALS. *We hypothesize that drugs targeting nuclear export pathways, the Ran-GTPase cycle, and nucleoporin chaperone activity, will rescue nuclear transport defects in sALS iPSN.*

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We successfully completed Aims 1 and 2 and published our findings in 2 separate publications:

1. Neuron (2020) (1)
2. Science Translation Medicine (2021) (2)

Regarding Summary results from Aim 1:

- We identified a reproducible subset of 7-8 nucleoporins (nups) which are reduced in *sALS* iPSN nuclei and nuclear pore complexes (NPCs) and similar changes in postmortem human sALS brain regions.
    - Specific nucleoporins are altered in *C9orf72* iPSN nuclei in an age dependent manner
    - This was based on a a study of a large cohort of >30 individual patient iPS cell lines obtained from our Answer ALS program.
    - Nucleoporin alterations in postmortem *sALS* patient motor cortex and thoracic spinal cord are identical to those in *sALS* iPSNs
        - These changes were not seen in unaffected brain regions such as occipital cortex from sALS autopsies
- These were quite similar to the same nups also found to be substantially reduced in C9orf72 iPSN nuclei and nuclear pore complexes (NPCs)
- For sALS – this injury to the nuclear pore complex (NPC) resulted in:
    - Loss of Ran GTPase gradients
    - Mis localization of TDP43, with a resultant loss of nuclear TDP43 and increased cytoplasmic TDP43
        - Associated functional evidence of TDP43 loss of function with various RNA species misspliced
- Together, the combined reduction in these 7-8 Nups negatively impacts the localization of nucleocytoplasmic transport proteins and neuronal survival.
- *Note: The results from Aim 1 were published in our Neuron, 2020 paper: Coyne AN, Zaepfel BL, Hayes L, Fitchman B, Salzberg Y, Luo EC, Bowen K, Trost H, Aigner S, Rigo F, Yeo GW, Harel A, Svendsen CN, Sareen D, Rothstein JD. G4C2 Repeat RNA Initiates a POM121-Mediated Reduction in Specific Nucleoporins in C9orf72 ALS/FTD. Neuron. 2020;107(6):1124-40.*

Regarding summary result for AIM 2:

- ESCRT3/CHMP7: We learned that a prominent chaperone family member, CHMP7, from the ESCRT3 family of nuclear membrane/NPC surveillance pathway, was engaged and was responsible for the injury. Based on this core new observation the main focus was then to knockdown expression of CHMP7 in sALS neurons and to determine if that "drug" would reverse the defects in the NPC, nuclear transport and the downstream functional defect associated with loss of NPC activity, such as TDP43 mis location and RNA splicing errors.
- Three distinct antisense oligonucleotides (ASO) independently and completely repaired the injury to the NPC and all downstream defects (NPC structure, nuclear transport, TDP43 mis localization, TDP43 based RNA altered splicing and sensitivity to excitotoxicity).
- The ASO were non-toxic to human spinal neurons when chronically delivered
- The repair of injury was effective, even after the injury had already initiated (pretreatment was not required).
- Overall these result set the stage for a new clinical candidate drug for ALS, due to efficacy in sALS and C9orf72 ALS/FTD iPS neurons
- *Note: The results from Aim 2 were published in our Science Translational Medicine, 2021 paper: Coyne AN, Baskerville V, Zaepfel BL, Dickson DW, Rigo F, Bennett F, Lusk CP, Rothstein JD. Nuclear accumulation of CHMP7 initiates nuclear pore complex injury and subsequent TDP-43 dysfunction in sporadic and familial ALS. Sci Transl Med. 2021;13(604).*

References

- 1. Coyne AN, Zaepfel BL, Hayes L, Fitchman B, Salzberg Y, Luo EC, Bowen K, Trost H, Aigner S, Rigo F, Yeo GW, Harel A, Svendsen CN, Sareen D, Rothstein JD. G4C2 Repeat RNA Initiates a POM121-Mediated Reduction in Specific Nucleoporins in C9orf72 ALS/FTD. Neuron. 2020;107(6):1124-40 e11. Epub 2020/07/17. doi: 10.1016/j.neuron.2020.06.027. PubMed PMID: 32673563.
- 2. Coyne AN, Baskerville V, Zaepfel BL, Dickson DW, Rigo F, Bennett F, Lusk CP, Rothstein JD. Nuclear accumulation of CHMP7 initiates nuclear pore complex injury and subsequent TDP-43 dysfunction in sporadic and familial ALS. Sci Transl Med. 2021;13(604). Epub 2021/07/30. doi: 10.1126/scitranslmed.abe1923. PubMed PMID: 34321318.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We published our studies:

- 1. Coyne AN, Zaepfel BL, Hayes L, Fitchman B, Salzberg Y, Luo EC, Bowen K, Trost H, Aigner S, Rigo F, Yeo GW, Harel A, Svendsen CN, Sareen D, Rothstein JD. G4C2 Repeat RNA Initiates a POM121-Mediated Reduction in Specific Nucleoporins in C9orf72 ALS/FTD. Neuron. 2020;107(6):1124-40 e11. Epub 2020/07/17. doi: 10.1016/j.neuron.2020.06.027. PubMed PMID: 32673563.

2. Coyne AN, Baskerville V, Zaepfel BL, Dickson DW, Rigo F, Bennett F, Lusk CP, Rothstein JD. Nuclear accumulation of CHMP7 initiates nuclear pore complex injury and subsequent TDP-43 dysfunction in sporadic and familial ALS. Sci Transl Med. 2021;13(604). Epub 2021/07/30. doi: 10.1126/scitranslmed.abe1923. PubMed PMID: 34321318

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

This is our final report- nothing to report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

These studies provide the first evidence of nuclear pore complex and associated nucleoporins (nup) alterations in sporadic ALS patient derived spinal neurons (iPS) along the consequential loss of NPC function including TDP-43 dysfunction and mis localization along with sensitivity to excitotoxic insult. Furthermore, we discovered that the fundamental reason for this injury was that CHMP7, a critical mediator of NPC quality control, is increased in nuclei of *C9orf72* and sporadic ALS induced pluripotent stem cell (iPSC) derived spinal neurons (iPSNs). Inhibiting the nuclear export of CHMP7, triggered Nup reduction and TDP-43 dysfunction and pathology in human neurons. Most importantly and of great therapeutic relevance was the observation that knockdown of CHMP7 alleviated disease associated Nup alterations, deficits in Ran GTPase localization, defects in TDP-43 associated mRNA expression, and alleviated downstream glutamate induced neuronal death. Thus, our data support a role for altered CHMP7 mediated Nup homeostasis as a prominent initiating pathomechanism for familial and sporadic ALS and highlights the potential for CHMP7 as therapeutic target.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

We published our studies:

- 1. Coyne AN, Zaepfel BL, Hayes L, Fitchman B, Salzberg Y, Luo EC, Bowen K, Trost H, Aigner S, Rigo F, Yeo GW, Harel A, Svendsen CN, Sareen D, Rothstein JD. G4C2 Repeat RNA Initiates a POM121-Mediated Reduction in Specific Nucleoporins in C9orf72 ALS/FTD. Neuron. 2020;107(6):1124-40 e11. Epub 2020/07/17. doi: 10.1016/j.neuron.2020.06.027. PubMed PMID: 32673563.

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Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Overall- no change:

Name:	*Jeffrey D. Rothstein MD, PhD*
Project Role:	*P*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Rothstein oversaw the entire project, reviewing experimental plans, experimental results and participated in the writing/publishing of all peer reviewed publications*
Funding Support:	*NIH*

Name:	*Alyssa Coyne*
Project Role:	*Post DOc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Coyne carried out and/or directed the completion of all studies in this application. She also collated all results and prepared initial manuscripts.*
Funding Support:	*NIH*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.