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TITLE: Early Detection of Alzheimer's Disease by Relayed NOE CEST MRI

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CONTRACTING ORGANIZATION:
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Jiadi Xu

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Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Baltimore, MD 21205-1832

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14. ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia, and currently, more than 5 million people have AD in the US alone. The accumulation of neuritic plaques comprised of amyloid-beta (Aβ) peptides and neurofibrillary tangles (NFT) of hyperphosphorylated tau are the major histopathological hallmarks of Alzheimer's disease. Therefore, the detection of the aggregated proteins involved in AD is expected to be a promising strategy for the early diagnosis of the disease. We recently developed a new chemical exchange saturation transfer (CEST) technique, UTE-CEST, which is able to obtain high-resolution rNOE-CEST MRI insensitive to motion. We hypothesize that the rNOE-CEST signal detected by CEST-UTE on AD mouse is associated with the Aβ and NFT accumulation due to the line broadening of amide and aliphatic proton signal in proteins introduced by protein aggregation. The optimized UTE-CEST method will be applied on two AD mouse models to verify that the technique is sensitive enough to detect the early protein aggregation seen in AD disease. Upon the successful completion of this proposal, we anticipate developing a new clinic-ready MRI technique that detect and evaluate the AD associated protein aggregation.

15. SUBJECT TERMS-

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

Alzheimer's disease (AD) is the most common cause of dementia, and currently, more than 5 million people have AD in the US alone. The accumulation of neuritic plaques comprised of amyloid-beta (Aβ) peptides and neurofibrillary tangles (NFT) of hyperphosphorylated tau are the major histopathological hallmarks of Alzheimer's disease. Therefore, the detection of the aggregated proteins involved in AD is expected to be a promising strategy for the early diagnosis of the disease. We recently developed a new chemical exchange saturation transfer (CEST) technique, UTE-CEST, which is able to obtain high-resolution rNOE-CEST MRI insensitive to motion. We hypothesize that the rNOE-CEST signal detected by CEST-UTE on AD mouse is associated with the Aβ and NFT accumulation due to the line broadening of amide and aliphatic proton signal in proteins introduced by protein aggregation. The optimized UTE-CEST method will be applied on two AD mouse models to verify that the technique is sensitive enough to detect the early protein aggregation seen in AD disease. Upon the successful completion of this proposal, we anticipate developing a new clinic-ready MRI technique that detect and evaluate the AD associated protein aggregation.

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

Alzheimer's Disease, plaque, amyloid-beta (Aβ), MRI, ultra-short echo time (UTE), chemical exchange saturation transfer (CEST), Nuclear Overhauser effect (NOE)

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.

Aim 1. To optimize and evaluate the MRI signal sensitivity and specificity of rNOE-CEST MRI techniques for detecting Aβ and NFT accumulation in mouse brain.

Major Task 1: A UTE-CEST sequence will be developed to acquire high-resolution rNOE-CEST CEST on an animal MRI scanner.

This task was completed in Apr. 30, 2019.

Major Task 2: Determine the sensitivity of protein CEST in detecting the protein aggregation on phantoms and two AD mouse models.

This task was completed by 100% before Oct. 20, 2019.

Aim 2. To verify the early detection of Aβ and NFT pathology in AD mouse by rNOE-CEST MRI.

Major Task 1: The optimized multi-slice UTE-CEST method will be applied on the APP and Tau AD mouse models for a longitudinal study (1.5 year) to verify that the technique is sensitive enough to detect the early protein aggregation occurring in AD brain. The individual measures of metabolism by *in vivo* MRS and quantitative MT (qMT) in each animal for each stage of disease will be performed to examine the CEST contributions from the metabolites and macromolecules.
This task started from May. 1 and was completed by 30% before Oct. 30, 2019.

Major Task 2: In order to monitor the initial stage of the protein aggregation, the histological analysis will be performed at four time points over the period of one year (4, 6, 8 and 10 month). After the final MRI experiments (18 month), all animals will be scarified for histological analysis to confirm the burden and the distribution of the Aβ and NFT in brain
This task was performed in parallel to task 1 and was completed by 10% before Oct. 30, 2019.

Aim 3. Implementation and optimization of the UTE-CEST sequence on a 3T clinical scanner

Major Task 1: The UTE-CEST sequence will be programed on the Philips Ingenia 3T Scanner. The width and power of the saturation pulses in the UTE-CEST will be optimized on a water phantom and several BSA phantoms to minimize the water direct saturation and MTC contaminations.
This task not start yet.

Major Task 2: Developing an image acquisition and post-processing pipeline for the correction of the B0 and B1 inhomogeneity on the 3T scanners when applying UTE-CEST sequence.
This task not start yet.

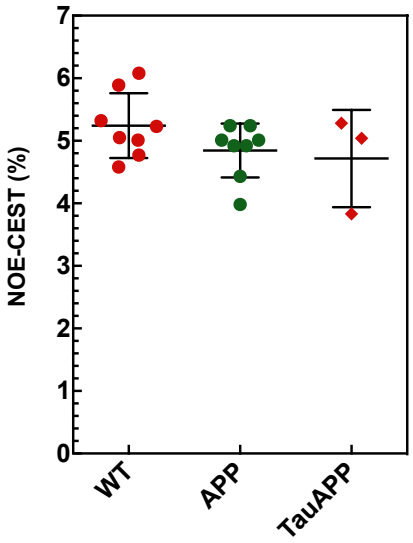
What was accomplished under these goals?

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.

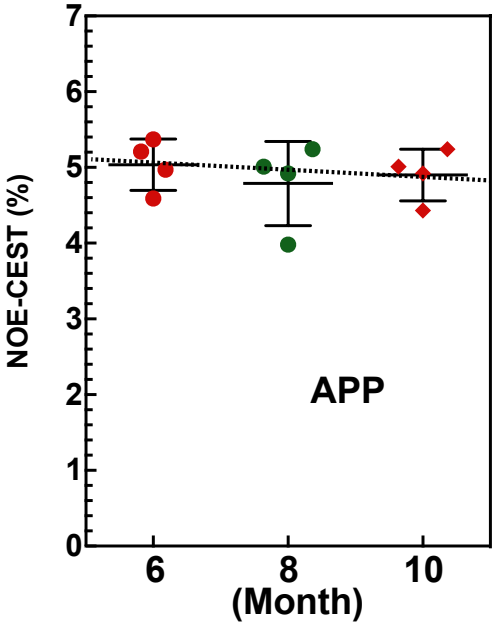
Longitudinal Study on AD mouse models:

During this reporting cycle, we mainly worked on the longitudinal study on our first AD cohort **(**WT, n=3; APP, n=4; and TauAPP, n=2) mice were breaded. Hence, we scanned the two more time points (8 month and 10 month) for the three mouse models with optimized UTE-CEST sequence. Histological analysis was performed on WT, APP and TauAPP mice with same age as those performed with MRI.

The APP mouse will develop significant amyloid plaques, while another TauAPP model is a novel tau mouse model developed by our co-investigator, in which wild-type tau is converted into pathological tau aggregates and NFT as well amyloid plaques will be developed. Their littermates will be used as wild-type (WT) control. The rNOE-CEST MRI was performed using the multi-slice UTE-CEST (msUTE-CEST) optimized in the previous grant period. The following images show the comparison between the three types of mice for the rNOE values in the cortex region (summary of all scans between 6-10 months). It can be seen that a clearly difference between WT and APP mice is observed (5.2% vs 4.8%), while the APP and Tau mice show similar rNOE-CEST signal.



The age dependent rNOE-CEST signal was also found in the APP mice as indicated from the longitudinal study (cortex). However, it needs further confirmation by increasing the number of mice.



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.

NONE

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.

NONE

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.

In the next reporting period, we will continue scan the current cohort (3 WT, 4 APP and 2 TauAPP) one every two months to monitor the rNOE-CEST signal change with respect to the age up to 16 month. In one time period, we will perform MRS and qMT to confirm the rNOE-CEST contributions from metabolite and MT difference. At the same time, we will perform pathological analysis on age matched cohort to confirm the burden of amyloid plaques and tau aggregates.

we will add new mice to the longitudinal scan to reach the number required for the significance.

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).

We developed one multi-slice ultra short echo time (UTE)-CEST sequence and established one automatic Matlab process pipeline for the multi-slice high-resolution rNOE images insensitive to motion. We demonstrated that the rNOE-CEST signal detected by CEST-UTE on AD mouse is associated with the Aβ accumulation due to the line broadening of amide and aliphatic proton signal in proteins introduced by protein aggregation. The current technique not only provide a new clinic-ready MRI technique that detect and evaluate the AD associated protein aggregation, but also can be used for detecting other metabolites in many tissues such as glycogen in liver.

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.

The current UTE-CEST method can be applied for the early diagnosis of the Alzheimer's disease (AD), and is also important for dynamically monitoring the effects of new therapeutic agents on the clearance of the aggregated proteins. The method may potentially be a marker for studying protein aggregation process in other diseases such as Huntington's disease and cirrhosis in the liver.

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.*

The project may lead to an MRI diagnosis technology that can transfer to entities in medical imaging industry.

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.*

Upon the successful completion of this proposal, we anticipate developing a new clinic-ready MRI biomarker that detect and evaluate the AD associated protein aggregation. Such a non-invasive translational technique would play a pivotal role for definitive diagnosis of AD and for differentiating AD from other dementias. Early diagnosis of AD has significant impact on its management, and may allow early therapeutic interventions, such as amyloid immunization or inhibitors of beta-secretase (BACE). Furthermore, the new biomarker can be applied to dynamically monitor the effects of new therapeutic agents on the clearance of the aggregated proteins in the AD patients after traumatic brain injury (TBI). Therefore, the proposed research is in line with the long-term goal of the PRARP's mission that reducing the burden on affected individuals. The proposed MRI biomarker provides a cost-effective and simple approach that allows the researchers in the health science to perform routine tests on the individuals after traumatic brain injury (TBI). Considering MRI has been routinely performed on the patients with TBI, the new rNOE-CEST MRI experiments will provide abundant information about the protein aggregation associated with AD disease without additional diagnosis instruments.

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:*

Nothing to Report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

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.

We found it is challenging to generate the required number of mice at the similar time period. Hence, we will perform our longitudinal study by using several cohorts of mouse. The cohort of mouse will be separated by 3-4 months depend on the breeding the mouse.

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

None.

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

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).*

1. Chen, L., Wei, Z., Chan, K.W.Y., Cai, S., Liu, G., Lu, H., Wong, P.C., van Zijl, P.C.M., Li, T.*, **Xu, J.***, 2018. Protein aggregation linked to Alzheimer's disease revealed by saturation transfer MRI. Neuroimage 188, 380-390. Published, *acknowledgement of federal support :* yes.
2. Chen L, Schär M, Chan KWY, Huang J, Qin Q, Weiss RG, van Zijl PCM, **Xu J***. High-resolution phosphocreatine mapping of human skeletal muscle by artificial neural network-based chemical exchange saturation transfer MRI at 3T. Nature communication: under review, *acknowledgement of federal support :* yes.
3. Chen L^+, Wei Z^+, Chan K, Huang J, Xu X, Wong P, Lu H, van Zij P, Li T*, **Xu J***. Glucose uptake and clearance in Alzheimer's disease mouse brain detected by on-resonance variable delay multiple pulse MRI, Journal of Cerebral Blood Flow & Metabolism: under review, *acknowledgement of federal support :* yes

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).*

NONE

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.*

1. **Xu J,** Chen L[+], Wei Z[+], Chan K, Huang J, Lu H, Wong P, van Zij P, Li T*, Protein aggregation in neuritic plaques revealed by saturation transfer MRI on a tauopathy mouse model, Alzheimer's Association international conference, 2019, Los Angeles, USA

- **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.

None

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

UTE-CEST method

- **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.

None

-

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.*

UTE-CEST sequence and the corresponding imaging processing software.

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".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: *Jiadi Xu*
Project Role: *PI*
Nearest person month worked: *2*
Contribution to Project: *Dr. Xu performed MRI experiment, pulse programing, and oversee the progress of the whole project including writing the papers and progress report.*

Name: *Peter van Zijl*
Project Role: *co-investigator*
Nearest person month worked: *1*

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:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (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://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) 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.*