MCKNIGHT BRAIN RESEARCH FOUNDATION (MBRF)
Meeting of the Research Committee
of the Board of Trustees

Wednesday, September 20th, 2023
4:00 pm ET – 5:00 pm ET

Members Attending: Dr. Madhav Thambisetty, Committee Chair; Dr. Mike Dockery, MBRF Chair; Dr. Sue Pekarske, Trustee; Dr. Patricia Boyle, Trustee; Dr. Roy Hamilton, Trustee

Also Attending: Dr. Lee Dockery, Chair Emeritus; Ms. Melanie Cianciotto, Corporate Trustee; Dr. Angelika Schlanger, Executive Director

AGENDA

4:00pm ET	1.	Call to Order/Roll Call	Dr. Madhav Thambisetty
ACTION	2.	Approval of Minutes, March 28, 2023	Dr. Madhav Thambisetty
	3.	Updated Activity Timeline	Dr. Madhav Thambisetty
4:05pm **ACTION**	4.	Leadership Council's Consensus Memo a. Cognitive Aging Memory Intervention (CAMI) Core Pilot Grant Program b. McKnight Brain Aging Registry (MBAR) c. Epigenetic and Genomics Core – Historical Review	Dr. Madhav Thambisetty
4:30pm	5.	Pilot Grant Report Template – Update	Dr. Angelika Schlanger
4:35pm	6.	Current Grants/Programs a. MBRF Innovator Awards in Cognitive Aging and Memory Loss (AFAR) i. 2022 - 2023 Annual Progress Report **ACTION** ii. 2023 Proposed Slate of Awardees **ACTION** iii. Discussion of Grant Renewal iv. Program Guidelines for 2024 v. Engaging with likeminded Funders b. MBRF Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss (ABF) i. 2022 - 2023 Progress and Final Reports	Dr. Madhav Thambisetty
4:55pm	7.	Other Business	
ACTION **5:00pm ET**	8.	Adjourn	Dr. Madhav Thambisetty

MINUTES
MCKNIGHT BRAIN RESEARCH FOUNDATION (MBRF)
RESEARCH COMMITTEE
CONFERENCE CALL
March 28, 2023

The Research Committee of the MBRF was called to order at 5:00 pm EST on March 28, 2023, by Dr. Madhav Thambisetty.

The following members were present:

Dr. Madhav Thambisetty, Chair of the Research Committee, Trustee
Dr. Mike Dockery, MBRF Chair
Dr. Sue Pekarske, Trustee

The following members were absent:
Dr. Patricia Boyle, Trustee
Dr. Richard Isaacson, Trustee

Others attending:

Dr. Lee Dockery, Chair Emeritus
Ms. Melanie Cianciotto, Corporate Trustee
Dr. Angelika Schlanger, Executive Director

1. Call to Order

Dr. Thambisetty welcomed the members of the committee to the call.

2. Minutes of the January 20, 2023 Meeting

The minutes of the January 20, 2023, Research Committee Meeting (Attachment 1) were reviewed and approved as amended. The changes are:

Item 4 a, the second sentence of the second paragraph should read "They feel making the match less restrictive for applicants from smaller institutions would allow for a broader, more diverse pool of candidates, including investigators from smaller institutions."

Item 5, the last sentence of the paragraph should read "He also shared the MBRF would not be in favor of having fellow or junior faculty members driving this process."

Action Item 1: The minutes of the January 20, 2023, Research Committee Meeting were approved as amended (Attachment 1).

3. Updated Activity Timeline

The committee reviewed the updated Activity Timeline (Attachment 2) for information.

4. Pilot Grant Applications

Dr. Thambisetty provided a summary of the Pilot Grant applications and review process. Five proposals were received during this cycle. The applications were reviewed by the Cognitive Aging and Memory Intervention Core – notably Dr. Ron Lazar and Dr. Bonnie Levin as well as at least two outside reviewers assigned to each proposal. The recommendation of the Cognitive Aging and Memory Intervention Core is to fund the requests of Dr. Joseph Signorile and Dr. Sonya Kaur. Dr. Signorile's study is titled "Cued High-Speed Multidirectional Yoga: Impact on Retinal Microvascular and Cognitive Measures" and has a total budget of $119,739. Dr. Kaur's study is titled "Feasibility of a Timed Bright Light Exposure Therapy to Improve Circadian Function" and has a total budget of $120,000.

The committee discussed the merits of the two recommended proposals. Dr. Signorile, who is a senior investigator, received the highest mean score of 3 (on a scale of 1 to 9, where 1 is the highest) for his project, and the committee noted its clinical relevance. Dr. Kaur, who is a junior investigator, received a mean score of "5" for her project. Despite this relatively lower score, the Trustees felt that pilot grant funding could have a positive impact on Dr. Kaur's career and could open the door to future funding from the National Institute of Health (NIH). The Trustees also discussed the weaknesses with the proposal that were identified by the reviewers. The committee discussed and reaffirmed the pilot program's stated goal to fund both a senior and junior investigator in each cycle, where possible.

The committee passed a motion to approve funding for the proposals endorsed by the Cognitive Aging and Memory Intervention Core. The motion also requests that Dr. Kaur's research project, in consultation with her mentors, be modified to overcome the weaknesses noted by the reviewers.

Due to time constraints, Dr. Schlanger will share the recommendation of the Research Committee with the full board to review and vote by email.

The committee also discussed the study submitted by Dr. Giovanna Pilonieta titled "A Single Arm Intervention Study to Evaluate Effects of Personality Traits on Efficacy of Processing Speed Training in Individuals with Subjective Cognitive Decline." The committee felt that the proposal had considerable potential, particularly with strong mentorship from the senior investigators on the research team. The committee would like to encourage Dr. Pilonieta to revise and resubmit her proposal during next year's Pilot Grant cycle.

Action Item 2: The committee passed a motion to approve funding for the two proposals endorsed by the Cognitive Aging and Memory Intervention Core (submitted by Dr. Kaur and Dr. Signorile). The motion also requests that Dr. Kaur's

research project, in consultation with her mentors, be modified to overcome the weaknesses noted by the reviewers.

Action Item 3: Dr. Schlanger will share the recommendation of the Research Committee with the full board to review and vote by email

Dr. Thambisetty provided a summary of his recent phone conversation with Dr. Ron Lazar. Dr. Lazar expressed that there is an increasing disconnect between the mission of the MBRF and the 4 MBIs. This centers around three issues:

- The MBRF does not provide a mechanism for direct salary support for Primary Investigators (PIs) for these initiatives.
- The MBRF's definition of translational research may be too restrictive, possibly excluding valuable projects from receiving pilot grant funds.
- Lack of awareness of the MBRF funding opportunities amongst the MBI researchers (e.g. MBRF Innovator Awards) and how to improve this awareness.

The committee feels the disconnect is bothersome and discussed next steps. The committee recommends having offline conversations with the Director of each MBI in advance of the MBRF Inter-Institutional Meeting Leadership Council Meeting. Dr. Schlanger will share the Innovator Awards funding opportunity by email with the Leadership Council, and the opportunity has already been advertised on the MBRF's social media platforms and in its recent newsletter.

5. Current Grants/Programs

a. MBRF Innovators Awards in Cognitive Aging and Memory Loss

Dr. Thambisetty shared that AFAR has agreed to streamline the process for the next grant cycle, including dividing the rankings into two groups (basic science and clinical-translational science), thereby ensuring that at least one clinical-translational project receives support in each cycle.

b. MBRF Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss

The committee reviewed the current draft of the RFA for the 2023 Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss (Attachment 3). The committee approved the following revisions to the RFA:

- Remove "solely" from the please note at the top of the document
- Under How to Apply #2, change "2022" to "2023"
- # 1 under Eligibility, second sentence add "may include" after "These"
- # 2 under Eligibility, remove "must" from the beginning of the first sentence and replace with "is"

Action Item 4: The committee approved the revisions to the current draft of the 2023 RFA for the Clinical Translational Research Scholarship in Cognitive Aging and Age Related Memory Loss (Attachment 3) as discussed.

6. Cognitive Aging Summit IV Planning Update

Dr. Thambisetty provided the committee with an update on the Cognitive Aging Summit IV Planning. The potential dates for the summit are March 19 – March 21, 2024.

7. Adjourn

Dr. Thambisetty asked if there was any further discussion. Hearing none, he called for adjournment of the meeting 6:15 p.m. EST.

Summary of Action Items:

Respectfully Submitted,

Melanie A. Cianciotto
Corporate Trustee

Addendum
McKnight Brain Research Foundation
Research Committee Meeting
March 28, 2023

Following the in-person meeting, the committee discussed, by email, funding a third pilot grant project, which was submitted by Dr. Farah Lubin "Ketogenic Diet Improvement of Age-Related Memory Impairments, Nominates Cell-type Specific O-GicNAc Deficiencies in the Aged Hippocampus." The committee reasoned that Dr. Lubin has been an active research investigator within the MBI at the UAB for many years and has distinguished herself in receiving the President's Diversity Champion Award at UAB. Dr. Lubin's proposal also received the highest mean score of "3," tied for the highest score with Dr. Signorile's project. Given that no pilot grants were awarded in 2022, it was felt that the MBRF could approve a third pilot grant in 2023. **Therefore, a motion was made and approved by the committee via email to also fund Dr. Lubin's proposal, for a total of three funded pilot grants in 2023.**

Research Committee Activity Timeline

2022-2023

Updated September 12, 2023

Duty (from Committee Charter)	Activity/Action	Outcome	Date	Comments
"Encourage and assess research at the McKnight Brain Institutes (MBIs)"	Review of the Annual Reports of the MBIs	Information for scientific review includes: scientific achievements, publications, presentations, collaborations	DONE February 5, 2020 DONE June 15, 2020 DONE Feb. 26, 2021 Annual Reports were reviewed by the Trustees on Feb. 9, 2022	Reviewers presented at Feb. 2021 Trustees Meeting. Follow up letters were written and sent to each of the MBIs. All Requests of MBIs have been addressed by MBIs. MBRF/MBI Task Force was established April 2021 to streamline Annual Report Recommendations. Recommendations were reviewed Oct 28, 2021 by Trustees. New Template was used for 2021 Annual Reports
	Review of all New Funding Requests from MBIs. Most Funding Requests should be reviewed by the Interventional Core Committee of the MBIs first.	UM submitted a request for $200,000 for Neurocognitive Post-Doctoral Fellowship over the next two years Christian Agudelo, MD, was selected	October 23, 2019 Trustees voted to fund -- payable over two years. Position Start Date – July 2020	The notification letter mentioned that future funding should come from other sources (See "The Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging" on page two)

		UA submitted a request for $244,400 for UM's participation in the Precision Aging Demonstration Pilot	The proposal was reviewed and approved by the Trustees on Feb 5, 2020. The budget was revised and approved June 2020	Dr. Mike Dockery notified UA of the Trustees' approval Trustees were notified of the revised budget and approved no-cost revisions
		A Funding Request "Centralized, telephone-based, computer-assisted…Spanish" for $129,000 was submitted in April 2021 by Dr. Ron Lazar	Reviewed by Cmte in July and not recommended	This request was reviewed by the Trustees in July 2021 and was denied. Suggestion was provided to Dr. Lazar to work though MBI Core Committee if he chooses to resubmit.
		UM submitted a request for $ 3 million to endow a Neurocognitive Training Fund in Brain Health and Aging. UM submitted a request for $250,000 to co-fund a fellowship over 5 years – The Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging"	July 1, 2021 October 2021 Research Cmte reviewed on October 21, 2021; Recommended funding; Trustees reviewed and approved funding October 28, 2021 Grant Notification Memorandum was dated Nov. 10, 2021	This request was denied by Trustees on July 28, 2021, but Dr. Lee Dockery was asked to pursue conversations with UM about how they might proceed. Dr. Dockery had several conversations and exchanges with UM with ideas for strengthening the program infrastructure. A memorandum notifying UM of the approval for funding the Evelyn F. McKnight Neurocognitive Clinical Scholar in Brain Health and Aging for a total of $250,000 ($50,000 over 5 years) to be matched by UM was sent by Dr. Mike Dockery to UM and agreed to and signed by Drs. Sacco and Rundek.

"Encourage and assess research at the McKnight Brain Institutes (MBIs)" continued	Review of Travel Award Fund: Originally established to fund research scholars and faculty to visit other McKnight institutions.	Few applications for travel. The funds allocated for travel have been used to fund the activities of focus groups: Epigenetics, MRI standardization and cognitive test battery working group	Reviewed at each Trustees' Meeting ON HOLD DUE TO UNIVERSITY TRAVEL RESTRICTIONS	Approved in 2009 In the amount of $100,000 Approximately $30,000 remains in the fund
	Inter-institutional Block Grants	Cognitive Aging Core Working Groups	N/A	5 Areas: Brain and Cognitive Health Cognitive Aging & Memory Cognitive Testing Battery Epigenetics MRI standardization
	Inter-institutional Block Grants	Bio-Informatics Core (Epigenetics)	Funding period: 9/1/2013-8/31/2015	Tom Foster, UF still lead scientist.
	Inter-institutional Block Grants	Neuroimaging Core	Funding period: 1/1/2015 to 12/31/2017 $931,759.00	
	Inter-institutional Block Grants	Cognitive Assessment and Brain Registry Core	Funding period: 9/1/2015-8/31/2017 Request for another extension was approved at the Feb 5, 2020, Trustees' meeting.	No-cost Extension Request submitted for April 30, 2021. Trustees approved the extension.
	Review of Pilot Grants (Funding Requests and Progress Reports)	1)A Novel Invention Tool – Levin 2)Revitalizing Cognition in Older Adults – Bowers	1)Funding Period: 5/1/2018-4/30/2020 2)Funding period: 5/1/2018-4/30/2020	1)Funding for 2-years for total of $120,000 2)Funding for 2-years for total of $120,000

				No-cost Extension Request submitted and approved for April 30, 2021.
		3)Transcutaneous Vagal Nerve Stimulation and Cognition Training – Williamson/Alexander	3)Approved July 2019 Funding period: 10/1/2019-9/30/2021 Deadline was extended	3)Funding for 2-years for total of $120,000
			Request for no-cost Extension	Trustees approved at their August 29, 2022 meeting
	Applications for 2021 Pilot Grants	5 Letters of Intent were Submitted	Research Cmte Reviewed LOIs for 2020 Jan. 29, 2021.	Trustees approved 3 grants
		3 Grants were approved	Feb. 26, 2021	
		With Dr. Gomes-Osman's subsequent departure from UM, the Core Committee recommended the next application in line to replace Dr. Gomes-Osman's. This was submitted by Dr. Sonya Kaur "Sleep Intervention…"	The Research Cmte did not recommend funding the next-in-line proposal in its July 2021 meeting "Reuniting the Brain and Body to Understand Cognitive Aging: The Nexus of Geroscience and Neuroscience" pilot grant August 2022	The Trustees denied funding and setting this precedent in its July 2021 meeting. Dr. Rundek was notified. Interim Report submitted. Trustees reviewed and approved on August 29, 2022
	Checked RFA for 2022 before it was posted to be sure it stresses Junior Faculty. It does.	Drs. Lazar and Levin shared that only 1 LOI was received for 2022 funding cycle.	January 31, 2022 Leadership Council Meeting attended by Drs. Thambisetty and Mike Dockery and A. Porter	Several reasons for only 1 LOI were cited. The Leadership Council drafted a new RFA to address these reasons and broaden the scope of the research for Trustee review at their February meeting

			February 23, 2022 September 12, 2022	Dr. Mike Dockery, on behalf of the Trustees, responded to the LC and the members of the Core Committee that they did not wish to change the focus of the pilot grant program by changing the RFA Dr. Mike Dockery, on behalf of the Trustees, and Angelika Schlanger attended the Leadership Council meeting and asked the Council to follow up with the MBRF on the status of the Cognitive Aging and Memory Intervention Core Workgroup, in terms of its membership and plans to respond to the Memo from February 23, 2022.
	2023 Pilot Grants	5 Applications Submitted on February 7, 2023 via Ron Lazar and Bonnie Levin. The research Committee approved three of the pilot grant applications.	March 28, 2023	Dr. Sonya Kaur (PI): "Feasibility of a Timed Bright Light Exposure Therapy to Improve Circadian Function" Dr. Farah Lubin (PI): "Ketogenic Diet Improvement of Age-Related Memory Impairments, Nominates Cell-type Specific O-GicNAc Deficiencies in the Aged Hippocampus" Dr. Joseph Signorile (PI): "Cued High-Speed Multidirectional Yoga: Impact on Retinal Microvascular

				and Cognitive Measures"
"Identify opportunities…to foster greater interest in cognitive aging and age-related memory loss (in the scientific community)"	Research Partnership with the Foundation for NIH and the NIA. 1st cycle-2009, 2nd cycle-2014 3rd cycle approved 2019 to begin Spring of 2020	Fund balance of $1 million from 2nd five-year partnership returned to MBRF Report received on all FNIH/MBRF activities RFA posted: "Network for Identification, Evaluation, and Tracking of Older Persons with Superior Cognitive Performance for Age" FNIH Report submitted For information only	DONE August 2019 FNIH Report in October 2019 had an error. A corrected report resubmitted on Feb. 5, 2020. Posted Feb 2020; Deadline LOI Sept. 1; Application October 1, 2020 First payment was made to FNIH by March 31, 2021. Will continue until 2025 Dr. Molly Wagster will be attending the March 23-25 Inter-institutional Meeting at UA. The Trustees have invited her to present at their	History: Established 2009 $5 M over 5 years from MBRF; match from NIA and partners was $23 M for total of $28 M (17 five-year grants funded) 2014 Partnership renewal funded one 5-year project for $15 million with $5 M from MBRF and $10 M from NIA Valerie connected with Julie Wolf-Rodda and Molly Wagster on promoting STARRS study. NIA will provide $14M to be pooled with MBRF $5 M. A 2.8 Match. RFA was shared with Communications Working Group for posting and with Leadership Council. Two grants were provided from the Research Partnership ""Network for Identification, Evaluation and Tracking of Older Persons with Superior Cognitive Performance for their Chronological Age" to Dr. Thomas

			meeting on March 23, and to the idea of inviting the grantees for a video presentation. Dr. Julie Gerberding, Julie Wolf-Rodda, FNIH, and Dr. Molly Wagster, NIA, attended MBRF Trustees Meeting on October 27, 2022, in DC Planning for CAS IV is underway. The date and location will be March 20-21, 2024 in Bethesda, MD	Perls, Boston University, and Dr. Emily Rogalski.
"Identify opportunities…to foster greater interest in cognitive aging and age-related memory loss (in the scientific community)"	MBRF Innovators Awards in Cognitive Aging and Memory Loss The McKnight Brain Research Foundation committed $4.5 million over the next five years to support outstanding mid-career scientists committed to researching the basic biological mechanisms underlying cognitive aging and memory loss.	Program was Approved by the Trustees Potential administrative and/or funding partners were approached American Federation of Aging Research (AFAR) was identified as an excellent partner organization. AFAR presented a proposal and draft contract for review Revised Agreement signed between AFAR and the MBRF	October 14, 2020 December 2020 January 2021 February 2021 2021 July 15, 2021 August 2021 Mid Oct. 2021 Dec. 15, 2021 March 2022 2022	AFAR Review Committee: Chair: Dr. Anna Maria Cuervo Members: Dr. Rafa de Cabo Dr. Thambisetty Dr. Boyle and Dr. Roz Anderson LOI Deadline – 9 LOIs Received LOI Review – 7 applicants asked to submit full application Application Deadline Award Announcement

			August 2, 2022 September 19, 2022 December 7, 2022 Deadline for 2023 Cycle is July 31, 2023	*LOI Submission and review was eliminated due to the small number of applicants in 2021* Application Deadline Application Review – 4 applied. Award Announcement
	Reserve & Resilience Workshop 2019 Reserve & Resilience Workshop Pilot Grants 2020 Reserve & Resilience Workshop 2021 Reserve & Resilience Workshop 2023	Over 300 Attendees (8 MBI researchers) Organizers requested $30,000 to support (1 – 3) pilot grants	September 9 and 10th, 2019 Bethesda In-Person Meeting CHANGED TO VIRTUAL MTG September 14 and 15, 2020; Report Submitted Jan. 2021 Oct 31/Nov 1 Bethesda Meeting will be a hybrid – part virtual and part person. The program is posted on reserveandresilience.com. Of note, Jen Bizon and Tom Foster are panelists. In-person meeting will take place on September 21 – 22, 2023 in Bethesda, MD on "Data Sharing." Panelists include Carol Barnes, Matt Huntelman, Thomas Foster, PhD, and Sara Burke.	This is an outcome from Cog. Aging Summit III held in 2017. Research Committee approved support in first and second years. Dr. Stern requested support for the Final R & R Workshop to take place Oct. 31/Nov. 1 in Bethesda. He did not request a specific amount but support MBRF provided last year was $30,000. Committee supports recommendation to fund at no more than $30,000. Dr. Stern requested support for meals totaling $30,000. Committee approved by email and full board will review on July 24, 2023.

"Encourage young investigators in this area of research"	McKnight Brain Research Foundation Clinical Translational Research Scholarship with American Academy of Neurology (AAN) and American Brain Foundation (ABF)	2021-2022 MBRF Reviewers are Dr. Boyle, Dr. Thambisetty, and Dr. Isaacson	Reviewers meet in Dec. Two Scholars are selected and alternates were identified. Awardees are notified in January. Funding starts July 1 of each cycle Edits to 2021 RFA were made and approved by Research Cmte. RFA was posted as of July 4, 2020, on AAN site. Advertising followed 2019 Plan for 2020 Award and begin in August, 2020. 8 applications for 2021 were received. October 14, 2020, Renewal for next five years was approved by the Trustees	<u>First Scholarships Awarded</u> January 2018 (McConnell, Albert) <u>Second Scholarships</u> Awarded January 2019 (Camargo, Sedaghat) <u>Third Scholarships</u> Awarded January 2020 (Baxter, Getz) <u>Fourth Scholarships</u> were Awarded in January 2021 to Dr. Wendy Yau Wai-Ying (Brigham and Women's) and ~~Dr. Matthew Burns (UF)~~ Dr. Reem Waziry (Publicly announced in April 2021 (Dr. Matthew Burns [UF] received a K-Award from NIA and had to decline the McKnight Scholarship.) <u>Fifth Scholarships</u> Advertising was conducted in August and September 5 Applications received Oct. 1. Review was in Dec. 2021
		Members of the 2022-23 Review Committee include Dr. Madhav Thambisetty and Dr. Patricia Boyle	2022-23 Deadlines September 1, 2022 Application Deadline Spring 2023 Announcement of Recipients	<u>Sixth Scholarships</u> New 2022-23 RFA Draft was reviewed and has been posted and advertised - 9 applications were reviewed

				2023 Scholars Announced (Drs. Eva Klinman, MD, PhD and Sheena Baratano, MD, PhD)
		Members of the 2023-24 Review Committee include Dr. Madhav Thambisetty, Dr. Patricia Boyle, and Dr. Roy Hamilton	The review committee met on Sept 7th, 2023.	5 applications were submitted and review – the committee raked on top clinical and one top basic research application.
"Encourage young investigators…" Continued	Poster Reception at 2019 Society for Neuroscience annual meeting (Chicago) MBRF/MBI Poster Reception 2020 Society for Neuroscience (SfN) annual meeting in DC October 24 – 28, 2020 canceled due to DC pandemic closing guidelines Society for Neuroscience will meet in San Diego Nov 12 - 16		October 20, 2019 August 29, 2022 September 5, 2022	First Poster Reception held in 2008. (50 submissions received) Sponsored by MBRF. Hosted by Directors of MBIs. Submissions open to researchers at MBIs and invited guests only MBRF Trustees Decided not to host the MBRF/MBI Poster session at the 2022 meeting. Dr. Mike Dockery updated the Leadership Council on Sept. 12, 2022 by Zoom. Dr. Mike Dockery wrote to the Leadership Council to ensure it will take place in 2023.

	Society for Neuroscience will meet in DC, Nov 11 - 15		September 1, 2022	Ms. Porter wrote to Dr. Molly Wagster to alert her that the poster reception will not take place this year. The poster session will take place on Nov 12, 2023. The session is being planned by Vicki Hixon and has been promoted to the MBIs.

To: Mike Dockery, MD, Chairman of the McKnight Brain Research Foundation

From: Interinstitutional McKnight Leadership Council (University of Florida: Jen Bizon, PhD, Sara Burke, PhD, Adam Woods, PhD; University of Miami: Tatjana Rundek, PhD, MD, Bonnie Levin, PhD; University of Alabama: Ron Lazar, PhD, FAHA, FAAN, Kristina Visscher, PhD; University of Arizona: Carol Barnes, PhD, Lee Ryan, PhD)

Subject: Recommendations for Inter-institutional Efforts including the Pilot Program and McKnight Brain Aging Registry

At our last inter-institutional breakfast with the trustees, two primary topics garnered considerable discussion: the interinstitutional pilot program and the next steps for the McKnight Brain Aging Registry (MBAR). It was suggested by the trustees that the Leadership Council work together to provide some consensus recommendations on both. We hope these recommendations are helpful and we look forward to working with you on the next steps for sustaining these significant inter-institutional efforts.

BRIEF REVIEW OF HISTORY

Cognitive Aging Memory Intervention (CAMI) Core. The CAMI-Core was created in 2016 from a proposal submitted to by the Cognitive Aging and Memory Intervention workgroup (members: Rundek (UMiami); Woods and Cohen (UFlorida); Alexander (UArizona) and Wadley (UAlabama)). With the vision that CAMI would "*facilitate the rapid implementation of interventions via pilot studies and subsequent clinical trials focused on preventing and treating age-related cognitive decline*" the Cognitive Aging and Memory Intervention working group proposed creation of "**a high-profile, nationally recognized Cognitive Aging and Memory Intervention (CAMI)-Core, which would serve as an interventional hub for the for MBIs and complement the existing Brain Aging registry, Cognitive Core, and Epigenetics Core. It would help to facilitate promising multisite pre-clinical and clinical interventions, with high potential for public impact and access.**" The original proposal from the Cognitive Aging and Memory Intervention working group was phased, with the goal of Phase 2 **"*to directly facilitate the rapid translation of pre-clinical or novel clinical findings through administration of pilot interventions administered across the four sites*."**

Following approval by the trustees, the pilot program was launched in 2017. To date, the MBRF has funded multiple rounds of pilot projects, with each project receiving a total of $120,000, spread across two years. The first round of proposals was managed by Drs. Woods (UFlorida) and Rundek (UMiami); the last two rounds were managed by Drs. Levin (UMiami) and Lazar (UAlabama). Funded intervention projects have included the following: 1) financial scamming (Levin, 2018); 2) NIRS (Bowers, 2018); vagus nerve stimulation (Williamson, 2019); exercise (Lazar, 2021); Diet (Hernandez, 2021); timed-bright light exposure therapy (Kaur 2023); diet (Lubin 2023); and yoga (Signorile 2023).

McKnight Brain Aging Registry. The MBRF has made other investments into inter-institutional efforts, most notably in the form of a block grant to create a McKnight Brain Aging Registry (MBAR) in 2015. The overall goals of this project were to 1) achieve greater understanding of successful cognitive and brain aging; 2) delineate factors linked to optimal cognitive/behavioral functioning in older adults; and 3) characterize cognition, behavior, brain structure and function in the oldest old. The original investigators on this project were Drs. Clinton Wright, Gene Alexander, and Kristina Visscher (funded as the Inter-Institutional Neuroimaging Core (2015) - $931,759). Subsequently the group expanded to include Drs. Adam Woods, Noam Alperin, and Ted Trouard. Dr. Tatjana Rundek replaced Dr. Wright. As the initially funded proposal from MBRF included only a minimal cognitive dataset, the group was later expanded to include Drs. Bonnie Levin and Virginia Wadley based on their interest and neuropsychology expertise. A second proposal to MBRF was led by Dr. Ron Cohen to enable the acquisition and analysis of cognitive and behavioral measures in the same cohort and Dr. Bonnie Levin led the cognitive component of this study (Inter-Institutional Cognitive Assessment and Brain Registry Core - $800,000). Salary support for investigators was disallowed from the beginning; MBRF funds were used to support recruitment,

neuroimaging costs, incentives, test materials, and study coordinator salaries for several years. In subsequent years, institutes or individual investigators have taken on costs to complete the data acquisition, analysis and achieve the following deliverables:

- Development of a battery of cognitive, behavioral and neuroimaging measures
- Recruitment of 200 participants (50 per site), 85 years of age or greater, exhibiting successful cognitive aging, without MCI
- Acquisition of these measures on all participants
- Establishing the McKnight Brain Aging Registry and associated database (REDCAP)
- Storage of raw neuroimaging (Hipergator) and other data, along with processed measures
- Primary manuscripts for the neuroimaging modalities and a manuscript presenting the cohort and normative data from the cohort
- Establishment of a proposal system for secondary analyzes and to provide access to necessary data

LEADERSHIP COUNCIL'S EVALUATION OF CURRENT STATE

While only a subset of current members of the Leadership Council were involved in the creation and/or administration of the CAMI-core or MBAR projects over the past decade; all members have reviewed the goals and outcomes of both initiatives. This information, together with their knowledge of the current state of both the field and inter-institutional collaboration (which has advanced significantly in the past 10 years), was used to construct the recommendations below. These recommendations reflect the Council's consensus view of how to maximize the impact of MBRF's continued investments in inter-institutional efforts.

Successes. Without question, the funding of the pilot interventions has been valuable in spurring collaborative research across institutes and reducing the barrier to entry for new scientists interested in building novel research programs within the field of cognitive aging. These projects have resulted in several new NIH grants, including several that extend across institutes. Similarly, the MBAR project has been a major undertaking with a significant investment of resources from not only MBRF but from each of the four institutions. This project has led either directly or indirectly to multiple individual R01 level grants, some of which are multi-site.

Challenges. In the past several years, submissions to the pilot program have been limited. The council's opinion is that this is in part because it is extremely challenging to conduct a high-quality intervention pilot across multiple sites within the scope of the award mechanism as designed. In addition, it has become clear after administering several rounds of these grants that coordinating a robust pilot mechanism across all four institutes requires a not-yet-established centralized administrative infrastructure that (1) retains core historical knowledge about the program year-to-year, (2) efficiently facilitates inter-institutional communications and (3) offers administrative support for the rotating Pilot Program Leaders. With respect to MBAR, although the coordinated effort has been leveraged successfully for extramural funding at multiple McKnight sites, a grant has not yet been secured by the MBAR team to provide a sustainable infrastructure for the cohort itself.

LEADERSHIP COUNCIL RECOMMENDATIONS

RECOMMENDATION 1. Prioritize securing extramural funding for MBAR in the next cycle of pilot awards: The Leadership Council recommends the trustees temporarily prioritize a call for proposals designed to provide a bridging infrastructure and accelerate acquisition of an extramural grant that leverages and sustains the MBAR dataset. Our discussions identified funded effort for a Primary Investigator to develop an extramural proposal and funding for a data management specialist to facilitate generation of preliminary data as two ways that submission of an extramural grant could be incentivized. Awarding such funding through a competitive process will ensure alignment with the overall MBRF mission and goals for the cohort as well as ensure a commitment to the extramural grant submission. We further suggest the following guidelines for this competition:

- Applications include an outline for an extramural proposal (R01 or equivalent) leveraging MBAR and include a timeline for submission (which should be within a 2-year time frame of the award onset).
- Although such a project will be inherently collaborative across institutes, a successful proposal should have one contact PI who will communicate with the trustees regarding progress/status of the MBAR effort and one institution identified where the data and specimen repository will be housed and managed. The proposal should clearly indicate the home institution's resources that can be leveraged to ensure long-term success of the repository.
- The PI should be allowed to request up to 15% effort for the duration of the two-year funding period.
- The proposal should include a long-term sustainability plan for MBAR, including how extramural funding sources will be leveraged to support core infrastructure long-term.
- The project should include a communications plan that specifies how approved investigators across sites can access data for new manuscripts and additional proposal development.

RECOMMENDATION 2: Broadening the focus of future pilot awards:

1) **Consider proposals that leverage MBAR.** MBAR has the potential to be a highly valuable resource. As described above, securing extramural funding to support a central MBAR infrastructure is a key priority. Once accomplished, we recommend that future cycles of the pilot program explicitly incorporate a request for proposals that are designed to provide foundational pilot data for successful inter-institutional collaborations related to MBAR.

2) **Consider proposals focused on identification of novel intervention targets**. Beyond the recommendations above, the Leadership Council discussed at length the fact that a multi-site interventional trial within the financial constraints of the current pilot program award is challenging and may limit the novelty and scope of the interventions proposed. Indeed, it is our consensus belief that there is a significant need in the field to **identify novel intervention targets** that can advance cognitive health in older adults. Moreover, AI/machine learning and other advanced data science methodologies offer new opportunities to leverage publicly available datasets to generate new hypotheses and interventional targets for successful aging. We therefore recommend expanding the scope of future pilot awards to include projects focused on identification of novel targets for intervention. To maintain the focus on translation to clinical trials, review criteria could include assessment of the early translational potential of proposals.

Broadening the focus of the acceptable proposals would likely increase the applicant pool, more effectively advance inter-institutional efforts, as well as ultimately be impactful in the field.

RECOMMENDATION 3: Creating an Infrastructure for the Inter-institutional Pilot Mechanism: In addition to the content recommendations above, the Leadership Council recommends several revisions to the implementation of the Pilot Program moving forward. While the CAMI-Core was originally awarded to a specific group of investigators, it is our understanding that the trustees now desire the Leadership Council to assume responsibility for the program's implementation. The Leadership Council is committed to working with the trustees to promote a vibrant inter-institutional community and continues to be enthusiastic about providing primary support for organizing the inter-institutional meetings. We are also willing to take the scientific lead in the pilot program which could include recommendations for individuals to reconstitute an inter-institutional pilot program committee and development of funding priorities. We ask the trustees to consider dedicating centralized administrative support to this program. Such a structure would retain historical knowledge year over year, streamline administrative hurdles and increase sustainability of the program.

We sincerely hope that these recommendations are beneficial. Much time and consideration have been invested to provide our most-informed guidance on how MBRF investments can be maximally impactful for advancing the foundation's central mission of promoting successful cognitive aging. We share your passion for this critical mission and are grateful for the opportunity to partner with you.

Epigenetic and Genomics Core
Historical Review

Prepared by Carol A. Barnes and T. C. Foster

The initial planning occurred in 2013:

The Mission Statement
The McKnight Brian Research Foundation Epigenetic and Genomics Core will pioneer a comprehensive program to test an epigenetic hypothesis of cognitive aging, working collaboratively with all the Evelyn F. McKnight Brain Institutes. The goal is to establish a shared Inter-Institute resource to provide a catalyst for discoveries in the area of epigenetics of cognitive aging. This is envisioned to be a "core without walls" to provide support for bioinformatic analysis of high-throughput DNA/RNA sequencing and epigenomics, bio-informatics, and cross-correlation of human and animal studies.

Strategic Priorities:

1. The study group concluded that an Epigenome-Wide Association Study (EWAS-Memory) would lead to foundational discoveries in the area of neuroepigenetics of aging by dentify the epigenome-transcription interface and its disruption in aging. Achieving this breakthrough will require the establishment of a highly collaborative bioinformatics initiative, utilizing an inter-institute bioinformatics core available to all McKnight Institutes and physically located at the University of Florida (Foster), the University of Alabama (Sweatt) with significant interactions with the UA McKnight Institute via EMBI members at The Translational Genomics Institute in Phoenix.

2. Establish the shared Inter-Institute resource to provide a catalyst for discoveries in the area of epigenetics of cognitive aging. This is envisioned to be a "core without walls" to provide support for bioinformatic analysis of high-throughput DNA sequencing and epigenomics, bio-informatics, and cross-correlation of human and animal studies.

3. The effort will focus on novel epigenetic target discovery to provide a basis for development of innovative new therapeutics.

4. The iInter-Institute core initiative will have as priorities: propelling discovery through Inter-Institute collaborations, McKnight mission-relevance, and high real-life therapeutic impact.

The first request for funding for the Epigenetic and Genomic Core proposed the creation of the infrastructure necessary to ensure that sequencing capability is available at each of the Institutes, and both Arizona and Gainesville sites developed advanced new methodologies for next generation sequencing and bioinformatic analysis. Arizona and Gainesville sites provided cognitively characterized rodent tissue that was analyzed at the separate sites to test our ability to coordinate tissue sharing, to see how the different methods used at the different sites compare, and to evaluate the strengths and and weaknesses of the methodologies proposed. We are unclear what the original funding for this Core was; however, the Epigenetic and Genomic initiative meetings were highly productive, focused, collaborative, and exceptionally innovative. The Core group identified a novel target area that would both propel new discoveries in cognitive aging and produce a high-profile scientific focal point with which the McKnight "brand" will be identified nationally and internationally. An innovative therapeutic approach to cognitive enhancement in aging is a strong, viable target outcome for the initiative.

With this background in mind, a specific proposal for a subsequent stage of advancing the Evelyn F. McKnight Epigenetics and Cognitive Aging Initiative was proposed.

The second request for funding was set at $415,000, which included several new hires for bioinformatics and statistical analysis, web-based design, microbiologist, software, Supercomputer resources, supplies and two meetings per year. Because of Dr. Dockery's email to Ron Lazar January 2, 2023, I (Barnes) believe the total resources invested (the combination of the first and second request) in this core was $600,000.

Accomplishments

During this second phase, we did engage Dr. Riva, with extensive experience in the development of software for HPC environments, and with the computational analysis of methylation in particular (see http://compbio.ufl.edu/dibig/dibig-software/).

Dr. Riva designed, implemented, tested, and deployed a computational infrastructure for epigenetics, with a specific focus on the high-throughput analysis of methylation. This infrastructure, consisting of a collection of software tools, pipelines, and Galaxy workflows, permits scientists to perform all the computational steps involved in the assembly and annotation of methylomes in a user-friendly, efficient, and reproducible way.

The developed analysis tools were designed to operate on datasets of different nature (Next-Gen sequencing, RNA-Seq, Chip-Seq, etc.) from a diversity of neural circuits, as well as from single-cell and real-time genomics. The overall purpose of the analysis is to identify novel gene regulatory pathways and mechanisms underlying neural circuit organization, learning and memory, age-related memory loss and neurodegeneration. Specific analysis paths and workflows can be determined in collaboration with faculty from the McKnight Brain Institutes. The infrastructure was deployed on the HiPerGator supercomputer, and included a combination of existing analysis tools and newly developed software programs, as needed.

Outcomes

In 2017 we published a paper **[1]** on the genomics of age-related cognitive decline that provided evidence for:

i) The ability of the Epigenetic and Genomics Core to work across institutes.

ii) The reliability of the different sequencing platforms (Illumina and Ion Torrent).

iii) The development of an analysis pipeline that was a "core without walls", which provided support for bioinformatic analysis of high-throughput sequencing.

iv) The input form the Epigenetic and Genomics Core was instrumental in the protocols for blood collection by the MBAR group.

v) The data were also used for a manuscript on the idea that mechanisms for genetic resilience or cognitive reserve preserve cognition in the face of aging and pathology **[2]**.

The remaining tissue (after the initial sequencing studies on the Ion Torrent and Illumina platforms) was stored at Alabama for potential DNA methylation experiments. However, this study never occurred (possibly because the funds were not available), and with the departure of Dr. Sweatt from Alabama, it appears that the samples were lost. Both Foster and Barnes have inquired with individuals at Alabama, and other members of the Sweatt lab who have since moved on, but no one can find the samples.

The epigenetic hypothesis of cognitive aging was tested, however, in several studies, using the Epigenome-Wide Association Study (EWAS-Memory) and the analysis pipeline developed by

the Epigenetic and Genomics Core to identify markers of the epigenome-transcription interface and its disruption during aging **[3-5]**.

In addition, we were able to obtain blood samples from cognitively characterized humans. We isolated extracellular vesicles and sequenced the microRNA, which was then analyzed for relationships with age and cognition **[6, 7]**, sex and inflammation **[8]**. This required development of new bioinformatics analyses based on microRNA, and included the addition of machine learning algorithms.

More recently, we have used DNA methylation in human blood as a biomarker of biological age. This work focused on older individuals with knee pain; however, due to the collection of a wide variety of measures, including blood DNA methylation we were able to relate epigenetic age with cognition, blood metabolites (e.g. vitamin D), socio-economic status, mobility, sleep, as well as pain **[9-13]**.

An update on this work was presented at the 13th Annual McKnight Brain Research Foundation Inter-Institutional Meeting **[14]**.

In summary, the interaction between the individuals in the Epigenetic and Genomics Core produced a pipeline for bioinformatics analysis of high throughput sequencing data, which was subsequently employed for use in several studies. This pipeline is no longer state of the art, because further development stopped in 2017 (although it is still useful, and could be updated with more support). The subsequent grants and collaborations that did result from this initial investment enabled individuals across the McKnight Institutes to fill the positions initially requested for statistical and bioinformatics analyses and resulted in development of newer analytical methods and publications.

Post script:
In 2017, the individuals involved at that point in the Epigenetic and Genomics Core with expertise in epigenetic and genomic methods (Foster, Lubin, Huentelman) proposed possible studies to examine blood collected in the MBAR Core. This resulted in a pre-meeting, prior to the 2018 MBRF annual meeting to discuss possible studies (Lubin-Discussion leader, members Barnes, Foster, Huentelman, Rundek).

A proposal developed by Foster, Lubin, and Huentelman was submitted to the MBAR SAC group ~3/8/2020 and again in 7/31/2020. A phone call between the Epigenetic and Genomics group and the MBAR SAC occurred 8/14/2020.

Our understanding of the outcome of these discussions was that the MBAR SAC group was able to have a central repository for the blood. However, no decision was made by them on what to do with it and much of the discussion revolved around how authorship for subsequent papers should be divided. The original Epigenetic and Genomics Core had always envisioned the MBAR group making use of the pipeline created – which is why the outreach by this group to MBAR in 2017 and 2018, and the followup call in 2020. So far, this collaboration has not been realized. Part of the issue may be ‘no funding’ for continuing the project in this way.

Conclusion
Barnes’ assessment is that the Epigenetic and Genomics Core fulfilled its original collaborative research goals, as discussed above, which resulted in publications and presentations as you see listed below. While I currently have no explanation for why the MBAR group has not moved forward with analysis of the blood collected from their participants –this could presumably be an

effort that could be proposed by MBAR to the MBRF for funding (they would have willing collaborators from the Epigenetic and Genomic Core, who submitted proposals to them for experiments that could be done).

I also have no explanation for the fact that the rest of the rat brain tissue that we believe resided in Alabama cannot be located – precluding additional investigations using the cognitively characterized tissue prepared by Barnes and Foster.

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Inter-Institutional Cognitive Aging and Memory Interventional (CAMI) Core Pilot Grant Program

Progress and Final Report Template

Progress Report:

The MBRF is looking forward to receiving an annual update on the progress of your pilot grant. Please compile a narrative progress report of no more than 1-2 pages that comprises the following:

- Summary of achievements and progress that has been made to date with the pilot project
- List any additional grant applications that have been submitted to support the project
- List any awards or grants that have been received as a result of the pilot grant
- List publications, abstracts, and/or presentations on the pilot project
- Describe any challenges that have surfaced, how you are addressing them, and whether any of them will prevent you from meeting the stated goals of the grant

Progress reports are due annually by June 1st. All reports should be emailed to the Executive Director at aschlanger@mcknightbrain.org by the Principal Investigator(s) for the project. Any no-cost extension requests should be included in this report.

Final Report for Completed Pilot Projects:

Please use the same format above to share a final report on the completed project no more than 3 months after the grant term end date or the project end date, whichever is sooner. In addition to addressing the topics outlined above, please share how the pilot project's outcomes have advanced the field of cognitive aging and how its findings will be leveraged for future research, including any planned future projects or studies. Additional pages are welcomed in the final report to discuss your outcomes. All reports should be emailed to the Executive Director at aschlanger@mcknightbrain.org by the Principal Investigator(s) for the project. Principal Investigator(s) should also present their final results at the annual Inter-Institutional meeting.

Any subsequent awards or papers published from these pilots after the grant term, please email the Executive Director to share this information.

Thank you for submitting a report on your pilot grant project. We look forward to learning about your progress.

PROGRESS REPORT

Grantee:	American Federation for Aging Research
Project Title:	McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss
Reporting Period:	July 1, 2022 – June 30, 2023
Submitted:	July 13, 2023

- **Program Summary**

The major goal of the program is to identify emerging scientific leaders by building a cadre of outstanding research scientists across the United States to lead transformative research in the field of cognitive aging. The program provides up to two 3-year awards of $750,000 (USD) each to advanced Assistant Professors and recently appointed Associate Professors (MDs and PhDs.) the investigator's institution needs to provide matching funds (at least 50%, cash or in-kind.) One award is to be made to support studies focusing on clinical translational research and another award toward understanding basic biological mechanisms underlying cognitive aging and age-related memory loss. The program targets full-time independent investigators at the rank of Assistant Professor or Associate Professor (or equivalent) with established independent research programs who have already demonstrated a firm commitment to cognitive aging research. It will add substantial start-up support for a period of three years to help these investigators develop and/or expand an outstanding research program in cognitive aging and memory loss.

- **Accomplishments in achieving the objectives**

2022 Program: AFAR received 5 applications for the 2022 program (9 Letters of Intent in 2021). It was agreed not to include a LOI level of review in 2022, as this is a very targeted request for applications and the pool of eligible candidates and ability for institutions to provide matching funds are limited. The program, however, attracts highly qualified investigators.

The review committee met September 19, 2022, to review the applications. The committee was comprised of the following members:

Ana Maria Cuervo, MD, PhD, *Chair*
Albert Einstein College of Medicine

Rozalyn Anderson, PhD
University of Wisconsin, Madison

Patricia Boyle, PhD
Rush University

Rafael de Cabo, PhD
National Institute on Aging

Madhav Thambisetty, MD, PhD
The McKnight Brain Research Foundation

Dr. Boyle was not able to attend the meeting due to illness but provided information on her top two choices. The other committee members identified two candidates and shared their recommendations with the MBRF board (Attachment A). The board approved the recommended candidates for funding:

Tara Tracy, PhD, Assistant Professor, Buck Institute for Research on Aging: *"Role of KIBRA in Age-Related Memory Loss"*
Grantee profile can be found here: https://www.afar.org/grantee-profiles/tara-tracy

and

Emilie Reas, PhD, Assistant Professor, University of California, San Diego*: "The mediating role of bloodbrain barrier dysfunction in effects of systemic inflammation on brain microstructure and memory"*

Grantee profile can be found here: https://www.afar.org/grantee-profiles/emilie-reas-1

AFAR staff worked with MBRF communications consultant on a press release which was distributed December 7, 2022. https://www.afar.org/imported/AFAR-Press-Release_2022-MBRF-Innovator-Awards_12.6.22.pdf

The grantees will be invited to the 2025 AFAR grantee conference.

2023 Program: AFAR shared two potential changes for consideration to the program with the MBRF board: 1. update the institutional commitment form to include a question about annual budget of the investigator's department (this may give us a better idea whether we only get applicants from well-sourced departments/institutions), and 2. Consider changing the matching funds requirement.

The MBRF board agreed with the first recommendation but kept the matching funds requirement at 50%. The 2023 guidelines and the RFA was posted on the AFAR website https://www.afar.org/grants/mcknight-award and the program announcement was widely disseminated, including several reminders, through eblasts, e-newsletters and shared through social media. The deadline for applications is July 31, 2023 with an anticipated start date of October 31, 2023.

Once the applications are received, we will review with Dr. Cuervo whether adjustments to the selection process need to be made, including possibly different rankings for the basic sciences applications vs. clinical translational applications and get an additional reviewer with a clinical research background. Once the 2023 applications are received, we will review the current list of reviewers and determine if additional members need to be invited with expertise not currently represented on the committee.

- **Opportunities for program refinement**

In the past two grant cycles of the program we identified two areas that we need to monitor and potentially refine:

- This is a significant award, but the number of applications is relatively low compared to other programs AFAR manages. There may be barriers for investigators to apply to this program reflecting on difficult academic environments. The barriers may include 1. Very targeted eligibility criteria; 2. Matching funds requirement, which may be especially difficult for investigators who are at institutions with limited resources. We can survey institutional leadership as well as applicants to provide feedback on the eligibility criteria and matching funds requirement.
- Achieve greater balance in basic science and clinical science applications (currently more applications in basic science). As a result, we broadened the eligibility criteria for MD investigators in 2022. For the 2023 program we may consider instituting two review tracks (basic and clinical/translational)

However, both years the committee commented on the high caliber of the candidates and the quality of the research proposals, so there may be a self-selecting process in place as well.

- **Outreach/Communications**

As mentioned above, AFAR disseminates the program announcement widely. In addition, we create grantee profiles that are posted on the AFAR website, are included in our newsletter and shared through social media. AFAR staff also worked with the MBRF communications consultant on a press release which was distributed December 7, 2022.

https://www.afar.org/imported/AFAR-Press-Release_2022-MBRF-Innovator-Awards_12.6.22.pdf

In the Fall of 2022 AFAR was contacted by *"Inside Philanthrophy"* regarding a response to a recent white paper and they were interested in writing a feature article on advancing research on cognitive aging. We reached out to MBRF and this led to a feature article in the November 29, 2022 issue of *Inside Philanthropy* on MBRF and AFAR and their efforts to advance research on cognitive aging: ***"As Americans Grow Older, These Funders are Advancing the Field of Cognitive Aging Research."*** (Attachment B)

- Awardee Progress reports

  For the 2021 grantees, the progress reports on the first year of the award are included in Attachment C. The 2022 grantees' reports are due September 1, 2023.

- Financial Report

  To be submitted under separate cover.

McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss Committee Recommendations

The selection committee met September 19, 2022, to review the 2022 applications for the MBRF Innovator Awards in Cognitive Aging and Memory Loss. Four applications received a full review. Each of the 5 committee members was asked to review and rank the applications prior to the review meeting. Dr. Boyle was not able to attend the meeting due to illness. During the meeting, the four applications were discussed in detail. One application received low overall rankings and received a brief review. The committee further discussed the top three applications, which would all be highly fundable, however, taking into consideration the program's goals and intent to support one award focusing on clinical translational research and another award toward understanding basic biological mechanisms underlying cognitive aging and age-related memory loss, they recommended the following two applicants:

Tara Tracy, PhD, Assistant Professor, Buck Institute for Research on Aging: *"Role of KIBRA in Age-Related Memory Loss"*

Project summary (provided by the applicant): Synapse function in the brain is critical for memory and cognitive processing, and yet we have a poor understanding of the mechanisms underlying synapse dysregulation in aging. Researchers have linked synapse plasticity deficits and memory impairment in aging, however, *a major gap in our knowledge in the brain aging field* is that little is known about what factors make synapses more vulnerable to dysfunction in aging. In this proposal, we will address this critical barrier by determining how the KIBRA (KIdney/BRAin) protein affects age-dependent susceptibility to synaptic and memory decline. A polymorphism in the WWC1 gene that encodes KIBRA is linked to cognitive decline in aging in humans. However, previous work has focused primarily on the role of KIBRA at synapses in postnatal and young adult mice. Moreover, the role of KIBRA in synapse function has not yet been explored in human neuron models. Our proposed study would be the first to mechanistically dissect how KIBRA levels influence susceptibility to memory loss in aging and to characterize KIBRA function in human neurons. We anticipate that the successful completion of our proposed studies will establish key mechanistic insights into how KIBRA affect synaptic vulnerability to age-related memory loss. Delineating this mechanism of synapse decline would be transformative and guide the development a therapeutic approach to enhance KIBRA-signaling at synapses as a treatment for age-related memory loss.

There was overall agreement by the committee that Dr. Tracy is a strong candidate who has clear support from her institution. While this is predominantly a basic science application, the hypothesis is derived from observations in humans.

- New approach that has potential clinical applications, really fits the program
- Limited to one strain of mice
- Novel protein, niche in this area
- Some of the correlations in the preliminary data very striking
- Associating this protein with cognitive aging

- While there is a significant basic component, hypothesis originated in human studies
- IPSC model – a lot of enthusiasm currently
- $7 million in start-up, publications
- Electrophysiology – calls it memory but she's measuring electrophysiological pulses
- Environment great, ideas good, blend to techniques very strong

Emilie Reas, PhD, Assistant Professor, University of California, San Diego: *"The mediating role of bloodbrain barrier dysfunction in effects of systemic inflammation on brain microstructure and memory"*

Project Summary (provided by the applicant): Sporadic Alzheimer's disease (AD) is an untreatable neurodegenerative memory disorder that profoundly impacts quality of life and increases caretaker and financial burden. As age is the strongest risk factor for AD, targeting biological aging has been proposed as a promising therapeutic strategy to prevent AD. Notably, low levels of chronic inflammation that emerge with age are a common thread among most age-related disorders, prompting coinage of the term "inflammaging". While neuroinflammation is strongly implicated in AD pathogenesis, peripheral inflammatory factors have also been associated with neurodegeneration and cognitive impairment, suggesting that the neurophysiological changes promoting cognitive decline and dementia may, in part, be systemic in origin. A critical outstanding challenge to disentangling the role of systemic aging in neurodegeneration and dementia is characterizing the pathways by which peripheral inflammatory signals compromise neural function. The brain enjoys a privileged environment, uniquely protected by a semipermeable blood-brain barrier (BBB) that tightly orchestrates influx of blood-borne nutrients and immune factors, and efflux of metabolic products. While the BBB is essential for maintaining brain homeostasis, it becomes more permeable with age and dysfunctional in many neurological disorders including AD. Indeed, BBB damage may partially account for the pronounced contribution of vascular dysfunction to AD risk and the frequent co-existence of vascular and AD neuropathology. Peripheral inflammation has been linked to endothelial BBB damage, immune reactivity, and immune cell infiltration [6], suggesting that systemic inflammation may disrupt the neurovascular unit by impairing BBB function, with further vulnerability to a neuroinflammatory response for those with concomitant BBB breakdown related to vascular disease. The proposed project will leverage a multimodal approach integrating advanced diffusion and permeability MRI with biofluid and neuropsychological measures to test the hypothesis that peripheral inflammation is associated with accelerated microstructural brain aging and memory impairment, a process mediated by BBB breakdown. To probe the specificity of this pathway to AD pathophysiology, we will assess modifying effects of an AD polygenic hazard score (PHS) that has been previously validated as a sensitive marker of preclinical AD. Findings will help to fill a crucial piece of the AD puzzle by clarifying the link between systemic inflammaging and neurodegenerative changes underlying cognitive decline. Ultimately, results may help to guide therapeutic approaches to preserve brain health into later life and to optimize cognitive aging trajectories.

The committee is also recommending that Dr. Reas is supported.

- Great translational significance
- Lots of talk about inflammaging – also relevant to cognitive aging/neurodegenerative disease
- How inflammatory signals are translated across the blood brain barrier is largely unknown
- Investigating how abnormalities of blood brain barrier allow peripheral inflammatory signals to reach the brain
- Fairly large study, 150 participants
- Question is of great translational importance – enthusiastic about ranking this highly, particularly with lack of clinical/translational applications in last cycle
- Restriction spectral imaging
- Less novel, other people doing similar types of research. This would not be a niche for her
- Divergent trajectories of memory impairment
- Novelty is trying to relate to longitudinal changes in aging as well as abnormalities in the blood brain barrier
- ADRCs are measuring everything they can get their hands on and connecting it with brain aging
- This individual has the full heft of ADRCs
- Is this more ADRD focused or brain aging focused?
- Looking at cognitive aging as opposed to pathological aging
- Weaknesses – not as novel, and may not be able to create own niche
- Strengths – really looking at longitudinal changes in aging

Dr. Thomas Longden was also considered a very strong candidate who could have been supported if funding were available. AFAR will explore whether other funders would consider supporting this application.

2022 MBRF Innovator Awards Committee

Ana Maria Cuervo, MD, PhD, *Chair*
Albert Einstein College of Medicine

Rozalyn Anderson, PhD
University of Wisconsin, Madison

Patricia Boyle, PhD
Rush University

Rafael de Cabo, PhD
National Institute on Aging

Madhav Thambisetty, MD, PhD
The McKnight Brain Research Foundation

2023 McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss

Application

Title of Proposal: Memory stability and flexibility across a lifetime. **Institution:** Icahn School of Medicine at Mount Sinai **Proposed Start Date:** 10/1/2023 **Proposed End Date:** 9/30/2026 **Total Funds Requested:** $740,751	**Name, title and address of official authorizing proposal:** Ms. Jessica R. Moise Grants and Contracts Officer Senior Associate Dean for Sponsored Programs **E-mail:** grants@mssm.edu **Phone:** (212) 824-8300 **Signature of Official:** Jessica Ruth Moise Digitally signed by Jessica Ruth Moise Date: 2023.07.31 15:02:57 -04'00'
Applicant: Denise J. Cai **Title:** Associate Professor **Degree(s):** B.S, Psychology; PhD, Behavioral Neuroscience **Address:** 444 E. 82nd St. Apt 1M, New York, NY 10028 **E-mail:** denise.cai@mssm.edu **Phone:** (626) 825-0235 **Signature of PI:** 	**Are you an Assistant Professor? Yes *No*** **If No, are you an Associate Professor? *Yes* No** **If Yes, indicate date of appointment:** Appointment date: 3/1/2022 **Do you have an R01 or equivalent funding? *Yes* No** **Will your institution provide 50% in cash or in-kind matching funds determined to be equivalent to the award: *Yes* No**

ABSTRACT: Provide a summary of your research proposal. Do not exceed space provided.

Key words: Learning & Memory, Aging
Model system used for the proposed research: Mice

There have been significant advances in the molecular, cellular, and systems mechanisms underlying the storage of single memories. Real-world memory, however, involves the integration of multiple memories across the lifetime, with one memory affecting how others are processed and stored. The central goal of this proposal is to investigate how the brain stably stores and flexibly updates memories across a lifetime. Aging is inevitable, but cognitive deficits may not have to be. During middle age, memory deficits are more subtle; and numerous studies have shown that middle-aged mice can stably remember individual memories (contextual and spatial memories) as well as young adult mice, but middle aged-mice have trouble flexibly updating their previous memory with new information. By tracking the neural activity of hundreds of neurons in freely behaving mice as they form multiple spatial maps during young adulthood and middle age, we will unveil how the brain stably stores and flexibly integrates memories across a lifetime. In addition to making fundamental insights into learning and memory processes, we hope to develop both biomarkers and behavioral markers that can predict subsequent age-related cognitive deficits and provide early intervention to prevent or slow down age-related cognitive decline.

Two fundamental and related questions surrounding the mechanisms responsible for information processing in the brain underlie the research project described here. **First, how does the brain stably store past spatial maps while flexibly learning new spatial maps?** To address this question, we will attempt to identify the rules that govern how information is accumulated in the brain over time and experience. **Secondly, how does memory stability and flexibility change with normal aging?** We will investigate how the processes governing memory accumulation over time affect both the aging brain's capacity and its ability to discriminate among similar experiences. Given the staggering number of memory disorders associated with age, it seems likely that at least part of these memory difficulties arise as a consequence of the burden of housing such a vast amount of information on a finite amount of 'disc space'. Addressing this question is therefore paramount to addressing age-related cognitive decline.

2023 MBRF/AFAR Grant Application - Page 3

2. **BUDGET**
Up to $250,000 in total cost per year may be requested.

Category	Year 1 MBRF/AFAR	Year 1 matching	Year 2 MBRF/AFAR	Year 2 Matching	Year 3 MBRF/AFAR	Year 3 matching	Grand Total
Personnel	165,113	$97,619	169,508	$97,619	$174,036	$97,619	$801,514
Equipment	$0	$0	$0	$0	$0	$0	$0
Supplies & Animals	$39,320	$4,880	$39,320	$4,880	$39,320	$4,880	$158,748
AFAR Conference	$0	$0	$0	$0	$2,000	$0	$2,000
Travel	$0	$4,500	$0	$4,500	$895	$4,500	$14,395
Other Expenses	$8,000	$18,000	$8,000	$18,000	$1,750	$18,000	$71,750
10% Indirect Cost	$22,115	$0	$22,554	$0	$22,672	$0	$67,341

TOTAL: $1,115,751

4. **BUDGET DETAIL AND JUSTIFICATION**

Personnel:

Dr. Denise Cai – Principal Investigator – 0.8 calendar months from AFAR + effort support from matching funds
Dr. Cai will oversee all experiments, including experimental design, behavioral training, *in vivo* calcium imaging, *in vivo* optogenetics, data analysis, and data presentation. She has extensive expertise in utilizing *in vivo* calcium imaging and optogenetics to characterize the functional role of neural circuits in memory and behavior in healthy and aging brains. The institution will cover 35% (4.2 CM) of Dr. Cai's salary as part f the match for this grant.

To be hired – Postdoctoral Researcher – 12 calendar months
A Postdoctoral Researcher will be hired to plan and execute experiments, analysis, and dissemination of results. The researchers will have complementary skills in *in vivo* calcium imaging, behavioral research, and dissemination of results by proven publications. They will lead the team with Dr. Cai and work with the Research Assistant to ensure all experiments are carried out successfully.

To be hired – Research Assistant – 12 calendar months
The RA to be hired assist the postdoctoral researcher and PI with experiments, data analysis, and lab management. The RA will likely have experience in calcium imaging with Miniscopes and animal husbandry.

Fringe benefits are charged at 31.5% on PI, postdoc, and research assistant salaries. A 3% **cost of living increase** is included in postdoc and research assistant salaries.

Supplies:

General supplies: $14,000 per year

- Behavior supplies = $6,000 per year
- Viruses, antibodies, reagents = $8,000 per year

Surgical supplies: $3,380 per year
We plan to perform surgeries on ~23 animals per year. Each surgical procedure requires the use of various drapes, cotton swabs, sterile gloves, drill bits for craniotomies, scalpels, and other small, low-cost (<$100) consumable materials. Additionally, we will have to periodically replace metabond (~$450), UV curable dental cement, surgical tools (razors, forceps, tweezers, etc), ophthalmic ointment, iodine, and other medications for general animal care during surgical procedures. In total, we expect to spend ~$147 per surgical procedure on consumables.

Calcium imaging supplies: $25,020 per year
Supplies for ~23 imaging animals per year, including animals that have to be excluded for off-target surgeries

- Implanted GRIN lenses, 1 per animal = $4,600
- Baseplates, 1 per animal = $920
- Data acquisition boards and required accessories = $2,400
- Coaxial cables = $900
- Commutators = $5,400
- Consumable components to build miniscopes: $12,600

While open-source Miniscopes are dramatically cheaper than commercial options, we often have to replace components that break or replace entire Miniscopes, which have an expected lifetime of less than 1 year.
Total = $44,200 per year

Animals:

Preliminary data were collected in C57BL/6J animals from Jackson Labs. Therefore, all animals for these experiments will be ordered from Jackson Labs for consistency. All animals will be double-housed for a week of habituation prior to surgery and 90 days after Miniscope surgery for virus expression and experimentation. We will use a total of 68 animals over 3 years (expected ~80% success rate). Each experimental animal will be double-housed with a female that will have ovariectomy surgery, reducing the risk of stress due to single-housing.

Total
Purchase 50 experimental mice aged 12 weeks x $51 = $2,550
Purchase 18 experimental mice aged 68 weeks x $353 = $6,354
Ovariectomy surgery ($65) for 34 experimental female mice = $2,210
Per diem, Habituation (7 days) + Experimentation (90 days): 68 cages x 97 days x $1.30/day = $8,575
Purchase 68 female mice for double housing ($30) + ovariectomy surgery ($65) = $6,460
All years = $26,149
$8,716 per year

Travel:

Domestic Travel: Funds will be used for the postdoctoral researcher and research assistant to attend and present posters at the Society for Neuroscience Annual Meeting (Years 1 - 3) and for Dr. Cai to attend and present at the AFAR meeting in Year 3.
Total = $4,500/year in Years 1 - 2, $5,395 in Year 3

AFAR Meeting:

Per grant requirement.
Total = $2,000 in Year 3

Data Management:

We request support to engage consultants to implement the Neurodata Without Borders framework, develop software pipelines to enable smoother data sharing and collaboration, and assist with data management and analysis.
Total = $12,000 per year

Publication & Figures:

Funds are requested to offset the cost of publication including journal publication fees, professional figure design, color figure fees, and poster printing for annual conferences (~$125 per poster x ~3 posters/year = $375/year). We expect to publish at least 2 papers during the course of this award, with an average cost of ~$5,000 each (e.g., Nature Communications, Cell Reports, etc.). We plan to commission professional figures, including introductory schematics, in the early phases of the award.
Total = $9,800 in Years 1 - 2; $7,750 in Year 3

Indirect costs:

Indirect costs are calculated as 10% of sponsor support.

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Does the research plan include use of human subjects? ☐ YES X NO

Does the research plan include use of animal subjects? X YES ☐ NO

Applicants should note that IRB certification (for human subjects) and/or Animal Use Committee approval (for animal subjects) must be provided to AFAR before a grant award can be made.

Applicants are urged to consult the AFAR website at **http://www.afar.org/research/funding/animal-use/** for advice on human and animal usage or this webinar recording from the Nathan Shock Centers of Excellence: https://nathanshockcenters.org/june2021webinar-1. The website includes a helpful set of guidelines for optimal use of rodents in aging research projects.

6. Indicate the candidate's % of time/effort that will be spent on the planned project:_6.7_% + matching salary support

7. a. Have you previously applied for an AFAR Grant? Yes_X__ No_____
   b. If yes, have you previously received any AFAR Grant? Yes_____ No __X___

If yes, please provide name of grant, year received and title of project: N/A

Research Proposal: Memory stability and flexibility across a lifetime

A. Novelty/impact and relevance to the field of cognitive aging and memory loss; why the project has potential to be transformative: In the last few decades, there have been significant advances in the molecular, cellular, and systems mechanisms underlying the storage of single memories. Real-world memory, however, involves the integration of multiple memories across the lifetime, with one memory affecting how others are processed and stored. The brain's ability to organize and integrate different experiences so that it can efficiently 'file' and 'cross-reference' information is critical for daily life. The central goal of this proposal is to investigate how the brain stably stores and flexibly updates memories across a lifetime. Aging is inevitable, but cognitive deficits may not have to be. While many studies have focused on cognitive deficits during late aging or age-related neurodegeneration (e.g., dementia, Alzheimer's Disease)[1-6], fewer studies have investigated the aberrant neural mechanisms underlying earlier stages of normal aging. During middle age, memory deficits are more subtle[7,8]; and numerous studies have shown that middle-aged mice can stably remember individual memories (contextual and spatial memories) as well as young adult mice, but middle aged-mice have trouble flexibly updating their previous memory with new information[8,9]. By tracking the neural activity of hundreds of neurons in freely behaving mice as they form multiple spatial maps during young adulthood and middle age, we will unveil how the brain stably stores and flexibly integrates memories across a lifetime. In addition to making fundamental insights into learning and memory processes, we hope to develop both biomarkers and behavioral markers that can predict subsequent age-related cognitive deficits and provide early intervention to prevent or slow down age-related cognitive decline[10].

Two fundamental and related questions surrounding the mechanisms responsible for information processing in the brain underlie the research project described here. **First, how does the brain stably store past spatial maps while flexibly learning new spatial maps?** To address this question, we will attempt to identify the rules that govern how information is accumulated in the brain over time and experience. **Secondly, how does memory stability and flexibility change with normal aging?** We will investigate how the processes governing memory accumulation over time affect both the aging brain's capacity and its ability to discriminate among similar experiences. Given the staggering number of memory disorders associated with age[11,12], it seems likely that at least part of these memory difficulties arise as a consequence of the burden of housing such a vast amount of information on a finite amount of 'disc space'. Addressing this question is therefore paramount to addressing age-related cognitive decline.

B. List of specific aims of the research plan: Aim 1: To determine how the brain stably stores previous spatial maps while flexibly learning new spatial maps to maximize memory capacity in young adult mice. **Aim 2:** To investigate how memory stability and flexibility change in middle-aged mice.

C. Background information needed to understand the importance of the problem: There are at least two competing theoretical models that can describe the rules that may account for how information is accumulated in the brain. The first predicts that each new memory is stacked atop the prior one orthogonally (i.e., in a perpendicular, unrelated manner, with few overlapping cells) and is unmodified across time[13-15]. This model suggests that the brain has a fixed capacity to store memories and that when capacity is reached memories will be lost or forgotten. The second model predicts that a non-orthogonal relationship exists between the number of memories stored and the size and interdependence of the neuronal networks dedicated to each memory[8,16-18]. In this second model, as memories accumulate, memories may have more representational similarity between the memory ensembles (two memories are neurally more similar), possibly to share neuronal resources as in order to maximize memory storage capacity. This is similar to data compression techniques used to maximize data storage on computer hard drives.

It is likely that the brain operates somewhere between these two extremes of representational similarity (orthogonal and non-orthogonal coding), although the rules the brain employs to move flexibly between these strategies are unknown. As an example, while computer compression techniques to maximally reduce disc space while retaining as much information as possible might be seen as the ideal, it is also burdensome with respect to the amount of processing required to achieve this end. For this reason, it may be better to accept a loss of information in order to reduce the amount of processing required. Understanding these trade-offs is key to determining why memory goes awry.

These are admittedly large, imposing questions. Therefore, we are proposing here to tackle these issues first through a constrained, focused investigation of 1) one specific brain region: the CA1 region of the hippocampus, known to play a necessary role in coding contextual and spatial information, and 2) how the CA1

accumulates one specific type of information: learning and remembering different spatial maps across a lifetime. From this, we hope to develop a method of extracting general principles of information processing that might be tested more broadly in other brain regions for processing other types of information. **Specifically, we will consider whether or not the accumulation of more/new spatial maps alters how the hippocampus forms each successive map and how the representation of previous maps is altered by new learning**.

We have known since the foundational work of O'Keefe and Dostrovky that hippocampal cells respond to specific spatial locations[19]. Collectively, these 'place cells' have been proposed to function as part of a cognitive map (i.e., a mental representation of the position and spatial relationships of an external environment) as well as contribute to episodic memory (e.g., memory of autobiographical events)[20,21]. Studies using immediate early gene tagging strategies (e.g., the TetTag system)[13,22-24], as well my prior work using calcium imaging[8,25] , support this view of the hippocampus, demonstrating that spatial memories are stably encoded by sparse populations of neurons in the hippocampus. When animals re-enter a context (i.e., an environment), many of the same neurons activated during initial coding of that context are re-activated during recall to support retrieval of the context memory. In contrast, entering a different environment activates a different population of neurons.

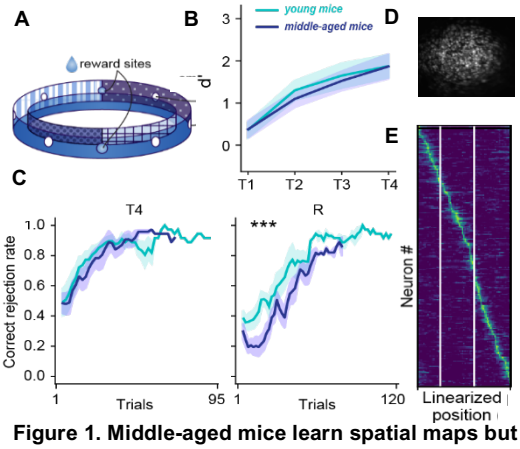

Figure 1. Middle-aged mice learn spatial maps but are impaired at integrating new information. A) Schematic of circular track for spatial navigation task. B) Across 4 days of training (T1-T4), young adult and middle-aged mice perform similarly on the circular track licking at reward ports and avoiding non-reward points. C) During the last day of training (T4), young and middle-aged mice have similar correct rejection rates of non-reward points. When the reward ports are changed (R), middle aged mice are impaired at rejecting reward ports that are not rewarded. D) Representative image of maximum projection of hippocampal calcium activity. E) Normalized firing rate of hippocampal neurons while a mouse performed on the circular track. White lines represent reward locations.

Surprisingly, these studies have found that cells jointly activated by two or more environments occur at chance levels, suggesting that different contexts or environments are encoded by largely independent/orthogonal neural ensembles (populations of cells). However, if every distinct context were encoded by an orthogonal ensemble of cells, with little overlap, as these studies suggest, our brains would "max out" the capacity of the hippocampus; there would only be a small number of environments that an animal could learn. This goes against evidence showing that animals are able to learn and remember large numbers of experiences[18,26]. Perhaps because many studies examining hippocampal encoding have generally looked at how the brain encodes a single contextual or spatial memory – or at most, how the brain distinguishes between two very different environments – they have been ill-equipped to capture the coding strategies employed by the brain under ordinary circumstances to accumulate large amounts of varied information. Moreover, there is growing evidence that memories are not static snapshots but are instead dynamically updated and integrated over time to facilitate efficiency, especially in brain regions such as the CA1 subregion of hippocampus[8,25,27-30]. This lack of attention to how the brain uses more versatile strategies to store and retrieve many memories is likely due to the technological hurdles required to visualize the large number of cells activated by one, let alone many, environments across long periods of time. The recent advent of microendoscopic calcium imaging techniques[31] and its further development by my team[8,25,32] has allowed us to surmount this barrier; we are now able to stably record hundreds to thousands of hippocampal cells simultaneously across the lifespan.

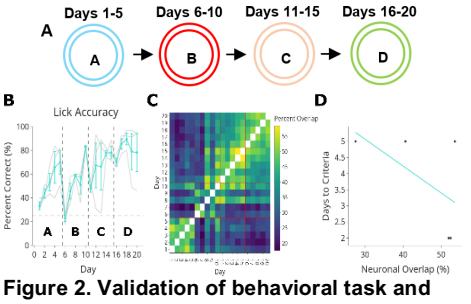

Figure 2. Validation of behavioral task and neuronal overlap analysis. A) Schematic of experimental design as mice learn 4 distinct circular tracks. B) Mice learn each new subsequent track faster. Horizontal dashed line indicates 25%, or chance performance. Vertical dashed lines indicate context switches C) Heatmap showing neuronal overlap between each day from an example mouse. Red horizontal/vertical lines indicate when mice switched circular tracks. D) Correlating days to criteria and neuronal overlap.

D. Preliminary data produced by the Principal Investigator: We will record hippocampal calcium activity with Miniscopes (**Fig 1D, E**) while mice perform a spatial navigation task (**Fig 1A**)[9]. Middle-aged mice can perform similarly to young adult mice for the initial spatial task (**Fig 1B**), however, they are impaired when they need to update their past spatial map with new reward information. In the first part of the spatial navigation task, middle-aged mice correctly lick at rewarded ports and correctly reject non-rewarded ports to the same level as young adult mice. However, when reward ports are switched, middle-aged mice continue to lick at the old reward ports that are no longer rewarded

whereas young adult mice learn to correctly reject the old ports (**Fig 1C**). We will use a modified version of this spatial navigation task – to ask how mice acquire multiple spatial maps, mice will learn to spatially navigate to reward ports associated with each of the 4 distinct circular tracks (**Fig 2A**). Each circular track is a physically separate track in a different location of the experimental room with distinct visual cues from the other tracks. We have shown that mice can achieve 75% accuracy in the first circular track within 5 days and they reach 75% accuracy faster in each successive track (**Fig 2B**). In this proposal, we will investigate how mice learn faster with each subsequent circular track. To ask if more or less representational similarity across tracks supports the faster learning of multiple spatial maps, we will measure representational similarity using multiple metrics. A common metric of representational similarity is the neuronal overlap between two memory representation: neurons that are active in one session that are reactivated in another session (**Fig 2C**)[8]. We will ask if neuronal overlap increases as mice accumulate more spatial maps, as a potential way to increase shared neural resources and increase efficiency of storage of multiple maps[18]. We will also correlate neural metrics to behavior as there is wide variability across animals. We will ask if the neural overlap between contexts is correlated with their ability to learn the reward ports associated with the new track (**Fig 2D**). While the data in Fig 2 are preliminary, they demonstrate feasibility of the task and analysis. We have also tested middle aged mice on the behavioral task shown in **Fig 2A**. While middle-aged mice perform similarly in the first track, they do not improve in their performance across tracks as the young adults do (**Fig 3**). To control for performance levels across young adult and middle-aged mice, we will train mice to 75% criteria in both young and aged mice before switching tracks in the proposed study. Lastly, we will use our novel dual-channel Miniscope to verify that we can stably image the same cells across these time periods (**Fig 4**). While Miniscopes have been used to track activity from hundreds of cells, if a cell is active at two timepoints, you know you are likely recording from the same cell. However, if a cell is active at one time point and not at another, it is unclear if the cell is not active or has shifted out of the field of view. Given the long duration of our experiments, it is critical that we can be confident in recording from the same cell across all timepoints. To ensure stability and reliability in recording the same cells, we will express a virus that has both a constiutively active tDTomato and dynamic GCaMP (**Fig 4**). We will only analyze GCaMP signals that have a constant tDTomato marker across the experiments. Mice are able to run along a track at the same speed with the dual-channel as a single-channel Miniscope (**Fig 4**)

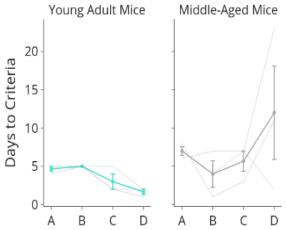

Figure 3. Middle-aged mice show a lack of increased rates of learning reward port locations in new circular tracks. Days to reach 75% criteria for young adult (4 months) and middle-aged (9-14 months) mice. Middle-aged mice did not show a decrease in days to reach 75% accuracy.

E. Experimental design, with key methodologies: Aim 1, Determine how the brain stably stores past memories while flexibly integrating new information to maximize storage capacity of cognitive maps in young adult mice. Animals: Young adult C57BL/6J Jackson mice (3-4 months old, n=12 per group x 2 group) will be used. Behavior: To assess how spatial maps are changing as mice learn multiple circular tracks, mice will undergo training for 20 minutes each day for five days within each of the four circular tracks (five days in track A, five days in track B, etc., **Fig 5**). After animals learn tracks A-D, they will return to the previous track C to study how prior memories are stably stored and retrieved compared to encoding a new track E. This fixed design (20 min/day, 5 day/track) allows for comparisons across all days to be combined for all mice and controls for time within each circular track. Miniscope recording: Six weeks prior to training, the virus GCaMP6f AAV1-hSyn-GCaMP6f-P2A-nls-dTomato will be targeted unilaterally to the dCA1 region of the hippocampus. A GRIN lens will then be implanted above the dCA1 region to allow for in vivo calcium imaging with the dual-channel Miniscope (**Fig 4**) as animals perform on the circular tracks (**Fig 1 and 2**). Analysis/Expected Outcomes: To ask if memory stability for each track changes for each subsequent track learned, we will compare both behavior and neural data across tracks (e.g., days 6-10 in track B vs days 11-15 in track C). We hypothesize that animals will learn each subsequent track faster (**see Fig 2**) and that neural representations will stabilize sooner. We will also measure memory retention with an intervening track (compare neural representation of day 11-15 on track C with day 21-25 on track C

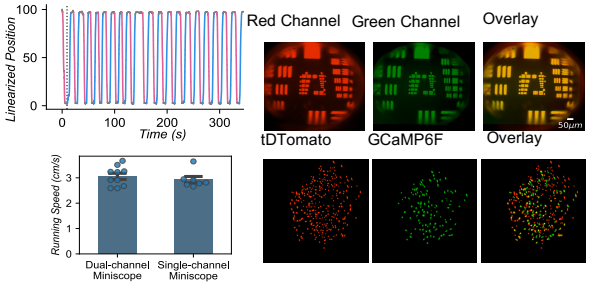

Figure 4. Validation of novel dual-channel Miniscope. Top L) Representative mouse running along a linear track plotted as the linearized position of the mouse across time (red is running left, blue is running right). Bottom L) There is no difference in running speed of mice wearing the dual-channel or single-channel Miniscopes Top R) Image of a resolution test target collected from red and green channels with a GRIN lens and the overlay of the 2 images. Bottom R) Representative field of spatial footprints of CA1 neurons in vivo shown for tDTomato, GCaMP6f and the overlay of the 2 images.

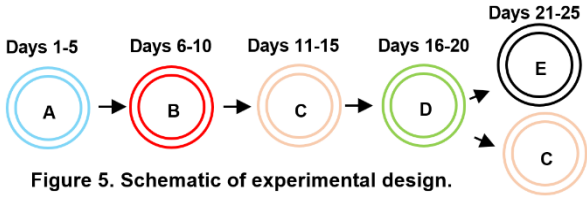

Figure 5. Schematic of experimental design.

again or a new track E). We expect that mice will reach faster accuracy in track C on day 21 compared to track E, showing that they remember track C. We will also measure representational similarity of neural data between tracks. We hypothesize that we will see orthogonal/independent coding on at least the first two tracks learned. However, as successive tracks are learned, we anticipate that the representational similarity between tracks will increase (e.g., representation similarity between C&D will be higher than A&B). The representational similarity increased, decreased, or unchanged will be incredibly informative. For example, it's possible that there is increased neuronal overlap across sessions, but the overlapping cells have very different firing patterns. That suggests that the hippocampus is increasing shared neural resources across tracks (shared cells) but could be distinguishing the different memories by their different firing patterns. Importantly, because each track is associated with distinct reward locations, we will also be able to ensure that any increase in representational similarity is not the consequence of a failure to discriminate between the tracks. We will calculate multiple metrics of stability including place cell stability, information content of place cells, population vector correlation, neuronal overlap, co-activity of neurons with ICA/PCA. Pitfalls: We have extensive preliminary data that all aspects of the proposed experiment are feasible and likely to produce insightful results on how the hippocampus accumulates spatial maps across time. It is possible that mice will have trouble with the increased weight of the dual-channel Miniscope, despite our strong preliminary data showing that they run at the same speed with the single- or dual-channel Miniscope. If this is the case, we will either counterweight the Miniscope with a helium balloon as we have done in the past or switch to a single-channel Miniscope. **In Aim 2, we will investigate how memory stability and flexibility changes in middle-aged mice.** Behavior: Young adult (3-4 months old) and middle-aged mice (13-14 month old) C57BL/6J Jackson mice will be used. (n=12 per group x 2 recall groups x 2 ages). Young adult and middle-aged mice will undergo training in four distinct circular tracks as in Aim 1 (**Fig 5**). While there are benefits to a fixed design as explained in Aim 1, in this experiment, mice must reach a criterion of 75% lick accuracy to progress to the next circular track. Since middle-aged mice may vary in the time it takes to learn each new track (**Fig 3**), this will ensure that all mice have sufficiently learned each circular track before progressing to the next. Miniscope recording & Analysis: Same as in Aim 1. Expected outcome: We hypothesize that middle-aged mice will have similar behavior and neural representation of the first track. Our published work also suggests we will see similar neuronal overlap between the first two tracks in young adult and middle-aged mice[8]. However, it is unclear how differences in stability and representational similarity will emerge across learning multiple tracks. It is possible we might find some similar metrics across young adults and middle-aged mice (e.g., increased neuronal overlap across tracks) while others differ (e.g., population vector correlation). They may both increase in neural overlap, sharing resources between tracks but in young adult mice, these overlapping cells have differential firing patterns in the different tracks whereas in the middle-aged mice, there may be more similar firing patterns, making it difficult to distinguish between tracks. Importantly, we expect variability in behavior and neural representations in our middle-aged mice and to separate the group into "good learners" and "poor learners" and relate their behavior to their neural activity. There are many different outcomes but all of them are informative of how the brain accumulates cognitive maps across a lifetime.

F. Brief discussion of implications for future research: It is unknown how the brain is able to accumulate a seemingly infinite number of memories across a lifetime. The compilation of these memories defines the human experience. Understanding these memory processes has profound implications not only for neuroscience but computer science as well (e.g., machine learning, artificial intelligence), and has potentially enormous clinical relevance across multiple disease areas, from drug discovery for neurodegenerative and psychiatric disease, to the development of neuroprosthetic devices (e.g., neural interfaces to address spinal cord injury and epilepsy).

G. Relation of this work to current research; why this work is complementary and not overlapping: The project described here is considerably different from research I have proposed, or would propose, for traditional grant funding mechanisms. First, we will explore an expansive, conceptually challenging question not directly linked to a specific disease area. While this research does have clinical relevance across a number of areas of human health and disease (diverse neurological and psychiatric disorders and illnesses), the project is not intended to lead directly to the discovery of therapeutics for a particular disease or disease subtype. This work is more explicitly theoretical and more broadly conceived than any of our currently funded work; it is related to other projects looking at spatial navigation and memory updating mechanisms in the lab but does not overlap with any current projects.

Scientific Rigor

Aim 1:

Animals: Young adult C57BL/6J Jackson mice (3-4 months old, n=12 per group x 2 groups) will be used. We will use equal numbers of males and females. This sample size will allow us to detect a large correlation coefficient (r) of -0.8 at 90%

Behavioral analyses (See Fig. 1): Behavioral performance on the circle track is computed using hit rates, correct rejection rates, and d'. The hit rate per trial is defined as the number of times a rewarded port was licked at least once on that trial divided by the number of rewarded ports (2 ports). Similarly, the correct rejection rate is defined as the number of times an unrewarded port was not licked at all on that trial divided by the number of unrewarded ports (6 ports). To calculate d', we use the loglinear approach to prevent infinite d' rates under edge cases and then followed the formula: d^'=z(H)-z(FA) where H is the loglinear transformed hit rate, FA is the false alarm rate (1 minus the correct rejection rate), and z(x) is the z-transform based on a standard normal distribution.

Neuronal overlap analysis (See Fig. 2): Each session will be compared to every other session in a pairwise manner, and neuronal overlap will be calculated as: (Matched neurons / total cells) * 100, where matched neurons are the number of neurons shared between session X and session Y(based on whether the cell was classified as active in both) and total cells are the number of active cells in session X plus the number of active cells in session Y minus the matched neurons.

Place cell analysis: To determine consistency between place fields, spatial neuronal activity rates will be calculated using 2-cm wide-spatial bins and a speed threshold of greater than 7 cm/s. Spatial neuronal activity will then be normalized by the occupancy of the mouse for each spatial bin. Spatial information will be calculated as previously described[9]. A neuron will be classified as a place cell if it has a statistically higher spatial information value than expected by chance. Rate remapping scores are calculated similarly to previous methods in order to determine the extent to which activity rates differed across time in a neuron's place field[9]. Place cell stability of a single neuron's spatial activity rate map is calculated by taking the Fisher Z-score of the Pearson correlation coefficient between the spatial activity rate maps at two time points. Spatial information content will be calculated as:

$$I = \sum_{i=1}^{N} p_i \frac{\lambda_i}{\bar{\lambda}} \log_2 \frac{\lambda_i}{\bar{\lambda}}, p_i = \frac{t_i}{\sum_{i=1}^{N} t_i}, \bar{\lambda} = \sum_{i=1}^{N} p_i \lambda_i$$

Population vector correlation will be calculated as:

$$PVO(x,y) = \frac{\sum_j^N \lambda_j^1(x)\lambda_j^2(y)}{\left(\sum_j^N \lambda_j^1(x)\lambda_j^1(x)\right)\left(\sum_j^N \lambda_j^2(y)\lambda_j^2(y)\right)}$$

Ensemble co-activity with PCA/ICA: Ensembles for each session are detected using a Python implementation of an unsupervised statistical framework based on principal component analysis (PCA) followed by independent component analysis (ICA)[33]. Binarized calcium activity is smoothed using a 5-bin moving average (~300 ms, slightly larger than a single theta cycle) and z-scored. Using this activity matrix, we performed PCA and compared the eigenvalues of each principal component to a surrogate distribution of eigenvalues, created using activity matrices where all neurons had their activities circularly shuffled relative to each other, repeated 500 times. The number of ensembles (independent components) is the number of principal components that had eigenvalues higher than 99% ($p < 0.01$) of the eigenvalues from the surrogate distribution. We then use this number as the number of independent components to be extracted with the fast-ICA algorithm. Each ensemble (independent component) has a weight vector representing the contribution of each neuron to the ensemble, and neurons were considered ensemble members if their weight exceeded 2 standard deviations above the mean of that ensemble[43,96]. We then used the outer product of the weight vector to produce the projection matrix *P*, which can be used to project neural activity into ensemble activation strength.

Statistics: Statistical significance is measured using two-tailed Wilcoxon signed-rank tests, paired and unpaired t-tests, one- or two-way analysis of variance (ANOVAs) with interaction terms, chi-square tests, Mann-Kendall trend tests, and Spearman correlations. Post-hoc tests on linear mixed models are done with pairwise t-tests using estimated marginal means (emmeans package in R). P-values are corrected for multiple comparisons using Sidak's or Benjamini-Hochberg's false discovery rate adjustments.

Aim 2:

Animals: Young adult (3-4 months old) and middle-aged mice (13-14 month old) C57BL/6J Jackson mice will be used (n=12 per group x 2 recall groups x 2 ages).

Analysis: Behavioral, neural, and statistical analysis will be performed as described in Aim 1.

Sex as a Biological Variable: We will examine males and females using an equal number of male/female subjects per group for all experiments, and sex will be included as a variable in all statistical analysis. Our preliminary data indicate that males and females are phenotypically similar in the spatial navigation tasks.

References

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Denise J. Cai, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): denisecai

POSITION TITLE: Associate Prof. in Neuroscience, Icahn School of Medicine at Mount Sinai (started 3/1/2022)

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
The University of California, San Diego	B.S.	06/2004	Psychology
The University of California, San Diego	Ph.D.	06/2010	Behavioral Neuroscience
The University of California, Los Angeles	Postdoctoral	06/2017	Neuroscience

A. Personal Statement

Our research goal is to understand how memories are stably stored and flexibly updated across time and experience. How does prior experience affect subsequent behaviors or update prior memories? We use a multilevel approach to investigate the dynamic neural mechanisms governing these processes in health and disease. My laboratory at the Icahn School of Medicine at Mount Sinai (ISMMS) combines cutting-edge cellular and behavioral techniques to gain critical insights into how memories are initially processed, stored, and retrieved, and how these processes are affected by trauma, stress, and aging. In particular, our expertise in electrophysiological approaches involving the use of activity-dependent tagging of neuronal ensembles, *in vivo* calcium imaging in freely-behaving animals, chemogenetics, optogenetics, and various behavioral assays relevant to the aims of this proposal put me in a unique position to advance our understanding of the neural circuits that process stressful experiences.

I am also one of the primary developers of an open-source Miniscope for *in vivo* calcium imaging and my recent paper was the first to describe and use this technology. I have also co-led a large effort to share this technology with the neuroscience community through an online wiki and have led workshops for 2000+ participants to learn how to build and use our system. 700+ labs globally have built our Miniscopes for performing calcium imaging in their own labs. My lab, in collaboration with labs at Duke, Mount Sinai, and UCLA, is also developing new versions of the Miniscope including multi-channel and optogenetics-capable versions with the goal of sharing all versions openly.

I have not published or created research products under another name.

<u>Ongoing projects that I would like to highlight include</u>:

- NIMH R56 MH132959-01
  Cai (PI)
  04/04/2023-02/28/2025
  Fear and anxiety circuit mechanisms in anterior hypothalamic nucleus
- NIMH DP2 MH122399-01
  Cai (PI)
  08/15/2019-06/30/2024
  How does the brain optimize storage capacity?
- NIMH R01 MH120162-01
  Cai (PI)
  12/03/2019-10/31/2024

Circuit mechanisms of retrospective memory linking

- Irma T. Hirschl/Monique Weill-Caulier Research Award
  Cai (PI)
  08/15/2019-06/30/2024
  Does hippocampal ensemble reactivation of trauma memories cause disrupted sleep in PTSD?

Selected Citations:

a. Zaki Y, Pennington ZT, Morales-Rodriguez D, Francisco TR, LaBanca AR, Dong Z, Carrillo Segura S, Silva AJ, Shuman T, Fenton A, Rajan K, & **Cai DJ** (2023) Aversive experience drives offline ensemble reactivation to link memories across days. ***bioRxiv.*** DOI:1101/2023.03.13.532469
b. Pennington ZT, LaBanca AR, Sompolpong P, Christenson Wick Z, Feng Y, Dong Z, Francisco T, Chen L, Fulton SL, Maze I, Shuman T & **Cai DJ** (2023) Dissociable contributions of the amygdala and ventral hippocampus to stress-induced changes in defensive behavior. ***bioRxiv***. DOI: 10.1101/2023.02.27.530077.
c. Dong Z, Mau W, Feng Y, Pennington ZT, Chen L, Zaki Y, Rajan K, Shuman T, Aharoni D, **Cai DJ**. (2022) Minian: An Open Source miniscope analysis pipeline. ***eLife.*** 10.7554/eLife.70661. PMID: 35642786; PMCID: PMC9205633
d. **Cai DJ***, Aharoni D*, Shuman T*, Shobe J* (*co-first author), Biane J, Song W, Wei B, Veshkini M, La-Vu M, Lou J, Flores S, Kim I, Sano Y, Zhou M, Baumgaertel K, Lavi A, Kamata M, Tuszynski M, Mayford M, Golshani P, Silva AJ. (2016) A shared neural ensemble links distinct contextual memories encoded close in time. ***Nature,*** 534(7605),115-118. 10.1038/nature17955. PMID: 27251287

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022–present	Associate Professor, Icahn School of Medicine at Mount Sinai
2017–2022	Assistant Professor, Icahn School of Medicine at Mount Sinai
2010–2017	Postdoctoral Fellow, UCLA Department of Neurobiology Advisor: Dr. Alcino Silva
2010	Adjunct Instructor, UCSD Department of Psychology
2006	Training Assistant, Short Course on Behavioral Neuroscience, MED Associates
2005–2010	Teaching Assistant (>20 courses), UCSD Department of Psychology
2005–2010	Graduate Student Researcher, UCSD Department of Psychology Advisors: Drs. Sara Mednick, Stephan Anagnostaras and Michael Gorman
2018–present	Reviewer: Cell, Nature, Nature Methods, Nature Communications, PLOS Computational Biology, Cognitive Computation, Current Opinion in Neurobiology

Honors and Awards

2023	Research Scholar Award, Friedman Brain Institute, ISMMS
2020–2022	Chair, Optogenetics Gordon Research Conference
2021	Irma T. Hirschl/Monique Weill-Caulier Research Award
2020	ISMMS Distinguished Scholar Award
2019	NIH Director's New Innovator Award
2019	One Mind Rising Star Award
2019	McKnight Memory and Cognitive Disorder Award
2018	NARSAD Young Investigator Award
2018	Brain Research Foundation Award
2018	Klingenstein-Simons Fellowship Award in Neuroscience
2018	Friedman Brain Institute Scholar
2018	Outstanding Teaching Award at Mount Sinai Graduate School
2018	Botanical Center Pilot Award
2017	American College of Neuropsychopharmacology Associate Member
2017	Center for the Neurobiology of Learning and Memory Fellow
2017	Allen Brain Next Generation Leaders
2015	Arnold Scheibel Distinguished Postdoctoral Fellow Award
2015	David Geffen School of Medicine Family Travel Award
2015	UCLA Integrative Center for Learning and Memory Young Investigator Award

2012–2014	Ruth L. Kirschstein Post-Doctoral National Research Service Award
2012–2013	Ruth L. Kirschstein NRSA Institutional Research Training Grant
2009–2010	UCSD Chancellor's Interdisciplinary Collaboratories Award
2009–2010	Dean of Social Sciences Travel Award
2007–2010	Norman Anderson Research Travel Awards
2008–2009	Student-elected Faculty Recruitment Representative
2006–2009	Faculty-elected Undergraduate Seminar Coordinator
2008	Student-elected Teaching Assistant Mentor

Other Experiences and Professional Memberships:

2022–	American College of Neuropsychopharmacology (Member)
2022	BRAIN Initiative: Targeted BRAIN Circuits Study Section (Reviewer)
2021–2022	NIH Learning, Memory, and Decision Neuroscience Study Section (Ad hoc Reviewer)
2017–2022	American College of Neuropsychopharmacology (Associate Member)
2007–	Society for Neuroscience
2007–	Molecular and Cellular Cognition Society

C. Contributions to Science

Interactions of memories across time

My work focused on the molecular, cellular, and circuit mechanisms that affect how memories are allocated in the neural circuit and how memories are linked across time. Specifically, I found that distinct memories encoded close in time are linked by sharing of an overlapping neural ensemble, such that the recall of one memory increases the likelihood of recall of the other temporally-related memory. I have also investigated age-related memory impairments in mice through longitudinal studies. Using Miniscope we identified neuronal ensembles in dorsal CA1 that were spatially tuned and stable across training sessions in a hippocampus-dependent spatial task. This spatial task included changing reward ports, and when reward locations were moved during a reversal session, a subset of these ensembles decreased their activation strength. This decrease in activation correlated with the animal's ability to learn new information. In middle-aged mice with impaired reversal learning, we demonstrated that higher remodeling correlated with their memory-updating performance. Our work using middle-aged mice has also characterized the cellular subtract of the impairment in aged mice; they do not have the expected increase in learning-induced excitability in CA1 ensemble cells, impairing processes like memory-linking. As memory deficits are common among the aging population, using longitudinal studies rather than blunt impairment we are introducing a naturalistic element to study changes in memory flexibility across a lifetime, which could be particularly useful for translational studies.

Recently, we showed that a strong aversive experience drives the offline ensemble reactivation of an aversive memory and a neutral memory formed two days prior, transferring the fear from the aversive to the neutral memory. These studies bring together several novel conceptual ideas: 1) demonstration that neural ensembles encoding a memory can be reactivated days later specifically due to an unrelated, but highly aversive experience; 2) that co-reactivation in the offline period between days can link experiences. We found that inhibiting hippocampal activity during the offline period after an aversive experience abolishes the transfer of fear from that aversive experience to prior neutral memories formed days ago. Reactivation of neural ensembles driving memory integration of previous experiences may provide a potential neural circuit mechanism that supports causal inference across longer time scales.

a. Zaki Y, Pennington ZT, Morales-Rodriguez D, Francisco TR, LaBanca AR, Dong Z, Carrillo Segura S, Silva AJ, Shuman T, Fenton A, Rajan K, & **Cai DJ** (2023) Aversive experience drives offline ensemble reactivation to link memories across days. ***bioRxiv.*** DOI:1101/2023.03.13.532469
a. Mau W, Morales-Rodriguez D, Dong Z, Pennington ZT, Francisco T, Baxter MG, Shuman T, **Cai DJ.** (2022) Ensemble remodeling supports memory-updating. ***bioRxiv.*** DOI: 10.1101/2022.06.02.494530
b. Chen L, Cummings KA, Mau W, Zaki Y, Dong Z, Clem RL, Shuman T, **Cai DJ.** (2020) The role of intrinsic excitability in the evolution of memory: significance in memory allocation, consolidation, and updating. ***Neurobiology of Learning and Memory***, 173:107266. PMID: 32512183
c. **Cai DJ***, Aharoni D*, Shuman T*, Shobe J* (*co-first author), Biane J, Song W, Wei B, Veshkini M, La-Vu M, Lou J, Flores S, Kim I, Sano Y, Zhou M, Baumgaertel K, Lavi A, Kamata M, Tuszynski M,

Mayford M, Golshani P, Silva AJ. (2016) A shared neural ensemble links distinct contextual memories encoded close in time. ***Nature,*** 534(7605),115-118. PMID: 27251287

Development and sharing of neuroscience technology and tools

As a member of the UCLA Miniscope project and tool developer, I have co-created open-source tools that can be built in-house at much lower costs. The Miniscope project includes full versions of the Miniscope system and the Miniscope resource ("wiki"), which allows users to create their own Miniscopes and innovate on our ideas. We have championed these efforts and held workshops around the world to teach others how to build and use their own Miniscopes. These workshops have been highly successful; we have taught users in Argentina, Israel, China, Germany, New Zealand, and many US locations. I have taught >2,000 scientists how to build their own Miniscopes, which are now in use in over 700 labs. We have further developed a wire-free Miniscope and an unpublished 2-channel Miniscope.

Additionally, we have published a calcium imaging analysis pipeline called Minian and behavioral tracking software, ezTrack. These open-source analysis tools are user-friendly, compatible with a variety of imaging systems, and well-supported through continuous refinement and updating. We are proud of the growing community using Minian (969 unique GitHub clones, 19K conda-forge downloads) and ezTrack (544 unique GitHub clones). Minian has been crucial for our lab and others to process calcium imaging data into a useful format for subsequent analysis.

a. Dong Z, Mau W, Feng Y, Pennington ZT, Chen L, Zaki Y, Rajan K, Shuman T, Aharoni D, **Cai DJ**. (2022) Minian: An Open Source miniscope analysis pipeline. ***eLife*****.** 10.7554/eLife.70661. PMID: 35642786; PMCID: PMC9205633
b. Pennington ZT, Dong Z, Bowler R, Feng Y, Vetere L, Shuman T, **Cai DJ.** (2019) ezTrack: An open-source video analysis pipeline for the investigation of animal behavior. ***Scientific Reports***, 9 (19979). PMID: 31882950
c. Shuman T*, Aharoni D*, **Cai DJ*, (*co-first author**), Lee CR, Chavlis S, Page-Harley L, Vetere LM, Feng Y, Chen YY, Molinedo-Gajate I, Chen L, Pennington Z, Taxidis J, Flores SE, Cheng K, Javaherian M, Kaba CC, Strahman M, Kakhurin KI, Masminidis S, Khakh B, Poirazi P, Silva AJ, Golshani P. (2020) Breakdown of spatial coding and neural synchronization in epilepsy. ***Nature Neuroscience,*** 23,229-238. PMID: 31907437
d. **Cai DJ***, Aharoni D*, Shuman T*, Shobe J* (*co-first author), Biane J, Song W, Wei B, Veshkini M, La-Vu M, Lou J, Flores S, Kim I, Sano Y, Zhou M, Baumgaertel K, Lavi A, Kamata M, Tuszynski M, Mayford M, Golshani P, Silva AJ. (2016) A shared neural ensemble links distinct contextual memories encoded close in time. ***Nature,*** 534(7605),115-118. PMID: 27251287

Sleep, memory, and stress

As a graduate student, I examined how sleep alters the encoding and consolidation of memories in both humans and mice. Using human subjects, I demonstrated that REM sleep specifically boosted memory integration by enhancing the assimilation of new information into past experience, thus creating a richer network of associations for future use. Furthermore, I also found that brain-states (i.e., sleep stages) differentially contributed to the consolidation of various memory domains. To better probe the neural mechanisms underlying the role of sleep on memory consolidation, I developed novel behavioral assays to run parallel studies in mice and humans. Consistent with the human results, I found sleep also enhances similar memory domains in mice. Specifically, contextual fear memory in mice was enhanced after a sleep phase but not an awake phase. In addition, I characterized the off-target effects of sleep deprivation methods, mainly stress, that led to memory impairments after sleep deprivation.

Our recent work has shown a process where stress-induced changes in defensive behaviors are mediated by the ventral hippocampus (vHC) and basolateral amygdala (BLA), altering neuronal activity within both the BLA and vHC. We found that plasticity within each brain region supports separate defensive behavior changes in response to stress, demonstrating the unique functions of these structures, and supporting the view that multiple memory systems underlie stress-induced defensive behavioral changes. By showing that different areas can induce different defensive behaviors, these findings could have important clinical implications as targeting one versus the other could treat different mental health conditions.

a. Pennington ZT, LaBanca AR, Sompolpong P, Christenson Wick Z, Feng Y, Dong Z, Francisco T, Chen L, Fulton SL, Maze I, Shuman T & **Cai DJ** (2023) Dissociable contributions of the amygdala and ventral hippocampus to stress-induced changes in defensive behavior. ***bioRxiv***. DOI: 10.1101/2023.02.27.530077.
b. **Cai DJ**, Mednick SA, Harrison EM, Kanady J, Mednick SC. (2009) REM, not incubation, improves creativity by priming associative networks. ***Proceedings of the National Academy of Sciences****,* 106(25), 10130-10134. PMID: 19506253
c. **Cai DJ**, Shuman T, Harrison EM, Sage JR, Anagnostaras SG. (2009) Sleep-deprivation and Pavlovian fear conditioning. ***Learning & Memory,*** 16, 595-599. PMID: 19794184
d. **Cai DJ**, Shuman T, Gorman MR, Sage JR, Anagnostaras SG. (2009) Sleep selectively enhances hippocampus-dependent memory in mice. ***Behavioral Neuroscience,*** 123(4), 713-719. PMID: 19634928

Complete List of Published Work: https://www.ncbi.nlm.nih.gov/pubmed/?term=denise+cai

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

Icahn School of Medicine at Mount Sinai
Full-Time
Other Support - Projects/Proposals

ACTIVE

*Title: How does the brain maximize storage capacity?

Major Goals: Our goal is to investigate how the brain optimizes its capacity to store information across a lifetime

*Status of Support: Active

Project Number: 1DP2MH122399-01

Name of PD/PI: D. Cai

Role: PI

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 9/1/2019 - 5/31/2024

*Total Award Amount (including Indirect Costs): $2,643,434

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
5. 2024	2.280

*Title: Circuit mechanisms of retrospective memory-linking

Major Goals: Our goal is to investigate the cellular and circuit mechanisms of memory linking by which neural events are retrospectively linked to aversive experiences in mice

*Status of Support: Active

Project Number: 5R01MH120162-04

Name of PD/PI: D. Cai

Role: PI

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/3/2019 - 10/31/2024

*Total Award Amount (including Indirect Costs): $2,595,694

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
4. 2023	2.280

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

5. 2024 3.000

*Title: Does hippocampal ensemble reactivation of trauma memories cause disrupted sleep in PTSD?
Major Goals: In our study, we will develop the tools to investigate the biological causes of disrupted sleep in
PTSD.
*Status of Support: Active
Project Number: N/A
Name of PD/PI: D. Cai
Role: PI
*Source of Support: Hirschl/Weill-Caulier Trust
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 1/1/2021 - 12/31/2025
*Total Award Amount (including Indirect Costs): $195,000
*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
2. 2023	0.204
3. 2024	0.600
4. 2025	0.600

*Title: Microcircuits governing conflicting memories of threat and safety
Major Goals: The goals are to test the role of hippocampal somatostatin interneurons in contextual discrimination of threat versus safety and identify circuit mechanisms underlying behavioral transitions from low to high fear
*Status of Support: Active
Project Number: 1R01MH132224-01A1
Name of PD/PI: R. Clem
Role: Co-I
*Source of Support: NIMH
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 5/15/2023 - 2/29/2028
*Total Award Amount (including Indirect Costs): $3,072,020
*Person Months (Calendar/Academic/Summer) per budget period:

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

Year (YYYY)	Calendar Months
1. 2024	0.750
2. 2025	0.750
3. 2026	0.750
4. 2027	0.750
5. 2028	0.750

*Title: Fear and anxiety circuit mechanisms in anterior hypothalamic nucleus
Major Goals: Acute severe stress plays a pivotal role in shaping mental health, predisposing individuals to an array of debilitating conditions including post-traumatic stress disorder and anxiety disorders. Here, we propose to explore the contribution of the anterior hypothalamic nucleus (AHN), a brain region that has previously received little attention, to the enhancements in fear and anxiety-like behavior provoked by stress.
*Status of Support: Active
Project Number: 1R56MH132959-01
Name of PD/PI: D. Cai
Role: PI
*Source of Support: NIMH
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 4/4/2023 - 2/28/2025
*Total Award Amount (including Indirect Costs): $1,324,592
*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	Calendar Months
1. 2024	2.280
2. 2025	3.000

PENDING

*Title: Prefrontal circuit mechanisms of valence-specific conditioning
Major Goals: The major goals of this project are to define the role of discrete prefrontal GABAergic subpopulations in emotional valence processing and understand their functional interactions with anatomically-defined networks.
*Status of Support: Pending
Project Number: 2R01MH116445-06A1
Name of PD/PI: R. Clem
Role: Co-I

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 1/1/2024 - 12/31/2028

*Total Award Amount (including Indirect Costs): $3,158,838

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	Calendar Months
1. 2024	0.750
2. 2025	0.750
3. 2026	0.750
4. 2027	0.750
5. 2028	0.750

*Title: Circuit Mechanisms of Retrospective Memory-Linking

Major Goals: We will investigate how strong aversive events can trigger the reactivation of previous memories encoded days prior, driving memory integration and the transfer of fear to the neutral experience; providing insight into the dynamic nature of real-world memory processing.

*Status of Support: Pending

Project Number: 2R01MH120162-06

Name of PD/PI: D. Cai

Role: PI

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 11/1/2024 - 10/31/2029

*Total Award Amount (including Indirect Costs): $4,168,078

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	Calendar Months
1. 2025	3.000
2. 2026	3.000
3. 2027	3.000
4. 2028	3.000
5. 2029	3.000

*Title: Sex Differences in Neural Circuit Mechanisms of Aggression

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

Major Goals: In this proposal, we will define sex differences in the neural circuit mechanisms of aggressive behavior in mice.

*Status of Support: Pending

Project Number: 1R01MH133299-01A1

Name of PD/PI: S. Russo

Role: Co-I

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 9/1/2023 - 8/31/2028

*Total Award Amount (including Indirect Costs): $3,365,286

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
1. 2024	1.200
2. 2025	1.200
3. 2026	1.200
4. 2027	1.200
5. 2028	1.200

*Title: Neuroeconomic mechanisms of counterfactual thinking

Major Goals: The goal of this project is to study the neural mechanisms underlying complex decision-making processes in mice.

*Status of Support: Pending

Project Number: 1R01MH136230-01

Name of PD/PI: B. Sweis

Role: Co-I

*Source of Support: NIMH

*Primary Place of Performance: Icahn School of Medicine at Mount Sinai

Project/Proposal Start and End Date: (MM/YYYY) (if available): 7/1/2024 - 6/30/2029

*Total Award Amount (including Indirect Costs): $4,195,948

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
1. 2025	0.600
2. 2026	0.600
3. 2027	0.600
4. 2028	0.600

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

5. 2029 0.600

*Title: Long-term Recording of Single-Cell Physiology Across Entire Living Brains
Major Goals: To bring technological breakthroughs to scalably record and analyze spatial and temporal structures of single-cell gene expression across the living brain underlying learning and neurodegeneration
Locations
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: C. Linghu, D. Cai
Role: MPI
*Source of Support: Chan Zuckerberg via Univ. of Michigan
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 3/1/2024 - 7/31/2025
*Total Award Amount (including Indirect Costs): $100,000
*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	**Calendar Months**
1. 2025	0.750

*Title: Optimization and Miniaturization of GRIN-SCAPE for High-Speed 3D Imaging of Cell-Type-Specific Activity Throughout Brain-Wide Circuits
Major Goals: We propose to develop a new kind of microscope that can capture high-speed, high-resolution, multi-color, 3D images through GRIN lenses that can be inserted into the brain to provide a clear view of deep brain regions, and can capture the activity and interactions of a wide range of cell types during behavior
*Status of Support: Pending
Project Number: N/A
Name of PD/PI: E. Hillman, D. Cai
Role: MPI
*Source of Support: NIH via Columbia University
*Primary Place of Performance: Icahn School of Medicine at Mount Sinai
Project/Proposal Start and End Date: (MM/YYYY) (if available): 4/1/2024 - 3/31/2028
*Total Award Amount (including Indirect Costs): $1,570,268

*Name of Individual: Cai, Denise
Commons ID: DENISECAI

*Person Months (Calendar/Academic/Summer) per budget period:

Year (YYYY)	Calendar Months
1. 2025	2.000
2. 2026	2.000
3. 2027	2.000
4. 2028	2.000

Other Support - In Kind Contributions

ACTIVE

None

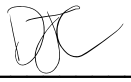
PENDING

None

OVERLAP

If all pending grants are funded, effort will be adjusted in accordance with NIH policy to not exceed 12 CM effort.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete, and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

Signature: 

Date: July 31, 2023

AMERICAN FEDERATION FOR AGING RESEARCH
55 West 39th Street, 16th floor, New York, NY 10018 (212) 703-9977

McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss

Institutional Commitment Form

Candidates for the McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss must be independent investigators with independent research space. To complete the application, this form must be completed by the Dean or the Department Chair. The form is NOT to be included in the application, but must be submitted directly to AFAR by the person completing the form (NOT the applicant), to afarapplication@afar.org as a Word or PDF file.

Name, title, and address of official completing this form:
Eric Nestler, MD, PhD
DEAN FOR ACADEMIC AFFAIRS
DIRECTOR, FRIEDMAN BRAIN INSTITUTE
PROFESSOR | Neuroscience
PROFESSOR | Pharmacological Sciences
PROFESSOR | Psychiatry
CHIEF SCIENTIFIC OFFICER

Icahn (East) Building Floor 10 Room 10-23
1425 Madison Ave
New York, NY 10029

E-mail: eric.nestler@mssm.edu
Phone: 212-659-5656

Signature of Official: _____

First and Last name of Applicant: Denise Cai

1. Does the candidate have independent investigator status at his/her institution?

☒ YES ☐ NO

2. Has the candidate's institution provided space and equipment specifically dedicated to his/her research program?

☒ YES
Please Describe:

Dr. Cai has lab and office space in the Hess Center Building at the Icahn School of Medicine at Mount Sinai

☐ NO
Please describe whose resources the candidate will use to execute the proposed project:

N/A

3. Did the candidate receive intramural start-up funds when offered his/her current position? (AFAR does not consider extramural funds from an outside organization/institution as 'start-up funds'.)

☒ YES
Please provide $ amount and details of start-up funds: $ 1,800,000

☐ NO

4. Does the candidate have designated administrative support (e.g. someone who helps with editing and submitting grants, tracks budgets, etc.)

☒ YES ☐ NO

5. What was the start date of the candidate's current position?

Month/Day/Year: 06/01/2017

6. Does your institution offer tenure:

☒ YES ☐ NO

a. If yes, is the candidate's current position a tenure track position?

☒ YES ☐ NO

b. If your institution does **not** offer tenure, please provide evidence of long-term institutional support

7. Does the candidate have teaching and/or clinical responsibilities in the current position?

☒ YES ☐ NO

If yes, indicate percentage of time: 10%

8. Describe overall annual research funding for the department that the investigator is primarily affiliated with.

Nash Family Department of Neuroscience
Federal Grants Total: $51,011,208

Non-federal Grants Total: $7,663,021

9. To demonstrate a commitment to the investigator, the institution is asked to support the investigator's project through matching funds. **Please provide a statement below stating that 50% ($375,000) in cash or in-kind matching funds will be committed to the project and investigator if an award is made.** Provide details and amounts for the matching funds. Matching funds can only be non-federal and cannot be used by more than one project. This could be cash and/or in-kind matching, and can include faculty effort, and goods and services paid from departmental funds. For an in-kind match, the selection committee will determine whether this is equivalent to a monetary match.

   Should this project be funded by the McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss, Icahn School of Medicine at Mount Sinai leadership have made the commitment to provide institutional matching funds of up to $375,000 in cash.

2023 McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss

Application

Title of Proposal: Counteracting age-associated cognitive decline via gut-brain signaling **Institution:** University of Pennsylvania **Proposed Start Date:** 10/01/2023 **Proposed End Date:** 09/30/2026 **Total Funds Requested:** $750,000	**Name, title and address of official authorizing proposal:** Michael Carman Jr. / Assistant Director **E-mail:** pennaors@lists.upenn.edu **Phone:** 215-898-7293 **Signature of Official:** Michael Carman Jr. Digitally signed by Michael Carman Jr. Date: 2023.07.28 14:46:56 -04'00'
Applicant: Christoph A Thaiss **Title:** Assistant Professor **Degree(s):** PhD **Address:** 3610 Hamilton Walk 301B Johnson Pavilion Philadelphia, PA 19104 **E-mail:** thaiss@pennmedicine.upenn.edu **Phone:** 215-746-7765 **Signature of PI:**	**Are you an Assistant Professor?** Yes No **If No, are you an Associate Professor?** Yes No **If Yes, indicate date of appointment:** 04/01/2018 **Do you have an R01 or equivalent funding?** Yes No **Will your institution provide 50% in cash or in-kind matching funds determined to be equivalent to the award:** Yes No

ABSTRACT: Provide a summary of your research proposal. Do not exceed space provided.

Key words: age-associated cognitive decline, gut-brain signaling, interoception, microbiome, inflammation, memory
Model system used for the proposed research: mouse

Aging is associated with a decline in memory function, which greatly affects the quality of life of a large proportion of elderly individuals. The rate of cognitive decline is highly heterogeneous, with some individuals retaining fully intact memories at old age, while others lose the ability to participate in public life due to a dramatic inability to form and recall memories. New strategies to understand and counteract the age-associated decline in memory function are thus urgently needed. **This proposal will explore the new hypothesis that age-associated cognitive decline is not solely brain-autonomous but regulated by interoceptive pathways originating in the gastrointestinal tract**.

Based on exciting unpublished findings, we hypothesize that communication pathways between the gastrointestinal tract and the brain progressively lose function with age, and that restoration of these pathways counteracts age-associated memory loss. We propose to chart a pathway that links molecules of microbial origin in the gastrointestinal tract, enteroendocrine cells in the intestinal epithelium, afferent sensory neurons, and the formation of memory engrams in the hippocampus. **Our approach provides a new framework for how age-related diseases of the brain may be treated by means of peripheral intervention from the gastrointestinal tract.**

1. **BUDGET**
   Up to $250,000 in total cost per year may be requested.

Category	**Year 1 MBRF/AFAR**	**Year 1 matching**	**Year 2 MBRF/AFAR**	**Year 2 Matching**	**Year 3 MBRF/AFAR**	**Year 3 matching**	**Grand Total**
Personnel	$127,403	$48,655	$131,224	$50,113	$135,160	$51,617	$544,172
Equipment	-	$50,000	-	$50,000	-	$50,000	$150,000
Supplies	$42,067	$26,345	$37,443	$24,887		$23,383	$184,828
AFAR Conference	-	-	-	-	$2,000		$2,000
Travel	$2,000	-	$2,000		$2,000		$6,000
Other Expenses	$55,803	-	$56,606		$57,409		$169,818
10% Indirect Cost	$22,727	-	$22,727	-	$22,728	-	$68,182
TOTAL	$250,000	$125,000	$250,000	$125,000	$250,000	$125,000	$1,125,000

- Personnel funds can be used for P.I., research assistant(s), technician(s), postdoc(s), or graduate student(s)
- $2,000 is budgeted to cover hotel, meals and other meeting incidentals related to attending the AFAR Grantee Conference in Santa Barbara, CA (not travel) and will be withheld from the final award amount
- Travel line item should include expenses for travel to the AFAR Grantee Conference in year 3 of the award. Allowable travel expenses are limited to reasonable expenses incurred by the grantee for domestic travel to attend a scientific meeting where the grantee is presenting research that has been supported by the award.
- Total budget requested from MBRF/AFAR may not exceed $750,000, including up to 10% for institutional overhead (up to $68,182)
- Institutional matching funds must be 50% of total funds requested from MBRF/AFAR; Indirect cost or overhead cost do not count towards the 50% matching funds.

(Note: AFAR does not provide funding for the purchase of personal computers or laptops or other costs not directly related to the research project, such as tuition, 'telecommunications' or similar.)

2. **BUDGET DETAIL AND JUSTIFICATION**

Personnel

Christoph A. Thaiss, Ph.D., Principal Investigator (effort: 5%). Dr. Thaiss has expertise in the fields of neuroscience, host-microbiome interactions, and metabolism. He also directs the Penn Gnotobiotic Animal Facility. Dr. Thaiss will conceptualize and plan all experiments, analyze the results in collaboration with the entire study team, ensure the quality of the experimental procedures, and mentor all study participants. He will also lead the interpretation and presentation of results, manuscript preparation, grant reporting, and public dissemination of findings and data.

Megan Liou, Ph.D., Postdoctoral Researcher (effort: 50%). Dr. Liou has extensive experience of more than seven years in working with host-microbial interactions. She earned her PhD from the University of California Davis working with Dr. Andreas Bäumler. Dr. Liou will be responsible for designing, performing and analyzing all experiments. In addition, Dr. Liou in an expert in bioinformatics analysis. She will furthermore participate in manuscript writing and presentation of the results.

Ashwarya Devason, B.S., Research Specialist (effort: 50%). Ms. Devason has recently graduated from Penn College and has extensive experience with all techniques proposed in this study. She has been a member of the Thaiss lab for the last three years and has gained extensive experience with gnotobiotic animal experiments, behavioral testing, mouse models of aging and cognitive decline, as well as next-generation sequencing. She will support all experiments and computational analyses

Other Direct Costs

Animals ($55,803 in the first year, and adjusted to increasing per diem charges thereafter)
We estimate that we need 550 mice per year for this study, including housing under SPF and gnotobiotic conditions. We will purchase young and old C57BL/6 mice from the Jackson Laboratory and will house genetically modified animals in our vivarium. Housing charges are $1.02/cage/day for the first year, with annual increases thereafter.

Next-generation sequencing ($25,400 in the first year, and adjusted thereafter)
Kits for samples processing, library preparation, and next-generation sequencing. Additional cost is required for computational analysis (UPenn PMACS).

In vivo and *in vitro* experiments ($13,500 per year)
Reagents, enzymes, and supplies for animal experiments, molecular biology analyses, cell culture, and imaging.

Materials and supplies ($3,167 in the first year, and adjusted thereafter)
Cost required for general lab supplies, plastics, glassware.

Matched Direct Costs

The Microbiology Department will cover 25% of the PIs salary, provide $50,000 per year for equipment purchases and service contracts, and around $25,000 to support supply purchases.

2023 MBRF/AFAR Grant Application - Page 4

3. Does the research plan include use of human subjects? ☐ YES ☒ NO
   Does the research plan include use of animal subjects? ☒ YES ☐ NO

   Applicants should note that IRB certification (for human subjects) and/or Animal Use Committee approval (for animal subjects) must be provided to AFAR before a grant award can be made.

   Applicants are urged to consult the AFAR website at **http://www.afar.org/research/funding/animal-use/** for advice on human and animal usage or this webinar recording from the Nathan Shock Centers of Excellence: https://nathanshockcenters.org/june2021webinar-1. The website includes a helpful set of guidelines for optimal use of rodents in aging research projects.

4. Indicate the candidate's % of time/effort that will be spent on the planned project:

   ____15____%

5. a. Have you previously applied for an AFAR Grant? Yes__x__ No_____

   b. If yes, have you previously received any AFAR Grant? Yes_____ No __x__

If yes, please provide name of grant, year received and title of project:

Research proposal

a. Novelty and Impact

This proposal explores innovative ways to counteract brain aging and cognitive decline by targeting the interoceptive system. Memory loss associated with aging and neurodegeneration significantly impacts the quality of life for a substantial portion of the elderly population[1]. Notably, the rate of cognitive decline varies greatly, with some individuals maintaining fully intact memories in old age, while others experience severe cognitive decline and an inability to form and retrieve memories. The underlying causes of this heterogeneity are not fully understood, highlighting the urgent need for novel strategies to mitigate age-related memory decline[2]. **We will approach this challenge by focusing on brain-extrinsic regulators of aging and cognitive decline.** In particular, we will focus on the interoceptive system, which transmits information from all tissues of the body to the brain.

Our primary hypothesis posits that interoceptive pathways originating in the gastrointestinal tract influence an individual's rate of age-associated cognitive decline. Our exciting unpublished findings indicate (1) a progressive decline in the functional communication pathways between the gastrointestinal tract and the brain with age, and (2) that restoration of these pathways can counteract age-associated memory loss. This discovery implies that gut-brain axis aging constitutes a major contributing factor to brain aging. In this study, we will explore the causes, consequences, and mechanisms by which aging of the gut-brain axis impacts cognitive decline. **Our approach offers a novel framework for treating age-related brain diseases by gastrointestinal intervention.**

b. Specific Aims

Our research project will consist of two major phases:

Aim 1: **Understanding gut-brain axis aging and its contribution to cognitive decline**

Aim 1.1: Charting the landscape of gut-brain axis aging

Aim 1.2: Evaluating the impact of gut-brain axis aging on brain aging and cognitive decline

Aim 2: **Identifying the upstream causes of gut-brain axis aging and interventions for rejuvenation**

Aim 2.1: Determining the aging intestinal interoceptome: deciphering the impact of the aging intestinal milieu on gut-brain axis function

Aim 2.2: Identifying gut-restricted interventions to rejuvenate interoceptive signaling and counteract age-associated cognitive decline

c. Background

The idea for this study is based on recent advances in our understanding of body-brain communication[3]. Brain health is not strictly brain-intrinsic, but strongly influenced by signals from the rest of the body, akin to the Roman poet Juvenal's famous quote *Mens sana in corpore sano* (a healthy mind in a healthy body). Indeed, it is known that elements of a healthy lifestyle, including regular exercise, a healthy diet, and sufficient sleep, ameliorate age-associated memory decline. However, a more detailed and mechanistic framework for how these lifestyle elements influence memory function is critically needed. **The innovation of this proposal is to provide and leverage a new conceptual approach to memory loss based on recent progress in the field of interoception.**

Our vision is that complex cognitive functions of the brain rely on interoceptive input derived from different internal organ systems. Interoception is the brain's ability to sense the internal state of the body[4]. It is achieved by the relay of information from internal organs to the brain via afferent sensory circuits of the vagus nerve and the spinal cord[3]. When coordinating physiological responses, the brain thus integrates information about body states like touch, movement, nutritional state, and many others. We have recently applied this concept to the mesolimbic reward system, which we found to integrate signals derived from the gastrointestinal tract (Dohnalová et al., *Nature*, 2022)[5]. **We now aim to examine the consequences of gut-brain axis aging, by focusing on age-associated decline in the formation and recall of memories**.

d. Preliminary Data

We have recently developed a new experimental pipeline to study the bidirectional communication between the gut and the brain, which will facilitate all parts of the proposed study. Our first applications of this pipeline have enabled us to uncover how gut-brain signaling controls motivated behavior (Dohnalová et al., *Nature*, 2022)[5] and how it regulates intestinal inflammation (Schneider et al., *Cell*, 2023)[6]. Now, we plan to apply these concepts and tools to investigate brain aging. Our objective is to elucidate how the functionality of the gut-brain axis changes throughout an individual's lifespan and how it impacts the likelihood of developing cognitive decline. Specifically, we aim to establish a comprehensive pathway that links age-associated molecular changes in the gastrointestinal tract, gut-innervating afferent sensory neurons, and the formation of memory engrams in the hippocampus (**Figure 1A**).

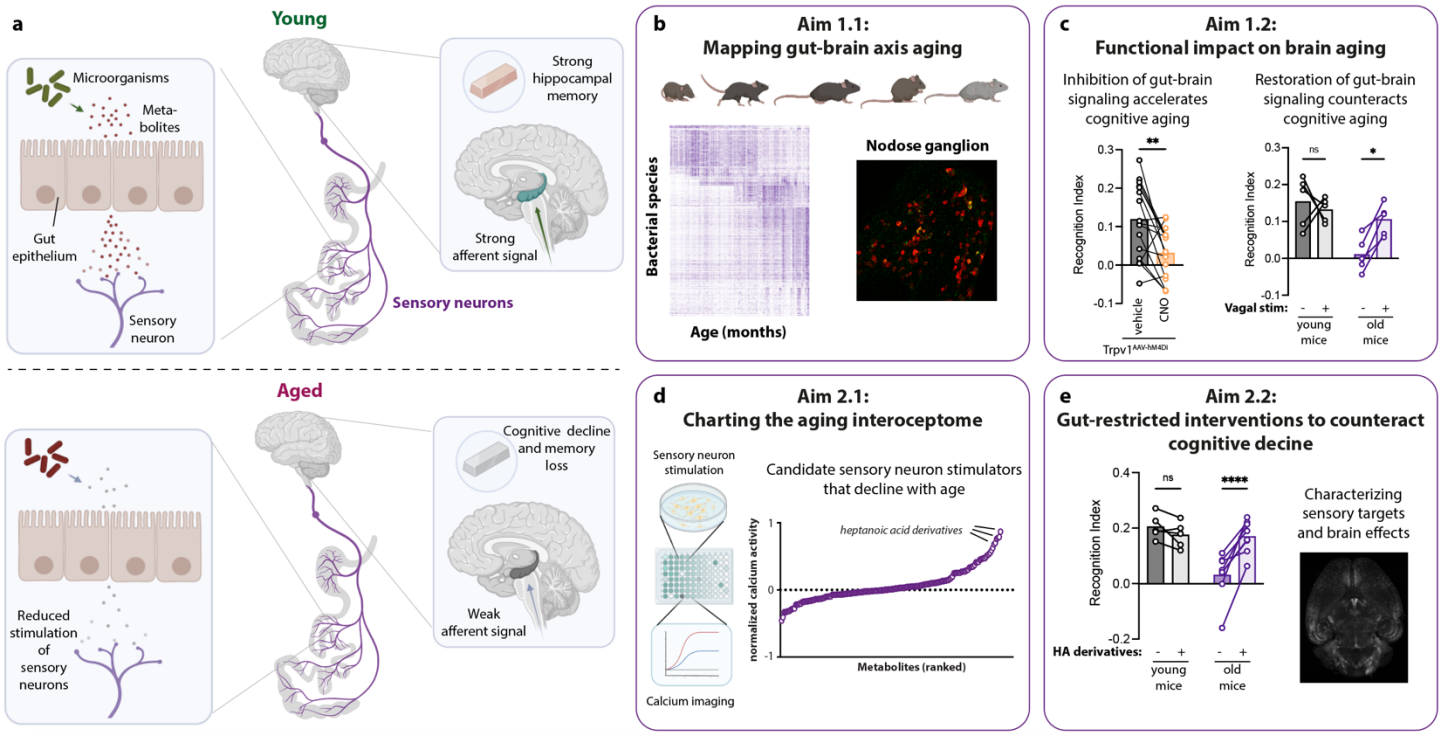


Figure 1. Overview of the proposed study. (**a**) We propose to explore a pathway linking intestinal microbes and their metabolites, sensory neuron activity, and the formation of hippocampal memory engrams. During aging, interoceptive input from the gut is reduced, resulting in decline of hippocampal function and memory loss. Restoration of gut-brain signaling may counteract age-associated cognitive decline. (**b-e**) Preliminary data showing our ability to characterize different aspects of the gut-brain axis over the lifespan (b), acceleration and deceleration of cognitive decline by modulation of the gut-brain axis (c), identification of candidate neurostimulatory molecules whose intestinal abundance changes with age (d), and intestinal interventions that counteract cognitive decline via the gut-brain axis (e).

e. Experimental design, key methodologies, and pitfalls

Aim 1.1 Charting the landscape of gut-brain axis aging

The interoceptive system is increasingly recognized as a crucial regulator of brain function[3]. Specifically, the signaling pathway connecting the intestinal microbiome, its metabolites, intestinal epithelial cells, gut-innervating sensory neurons, and interoceptive brain regions plays a pivotal role in various aspects of systemic physiology and disease[7]. However, the extent to which body-brain interactions age throughout the lifespan and contribute to neurodegeneration and cognitive decline remains unclear. In the initial phase of our study, we will construct **the first comprehensive atlas illustrating the aging process of the neuronal gut-brain axis**.

To accomplish this, we will leverage an aging cohort of C57BL/6 mice that we have monitored throughout their lifespan. Through a comprehensive multi-omic analysis, we will examine the following elements: (1) Metagenomic sequencing of the microbiome, (2) transcriptomics of intestinal epithelial cells, (3) single-nucleus sequencing of sensory afferent neurons, and (4) single-nucleus sequencing of sensory terminals located in the brainstem and thalamus.

To demonstrate the feasibility of our approach, we have conducted a pilot experiment that has already yielded

valuable insights into the functional changes occurring in microbiome-gut-brain signaling during aging (**Figure 1B**). Expanding on this preliminary work, we will also integrate functional readouts, such as examining vagus nerve responses to stimulation by dietary and microbial metabolites, throughout the lifespan.

Major concept for Aim 1.1: The key concept driving this subaim is to characterize the cellular and molecular components of the interoceptive system that establishes the connection between the gastrointestinal tract and the brain. While significant progress has been made in understanding these elements in recent years, no information is currently available regarding how this system evolves over an organism's lifespan. Our study will provide the first comprehensive map of gut-brain axis aging, shedding light on its dynamic changes and potential implications for neurodegenerative processes.

Aim 1.2 Evaluating the impact of gut-brain axis aging on neurodegeneration and cognitive decline

We will investigate the influence of gut-brain axis aging on age-associated cognitive decline and the susceptibility of the brain to neurodegeneration. To accomplish this, we will conduct two types of functional tests:

First, we will conduct **accelerated aging experiments** ("loss of function assays") to determine whether dysfunction in the gut-brain axis is sufficient to drive cognitive decline in young mice. This will involve chemogenetic silencing of gut-innervating neurons, toxin-mediated ablation of these neurons, and microbiome transfers from aged to young mice.

Second, we will carry out **rejuvenation experiments** ("gain of function assays") to examine whether restoring the gut-brain axis can rescue cognitive decline in aged mice. This will involve chemogenetic activation of gut-innervating sensory neurons in aged mice and transfers of microbiomes from young to aged mice.

In both types of experiments, we will conduct behavioral analyses focused on cognition, utilizing tests such as novel object recognition and the Barnes maze, to identify behavioral correlates of brain aging. Our preliminary experiments demonstrate that disrupting the gut-brain axis in young mice accelerates cognitive aging, while restoring it in aged mice decelerates cognitive decline (**Figure 1C**). Furthermore, we will perform cellular and molecular analyses of neurons in the hippocampus, a critical brain region involved in memory formation. Specifically, we will explore how interoceptive input from the gastrointestinal tract influences the formation of memory ensembles, known as "engrams," in the hippocampus.

Major concept for Aim 1.2: Hippocampal memory engram formation declines with age and this loss is accelerated by neurodegeneration[8]. However, the underlying causes remain largely unclear. This part of our study will investigate whether the excitability of hippocampal neurons is regulated by interoceptive input originating from the periphery, and whether the deterioration of gut-brain signaling over the lifespan is a driver of cognitive decline.

Aim 2.1 Determining the aging intestinal interoceptome

In addition to our characterization of gut-brain axis aging and its impact on the aging brain, we will delve into the **age-related alterations occurring within the gastrointestinal milieu, which contribute to the functional decline of intestinal interoception**. To achieve this, we will utilize samples collected from a longitudinal cohort of C57BL/6 mice that we follow over the lifespan and employ untargeted metabolomics to obtain a comprehensive understanding of how metabolite abundances change in the intestine with age.

In addition, we have developed a new screening system to assess the impact of thousands of gastrointestinal molecules on the activity of gut-innervating neurons. This system will now allow us to achieve three major insights: (1) identifying the complete array of intestinal molecules that are sensed by gut-innervating neurons (the "interoceptome"), (2) determining how the stimulation of sensory neurons by intestinal molecules evolves with age, and (3) identifying specific molecular candidates that drive the aging of the gut-brain axis and contribute to cognitive decline. Our preliminary studies have established the feasibility of this approach and identified the first candidate molecules (**Figure 1D**).

Major concept for Aim 2.1: The complete repertoire of molecules that are perceived by peripheral sensory neurons remains elusive – a major missing piece in sensory neurobiology[9]. Our approach will not only add many new agonists of the interoceptive repertoire but will also define how this repertoire changes over the lifespan. This approach holds the potential to identify candidate triggers of functional decline in interoception, which, in turn, plays a pivotal role in various aspects of brain aging.

Aim 2.2 Identifying gut-restricted interventions to rejuvenate interoceptive signaling and counteract age-associated cognitive decline

The identification of neurostimulatory intestinal molecules that decline in abundance with age, as well as neuroinhibitory molecules that increase with age, presents a unique opportunity to address a critical challenge: **discovering interventions targeting the intestine that can restore gut-brain signaling and effectively counteract cognitive decline**. Encouragingly, our preliminary experiments have already shown promise in identifying such candidates (**Figure 1E**). Building upon this progress, we will now systematically identify the receptors for these molecules, investigate their impact on neuronal signaling to the brain, assess hippocampal responses to changes in their intestinal abundance, and evaluate their effects on cognitive behavior and memory formation.

Major concept for Aim 2.2: The central concept driving this subaim is the exploration of an exciting hypothesis that challenges the traditional approach of targeting the brain directly to counteract neurodegeneration. Instead, we propose that age-associated cognitive decline is not solely governed by intrinsic brain factors but is also regulated by interoceptive pathways originating in the gastrointestinal tract. By pursuing this milestone, we aim to overcome the significant challenge posed by the limited accessibility of the brain for therapeutic interventions. The identification and targeting of specific intestinal molecules, which play a crucial role in interoceptive signaling, present a promising avenue for addressing neurodegeneration and age-associated cognitive decline.

f. Brief discussion of implications for future research

Our study will have four major implications:

First, it will establish a **new conceptual framework** of viewing age-associated cognitive decline as a result of impaired body-brain communication.

Second, our study will generate **several large-scale resources** that will benefit the broader aging research community. These include a comprehensive characterization of gut-brain axis aging over the lifespan of C57BL/6 mice across multiple levels (metagenome, metabolome, transcriptome of cells in the intestine, afferent neurons, and affected brain regions).

Third, our efforts will spark **new collaborative efforts**. Our vision is that complex cognitive functions of the brain rely on interoceptive input derived from internal organ systems. We hope to join forces with several research teams with expertise in the molecular pathology of neurodegeneration and other diseases of the aging brain.

Fourth, the pathway explored in this project may serve as the stepping-stone for the **development of gut-focused interventions** that counteract age-associated memory loss through body-brain signaling. This would run counter to the trend of trying to combat neurodegeneration with brain-penetrant drugs, which are difficult to develop and assess at large scale. We have recently coined the term “interoceptomimetics” for molecules that stimulate the transmission of body-derived signals to the brain for therapeutic purposes[5]. This study will be among the first to explore this unchartered territory, laying the groundwork for future exploration of oral **interoceptomimetic molecules as potential therapeutics against brain aging and memory loss**.

g. Relation of this work to current research

The proposed study has no overlap with any of our currently ongoing or other funded work. It will serve as a catalyzer for numerous follow-up studies that will explore each aspect of the proposed pathway in more detail. Over the past 5 years, my group has developed a large toolbox to study the impact of gut-brain interactions on numerous aspects of physiology. With this project, we will for the first time apply this toolbox to the study of age-associated cognitive decline.

Our study will constitute a foundational resource for the aging research community, providing it with new concepts and data pertaining to body-brain interactions. We will contribute comprehensive, multi-modal datasets that describe various aspects of gut-brain axis aging over the lifespan, including longitudinal metagenomics, metabolomics, and single-cell RNA-sequencing. These datasets will be made available to the aging research community for exploration. We anticipate fruitful collaborations with experts in brain circuits that regulate cognitive decline, **facilitating the integration of brain-intrinsic and brain-extrinsic regulators of cognitive aging**. This holistic approach promises to provide a more comprehensive understanding of diseases of the aging brain. Importantly, this endeavor may uncover peripheral intervention targets for cognitive decline, providing an entirely new perspective on potential treatments.

Scientific Rigor

Given the complexity of the interplay between the microbiome, the peripheral nervous system, and cognitive brain function, we will control as many experimental variables as possible in our animal studies. For example, we will use littermate controls in each experiment from multiple breeding cages, to eliminate cage effects and breeding legacy effects. Control animals will always receive mock treatments with vehicle substances.

For statistical comparisons between two groups, we will use Mann-Whitney *U*-tests. Our preliminary data show that we cannot assume normal distribution of data obtained across all modalities, which is why we rely on tests that do not assume normal distribution. For comparisons between three or more groups, we will use ANOVA with corrections for multiple comparisons between groups. For identifying elements with statistically significant changes in large data sets, such as next-generation sequencing data sets, we will use FDR correction according to the Benjamini-Hochberg method.

Power analyses will be used to determine sample sizes for each experiment with power=0.80 and alpha=0.05. Assumptions about effect sizes will be based on our preliminary data and pilot studies (see **Figure 1**). For between-subject analyses, mice will be randomly assigned to experimental condition. For within-subject analyses, all mice will receive all experimental conditions. We anticipate using n=10 mice in each group based on the following calculations. Assuming effect sizes as those observed in **Figure 1**, we reach 98% power using a significance level of 0.05 in a two-sample Mann-Whitney *U*-test (**Figure 2A**). For time course experiments, such as rates of cognitive decline, rates of gene expression changes, or changes in metabolite abundance, we simulated various curve slopes with zero offset (**Figure 2B**), demonstrating that the minimum effect size for 100% power is a difference in slope of 0.4 s.d. (**Figure 2C**). Since our initial experiments show a slope of 0.7 s.d., we are well powered to detect statistically significant differences.

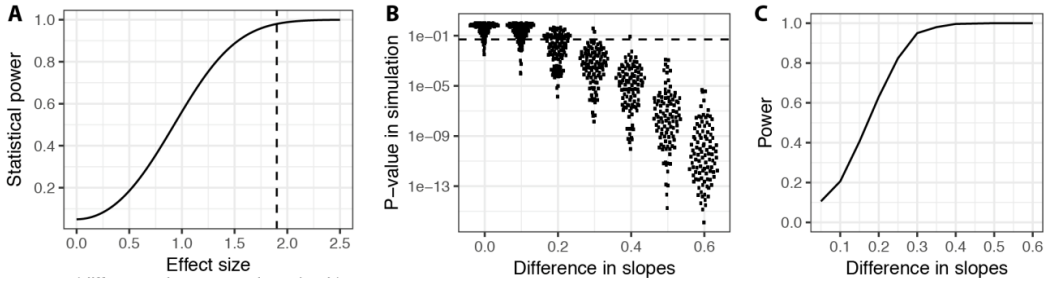


Figure 2. Power analysis. (**A**) Power curve for effect sizes in two-sample comparisons. (**B**) Simulations of p-values for various slopes. (**C**) Power curve for various weight slopes as in (B), with 500 trials per effect size.

Several studies have reported that cognitive decline differs between male and female mice[10-13]. All preliminary data shown here has been collected in both males and females and all proposed experiments will be conducted in both sexes, powered to detect any differences that may exist. If necessary, the estrous cycle of females will be monitored by vaginal cytology[14] so that any deviations due to hormonal effects can be identified. We are cognizant of the fact that cognitive decline is the result of a complex interplay between multiple factors, including systemic metabolism, sleep, hydration, satiety, and many other aspects of physiology. This proposal addresses the specific contribution of gut-derived metabolites on cognitive performance.

As young animals, we will use mice between 2-3 months of age. Our preliminary data (**Figure 1**) show that these mice are cognitively normal and achieve consistent preference for novelty in the novel object recognition task. For aged animals, we will use mice that are 20 months of age or older. At this age, according to our preliminary experiments, novel object preference is lost, and both objects are explored with equal times. We have made similar observations of impaired memory in 20-months-old animals in other assays of cognitive function, such as Barnes maze.

References

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2 Jagust, W. Vulnerable neural systems and the borderland of brain aging and neurodegeneration. *Neuron* **77**, 219-234, doi:10.1016/j.neuron.2013.01.002 (2013).

3 Chen, W. G. *et al.* The Emerging Science of Interoception: Sensing, Integrating, Interpreting, and Regulating Signals within the Self. *Trends Neurosci* **44**, 3-16, doi:10.1016/j.tins.2020.10.007 (2021).

4 Nord, C. L. & Garfinkel, S. N. Interoceptive pathways to understand and treat mental health conditions. *Trends Cogn Sci* **26**, 499-513, doi:10.1016/j.tics.2022.03.004 (2022).

5 Dohnalova, L. *et al.* A microbiome-dependent gut-brain pathway regulates motivation for exercise. *Nature* **612**, 739-747, doi:10.1038/s41586-022-05525-z (2022).

6 Schneider, K. M. *et al.* The enteric nervous system relays psychological stress to intestinal inflammation. *Cell*, doi:10.1016/j.cell.2023.05.001 (2023).

7 Agirman, G., Yu, K. B. & Hsiao, E. Y. Signaling inflammation across the gut-brain axis. *Science* **374**, 1087-1092, doi:10.1126/science.abi6087 (2021).

8 Josselyn, S. A. & Tonegawa, S. Memory engrams: Recalling the past and imagining the future. *Science* **367**, doi:10.1126/science.aaw4325 (2020).

9 Prescott, S. L. & Liberles, S. D. Internal senses of the vagus nerve. *Neuron* **110**, 579-599, doi:10.1016/j.neuron.2021.12.020 (2022).

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11 Short, A. K. *et al.* Sex-dependent effects of chronic exercise on cognitive flexibility but not hippocampal Bdnf in aging mice. *Neuronal Signal* **6**, NS20210053, doi:10.1042/NS20210053 (2022).

12 Cuervo-Zanatta, D., Garcia-Mena, J. & Perez-Cruz, C. Gut Microbiota Alterations and Cognitive Impairment Are Sexually Dissociated in a Transgenic Mice Model of Alzheimer's Disease. *Journal of Alzheimer's disease : JAD* **82**, S195-S214, doi:10.3233/JAD-201367 (2021).

13 Yanguas-Casas, N., Crespo-Castrillo, A., Arevalo, M. A. & Garcia-Segura, L. M. Aging and sex: Impact on microglia phagocytosis. *Aging Cell* **19**, e13182, doi:10.1111/acel.13182 (2020).

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BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Christoph A. Thaiss

eRA COMMONS USER NAME (credential, e.g., agency login): thaissc

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Bonn, Germany	B.Sc.	08/2010	Molecular Biomedicine
Yale University, USA & ETH Zurich, Switzerland	M. Sc.	08/2012	Microbiology, Immunology
Weizmann Institute of Science, Israel	Ph.D.	08/2017	Science

A. Personal Statement

My scientific fascination centers on the shared etiologies of the most common human diseases, including neurodegeneration, chronic inflammatory diseases, cancer, and cardiometabolic diseases. There are currently two major theories for why these diseases arise: (1) that these are diseases of aging, caused by byproducts of normal physiology that accumulate over time, and (2) that these are diseases of environmental mismatch, caused by environmental and lifestyle factors that the human body has not evolved to deal with. We propose to add a third common mechanism: dysfunctional body-brain communication.
The interoceptive system of the body mediates the transfer of information between the body and the brain, which is critically required for the function of whole-organism physiology. Based on findings from our group and other groups in the field, we postulate that interoceptive dysfunction is at the core of many of the most common human diseases.
In this project, we will focus on the impact of body-brain communication on brain aging and cognitive decline. It is my scientific mission to uncover these influences on a mechanistic level, to decipher the molecular pathways by which they influence disease susceptibility, and to use the design principles of body-brain and host-environment interactions for the rational and innovative development of new therapies.

Ongoing and recently completed projects that I would like to highlight include:

DP2-AG-067492-01 (PI: Thaiss) 09/01/2019 – 08/31/2024
NIH Director's New Innovator Award
A conserved function of amyloid proteins in host-microbiome interactions

Pew Scholar Award (PI: Thaiss) 07/01/2020 – 06/30/2024
Pew Charitable Trust
A conserved tissue housekeeping function of the immune system

R01-DK-129691 (PI: Heuckeroth, co-I: Thaiss) 08/27/2022 – 05/31/2025
NIH/NIDDK
Defining non-genetic mechanisms that prevent death in a Hirschsprung disease mouse model

HFSP Program Grant (PIs: Aguzzi, Thaiss) 07/01/2022 – 06/01/2025
Human Frontiers Science Program
Mapping gut-to-brain transmission of prion protein

Highlighted citations from the past year:

1. Schneider KM, Blank N, Alvarez Y, Thum K, Lundgren P, Litichevskiy L, Sleeman M, Bahnsen K, Kim J, Kardo S, Patel S, Dohnalová L, Uhr GT, Descamps HC, Kircher S, McSween A, Rezazadeh Ardabili A, Nemec KM, Jimenez MT, Glotfelty LG, Eisenberg JD, Furth EE, Henao-Mejia J, Bennett FC, Pierik MJ, Romberg-Camps M, Mujagic Z, Prinz M, Schneider CV, Wherry EJ, Bewtra M, Heuckeroth RO, Levy M, **Thaiss CA**, The enteric nervous system relays psychological stress to intestinal inflammation, ***Cell***, 2023
2. **Thaiss CA**, A microbiome exercise, ***Science***, 2023
3. Dohnalová L, Lundgren P, Carty JRE, Goldstein N, Wenski SL, Nanudorn P, Thiengmag S, Huang K-P, Litichevskiy L, Descamps HC, Chellappa K, Glassman A, Kessler S, Kim J, Cox TO, Dmitrieva-Posocco O, Wong AC, Allman EL, Ghosh S, Sharma N, Sengupta K, Cornes B, Dean N, Churchill GA, Khurana TS, Sellmyer MA, FitzGerald GA, Patterson AD, Baur JA, Alhadeff AL, Helfrich EJN, Levy M, Betley JN, **Thaiss CA**, A microbiome-dependent gut-brain pathway regulates motivation for exercise, ***Nature***, 2022
4. Dmitrieva-Posocco O, Wong AC, Lundgren P, Golos AM, Descamps HC, Dohnalová L, Cramer Z, Tian Y, Yueh B, Eskiocak O, Egervari G, Lan Y, Liu J, Fan J, Kim J, Madhu B, Schneider KM, Khoziainova S, Andreeva N, Wang Q, Li N, Furth EE, Bailis W, Kelsen JR, Hamilton KE, Kaestner KH, Berger SL, Epstein JA, Jain R, Li M, Beyaz S, Lengner CJ, Katona BW, Grivennikov SI, **Thaiss CA**[#], Levy M[#], Beta-hydroxybutyrate suppresses colorectal cancer, ***Nature***, 2022

I have only published under the name “Christoph A Thaiss”.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2018	Assistant Professor, Department of Microbiology, University of Pennsylvania
	Member, Biomedical Graduate Studies, University of Pennsylvania
	Co-director, Penn Gnotobiotic Animal Facility
2013	Visiting Scientist, Stanford University
2012	Visiting Scholar, Broad Institute of MIT and Harvard

Honors

2023	Science & Noster Microbiome Prize
2021	Highly Cited Researcher (top 1%), Clarivate
2020	Pew Biomedical Scholar
2020	Kathryn W. David Aging Brain Scholar
2019	NIH Director’s New Innovator Award
2018	Science & SciLifeLab Grand Prize for a Young Scientist
2018	Agilent Early Career Professor Award
2017	Harold M. Weintraub Graduate Student Award
2017	Innovation Award of the German Medical Association
2017	John F. Kennedy prize for excellent doctoral work, Weizmann Institute of Science
2016	Systems Biology Student Award, Weizmann Institute of Science
2015	Delegate and Master Class Speaker at the 65th Lindau Nobel Laureate Meeting
2015	Immunology Department Student Award, Weizmann Institute of Science
2014	Best presentation award, Timelines in Biology, Weizmann Institute of Science
2013	Boehringer Ingelheim Fonds PhD Fellowship
2012	Short-term scholarship for studies at the Broad Institute of MIT and Harvard
2012	Medal of ETH Zurich (Swiss Federal Institute of Technology) for the best Master’s Thesis
2010	Visiting Fellowship and Stipend, Yale Graduate School of Arts and Sciences
2007	Fellowship of the German National Academic Foundation

C. Contributions to Science

1. Regulators of immunity, inflammation, and cancer

We have pioneered the application of high-throughput genomics and metabolomics approaches to the study of immune cell function. We have used single-cell RNA-sequencing to characterize the population of intestinal innate lymphoid cells (ILCs) and to identify those subsets of ILCs that are uniquely responsive to intestinal microbial colonization. Together with the analysis of epigenetic landscapes in distinct ILC subgroups, this work has uncovered the spectrum and regulatory landscapes of ILCs and their genomic responses to the intestinal microbiota.

In a different study, we have used metabolomics tools to identify specific microbiota-derived metabolites that act as signaling molecules and shape the innate immune response of the host. In particular, we have studied metabolites that control innate immune platforms and cytokine production by intestinal epithelial cells, which regulate the secretion of antimicrobial peptides. Thus, the signaling circuits of microbial metabolites, epithelial responses, and antimicrobial mechanisms form a feedback look that regulates the colonization niche of commensal bacteria in the gastrointestinal tract. These studies may serve as blueprints for the systematic elucidation of genomic and metabolic elements that control immune functions.

We have also applied the concept to metabolite regulation of disease to the study of colorectal cancer. We have identified a new diet-metabolite-epithelial cell pathway that potently suppresses the development and progression of colorectal cancer. These findings have inspired two clinical trial that are investigating the efficacy of this pathway in human colorectal cancer patients.

a. Levy M*, **Thaiss CA***, Zeevi D, Dohnalová L, Zilberman-Schapira G, Mahdi JA, David E, Savidor A, Korem T, Herzig Y, Pevsner-Fischer M, Shapiro H, Christ A, Harmelin A, Halpern Z, Latz E, Flavell RA, Amit I, Segal E, Elinav E, Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. ***Cell***, 2015, PMID: 26638072. * denotes authors with equal contribution
b. **Thaiss CA***, Zmora N*, Levy M*, Elinav E, The microbiome and innate immunity. ***Nature***, 2016. PMID: 27383981. * denotes authors with equal contribution
c. Gury-BenAri M*, Thaiss CA*, Serafini N, Winter DR, Giladi A, Lara-Astiaso D, Levy M, Salame TM, Weiner A, David E, Shapiro H, Dori-Bachash M, Pevsner-Fischer M, Lorenzo-Vivas E, Keren-Shaul H, Paul F, Harmelin A, Eberl G, Itzkovitz S, Tanay A, Di Santo JP, Elinav E, Amit I, The spectrum and regulatory landscapes of intestinal innate lymphoid cells are shaped by the microbiome. ***Cell***, 2016. PMID: 27545347. * denotes authors with equal contribution
d. Dmitrieva-Posocco O, Wong AC, Lundgren P, Golos AM, Descamps HC, Dohnalová L, Cramer Z, Tian Y, Yueh B, Eskiocak O, Egervari G, Lan Y, Liu J, Fan J, Kim J, Madhu B, Schneider KM, Khoziainova S, Andreeva N, Wang Q, Li N, Furth EE, Bailis W, Kelsen JR, Hamilton KE, Kaestner KH, Berger SL, Epstein JA, Jain R, Li M, Beyaz S, Lengner CJ, Katona BW, Grivennikov SI, **Thaiss CA**[#], Levy M[#], Beta-hydroxybutyrate suppresses colorectal cancer. ***Nature***, 2022. PMID: 35477756. [#] co-corresponding authors

2. Host-microbiota interactions in obesity

A central characteristic of the modern lifestyle that is strongly associated with the rising incidence in metabolic and inflammatory diseases is the change in dietary patters. Our recent work has addressed two fundamental but poorly understood phenomena associated with obesity.

First, we have addressed the question of why weight loss therapies in humans are frequently accompanied by post-dieting weight regain, a phenomenon typically referred to as the “yo-yo effect” of recurrent obesity. We have found that a period of obesity introduces long-lasting structural alterations in the commensal microbial community in the intestine, which persist even after successful dieting and return to normal weight. During the persistence of this abnormal microbiome, the host is susceptible to enhanced secondary weight gain. The underlying mechanism involves microbiome-mediated degradation of dietary flavonoids, which is enhanced during obesity and the post-obesity period. This, in turn, leads to continuously low levels of intestinal flavonoids and reduced energy expenditure in adipose tissue. This work has identified long-term “memory-like” properties of the metabolome that persist even after reversal of the initial environmental stimulus and influence host responses to subsequent environmental exposures.

In addition, we have investigated the mechanisms linking obesity to several inflammatory manifestations and enhanced susceptibility to infection. We found that hyperglycemia, rather than obesity, causes loss of intestinal barrier integrity, influx of immunostimulatory microbial products into the systemic circulation, and systemic dissemination of pathogenic bacteria upon enteric infection. These manifestations are caused by glucose-mediated metabolic reprogramming of intestinal epithelial cells and can be counteracted by therapeutic approaches lowering glucose levels in the circulation or inhibiting epithelial glucose metabolism.

Furthermore, we have used single-cell RNA-sequencing to identify lipid-associated macrophages in adipose tissue, their regulation by the receptor Trem2, and their importance for metabolic homeostasis during obesity.

a. **Thaiss CA***, Itav S*, Rothschild D*, Meijer M, Levy M, Moresi C, Dohnalová L, Braverman S, Rozin S, Malitsky S, Dori-Bachash M, Kuperman Y, Biton I, Gertler A, Harmelin A, Shapiro H, Halpern Z, Aharoni A, Segal E, Elinav E, Persistent microbiome alterations modulate the rate of post-dieting weight regain. ***Nature***, 2016. PMID: 27906159. * denotes authors with equal contribution
b. **Thaiss CA**, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, Braverman S, Tengeler AC, Barak O, Elazar M, Ben-Zeev R, Lehavi-Regev D, Katz MN, Pevsner-Fischer M, Gertler A, Halpern Z, Harmelin A, Aamar S, Serradas P, Grosfeld A, Shapiro H, Geiger B, Elinav E, Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. ***Science***, 2018. PMID: 29519916.
c. **Thaiss CA**, Microbiome Dynamics in Obesity. ***Science***, 2018. PMID: 30467161
d. Jaitin DA*, Adlung L*, **Thaiss CA***, Weiner A, Li B, Descamps H, Lundgren P, Bleriot C, Liu Z, Deczkowska A, Keren-Shaul H, David E, Zmora N, Eldar SM, Lubezky N, Shibolet O, Hill DA, Lazar MA, Colonna M, Ginhoux F, Shapiro H, Elinav E, Amit I, Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. ***Cell***, 2019. PMID: 31257031. * denotes authors with equal contribution

3. Environmental control of physiology

We have discovered diurnal oscillations of the microbiome on the level of taxonomic composition, biogeographical distribution, metagenomic function, and metabolite secretion. We furthermore found that these daily bacterial fluctuations are controlled by the host circadian clock through rhythmic feeding behavior, and that oscillatory proliferation patterns are driving the 24-hour rhythmicity. In addition, these diurnal rhythms influence the epigenetic and transcriptional programs of the host circadian clock in multiple tissues and determine metabolite oscillations in the systemic circulation. As such, the circadian programs of the host and microbiome are closely linked.

These discoveries have numerous implications for metabolic conditions that are commonly associated with disruption of the circadian clock, one of the hallmarks of the modern human lifestyle. Indeed, we found that metabolic derangements associated with chronic jetlag have a microbiome component, since jetlag in humans is associated with dysbiosis, an alteration in the composition and function of the microbiome, and with microbiome-driven adiposity and hyperglycemia in mice. Together, these studies have identified community-wide diurnal oscillations in the intestinal microbiome, which have profound implications for the circadian biology of the host. Thus, environmental influences such as the daily cycles of light and dark can strongly influence host-microbiome mutualism. The microbiome functions as an integrative signaling hub that links variations in environmental conditions to host physiology.

In recent work, we have extended the scope of our investigations into the circadian impact on host physiology by exploring the role of aging on macrophage circadian clocks. We discovered that circadian gene regulation and function in macrophages decline with age, with important consequences for innate immune functions such as phagocytosis and immune cell trafficking.

We have furthermore discovered a subpopulation of lipogenic brown adipocytes that orchestrates organismal response to repeated temperature challenges – a phenomenon we refer to as “thermogenic memory”.

a. **Thaiss CA**, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, Abramson L, Katz MN, Korem T, Zmora N, Kuperman Y, Biton I, Gilad S, Harmelin A, Shapiro H, Halpern Z, Segal E, Elinav E, Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis. ***Cell***, 2014. PMID: 25417104.
b. **Thaiss CA***, Levy M*, Korem T*, Dohnalová L, Shapiro H, Jaitin DA, David E, Winter DR, Gury-BenAri M, Tatirovsky E, Tuganbaev T, Federici S, Zmora N, Zeevi D, Dori-Bachash M, Pevsner-Fischer M, Kartvelishvily E, Brandis A, Harmelin A, Shibolet O, Halpern Z, Honda K, Amit I, Segal E, Elinav E, Microbiota diurnal rhythmicity programs host transcriptome oscillations. ***Cell***, 2016. PMID: 27912059. * denotes authors with equal contribution
c. Blacher E*, Tsai C*, Litichevskiy L*, Shipony Z, Iweka CA, Schneider KM, Chuluun B, Heller HC, Menon V, **Thaiss CA**[#], Andreasson KI[#], Aging disrupts circadian gene regulation and function in macrophages, ***Nature Immunology***, 2021. [#] co-corresponding authors
d. Lundgren P, Sharma P, Dohnalová L, Coleman K, Uhr GT, Kircher S, Litichevskiy L, Bahnsen K, Descamps HC, Demetriadou C, Chan J, Chellappa K, Cox TO, Heyman Y, Pather SR, Shoffler C, Petucci C, Shalem O, Raj A, Baur JA, Snyder NW, Wellen KE, Levy M, Seale P, Li M, **Thaiss CA,** A subpopulation of lipogenic brown adipocytes drives thermogenic memory, ***Nature Metabolism***, *in press*

4. Body-brain communication in health and disease

A major focus of study in my group is the systematic identification of body-brain circuits that regulate brain function. We have recently uncovered a pathway linking intestinal microorganisms and their metabolites, gut-innervating sensory neurons, and dopamine levels in the striatum that regulates exercise performance. We have also characterized a new pathway by which the perception of psychological stress in the brain drives exacerbated intestinal inflammation, via alterations in the enteric nervous system.

a. Dohnalová L, Lundgren P, Carty JRE, Goldstein N, Wenski SL, Nanudorn P, Thiengmag S, Huang K-P, Litichevskiy L, Descamps HC, Chellappa K, Glassman A, Kessler S, Kim J, Cox TO, Dmitrieva-Posocco O, Wong AC, Allman EL, Ghosh S, Sharma N, Sengupta K, Cornes B, Dean N, Churchill GA, Khurana TS, Sellmyer MA, FitzGerald GA, Patterson AD, Baur JA, Alhadeff AL, Helfrich EJN, Levy M, Betley JN, **Thaiss CA**, A microbiome-dependent gut-brain pathway regulates motivation for exercise, ***Nature***, 2022. PMID: 36517598
b. **Thaiss CA**, A microbiome exercise, ***Science***, 2023. PMID: 37410844
c. Schneider KM, Blank N, Alvarez Y, Thum K, Lundgren P, Litichevskiy L, Sleeman M, Bahnsen K, Kim J, Kardo S, Patel S, Dohnalová L, Uhr GT, Descamps HC, Kircher S, McSween A, Rezazadeh Ardabili A, Nemec KM, Jimenez MT, Glotfelty LG, Eisenberg JD, Furth EE, Henao-Mejia J, Bennett FC, Pierik MJ, Romberg-Camps M, Mujagic Z, Prinz M, Schneider CV, Wherry EJ, Bewtra M, Heuckeroth RO, Levy M, **Thaiss CA**, The enteric nervous system relays psychological stress to intestinal inflammation, ***Cell***, 2023. PMID: 37236193

Complete list of publications:
https://www.ncbi.nlm.nih.gov/pubmed/?term=thaiss+ca

For New and Renewal Applications – DO NOT SUBMIT UNLESS REQUESTED
PHS 398 OTHER SUPPORT

There is no "form page" for reporting Other Support. Information on Other Support should be provided in the format shown below.

*Name of Individual: CHRISTOPH THAISS
Commons ID: THAISSC

Other Support – Project/Proposal

ACTIVE

*Title: Defining non-genetic mechanisms that prevent death in a Hirschsprung disease mouse model

Major Goals: (1) Test the hypothesis that changing diet and/or bowel microbes influences survival in sl/sl mice, (2) Define mechanistic differences between the bowel barrier for sl/sl at CHOP and UQAM, and (3) Test the hypothesis that mucosa-associated microbes and luminal metabolites more abundant at CHOP than at UQAM enhance epithelial protective barriers to prevent inflammation and sepsis.

*Status of Support: Active

Project Number: Sub to R01-DK-129691

Name of PD/PI: ROBERT HEUCKEROTH

Name of co-I: CHRISTOPH THAISS

*Source of Support: CHILDREN'S HOSPITAL OF PHILADELPHIA (NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES/NIH/DHHS)

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2021 - 05/2025

*Total Award Amount (including Indirect Costs): $1,091,496

*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2024	1.2 Calendar
4. 2025	1.2 Calendar

*Title: A conserved function of amyloid proteins in host-microbiome interactions

Major Goals: The goal of this study is to determine the impact of amyloid proteins on host-microbiome interactions in homeostasis and disease.

*Status of Support: Active

Project Number: DP2-OD-027726-01

Name of Individual: CHRISTOPH THAISS
Commons ID: THAISSC

Name of PD/PI: CHRISTOPH THAISS
*Source of Support: NIH
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 9/15/19 – 6/30/24
*Total Award Amount (including Indirect Costs): 2,434,500
*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2024	3.00 Calendar

*Title: A conserved tissue housekeeping function of the immune system
Major Goals: This project will explore tissue maintenance functions of immune cells in different organs.
*Status of Support: Active
Project Number: Pew Scholar Award
Name of PD/PI: CHRISTOPH THAISS
*Source of Support: The Pew Charitable Trust
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 8/1/20 – 7/31/24
*Total Award Amount (including Indirect Costs): $300,000
*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2024	.01 Calendar

Title: Mapping gut-to-brain transmission of prion protein
Major Goals: To identify the full landscape and biology of membrane receptors actively interacting with PrP.
*Status of Support: Active
Project Number: RGP0001/2022
Name of PD/PI: CHRISTOPH THAISS
*Source of Support: Human Frontier Science Program
*Primary Place of Performance: University of Pennsylvania
Project/Proposal Start and End Date: (MM/YYYY) (if available): 6/1/22 – 5/31/25
*Total Award Amount (including Indirect Costs): $450,000
*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2024	1.0 Calendar
4. 2025	1.0 Calendar

Name of Individual: CHRISTOPH THAISS
Commons ID: THAISSC

PENDING

*Title: Intestinal regulation of exercise performance

*Major Goals: This proposal explores the impact of the intestinal microbiota on the activity of brain neurons that influence exercise performance. The results of these experiments will enhance our understanding of how exercise performance can be controlled, and possibly enhanced, from the gastrointestinal tract.

Contact PD/PI: THAISS, CHRISTOPH

*Status of Support: Pending

Project Number: 1R01NS134976-01

Name of PD/PI: CHRISTOPH THAISS (thaiss)

*Source of Support: NATIONAL INSTITUTES OF HEALTH

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2023 - 08/2028

*Total Award Amount (including Indirect Costs): $3,549,170

*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2024	2.4 Calendar
2. 2025	2.4 Calendar
3. 2026	2.4 Calendar
4. 2027	2.4 Calendar
5. 2028	2.4 Calendar

*Title: Gut-brain communication in enteric infection

*Major Goals: This project will greatly enhance our understanding of gut-brain communication during infection and may teach us about new host protection pathways that are engaged by the brain during pathogenic infection.

*Status of Support: Pending

Project Number: N/A

Name of PD/PI: CHRISTOPH THAISS (thaiss)

*Source of Support: BURROUGHS WELLCOME FUND

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2023 - 06/2028

*Total Award Amount (including Indirect Costs): $500,000

*Person Months (Calendar/Academic/Summer) per budget period.

Name of Individual: CHRISTOPH THAISS
Commons ID: THAISSC

Year (YYYY)	Person Months (##.##)
1. 2024	1.33 Calendar
2. 2025	1.33 Calendar
3. 2026	1.33 Calendar
4. 2027	1.33 Calendar
5. 2028	1.33 Calendar

*Title: Isotope tracing of the metabolic fate of NAD precursors in Young and Old Adults

*Major Goals: Will oversee the microbiome sequencing from fecal samples and provide guidance on potential associations with NAD metabolism and other outcomes in human subjects. Microbiome sequencing will include both 16S sequencing and metagenomics for up to 96 samples.

*Status of Support: Pending

Project Number: N/A

Name of PD/PI: JOSEPH A BAUR PH.D. (baur)

*Source of Support: MASSACHUSETTS GENERAL HOSPITAL (NATIONAL INSTITUTES OF HEALTH)

*Primary Place of Performance: University of Pennsylvania

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2023 - 08/2027

*Total Award Amount (including Indirect Costs): $267,401

*Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2024	0.3 Calendar
2. 2025	0.3 Calendar
3. 2026	0.3 Calendar
4. 2027	0.3 Calendar

THAISS LAB PERSONNEL RECEIVING EXTERNAL SUPPORT

Megan Liou
Home Institution: University of Pennsylvania
Funding Source: Hartwell Foundation

Alexis Cowan
Home Institution: University of Pennsylvania
Funding Source: Princeton University, Ludwig Institute for Cancer Research

Name of Individual: CHRISTOPH THAISS
Commons ID: THAISSC

Niklas Blank
Home Institution: University of Pennsylvania
Funding Source: German National Academic Foundation

IN-KIND

NONE

***Overlap:** There is no potential overlap with the active or pending projects and activities, other positions, affiliations, resources and in terms of the science, budget or committed effort. If any of the Pending applications are funded, Dr. Thaiss will adjust her effort accordingly as not to exceed twelve calendar months of funding

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: _____

Date: _____

AMERICAN FEDERATION FOR AGING RESEARCH
55 West 39th Street, 16th floor, New York, NY 10018 (212) 703-9977

McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss

Institutional Commitment Form

Candidates for the McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss must be independent investigators with independent research space. To complete the application, this form must be completed by the Dean or the Department Chair. The form is NOT to be included in the application, but must be submitted directly to AFAR by the person completing the form (NOT the applicant), to afarapplication@afar.org as a Word or PDF file.

Name, title, and address of official completing this form:

Frederic Bushman, PhD
Professor and Chair, Department of Microbiology
3610 Hamilton Walk; Johnson Pavilion 225
Philadelphia, PA 19104

E-mail: bushman@pennmedicine.upenn.edu
Phone: 215-573-8732

Signature of Official: _____

First and Last name of Applicant: Christoph Thaiss, PhD

1. Does the candidate have independent investigator status at his/her institution?

☑ YES ☐ NO

2. Has the candidate's institution provided space and equipment specifically dedicated to his/her research program?

☑ YES
Please Describe:

☐ NO
Please describe whose resources the candidate will use to execute the proposed project:

3. Did the candidate receive intramural start-up funds when offered his/her current position? (AFAR does not consider extramural funds from an outside organization/institution as 'start-up funds'.)

☑ YES
Please provide $ amount and details of start-up funds: $2,000,000; no expiration

☐ NO

4. Does the candidate have designated administrative support (e.g. someone who helps with editing and submitting grants, tracks budgets, etc.)

☑ YES ☐ NO

5. What was the start date of the candidate's current position?

Month/Day/Year: 3/1/2018

6. Does your institution offer tenure:

☑ YES ☐ NO

a. If yes, is the candidate's current position a tenure track position?

☑ YES ☐ NO

b. If your institution does **not** offer tenure, please provide evidence of long-term institutional support

7. Does the candidate have teaching and/or clinical responsibilities in the current position?

☑ YES ☐ NO

If yes, indicate percentage of time: 10% teaching

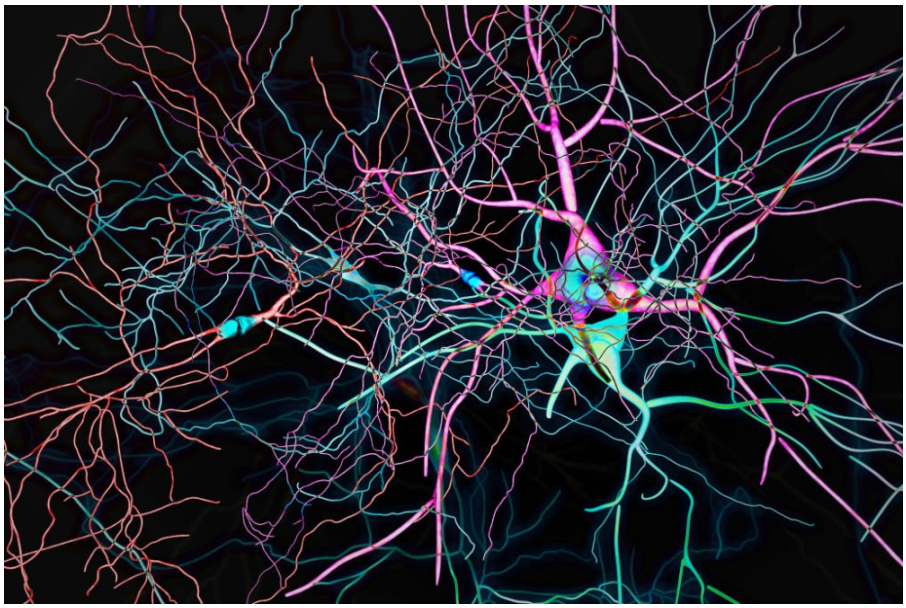
8. Describe overall annual research funding for the department that the investigator is primarily affiliated with. FY23 total expenditures: $26.6M; 85% Federal/15% non-Federal

9. To demonstrate a commitment to the investigator, the institution is asked to support the investigator's project through matching funds. **Please provide a statement below stating that 50% ($375,000) in cash or in-kind matching funds will be committed to the project and investigator if an award is made.** Provide details and amounts for the matching funds. Matching funds can only be non-federal and cannot be used by more than one project. This could be cash and/or in-kind matching, and can include faculty effort, and goods and services paid from departmental funds. For an in-kind match, the selection committee will determine whether this is equivalent to a monetary match.

Dr. Thaiss' institutional startup fund has remaining funds to support this matching request. If at time of award, available funding to Dr. Thaiss drops below this requirement, the Department will contribute funding as needed.

As Americans Grow Older, These Funders are Advancing the Field of Cognitive Aging Research

Mike Scutari | November 29, 2022



NAEBLYS/SHUTTERSTOCK

As people live longer, society will grapple with unprecedented demand for prevention, diagnosis and

treatment pertaining to certain neurological conditions. For example, researchers expect the number of people living with Alzheimer's disease to triple by 2025.

Thirteen percent of people over the age of 65 develop the disease, but a portion of the remaining 87% will struggle with cognitive decline due to the normal aging process. They may forget names, become disoriented by simple tasks, or find it difficult to manage their finances. The effects of cognitive decline will have an adverse impact on millions of individuals' quality of life and exact a toll on the public healthcare system.

With this coming wave of increased need in mind, neurological research funders are pushing the limits of what the field can do to support an aging population. The McKnight Brain Research Foundation (MBRF) and the American Federation for Aging Research (AFAR) are two such grantmakers tackling these looming challenges by ramping up support for cognitive aging research. "Our focus is on how to permit people to remain cognitively healthy for as long as they can," said MBRF Chair Emeritus J. Lee Dockery, MD. "We're the only organization that differentiates age-related cognitive decline and memory loss from other neurodegenerative disorders."

AFAR acknowledges that the more researchers and physicians understand the basic biology of aging, the better equipped they'll be to delay many chronic diseases, including stroke, Alzheimer's disease, cancer and diabetes. Its goal is to "establish the field of aging researchers who are looking into the biological mechanisms of why and how we age, and how that

affects the diseases of aging, including cognitive decline," said Executive Director Stephanie Lederman. "There has been a considerable amount of research to the point now that we are on the verge of some important breakthroughs. It's a very exciting time."

Long-standing commitments to cognitive aging research

The Orlando-based MBRF defines cognitive aging as "a natural process that can have both positive and negative effects, and these effects vary widely from person to person." It was established in 1999 by Evelyn F. McKnight, the widow of former 3M Chairman William L. McKnight, who founded the Minneapolis-based McKnight Foundation in 1953. The MBRF has funded more than $180 million in cognitive aging research since its inception.

The foundation has established Evelyn F. McKnight Brain Institutes at the University of Alabama at Birmingham, the University of Arizona, and the University of Miami; and the Evelyn F. and William L. McKnight Brain Institute at the University of Florida. Its partners include the National Institute on Aging through the Foundation for the National Institutes of Health and AFAR.

The New York City-based AFAR was launched in 1981 by cardiologist and American Heart Association cofounder Irving Wright. "At the time, there was very little knowledge in the field," Lederman told me. "But he knew that baby boomers would be getting older, so it was important to better understand how we could keep people healthier for a longer period of time." In

the intervening years, it has awarded more than $193 million in research grants to over 4,350 investigators. AFAR maintains a list of funding opportunities on its site.

Distinguishing cognitive decline from other conditions

Madhav Thambisetty, MD, Ph.D. is an adjunct professor of neurology at the Johns Hopkins University School of Medicine and leads a research group working on drug discovery in Alzheimer's disease at the National Institute on Aging. Thambisetty said that when he joined the McKnight board as a trustee in 2015, "the biggest challenge was, how do we define the distinction between what is normal aging and what is Alzheimer's disease?"

Getting this distinction right or wrong can lead to downstream consequences. Physicians may misdiagnose a patient and prescribe expensive treatments that do not address the underlying condition, or individuals may worry they have Alzheimer's when, in fact, they're simply experiencing the effects of aging. This uncertainty can compel individuals to put their faith in unproven treatments or outright scams to stay mentally sharp. "Don't forget the jellyfish," Lederman said, alluding to a jellyfish memory supplement that turned out to be a hoax.

The lack of a clear delineation between normal aging and conditions like Alzheimer's can also stymie efforts to generate private support since many funders seek to galvanize treatments for established diseases. "So much of the funding opportunities for diseases are from benefactors who've had a relative or someone that

they know who has been a victim of a disease, so they support that cause, and it's justified that they do so," Dockery said. "But we don't have many partners that are interested in funding and investigating aging itself."

"It doesn't happen overnight"

McKnight and AFAR are working to fix this disconnect. Cognizant of funders' desire to bring promising treatments to market, Thambisetty said that McKnight's grantmaking strategy "ensures that the people that we fund, whether individual researchers or the McKnight Brain Research Institutes, have a clear translational focus in all of the research that they do."

McKnight stakeholders also acknowledge that translational research cannot exist without fundamental basic research, which is typically defined as studies that span disciplines and lead to new knowledge. As a result, Thambisetty said, the foundation "tries to balance our funding portfolio to encourage basic science that has a promise of clinical translation."

Administered in partnership with AFAR, the McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss bridges these two research approaches. "One of the awards is for basic science and the other is for clinical translational research," Thambisetty said. "We recognize that both have to be promoted, but one can inform the other, and they can converge and advance meaningful translational findings."

Looking back on his tenure so far, Thambisetty believes the field of cognitive aging has made tremendous progress since he joined the McKnight board seven years ago. “We are now at the stage where we may have relatively noninvasive, highly accurate tests that allow us to make the distinction between who has pathology in their brain, who’s likely to have Alzheimer’s disease, and who may not have the telltale pathological signs of the disease,” he said.

AFAR, which also funds basic and translational research, embraces the term “geroscience,” which it defines as “a research paradigm based in addressing the biology of aging and biology of age-related diseases.” The idea here is that donors will be more supportive of age-related research once they realize that the biological processes of aging are the greatest risk factors for many chronic diseases. “Interventions that slow the aging processes would dramatically lower healthcare costs, perhaps more than the cure of any single disease, while significantly improving quality of life,” its site reads.

I spoke with McKnight and AFAR reps for an upcoming IP white paper on the state of funding for neurological research organizations. In practically every conversation, leaders implored fundraisers to mix hope-based messaging with healthy doses of pragmatism and patience. “It’s a matter of education,” Lederman said. “So many of the breakthroughs we’ve seen took years to be developed with support from the private sector. It doesn’t happen overnight. That’s just the nature of research.”

As Americans Grow Older, These Funders are Advancing the Field of Cognitive Aging Research

This Research Funder Is on a Mission to Cure — and Destigmatize — Epilepsy

“A Period of Rapid Change.” How the ALS Association Shepherded a New Treatment to Market

“Explore the Gaps.” A Closer Look at the Kavli Foundation’s Neuroscience Grantmaking

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Philanthropy Needs a New Norm Eight Top Black Philanthropists c

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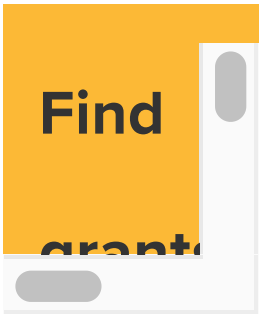

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SUMMARY OF PROGRESS

In this study, we aim to understand how changes in a structure called the extracellular matrix (ECM), affect learning and memory during aging. The extracellular matrix is a meshwork of many different proteins and sugars that provides support to the synaptic connections between neurons in the brain. Decline of this support could play a key role in determining which brain regions are most vulnerable to synapse loss, which is critically connected to learning and memory. In work completed so far, we analyzed how the ECM changes in different brain regions in mice during aging. We showed that ECM structure is different in distinct brain regions even in young mice and that aging impacts the ECM in some brain regions more than others, beginning in middle age. We also found that non-neuronal cells called glial cells contact the ECM and may play a role in some of the changes to its structure during aging. To understand how ECM changes are connected to learning and memory, we developed a mouse behavioral task that requires mice to learn and remember the location of rewarding food pellets. We showed that by late middle age, mice cannot remember reward locations for as long as young mice. We are now working to show which changes in glia, ECM, and synapses are most closely linked to these decreases in learning and memory. Moving forward, we will also analyze tissue from a tissue bank from aged, cognitively-characterized macaques to understand which changes in ECM structure are most likely also occurring in aging humans. Our studies promise to identify previously unrecognized phenomena that contribute to cognitive decline during aging and potential strategies for preserving learning and memory.

TRAINING / SCIENTIFIC PRESENTATIONS

Dr. Daniel Gray, the lead postdoctoral fellow contributing to this research, presented preliminary study findings at a McKnight Foundation meeting in March 2022. He also attended the Federation for European Neuroscience meeting (July 2022) as well as the Society for Neuroscience (SFN) Annual Meeting (November 2022). At SFN, he presented a poster with our findings from this study and received a great deal of interest in the work and constructive feedback (abstract attached). Daniel has been mentoring two undergraduate students, Abigail Gutierrez and Claribel Charway, who are playing essential roles in assisting with mouse behavior and histology. Abigail received a UCLA CARE Fellowship to support full time research during the summer of 2022 and both undergraduates are receiving academic credit / honors credit for research completed during the academic year. Daniel has recently started mentoring a third undergraduate student, Palak Parikah, and has done an excellent job of helping his team of undergraduate students establish a foundation of experimental and critical reasoning skills and in fostering their independence and interest in pursuing additional training in research. For his own training, Daniel has regularly helped me with refereeing manuscripts, preparing grant applications, and preparing scientific manuscripts. He has continued to deepen his expertise in imaging, molecular biology, and mouse behavior, building a potent suite of skills to complement his expertise in analysis of cognitive aging in non-human primates, derived from his graduate training.

I have presented portions of our research from this study at the Glial Biology in Medicine Conference (virtual Nature Conference, Oct 2021), the International Dementia Conference Series (March 2022, virtual seminar series organized by the Karolinska Institute) and a Neuroscience Seminar (UMass Medical School, May 2022). A key component of my own ongoing training has been participation in faculty-targeted mentoring workshops, which include strategies for building inclusive, diverse laboratory training environments. I have also participated in workshops that cover how to recognize and counter microaggressions.

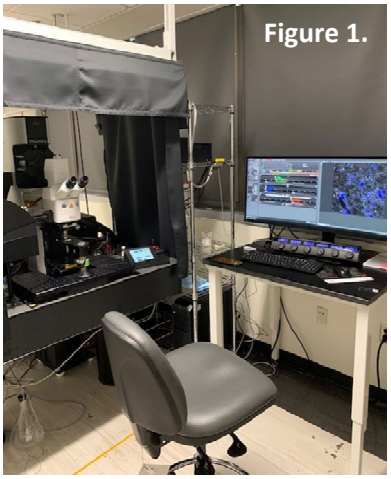
Figure 1.

LABORATORY EQUIPMENT

We have acquired, installed, and are actively using the Leica Stellaris 8 DIVE multiphoton / confocal microscope that was described in our application (**Fig. 1**). Contributions from UCLA's Physiology Department and the UCLA School of Medicine Dean's office toward purchase of this microscope constituted a significant portion of the institutional match for this funding period. The confocal capabilities of this microscope have significantly enhanced and sped up acquisition of images for this study. The fluorescence lifetime capability of the microscope has enabled new methods for clarifying what signals arise from autofluorescent aggregates in tissue from aging mice, rats, and macaques.

The multiphoton capabilities of the microscope have enabled us to analyze microglial cell process motility in acute brain sections and we are currently equipping the microscope for experiments that will involve head fixed mice. With tools we hope to generate that will tag specific ECM components and synaptic components, this will allow for future experiments that examine microglial-ECM interactions, microglial-synapse interactions, and synapse-ECM associations *in vivo* in young and aging mice.

SCIENTIFIC PROGRESS

For the most recent funding period, we would like to highlight the following progress:

1) *Histological analysis of ECM components (**relevant to All Aims**).* Using tissue from young and aged *CX3CR1*$^{EGFP/+}$ mice, high-resolution confocal imaging, and 3D reconstruction in Imaris, we have quantified changes in two key ECM components – hyaluronan and aggrecan - in multiple brain regions. In young adult mice, hyaluronan is abundant and filamentous in the ventral tegmental area (VTA) and much more fragmented in the nucleus accumbens (NAc). In the NAc, there were no significant changes in the abundance or structure of hyaluronan during aging. Instead, in the VTA, we found significant decreases in size and abundance of hyaluronan filaments, with hints that these changes begin to emerge as early as 13mo of age (**Fig. 2** and **3**). From these same mice, we have completed immunostaining and image

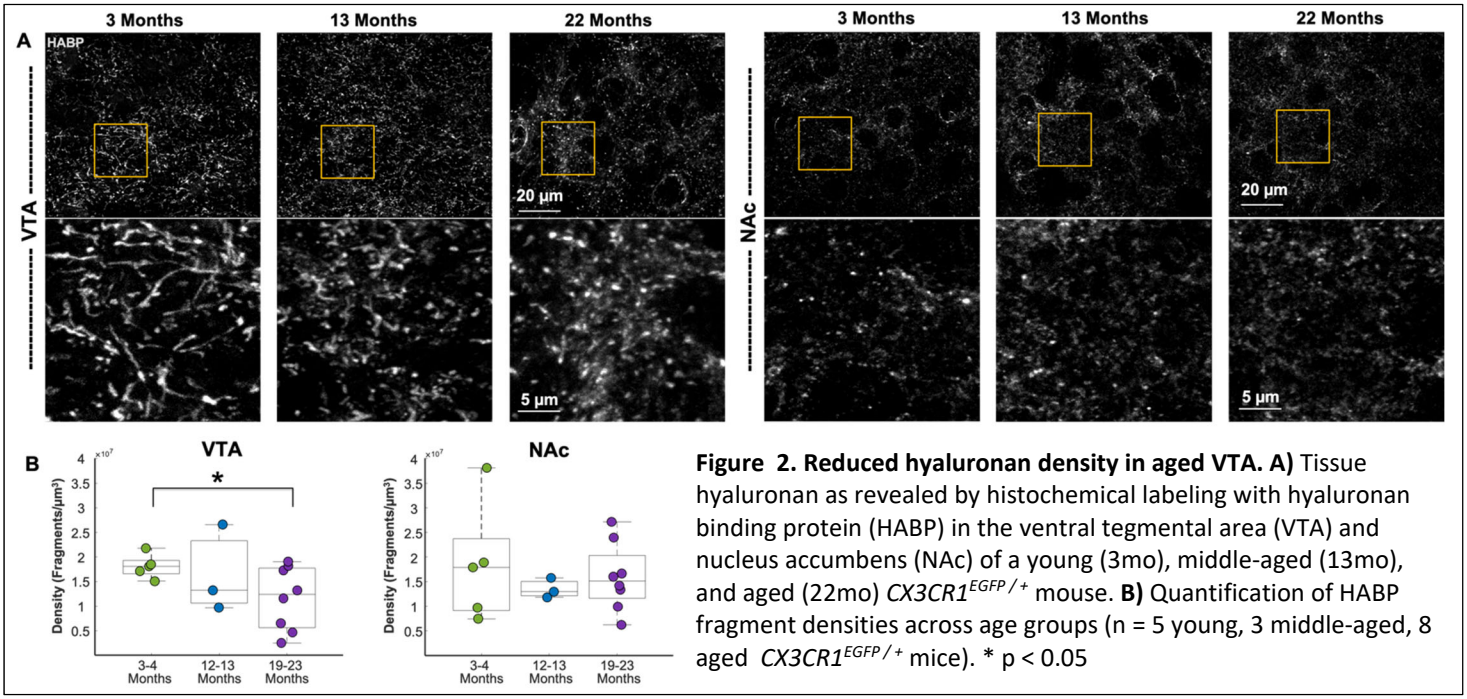

Figure 2. Reduced hyaluronan density in aged VTA. A) Tissue hyaluronan as revealed by histochemical labeling with hyaluronan binding protein (HABP) in the ventral tegmental area (VTA) and nucleus accumbens (NAc) of a young (3mo), middle-aged (13mo), and aged (22mo) *CX3CR1*$^{EGFP/+}$ mouse. **B)** Quantification of HABP fragment densities across age groups (n = 5 young, 3 middle-aged, 8 aged *CX3CR1*$^{EGFP/+}$ mice). * p < 0.05

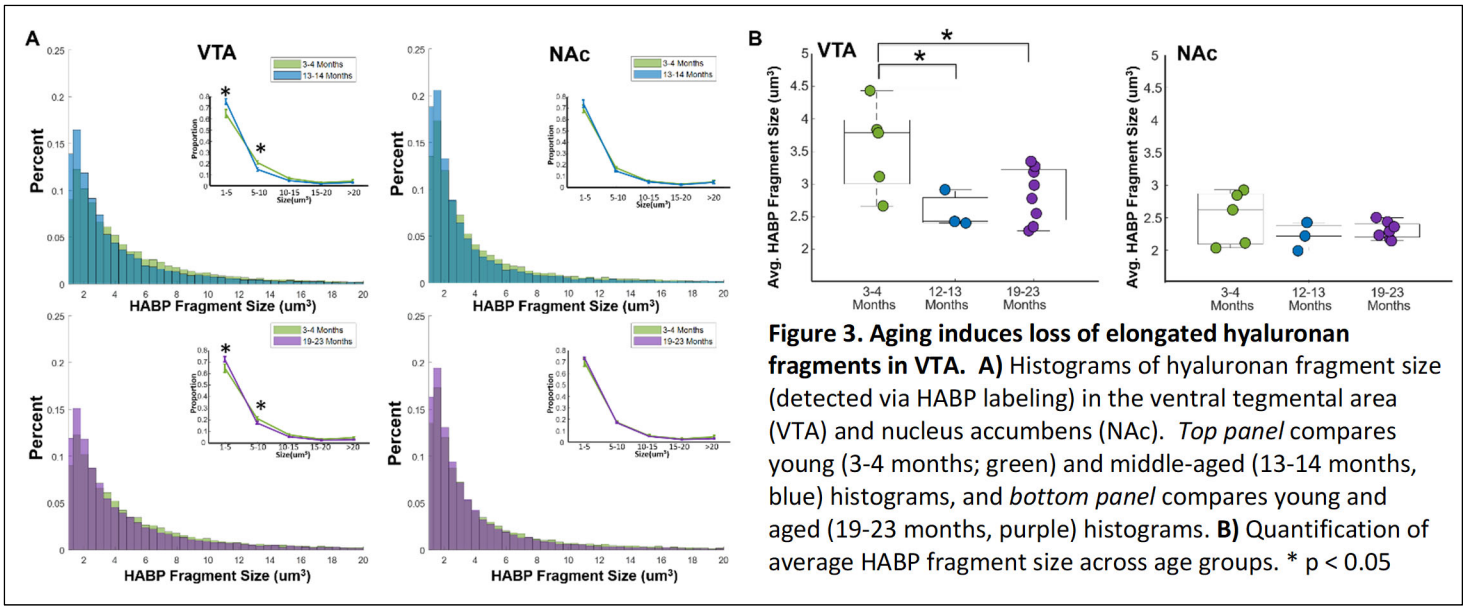

Figure 3. Aging induces loss of elongated hyaluronan fragments in VTA. A) Histograms of hyaluronan fragment size (detected via HABP labeling) in the ventral tegmental area (VTA) and nucleus accumbens (NAc). *Top panel* compares young (3-4 months; green) and middle-aged (13-14 months, blue) histograms, and *bottom panel* compares young and aged (19-23 months, purple) histograms. **B)** Quantification of average HABP fragment size across age groups. * p < 0.05

acquisition for the prefrontal cortex and hippocampus and analysis of hyaluronan structure and abundance is 60-70% complete. In addition, immunostaining and image acquisition for aggrecan has also been carried out in tissue from these same mice. Similar to hyaluronan, aggrecan shows prominent regional differences in its abundance and distribution in young adult mice (*not shown*), being most abundant in cortex and hippocampus and least abundant in the VTA and NAc. Analysis of aging-induced changes in aggrecan structure and abundance is ongoing. Additional analyses that we plan to carry out include defining spatial relationships between hyaluronan, aggrecan, and local synapses. **These data acquired so far confirm that ECM structure and composition varies significantly across brain regions and that aging impacts ECM architecture differently in distinct brain regions. These observations highlight the importance of defining whether local ECM structure is a critical determinant of which neurons and synapses are most vulnerable to decline during aging.**

2) *Histological analysis of microglial interactions with key ECM components (**relevant to All Aims**).* The observation that hyaluronan becomes more fragmented in VTA during aging is intriguing because hyaluronan of different molecular weights can interact with and signal through distinct receptors, many of which are expressed by microglia (e.g. TLR4, CD44, and LYVE1)[1,2] In the same tissue described above, we reconstructed microglial cells and analyzed the degree of contact between microglia and hyaluronan (**Fig. 4**). This analysis revealed significant decreases in putative contacts between VTA microglia and hyaluronan beginning as early as 13mo of age. This decrease was not observed in NAc microglia, which displayed fewer contacts with hyaluronan to begin with, even in young adult mice. These observations raise the possibility that, in addition to microglia shaping the ECM, the changing ECM could play an important role in regulating microglial cellular architecture. For example, microglia may use contact points with hyaluronan to stabilize portions of their cell processes and loss of microglial-hyaluronan contact points could drive simplification of microglial morphology observed during aging[3]. Moving forward, we will also analyze: 1) whether microglial-hyaluronan contacts occur preferentially at microglial somas, proximal cell processes, or distal cell processes, 2) whether there are correlations between morphological complexity of individual microglial cells and local hyaluronan, and 3) whether microglia exhibit a preference for contact with fragmented versus filamentous hyaluronan. We have also acquired images from hippocampus, retrosplenial cortex, and prefrontal cortex and analysis of microglial-hyaluronan interactions in these regions is ongoing Similar analysis of microglial interaction with aggrecan is also underway. **Revealing that ECM interactions play a critical role in stabilization of microglial morphology would have critical implications for changing ability of these cells to survey surrounding tissues and synapses during aging.**

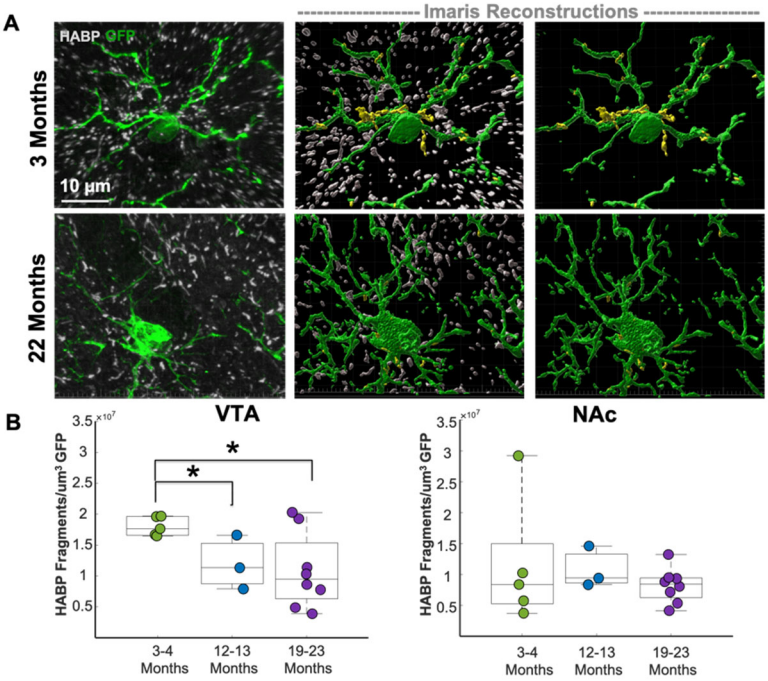

Figure 4. Fewer microglia-hyaluronan contacts in VTA of aged mice. **A)** Immunohistochemistry for hyaluronan binding protein (HABP) in the VTA of a young (3mo) and aged (22mo) *CX3CR1$^{EGFP/+}$* mouse. *Middle and right panels* show Imaris reconstruction of microglia and HABP followed by filtering for HABP fragments contacted by microglia. **B)** Quantification of the average number of HABP fragments contacted by microglia, relative to microglial volume across age groups. * = $p < 0.05$

3) *Unbiased proteomic analysis of changes in ECM composition during aging (**relevant to Aim 3**).* Using a tissue fractionation kit from Millipore, we carried out proteomic analysis of midbrain (MB) and striatum (STR) tissue from young and aged wildtype mice. Initial analyses revealed that ECM components are primarily found within the lowest solubility fractions (**Fig. 5**). Using this cellular fractionation strategy will

allow us to quantify both changes in overall abundance of specific ECM components during aging, as well as changes in solubility of individual ECM components, which is important for their functional impact[4].

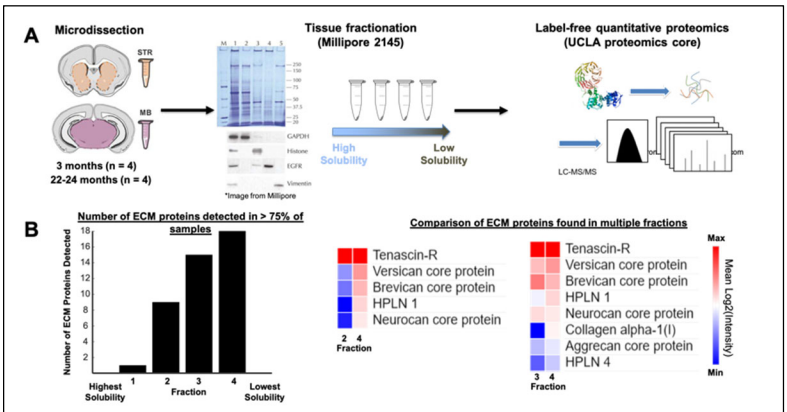

Figure 5. Tissue fractionation for enrichment of ECM proteins prior to proteomics. A) Proteomics Workflow. **B)** The lowest solubility fraction contained the most detectable ECM proteins.

Comparative analysis revealed that hyaluronan binding protein 2 (Hapln2) levels were significantly elevated in midbrain tissue from aged mice (**Fig. 6**). It is also elevated in striatal tissue, although this increase did not reach significance. Hapln2 is an important linking protein that mediates interaction of multiple proteoglycans with hyaluronan scaffolds throughout the tissue. **Hence, this unbiased approach also points to important changes within hyaluronan scaffolds during aging, particularly in midbrain.** It is possible that upregulation of Hapln2 represents a compensatory response to loss of signaling normally mediated by filamentous, high molecular weight hyaluronan. We will use immunostaining with antibodies for Hapln2 to determine whether spatial interactions between hyaluronan and Hapln2 are altered during aging (e.g. – is Hapln2 still bound to hyaluronan and tethering proteoglycans onto the hyaluronan scaffold or is Hapln2 aggregating independently of hyaluronan? Do microglia also exhibit specific interactions with this link protein?). We have also collected and fractionated tissue samples from hippocampus and prefrontal cortex from the same young and aged mice and mass spec / proteomics analysis of those samples is underway. This will reveal whether upregulation of Hapln2 is a common phenomenon observed in most CNS regions during aging. Other interesting observations that emerged from our initial proteomics include: significant increases in collagen alpha 2 (VI) in midbrain and potential decreases in brevican, tenascin-R, and glypican-1 in both regions. Analysis to look for correlations between abundance and solubility of ECM components and: 1) local inflammation, 2) local synaptic proteins, 3) ECM synthetic enzymes, and 4) ECM sensing receptors is also underway. **To our knowledge, these data represent the first proteomic mapping of ECM composition in multiple brain regions during aging. These data are made more powerful by incorporating the ability to assess changes in solubility in addition to changes in overall protein abundance.** In future experiments, we will use this workflow to carry out proteomic mapping of the ECM and synapse integrity in behaviorally characterized aging mice.

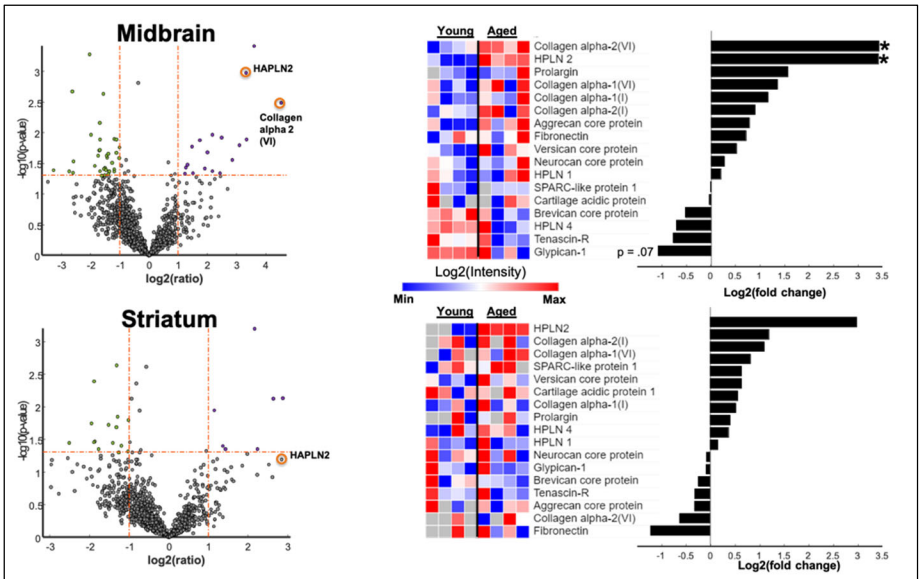

Figure 6. Hyaluronan and proteoglycan link protein 2 (Hapln2) levels increase in the midbrain during aging. Aging-induced changes in abundance of specific ECM components in the low solubility fraction from midbrain and striatum (n= 4 young, 3mo old and 4 aged, 22-24mo old wildtype mice). * $p < 0.05$

4) *Assessment of spatial and reward-based learning in young adult and aging mice (**relevant to Aims 2 and 3**).* We have now moved two full cohorts of young and aged mice through our learning paradigm. The paradigm, which we have refined over the past year, consists of a habituation phase, a training phase, and a test phase. During habituation, mice are allowed 5 minutes to explore an open arena containing a single sand well baited with a palatable food pellet reward. Mice undergo 5 habituation sessions. In the training phase (**Fig. 8**), mice are placed in the arena for a 5-minute encoding period with one rewarded sand well. They are then returned to the arena following a 30-minute delay for a retrieval period in which the same

rewarded sand well is present along with 3 distractor wells placed throughout the arena. Mice are allowed to forage for 2 minutes during the retrieval period. Mice undergo 1 training session per day for 10 total training sessions. The rewarded location is randomized between mice within a day and varies from day to day for any individual mouse. The protocol for the test phase is identical to the training phase with two exceptions. First, the delay period between encoding and retrieval varies between either 2 hours or 24 hours, and second, mice undergo a 2-minute probe trial in which 4 unbaited wells are present, 1 of which is in the location that was rewarded during encoding. This probe trial allows us to measure the proportion of foraging time a mouse spent at the previously rewarded location compared to the unrewarded locations (e.g., a discrimination ratio) and rules out that mice are using olfactory cues alone to navigate to rewarded wells. Mice undergo one test session per day for a total of four 2-hour delay sessions and four 24-hour delay sessions, and no rewarded location is used more than once.

Our results indicate that both young and aged mice require between 10-15 sessions to begin foraging for food consistently, and consume the same number of treats on average within a session once they have begun to forage consistently. These observations indicate that young and aged mice are similarly motivated to engage in the task. Analysis from the test phase of the task indicates that, compared to young mice, aged mice spend a greater proportion of foraging time at previously rewarded locations during unbaited probe trials after a 2-hour delay. This is not the case following a 24-hour delay (**Fig. 8C**). Difference measures between the 24- and 2-hour delays demonstrate that aged mice all performed more poorly following a 24-hour delay, whereas most young mice performed better after a 24-hour delay (**Fig. 8C**). We attribute this improvement in the young mice to increased motivation during the 24-hour probe trial, as it represents the first foraging experience within a day whereas the 2-hour delay probe trial is the second foraging bout within a day.

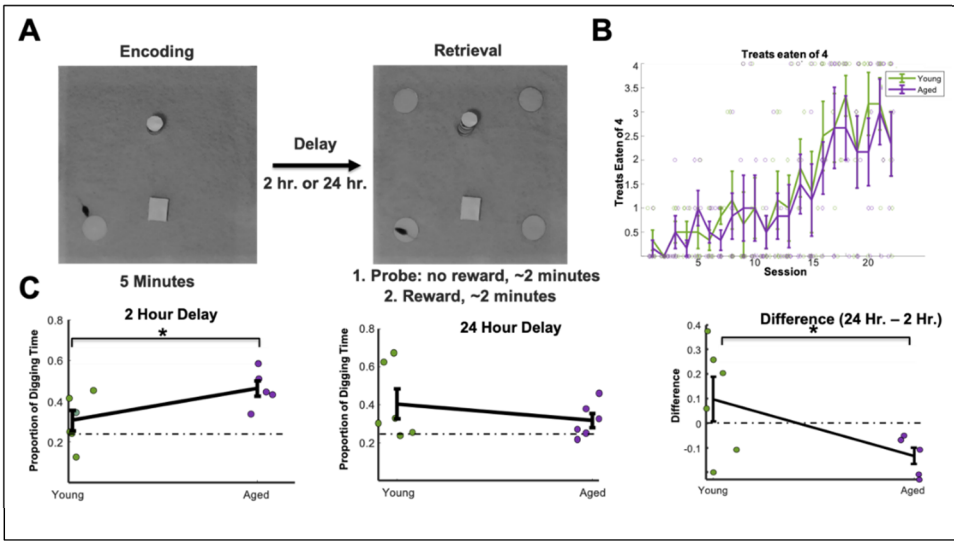

Figure 8. Deficits in spatial- and reward-based learning / memory are evident by late middle age in mice. A) Schematic of foraging-based spatial reference memory task. **B)** Number of treats eaten out of 4 across sessions for young (4-5mo old, green) and aged (17-18mo old, purple) mice. **C)** Proportion of time spent investigating the rewarded feeder during probe trials following a 2 or 24 hour delay as well as the difference in behavior between these delay lengths (24 Hr. – 2 Hr.). * $p < 0.05$.

We have collected tissue from these mice, preserving one hemisphere for histological analysis (hyaluronan, aggrecan, microglial-ECM contact, synapse integrity, patterns of neuronal activation) and one hemisphere for analysis of gene expression (ECM synthetic enzymes, ECM degradative enzymes, ECM sensing receptors). We are planning to use Vizgen Merfish for spatial transcriptomics rather than 10x Genomics Visium, as it allows for single-cell resolution to characterize the impact of behavioral engagement on gene expression patterns. Tissue from future cohorts of mice will also be prepared for quantitative proteomic analyses as described above to obtain unbiased quantification of protein-level changes following behavioral engagement. **Together these data will provide currently unavailable insight into how cellular and molecular level changes in multiple brain regions (ECM composition, microglia-ECM interactions, ECM-synapse interactions, and synapse integrity) align with cognitive performance in individual mice. These experiments will also reveal cellular and molecular targets that look most promising for preserving synapse integrity and cognition during aging.**

We have decided to include, as an additional component of this behavior experiment, a group of control mice in every cohort that does not undergo the behavioral assessment but lives in the same vivarium as the behaviorally-trained mice. Because the behavioral paradigm consists of 5 weeks of training/testing, and is entirely reward-based, the behavior could be considered a form of environmental enrichment. The inclusion of a no-behavior control will allow us to study the potential impact that behavioral enrichment has on ECM and microglial properties in both young and aged mice.

CHANGES TO ORIGINALLY PROPOSED EXPERIMENTS

1) To further probe the relationship between microglial-ECM interactions and cognition, we originally planned to acquire *Itgb1*$^{fl/fl}$ mice and *CtsS* knockout mice to define how altering microglial sensing of the ECM (via Itgb1 removal) or degradation of the ECM (via CtsS removal) shaped synapse integrity and behavioral performance. Given the prominent changes we observed in hyaluronan scaffolds, we are weighing a potential pivot to deletion of microglial receptors known to interact with hyaluronan (Tlr4, CD44). We have also decided to pursue pharmacological manipulation of the hyaluronan matrix to determine the impact on microglial morphology, process motility, gene expression, and interactions with synaptic structures. To accomplish this, we have acquired the compound 4-methylumbelliferone (4-MU), which acts as a competitive substrate for uridine diphosphate glucuronyltransferase, a key enzyme in hyaluronan synthesis. 4-MU is delivered to the mice as a dietary supplement and previous studies have demonstrated that between 1 and 2 months of treatment is sufficient to significantly reduce brain hyaluronan levels[5,6]. Following treatment, tissue from 4-MU and control mice will be used for immunohistochemistry, RNA sequencing, and/or proteomic analyses using protocols similar to those described above. In addition, we will use these mice to perform live imaging experiments of microglial process motility and surveillance to assess the impact that the state of the hyaluronan matrix has on microglial physiology and synapse interactions. We anticipate completing 4MU based experiments within the next 4-6 months. These experiments and further analysis of proteomic data in behaviorally-characterized mice will be used to finalize which transgenic manipulations we elect to pursue.
2) We initially proposed to analyze microglial-ECM interactions and synapse integrity in behaviorally characterized rats with the possibility to expand into analysis of non-human primates in future studies. We have decided to prioritize analysis of macaque tissue, as this analysis has greater potential to reveal which observations in mice are likely to be most translationally relevant. Through collaboration with Carol Barnes, we have transferred to our lab brain sections from her tissue bank of cognitively characterized young, middle age, and aged macaques. We have acquired necessary slides and reagents for histological analysis of microglia, ECM, and synaptic structures in these tissues. With the fluorescence lifetime capability of our Leica Stellaris microscope, we anticipate being able to very cleanly separate signals from labeled structures and the prominent autofluorescent aggregates present in tissue of aged macaques. We anticipate completing staining, imaging, and analysis of these tissues within the next 4-6 months.

NEW COLLABORATIONS. We have been working with Dr. Sotiris Masmanidis (Department of Neurobiology, UCLA) to use machine learning based analysis of gait in young and old mice (**Fig. 9**). Initial experiments suggest important relationships between changes in gait (e.g. increased asymmetry of gait) and cognitive performance in aging mice run through our spatial reference memory task. We plan to continue exploring this relationship and will collect gait data for all mice run through the spatial reference memory task prior to euthanasia.

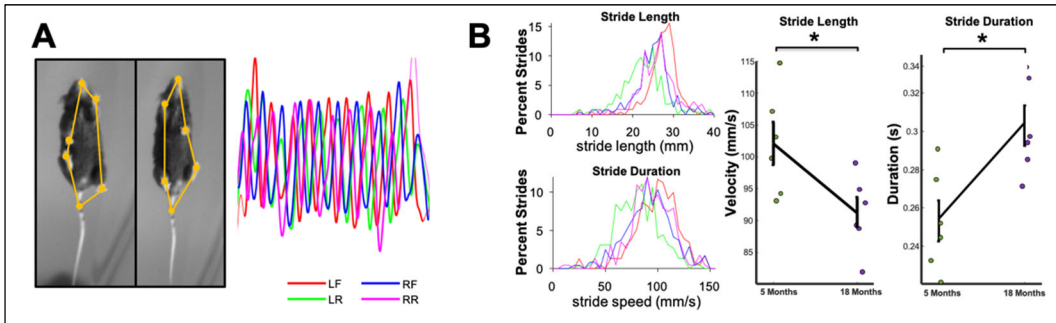

Figure 9. Detailed analysis of mouse gait. A) Representative image of gait-tracking analysis using SLEAP.ai software and individual paw tracking data during bouts of locomotion. **B)** Histograms of stride length and duration for each paw from a single aged mouse and comparisons of stride length and duration between 4 month (n=6) and 17 month (n=6) mice. * $p < 0.05$.

PLANS FOR ADDITIONAL FUNDING

1) Abigail Gutierrez has been instrumental in helping run mouse behavior and in analysis of the relationship between mouse behavior and neuronal activity (via immunostaining for Fos). She plans to work as a

research technician for 1-2 years following graduation while applying to MD/PhD graduate programs. We will apply for an NIH diversity supplement to support her post baccalaureate training.

2) Together with Dr. Masmanidis, we plan to apply for a Larry Hillblom Network Grant to further explore the relationship of gait and changes in cognition. In particular, we would hope to develop a mouse paradigm for assessing dual task walking, which has high validity in humans for predicting future cognitive decline. We could then align this with information about glial, ECM, and synapse changes, thus revealing how an important behavioral biomarker (impaired performance in dual task walking) is linked to cellular and molecular players that could be the most promising therapeutic / preventative targets.
3) Dr. Gray's long-term career goal is to establish his own independent research program with a focus on cellular and molecular determinants of cognitive function during aging. We plan to submit a K99/R00 application within the next year to begin laying the groundwork for his eventual transition to independence.

PLANS FOR SCIENTIFIC MANUSCRIPTS. We anticipate submitting an initial manuscript detailing our spatial reference memory task and relationship to changes in microglial density in the spring / summer of 2023.

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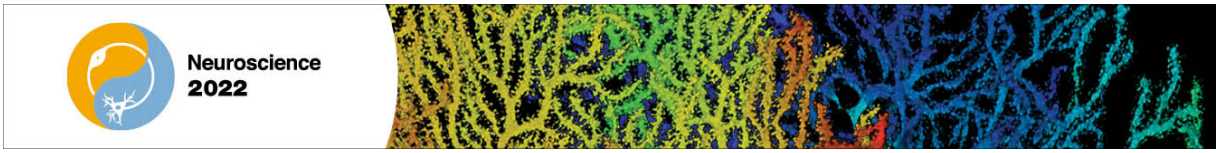

(https://www.abstractsonline.com/pp8/#!/10619)

Session 273 - Microglial Mechanisms of CNS Injury and Disease

Add to Itinerary

273.11 / C46 - Microglial regulation of the brain extracellular matrix may play critical roles in age-associated memory dysfunction

November 14, 2022, 8:00 AM - 12:00 PM | SDCC Halls B-H

Presenter at Poster
Mon., Nov. 14, 2022 9:00 AM - 10:00 AM

Session Type
Poster

Grant Support
AFAR Grant MCKNIGHT21003

Citation
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Authors
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Disclosures
D.T. Gray: None. **A. Guiterrez:** None. **C. Charway:** None. **M. Haratian:** None. **C.A. Barnes:** None. **L.M. De Biase:** None.

Abstract
The brain extracellular matrix (ECM) is a complex network of proteins and sugars secreted by multiple cell types that provides neurons, glia, and vasculature with structural and biochemical support. Accumulating evidence indicates that microglia, the innate immune cells of the brain, regulate the ECM through release of degradative enzymes, by directly engulfing ECM proteins, and by signaling to other cell types that regulate the ECM. Several studies have shown that aging is accompanied by region-specific changes in the abundance of specialized ECM structures (e.g., perineuronal nets), and proteins that synthesize ECM scaffold proteins. Our group has shown that microglia also display regional differences in their responses to aging, raising the possibility that these cells play critical roles in determining which populations of neurons become most vulnerable to functional decline across the lifespan. However, whether age-associated changes in microglia, the ECM, or microglial regulation of the ECM have protective or detrimental impacts on cognition later in life has not been clearly determined. Here, we present findings from novel, label-free quantitative proteomics analysis of midbrain (MB) and striatal (STR) tissue from young (n=4, 3mo) and aged (n=4, 24mo) mice that was separated into high- and low-solubility fractions to better isolate ECM proteins. This previously unavailable map of the aging ECM reveals age-associated increases in critical ECM proteoglycans (e.g., aggrecan; MB fold change: 0.7, STR fold change: 4.1), link-proteins (e.g., hyaluronan and proteoglycan link protein 2; MB fold change: 3.3, STR fold change: 8.2), and proteins associated with the vasculature-associated basement membrane (e.g., vitronectin; MB fold change: 2.4, STR fold change: 1.8). These findings suggest that aging is accompanied by dysregulation in the synthesis and/or turnover of ECM proteins and that the magnitude of these changes varies across brain regions. These changes may critically impact brain processes the ECM is known to regulate, including synaptic plasticity and vascular integrity. Next, using both rodent and nonhuman primate models of brain aging, we show that age-related changes in microglia are associated with changes in the ECM in older brains, and that these relationships may negatively impact cognitive function. Finally, we highlight preliminary data and future directions from ongoing experiments that combine behavioral assessments, high-resolution imaging, molecular biology, and proteomic analyses to specifically test the hypothesis that aberrant

microglial remodeling of the ECM is a central driver of age-related memory impairments.

Villeda, Saul, 2022, McKnight Innovator Award

LAY SUMMARY OF PROGRESS

Evidence indicates that caloric restriction can counter age-related decline in neurogenic and cognitive processes in the aged brain. Despite the evident benefit of caloric restriction, its application is hindered in the elderly by technical barriers. The research described in this progress report aims to challenge prevalent views of brain aging as a rigid process by investigating the potential of circulating blood factors to confer benefits of caloric restriction while circumventing pre-existing limitations in the elderly. Behavioral data generated in my lab indicate that late-onset, short-term caloric restriction initiated late in life in aged mice can rejuvenate cognitive function in the aging hippocampus – a brain region highly vulnerable to the effects of aging. Furthermore, using systemic administration of blood plasma derived from late-onset, short-term caloric restricted aged mice, we demonstrate that the benefits of caloric restriction on hippocampal-dependent learning and memory can be transferred to *ad libitum* fed aged mice through circulating factors in blood. Studies building on this foundational evidence will have significant translational potential, identifying molecular pathways that could be targeted for novel therapies to restore age-related cognitive dysfunction and potentially treat dementia-related neurodegenerative disorders.

SPECIFIC AIMS/OBJECTIVES

The goal of the proposal is to investigate the rejuvenating potential of calorie restriction (CR)-induced blood factors on the aged hippocampus at the molecular, cellular, and cognitive level. *We hypothesize that the rejuvenating effects of CR on cognitive function can be transferred through circulating blood factors.* We are testing this theory with two Specific Aims:

1. Determine molecular mechanisms downstream of CR that underlie cognitive rejuvenation in the aged brain.
2. Investigate the rejuvenating potential of CR-induced blood factors on cognitive function in the aged brain.

ACCOMPLISHMENTS/RESEARCH PERFORMED

The following data has been generated by my research group in support of the specific aims highlighted in the objectives of the project.

Late-onset, short-term CR improves hippocampal-dependent learning and memory in aged mice. Previously, our lab assessed the potential of short-term CR to rescue age-related impairments in hippocampal-dependent learning and memory using novel object recognition (NOR) behavioral paradigms. Preliminary data included in our original submission demonstrated that CR aged animals exhibited enhanced object and spatial learning and memory compared to *ad libitum* (AL) aged mice. These behavioral data indicated that short-term, late-onset CR is sufficient to rejuvenate hippocampal-dependent cognitive function at old age. To build upon these preliminary data, we have performed a more extensive cognitive assessment of hippocampal-dependent learning and memory in CR aged mice using NOR, radial arm water maze (RAWM) and contextual fear conditioning behavioral paradigms (**Figure 1**).

Aged (22-month-old) mice (late-onset) were subjected to a 30% reduction in daily caloric intake for 4 weeks (short-term). Age-matched aged mice placed on AL diet served as control. Changes in hippocampal-dependent learning and memory were measured using NOR, RAWM, and contextual fear conditioning (**Figure 1A**). For NOR, during the training phase a subject is allowed to interact with

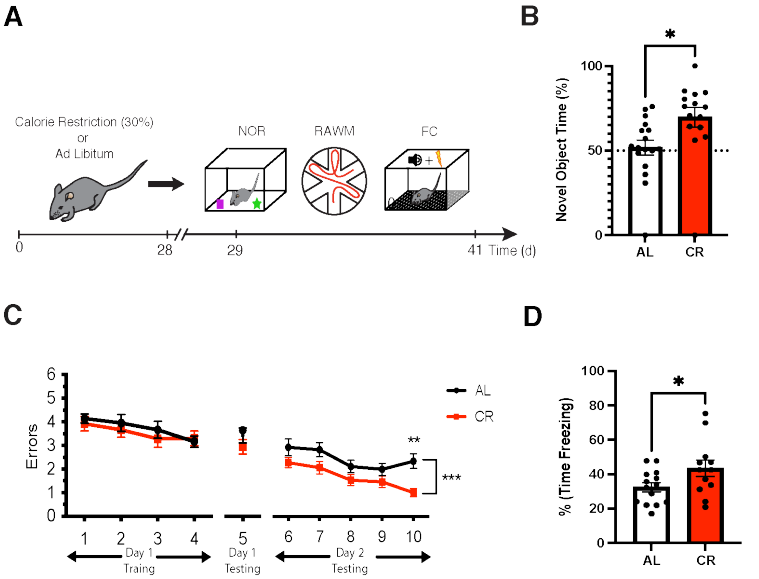

Figure 1. Short-term, late-onset calorie restriction improves cognitive function in aged mice. A. Novel object recognition (NOR), radial arm watermaze (RAWM), and fear conditioning (FC) used to assess hippocampal-dependent learning and memory in aged (22 months) mice following short-term (4 weeks) caloric restriction (CR, 30% decrease in food intake) initiated late in life (21 months) or ad libitum (AL) feeding. **B.** Object recognition memory assessed by NOR as percent time spent exploring a novel object 24 hours after training. **C.** Spatial learning and memory assessed by RAWM as number of entry errors committed during each block of the training and testing phases. **D.** Contextual fear memory was assessed by quantify the percent freezing time 24 hours after training. N=14 mice per group. Data represented as mean ± s.e.m; *P<0.05; **P<0.01; t-test (**b,d**), (two-way ANOVA with Šídák's correction for multiple comparisons **c**).

two identical objects in the exploration arena during the training phase. During the testing phase 24 hours later, the subject is placed back into the arena and one of the familiar objects is replaced with a novel object. Exploration time with the familiar and novel object is then quantified using the Smart Video Tracking Software (Panlab; Harvard Apparatus). Consistent with preliminary data, aged mice that underwent late-onset, short-term CR spent significantly more time with the novel object compared to AL control aged mice (**Figure 1B**).

For the RAWM paradigm, a pool of water was divided into 6 arms with a platform placed in a goal arm that was kept constant. A series of spatial cues surrounded the pool such that animals used spatial orientation to learn the location of the platform. During the training phase on day one, mice were trained for 15 trials, with trials alternating between a visible and hidden platform. During the testing phase on day two, mice were tested for 15 trials with a hidden platform. Entry into an incorrect arm was scored as an error, and errors were averaged over training blocks (three consecutive trials). In the RAWM, all aged mice showed similar spatial learning capacity. However, late-onset, short-term CR aged mice committed fewer errors compared to AL control aged mice (**Figure 1C**).

For the contextual fear-conditioning paradigm, mice learned to associate the environmental context (a fear conditioning chamber) with an aversive stimulus (a mild foot shock; unconditioned stimulus). Conditioned fear was measured following each chamber exposure as freezing behavior using a FreezeScan video tracking system and software (Cleversys, Inc). On day one each mouse was placed in a fear-conditioning chamber and allowed to explore for two minutes before delivery of a 30-second tone (70 dB) ending with a two-second foot shock (0.6mA). Two minutes later, a second CS-US pair was delivered. On day two each mouse was placed in the fear-conditioning chamber containing the same exact context, but with no administration of a CS or foot shock. All aged mice exhibited similar baseline freezing (data not shown). Interestingly, late-onset, short-term CR aged mice demonstrated increased freezing in contextual memory testing compared to AL control aged mice (**Figure 1D**). 24 hours after behavior was completed, one brain hemisphere was collected and processed for histological analysis. And the contralateral hemisphere was processed for transcriptional profiling and biochemical analysis. Together, these behavioral data further validate that late-onset, short-term CR is sufficient to rejuvenate hippocampal-dependent learning and memory in aged mice.

Systemic administration of blood plasma derived from late-onset, short-term CR aged mice improves hippocampal-dependent learning and memory in AL aged mice. To investigate the role of CR-induced circulating blood factors in rejuvenating the aged brain, blood plasma was isolated from aged mice that either underwent late-onset, short-term CR or were fed AL diet. Aged mouse blood was collected by intracardial bleed and followed by centrifugation at 1,000 g for 10 minutes for plasma preparation. Plasma was aliquoted for systemic administration studies and for future mass spectrometry analysis. Prior to use for systemic administration, plasma was dialyzed using 3.5-kDa D-tube dialyzers (EMD Millipore) in saline to remove any remaining anticoagulant. Subsequently, aged AL mice were systemically treated with either CR or AL control blood plasma (100 µL/injection) via intravenous tail vein injection eight times over 24 days. Hippocampal-dependent function was then assessed using NOR and RAWM (**Figure 2A**).

Remarkably, systemic administration of CR blood plasma was sufficient to reverse age-related cognitive decline at old age. For NOR, aged mice that received blood plasma from late-onset, short-term CR aged mice spent significantly more time with the novel object compared to aged mice that received blood plasma from aged AL mice (**Figure 2B**). For RAWM paradigm, aged mice receiving systemic administration of CR or AL aged blood plasma showed similar spatial learning capacity. However, during the testing phase, aged mice

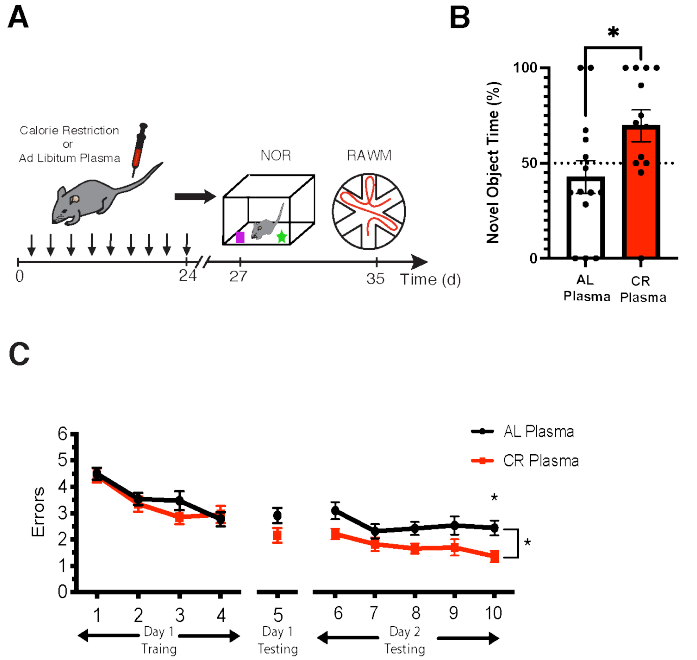

Figure 2. Systemic administration of calorie restriction-induced blood factors improves cognitive function in aged mice. A. Plasma was collected from aged (22 months) mice on CR or AL diet and administered to aged mice on AL diet 8 times over 24 days (100 µl per intravenous injection). Hippocampal-dependent learning and memory was then assessed with NOR and RAWM. **B.** Object recognition memory assessed by NOR as percent time spent exploring a novel object 24 hours after training. **C.** Spatial learning and memory assessed by RAWM as number of entry errors committed during each block of the training and testing phases. N=14 mice per group. Data shown as mean±s.e.m.; *P<0.05, **P<0.01, t-test (B); two-way ANOVA with Šídák's correction for multiple comparisons (C).

receiving CR aged blood plasma committed fewer errors compared to aged mice receiving AL aged blood plasma (**Figure 2C**). 24 hours after behavior was completed, one brain hemisphere was collected and processed for histological analysis. And the contralateral hemisphere was processed for transcriptional profiling and biochemical analysis. Collectively, these behavioral data indicate that the benefits of late-onset, short-term CR on cognitive function can be transferred through circulating factors in blood at old age.

NEW FUNDING
1. NIH/NIA R01AG077770: Pro-youthful role of Gpld1 on regenerative and cognitive function in the aged brain.
2. NIH/NIA R01AG077816: Systemic mechanisms of brain rejuvenation.

2023 McKnight Brain Research Foundation Innovator Awards in Cognitive Aging and Memory Loss Committee Recommendations

The selection committee met September 7, 2023, to review the 2023 applications for the MBRF Innovator Awards in Cognitive Aging and Memory Loss. Five applications received a full review by the Selection Committee. In 2023, Dr. Roy Hamilton, MBRF Trustee and Professor of Neurology at the University of Pennsylvania, joined the committee.

The committee members were asked to review and rank the applications prior to the review meeting. Dr. Boyle reviewed the two applications that were designed as more clinically focused, and Dr. Hamilton was asked to review the three more basic science-focused applications. Dr. Hamilton had a conflict of interest with one of the applications and did not score or participate in the discussion. The committee was asked to rank the applications in two separate groups – basic and clinical. During the meeting, each of the five applications were discussed in detail. The committee identified two applications belonging in different categories and ultimately recommended the following two applicants:

Basic Science-focused Application:
Denise Cai, Ph.D., Associate Professor, Icahn School of Medicine at Mount Sinai: *Memory stability and flexibility across a lifetime.*

Project summary (provided by the applicant): There have been significant advances in the molecular, cellular, and systems mechanisms underlying the storage of single memories. Real-world memory, however, involves the integration of multiple memories across the lifetime, with one memory affecting how others are processed and stored. The central goal of this proposal is to investigate how the brain stably stores and flexibly updates memories across a lifetime. Aging is inevitable, but cognitive deficits may not have to be. During middle age, memory deficits are more subtle; and numerous studies have shown that middle-aged mice can stably remember individual memories (contextual and spatial memories) as well as young adult mice, but middle-aged mice have trouble flexibly updating their previous memory with new information. By tracking the neural activity of hundreds of neurons in freely behaving mice as they form multiple spatial maps during young adulthood and middle age, we will unveil how the brain stably stores and flexibly integrates memories across a lifetime. In addition to making fundamental insights into learning and memory processes, we hope to develop both biomarkers and behavioral markers that can predict subsequent age-related cognitive deficits and provide early intervention to prevent or slow down age-related cognitive decline. Two fundamental and related questions surrounding the mechanisms responsible for information processing in the brain underlie the research project described here. **First, how does the brain stably store past spatial maps while flexibly learning new spatial maps?** To address this question, we will attempt to identify the rules that govern how information is accumulated in the brain over time and experience. **Secondly, how does memory stability and flexibility change with normal aging?** We will investigate how the processes governing memory accumulation over time affect both the aging brain's capacity and its ability to discriminate among similar experiences. Given the staggering number of memory disorders associated with age, it seems likely that at least part of these memory difficulties arise as a consequence of the burden of

housing such a vast amount of information on a finite amount of 'disc space'. Addressing this question is therefore paramount to addressing age-related cognitive decline.

There was overall agreement by the committee that Dr. Cai is a strong candidate with an exciting and innovative model. While this was initially identified as a clinical application, the translation is in early stages and should be considered a basic science proposal as it is asking fundamentally important questions about memory and aging.

- Strong preliminary data
- Research could be impactful
- Applicant has very strong record of success securing funding before and a good publication record (despite the impact that the pandemic had in NYC labs) and is on an upward trajectory
- Very innovative idea
- Some issues with design regarding age and number of animals – needs an additional age group.

The committee recommends funding this candidate and providing her with some feedback on the animal model and age groups and on the importance of including both sexes. Dr. Cai should be able to do this within her current budget and start -up package.

Clinically-focused application:
Christopher Thaiss, PhD, Assistant Professor, University of Pennsylvania: *Counteracting age-associated cognitive decline via gut-brain signaling*

Project Summary (provided by the applicant): Aging is associated with a decline in memory function, which greatly affects the quality of life of a large proportion of elderly individuals. The rate of cognitive decline is highly heterogeneous, with some individuals retaining fully intact memories at old age, while others lose the ability to participate in public life due to a dramatic inability to form and recall memories. New strategies to understand and counteract the age-associated decline in memory function are thus urgently needed. **This proposal will explore the new hypothesis that age-associated cognitive decline is not solely brain-autonomous but regulated by interoceptive pathways originating in the gastrointestinal tract**. Based on exciting unpublished findings, we hypothesize that communication pathways between the gastrointestinal tract and the brain progressively lose function with age, and that restoration of these pathways counteracts age-associated memory loss. We propose to chart a pathway that links molecules of microbial origin in the gastrointestinal tract, enteroendocrine cells in the intestinal epithelium, afferent sensory neurons, and the formation of memory engrams in the hippocampus. **Our approach provides a new framework for how age-related diseases of the brain may be treated by means of peripheral intervention from the gastrointestinal tract.**

The committee is also recommending with great level of enthusiasm that Dr. Thaiss is supported.

- This was everyone's top-ranked proposal
- The concept is not new but the approach is very innovative
- Very well written proposal

- Impressive platform that has already shown this work can be done to address other biological questions
- Great translational significance
- Candidate is a rock star, new to aging, would be an asset if we could bring him to the field

Discussion:

This is the last cohort selected through the current grant. If the foundation considers continuation of the program there are several things we could explore:

- Increase the number of applications
  - AFAR has broad reach (over 7,000 contacts on our scientific mailing lists) but the MBRF Innovator Awards program has a very narrow set of eligibility requirements. We do get excellent candidates and the quality of the applications has increased every year but should we aim to reach more?
  - Have former McKnight Fellows give some talks/do some promotions/webinars conference workshops
  - Reach out to program officers at NIH to make them aware of this unique opportunity for their best mid-career awardees
- Matching Fund requirement
  - Is the matching fund requirement a barrier? AFAR has surveyed deans and department chairs about the matching funds and there was a mixed response. Well-funded schools can do it, but not schools that lack resources, so possibly there is some inequity.
  - Matching funds limits who can apply
  - Explore other ways institutions demonstrate commitment to the candidate
    - Assigned independent space, etc. More specific at this career stage?
    - Protected time – ask the institutions to confirm that they are aware of committed effort needed for the project and that this time is protected.
    - AFAR could do additional outreach/surveying if MBRF considers continuation of the program.
- Review other eligibility/selection criteria
  - R01 funding requirement/equivalent - do we need it?
  - Is this an award for well-funded investigators or for researchers who need the funds?

2023 Committee

Ana Maria Cuervo, MD, PhD, *Chair*
Albert Einstein College of Medicine

Rozalyn Anderson, PhD
University of Wisconsin, Madison

Patricia Boyle, PhD
Rush University
The McKnight Brain Research Foundation

Rafael de Cabo, PhD
National Institute on Aging

Roy H. Hamilton, MD, MS
University of Pennsylvania Perelman School of Medicine
The McKnight Brain Research Foundation

Madhav Thambisetty, MD, PhD
The McKnight Brain Research Foundation

Interim Report

Michael Kleiman

2805 - Assessing trajectories of discrete measures of speech behavior in age-related decline

Restatement of Specific Aims and progress achieved for each Aim

Aim 1: The first specific aim is to examine the trajectory of age-related speech degradation across age groups using discrete measures of speech behavior. To date, 15 participants with no cognitive impairment have been recruited for this study. Ten participants per half-decade age group were proposed to be recruited, and good progress has been made (Figure 1). For participants between 55 and 69, and 80 and 84, we have recruited approximately one third of the proposed participants. As expected, it has been more difficult to recruit participants above age 85 for two reasons; a) there are fewer participants available in the population able to participate in research at these older age ranges due to health concerns, and b) it is more common for cognitive impairment to manifest in later ages so they are less likely to test as cognitively normal. To address this, we will more aggressively recruit participants at these older ages. Three participants below age 55 were also recruited from other studies, which while not associated with this project may provide useful comparisons to older individuals.

Data collection has gone according to plan, and data quality is high. We have learned that one of the spontaneous speech questions we used early on ("Recall a recent dream") resulted in very short responses, so it was replaced with a more useful question ("Recall everything you ate yesterday") which was better able to provide the detailed responses required for our analyses. Another spontaneous speech question ("Please describe your morning routine") was recorded for all participants. Useful speech data was produced by all participants. Transcriptions took more effort than expected, with the Research Assistant regularly exceeding the allocated time. To mitigate, the PI (Dr. Kleiman) took over transcription correction to ensure accuracy and reduce effort required by the RA. Analysis for this Aim has not yet been performed but will begin once at least half of each age group has been recruited.

Aim 2: The second specific aim is to identify social and medical determinants of age-related speech degradation, based on previously collected cognitive, functional, neuropsychological, medical, demographic, lifestyle, and nutritional data. As this data is already available for every participant, no additional data collection is necessary. Analysis has not yet been performed but will begin once at least half of each age group has been recruited.

Aim 3: The third specific aim is to determine neurological correlates of age-related speech

degradation using neuroimaging (structural, diffusion, functional MRI). Neuroimaging scans will be obtained within six months of enrollment in this study. To date, MRI scans from 11 of the 15 participants have been collected, with the remaining four on track to be collected before the 6-month deadline. Analysis for this Aim has not yet been performed but will begin once at least half of each age group has been recruited.

Analysis: Part of the analysis pipeline, including data cleaning and preparation, has been completed. Pipelines for approximately half of available features is complete, including lexical diversity and complexity analysis and part of speech analysis. Pipelines to be completed include disfluency detection (automated) and audiometric preprocessing (e.g., laryngeal waver).

Describe obstacles, if any, in achieving each Specific Aim
See above

Plans for Next Year
Key plans for the remaining award period are the completion of recruitment (target of 10 participants per half-decade age group for a total of 80) and the development of the analysis pipeline as well as dissemination of results. Recruitment for older aged participants is to be given priority over younger age groups. Additional analysis pipelines are also expected to be developed in the near future, followed by preliminary data analysis once at least half of each participant age group is recruited and assessed.

Coursework and other educational opportunities and activities
To date, completed courses include a responsible conduct of research workshop, tutorials on neuroanatomy and neuroimaging analysis including FreeSurfer, fMRI, and DTI. In addition, the Harvard Annual Dementia Review course and the Alzheimer's Association International Conference was attended. Dr. Kleiman has also shadowed and observed clinicians and psychometricians administer clinical examination, neuropsychological testing, functional assessments, and cognitive interviews.

Publications, presentations, awards, resulting from current American Academy of Neurology support
No results have yet been generated from this project, and so no dissemination in publications or presentations has yet occurred. Preliminary results are expected to be ready for presentation at the 2023 Psychonomics Society meeting in November 2023, as well as the 2024 AAN meeting. Dr. Kleiman will not attend the April 2023 annual meeting of the American Academy of Neurology or the May 2023 meeting of the Association for Computational Linguistics (ACL) due to the birth of his first child, expected in late April 2023. He will instead attend and present at the 2024 meetings.

Please include a one page research summary written for a lay audience (minimum of 150 words). Please gear the summary to an audience with a 2nd grade reading level

This will be used by the American Academy of Neurology to promote the Research Program to current and potential donors.

Aging is a part of our lives -- there's no avoiding it. And just as our bodies age along with us, so do our brains. Our cognitive processes slow down ever-so gradually as we grow older, however (because these changes are so gradual) there aren't many great ways to measure exactly how these changes progress over time. My approach is to measure speech and language using new advances in artificial intelligence technology, so we can paint a better picture of what normal cognitive aging looks like. First, I ask participants to perform a handful of simple tasks, such as describing everything happening in a picture. Then, using the same technology found in Siri and Alexa, I take the recorded audio and create a transcript, and from this transcript I can measure a huge number of things, like the complexity of the word choices, how detailed the descriptions are, the rate of speech production, and how often pauses like "um" or "uh" are used. I also use the audio recording itself, which can give me information on physical characteristics such as wavering and pitch. From all of this, I analyze the data to determine what subtle aging looks like. For example, while language complexity probably wouldn't be affected much with age, we might find that the length of pauses before those complex words or phrases might increase, as it might take just a bit more time to find the right words. I also look for comparisons to the participant's demographics like sex and race, or other diseases they may have, so we can create a more detailed model.

Of course, there are other benefits to this research than just understanding how we age. In order for us to better identify abnormal decline, such as dementia in its earliest stages, we need to understand what normal decline looks like. My hope is that from this research we are able to better map the progression of normal decline, allowing us to notice the early signs of Alzheimer's disease and other dementias earlier than currently possible.

Mentor Comments

Thank you for being a mentor for Michael Kleiman on their American Academy of Neurology award.

Please comment on your mentee’s progress in achieving their specific research aims and their overall progress on the project.

I reviewed Dr Kleiman’s report and agree with its contents. Dr Kleiman has been working diligently on his project and has made great gains. We have been reviewing the early data and planning presentations and publications. This award has been important in his career developments and was key to his promotion from Instructor to Research Assistant Professor. I continue to support Dr Kleiman’s effort on the important project.

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July 25, 2023

Interim Expenditure Report

American Academy of Neurology Institute

McKnight Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss funded by the McKnight Brain Research Foundation Grant Agreement

PI: Michael Kleiman, PhD
TITLE: Assessing trajectories of discrete measures of speech behavior in age-related decline

For period 07/01/2022 – 06/30/2023

EXPENDITURES	
Salaries	$ 34,587.31
Fringe Benefits	$ 12,076.20
TOTAL COSTS	$ 46,663.51

If you should have any questions, please contact:
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Reem Waziry, MBBCh MPH PhD
Clinical Translational Research Scholarship | Final Scientific Report

PROJECT START DATE: 07/01/2021
REPORT DATE: 09/01/2023

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Pages: 172
References: 336

PROJECT SUMMARY AND AIMS

Despite advances in management of vascular health, risk of vascular disease and related cognitive morbidities including stroke continue to rise in aging populations. The pathogenesis of stroke is not fully understood despite the established evidence on "*classic risk factors*" that contribute to onset of these disorders and their cognitive related sequalae. Thus, traditional interventions or *"business as usual"* that determine risk or prognosis by calendar years are suboptimal for achieving primary and secondary prevention of vascular disorders and associated cognitive aging and age-related memory loss.

Biologically older individuals are at higher risk of developing first-time stroke and have higher burden of cerebrovascular and cardiovascular disease. In addition, inflammation and key genetic factors such as APOE allele carriership are suggested to play key roles in the susceptibility of an affected individual to age with intact cognition or progression to cognitive impairment and dementia. Therefore, Novel biomarkers measured in accessible body tissues are pivotal to improve precision and sensitivity to achieve optimal vascular and brain health in aging. In this project we aimed to delineate the role of aging biology in vascular and brain health with emphasis on stroke, dementia and cognitive health following stroke.

SUMMARY OF FINDINGS

- Blood-based aging physiology and some DNA methylation biomarkers are strongly associated with vascular disorders including stroke and are more precise and sensitive biomarkers of aging. **(Waziry et al. Neurology 2023)**

- Saliva-based telomere length and blood-based DNA methylation and physiology biomarkers likely represent different aspects of biological aging and accordingly vary in their precision as novel biomarkers for optimal vascular health. **(Waziry et al. Neurology 2023)**

- Variations in aging biology may influence individuals' ability to live healthfully in older age. **(Waziry et al. Neurology 2023)**

- Better understanding of advanced stages of Alzheimer's dementia (AD) and timely monitoring of its preventable complications are necessary to improve survival and quality of life in the AD population. **(Waziry et al. Neurology Clinical Practice 2023)**

- Alzheimer's dementia (AD) and more broadly neurodegenerative disorders should be recognized as chronic '*life-limiting illnesses*' rather than '*terminal diseases'* in light of the phases of disease progression, the varying survival following AD and promising advances in AD therapeutics. **(Waziry et al. Neurology Clinical Practice 2023)**

- Factors routinely collected for stroke patients are a useful resource for monitoring dementia progression in this population. Cardiovascular factors, stroke location, stroke-related disability and chronic brain

changes are key predictors of progression to dementia after ischemic stroke. **(Waziry et al. J Alzheimer Disease. 2022)**

- Risk of dementia following ischemic stroke is likely not affected by *APOE* ε4 carriership as a risk factor gene. These findings provide evidence support to guide eligibility of patients for inclusion in clinical trials on Alzheimer's and Related Dementias among stroke survivors. **(Waziry et al. 2023.** *Manuscript under review***)**

- In the relationship between stroke and cognition, inflammatory pathways including cytokines play a synergistic interaction role. In contrast, biological aging mediates the relationship between stroke and cognition. These results indicate different mechanistic roles of aging and vascular disease on cognitive aging and age-related memory loss. **(Waziry et al. 2023.** *Manuscript under review***)**.

PROJECT SCIENTIFIC OUTPUTS
Publications, presentations and awards

- **Publications**

1. **Waziry R**, Gu Y, Boehme A, Williams O. *Measures of Aging Biology in Saliva and Blood as Novel Biomarkers for Stroke and Heart Disease in older adults.* **Neurology. 2023** (In Press)

2. **Waziry R**, Williams O. *Is Alzheimer's A Terminal Disease?* **Neurology Clinical Practice. 2023** (In Press)

3. **Waziry R**, Claus JJ, Hofman A. Dementia Risk Following Ischemic Stroke: *A Systematic Review and Meta-Analysis of Factors Collected at Time of Stroke Diagnosis.* **J Alzheimer Disease**. 90.4: 1535-1546. **2022** (Published)

4. **Waziry R**, Hofman A, Ghanbari M, Tiemeier H, Ikram MA, Viswanathan A, Klap J, Ikram MK, Goudsmit J. *Biological Aging for Risk Prediction of First-Ever Intracerebral Hemorrhage and Cerebral Infarction in Advanced Age.* **J Stroke Cerebrovasc Dis.** 31.8: 106568. **2022** (Published)

5. **Waziry R**, Dufouil C, Hofman A, Ikram MK, Debette S, Wolters FJ, Fani L, Romero R, Aparicio HJ, Lioutas V, Viswanathan A, Kevin S, Goudsmit J, Seshadri S, Beiser A, Ikram MA. *Risk of dementia following first-ever hemorrhagic or ischemic stroke in the general population.* **2023** (Manuscript under review)

6. **Waziry R**, Gu Y, Williams O, Hägg S. *Connections between Cross-Tissue and Intra-Tissue Biomarkers of Aging Biology in Older Adults.* **2023** (Manuscript under review)

7. **Waziry R**, Williams O. *Causes of Death following Alzheimer's Disease and Variations According to Calendar Period.* **2023** (Manuscript under review)

8. **Waziry R**, Miles C, Hagg S, Goudsmit J, Kraus W, Williams O. *The Role of Inflammation and Biological Aging in the Relationship between Stroke and Cognition in Older Adults.* **2023** (Manuscript under review).

▶ **Conference Presentations**

9. **Waziry R.** *Measures of Aging Biology in Saliva and Blood as Novel Biomarkers for Healthy Aging.* Oral Presentation at the Gerontological Society of America Annual Meeting, November **2023.** Tampa, FL.

10. **Waziry R.** *The Relationship between Cross-Tissue and Intra-Tissue Measures of Aging Biology in Older Adults.* Poster Presentation at the Gerontological Society of America Annual Meeting, November **2023.**Tampa, FL.

11. **Waziry R.** *Improving Precision for Early Detection of Stroke Risk: Biological Aging Physiology and DNA methylation Crosstalk.* The Annual meeting of the American Academy of Neurology, April **2022.** Seattle.

12. **Waziry R.** *Blood-based Biological Aging Measures Are Easy-to-access Biomarkers for Detection of Incident Stroke,* The International Stroke Conference **2022.** New Orleans, LA.

13. **Waziry R.** *Risk of Dementia Following First-ever Hemorrhagic or Ischemic Stroke in The General Population,* The International Stroke Conference **2022.** New Orleans, LA.

14. **Waziry R.** *Dementia Risk Following Ischemic Stroke: A Systematic Review and Meta-analysis Of Factors Collected at Time of Stroke Diagnosis*, The International Stroke Conference **2022.** New Orleans, LA.

15. **Waziry R.** *Biological Age for Prediction of First-ever Intracerebral Hemorrhage And Cerebral Infarction In Advanced Age*, The International Stroke Conference **2022.** New Orleans, LA.

▶ **Lectures**

16. **Waziry R.** *Can we age free of dementia and Alzheimer's disease?* **April 2022.** Northwestern University. https://planitpurple.northwestern.edu/event/588035

17. **Waziry R.** *Can we age free of dementia and Alzheimer's disease?* **February 2022.** Rush University Medical Center

▶ **Invited Session Chair**

18. **Waziry R.** Biology of Aging Research. the Gerontological Society of America Annual Meeting, **November 2023.** Tampa, FL.

- **Awards**

19. NIH K99/R00 Pathway towards independence career development award

- **Coursework and other educational opportunities and activities**

20. Summer Bootcamp “Quantitative Genomics Training: Methods and tools for whole-genome and transcriptome analyses ". 14 – 15 June 2023. The Columbia University Sharp Training Program

Title page: Measures of Aging Biology in Saliva and Blood as Novel Biomarkers for Stroke and Heart Disease in older adults

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Supplementary tables: 6

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ABSTRACT

Background and Objectives. The role of aging biology as a novel risk factor and biomarker for vascular outcomes in different accessible body tissues such as saliva and blood remain unclear. We aimed to 1) assess the role of aging biology as a risk factor for stroke and heart disease among individuals of same chronological age and gender and 2) compare aging biology biomarkers measured in different accessible body tissues as novel biomarkers for stroke and heart disease in older adults.

Methods. The present study included individuals who consented for blood and saliva draw in the Venous blood study and Telomere length study of the Health and Retirement Study (HRS). HRS is a population-based, nationally representative longitudinal survey of individuals aged 50 and older in the United States. Saliva-based measures included telomere length. Blood-based measures included DNA methylation and physiology biomarkers. Propensity scores matched analyses and Cox regression models were conducted.

Results. The present study included individuals aged 50 and older, who consented for blood (N=9,934) and saliva (N=5,808) draw in HRS. Blood-based biomarkers of aging biology showed strong associations with incident stroke as follows: compared to the lowest tertile of blood-based biomarkers of aging, biologically older individuals had significantly higher risk of stroke based on DNA methylation Grim Age (aHR=2.64, 1.90, 3.66, P < 0.001) and Physiology-based Phenotypic Age (aHR=1.75,1.27, 2.42, P<0.001) respectively. In secondary analysis, biologically older individuals had increased risk of heart disease as follows: physiology based Phenotypic Age (aHR= 1.77, 95% CI 1.49,2.11, p <0.001) and DNA methylation Grim Age (aHR=1.61, 95% CI 1.36, 1.90, P<0.001).

Discussion. Compared to saliva-based telomere length, blood-based aging physiology and some DNA methylation biomarkers are strongly associated with vascular disorders including stroke and are more precise and sensitive biomarkers of aging. Saliva-based telomere length and blood-based DNA methylation and physiology biomarkers likely represent different aspects of biological aging and accordingly vary in their precision as novel biomarkers for optimal vascular health. Variations in aging biology may influence individuals' ability to live healthfully in older age.

INTRODUCTION

Despite advances in management of vascular health, risk of vascular disease including stroke continues to rise in aging populations [1]. The pathogenesis of stroke is not fully understood despite the established evidence on "classic risk factors" that contribute to onset of these disorders [2, 3]. The role of aging biology as a novel risk factor and biomarker for vascular disease including stroke in different body tissues such as saliva and blood remain unclear. Novel biomarkers measured in accessible body tissues are pivotal to improve precision and sensitivity to achieve primary prevention of vascular disease including stroke [4].

Aging is a major risk factor for vascular disorders including stroke [2]. However, aging based on chronological age is not a sufficient indicator of morbidity and overlooks varying aging effects between individuals as calendar time does not vary for individuals of the same chronological age [3]. Furthermore, evidence on the role of biological underpinnings in relation to stroke and heart disease in different body tissues remain sparse. New methods make it possible to infer the state of biological aging in an individual by combining multiple molecular and physiological parameters in algorithms to predict biological age [12]. Biological aging biomarkers based on Telomere length, DNA methylation and physiological biomarkers are novel approaches to identify and monitor persons at risk of vascular disease including stroke. These biomarkers, which are potentially modifiable by lifestyle and behavioral factors, may be promising biomarkers for diagnosis, targeted neuroprotective therapies, and for identifying high risk groups for preventative interventions.

In the present investigation, we aimed to 1) assess the role of aging biology as a novel risk factor for stroke and heart disease among individuals of same chronological age and gender and 2) compare aging biology biomarkers measured in different accessible body tissues as novel biomarkers for vascular outcomes in older adults.

METHODS

Study population

The Health and Retirement Study (HRS) is a nationally representative longitudinal survey that recruited more than 37,000 individuals aged 50 and older in the U.S. The survey has been conducted every two years since 1992 with a focus on issues related to changes in health and economic circumstances in aging at both the individual and population levels [4]. HRS data are linked to records from Social Security, Medicare, Veteran's Administration, the National Death Index and employer-provided pension plan information. Genetic ancestry in HRS is identified through PC analysis on genome-wide SNPs [4]. HRS is coordinated by the Institute for Social Research at the University of Michigan.

Standard Protocol Approvals, Registrations, and Patient Consents

The present study included individuals who consented for blood and saliva draw in the Health and Retirement 2016 Venous blood study and 2008 Telomere length study respectively [4-6]. The HRS (Health and Retirement Study) is sponsored by the National Institute on Aging (NIA U01AG009740) and is conducted by the University of Michigan. The Health and Retirement study is reviewed and approved by the University of Michigan's Health Sciences IRB. Our study was also approved by the Institutional Review Board of Columbia University. The present study has been conducted in accordance with STROBE guidelines for observational studies [7].

Biological aging measurements

We assessed biological aging based on A-Telomere length, B-DNA Methylation and C-Physiology measures.

A- Telomere Length measurement

Telomere length data were available from 5808 HRS respondents who consented for saliva sample draw during the 2008 interview wave. Telomere length data were available from 5808 HRS respondents who consented for saliva sample draw during the 2008 interview wave, among whom about half had biomarker information in HRS VBS. Assays were performed by Telome Health (Telomere Diagnostics). Quantitative PCR (qPCR) was used to assay average telomere length. For each patient's sample, telomere sequence copy number (T) was compared to a single-copy gene copy number (S). Telomere length mean was proportional to the T/S ratio. Funding was provided through the National Institute on Aging (NIH U01 AG009740 and RC4 AG039029) [6]

The Venous blood substudy (VBS) and assay protocol in 2016 Health and Retirement Study

All respondents, with the exception of proxy and nursing home respondents, who completed the HRS interview in 2016 (visit 13) were asked to consent for blood draw [5]. There was an excellent response rate to the blood collection protocol in 2016. The consent rate was 78.5%, among which 82.9% had a completed collection [5]. The final VBS sample included 9,934 individuals. Physiology based biomarkers were assessed on the whole sample of participants who consented for blood draw, while DNA-methylation was assessed in a subsample randomly selected and fully representative of the whole sample

[5]. DNA methylation assays were available for 4,104 individuals who participated in the 2016 Venous Blood Study and 4,018 samples passed the QC [8] . The VBS DNA methylation subsample is considered fully representative of the HRS sample with 58% females and a median age of 68.7 years [5]. We included in the present analysis individuals had complete data for both physiologic and DNA methylation in 2016.

Blood samples were centrifuged and shipped overnight to CLIA-certified Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing was done within 24 hours of arrival at the lab (within 48 hours of collection). All assays were done at the University of MN Advanced Research and Diagnostic Laboratory (ARDL) under the direction of Bharat Thyagarajan.

B-DNA Methylation

DNA methylation assessment included the following clocks: Horvath DNA methylation clock [9]; Hannum [10]; PhenoAge [11]; and GrimAge [12]. The chronological classification of the clocks can be summarized as follows: the first-generation clocks were developed using machine-learning to predict chronological age. These clocks demonstrated two important proofs of concept: They recorded increases in clock-age within individuals as they grew older [13, 14]; and more advanced clock-age estimates (i.e. clock ages older than chronological age) were associated with increased mortality risk among individuals of the same chronological age[15]. Second generation DNA methylation clocks were developed from analysis of mortality risk, incorporating information from DNA methylation prediction of physiological parameters [12, 15]. These second-generation clocks are more predictive of morbidity and mortality[11] and are proposed to have improved potential for testing impacts of interventions to slow aging [16]. We analyzed the first-generation clocks proposed by Horvath [17] and Hannum et al.[10], referred to as the Horvath Clock and Hannum Clock, respectively, and second-generation clocks proposed by Levine et al.[15] and Lu et al.[12], referred to as the PhenoAge Clock and GrimAge Clock, respectively. The Horvath epigenetic clock is widely used. It predicts age using 353 CpG sites in the DNA methylation profile and has been used to calculate “age acceleration” in various tissues and environments. The Hannum et al. clock was trained and tested on blood-derived DNA, it comprises 71 CpG sites selected from the Illumina 450k array that capture changes in chronological age, which is partly driven by age-related shifts in blood cell composition. Phenotypic age was developed using data from whole blood, it correlates strongly with age in every tissue and cell tested and was based on 513 CpGs. These DNA methylation clocks are built using a supervised machine learning methods trained against chronological age to identify an informative and sparse predictive set of CpGs.

C-Physiology measures

Among available and validated measures of biological aging based on physiologic parameters, we opted to use Phenotypic Age described in detail by Liu et al [18]. This clock uses the following 9 parameters: albumin, creatinine, glucose, [log] C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count. These parameters were previously validated as predictors of aging in comparable settings [18].

Assessment of outcomes

The primary outcome included presence or absence of stroke. Secondary outcomes included presence or absence of any heart disease. Outcomes were assessed through self or proxy report. Although imperfect, the high correlation between self-reported strokes and hospital records is well documented [2, 19-22]. Stroke subtype specification was not available for the present investigation.

Stroke events were ascertained as first reported event that is nonfatal or fatal based on self or proxy-report (for fatal events) of a doctor's diagnosis (e.g., "Has the doctor ever told you that you had a stroke or transient ischemic attack?", "Has the doctor ever told you that you had a heart problem"). New stroke cases were ascertained using the same question but specific to the wave ("e.g., reports stroke this wave") [23]. Participants were also asked about the stroke month and year and when such information was not available, the median stroke month for events reported by other participants in the same interview wave. Individuals who reported a possible stroke/TIA were not included.

Heart disease that was assessed as "Has a doctor ever told you that you have had a heart attack, coronary heart disease, angina, congestive heart failure, or other heart problems?" [23]. Including the combined heart assessment in the present analysis was determined as priori as it has the advantage of minimizing potential recall bias in relation to heart disease subcategory among respondents [24]. Measures of health in HRS including stroke and heart disease have been widely validated in the literature and are suggested to have high rates of construct validity in older adults [25-30].

Statistical analysis

Summary statistics were assessed as median (IQR) for continuous variables and percentages for categorical variables. In addition, tertiles of biological aging were assessed. Survey weights for variables included were used in descriptive analysis [31]. Biomarkers were calculated as standardized residuals regressed on age at time of measurement for all eligible individuals for each biomarker.

The direct relationship between biological aging measures and stroke and heart disease, as separate binary outcomes, were assessed in a multi-model approach using propensity scores matched analysis and Cox regression models. We conducted matching of individuals with stroke and heart disease with comparison matched on age and sex using propensity scores with probit link to the nearest neighbor and without replacement [32]. Models were conducted conditional on matched pairs [33]. Cox regression models were used to account for time to outcomes using age scale between age at biomarker measurement and the subsequent follow-up visits among individuals for which this data was available. Models were tested for proportionality assumption [34-36]. Models were adjusted for age, race and gender including the matched samples to ensure adequate control for confounding effects and to include covariates with the most complete data for the included participants [37]. All analyses were conducted using Stata SE V.16.0.
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Sensitivity analyses

To minimize potential biases due to reverse causality, we repeated the analysis after adjusting for prevalent disease prior to the biomarker measurement. Prior prevalent disease included presence of any of the following: prior stroke, high-blood pressure, heart disease, diabetes mellitus, lung disease or cancer. We tested the models using Telomere length (logged) and Telomere length T/S ratio greater than 2.0 [38]. Potential differences in the relationship between stroke or heart disease and aging according to education attainment were assessed in a stratified analysis (college education or higher versus less education attainment).

RESULTS

Venous Blood Study (VBS)

The VBS sample included 9,934 participants from the HRS 2016 wave who consented for blood draw. The stroke sample for the present study included 310 and 3,342 individuals with prevalent stroke and comparison at wave 13 and 291 and 3, 222 individuals with incident strokes and comparison in wave 14 respectively (table 1, etable 1).

The heart disease sample for the present study included 957 and 2, 694 individuals with prevalent heart disease and comparison at wave 13 and 1002 and 2, 510 individuals with incident heart and comparison at wave 14 respectively (etables 1, 2).

Telomere Subsample, Saliva

The Telomere sample included 5,808 participants from the HRS 2008 wave who consented for saliva draw. The stroke sample for the present study included 478 and 5, 324 individuals with prevalent stroke and comparison at 2008 wave, 395 and 4, 818 individuals with incident strokes and comparison in 2010 respectively (table 1, etable 1).

The heart disease sample for the present study included 1,443 and 4, 360 individuals with prevalent heart disease and comparison at 2008 wave, and 1,500 and 3, 739 individuals with incident heart and comparison in 2010 wave respectively (etables 1,2).

Relationship between biological aging and Stroke

In propensity score matched analysis, compared to the lowest tertile, the adjusted odds ratios of biological aging with stroke showed higher magnitudes DNA methylation Grim Age clock aOR= 2.37, (95% CI 1.55 , 3.63 , p < 0.001) and aOR= 2.01 (95% CI 1.34 , 3.03 , P <0.001) for physiology based Phenotypic Age .Telomere length measured in saliva was not associated with stroke (aOR 1.12 ; 95% CI 0.94, 1.33, P = 0.19) (Table 2, etable 3, Figure 1).

In Cox regression models, one year increase in biological age based on DNA methylation was associated with higher risk of new stroke occurrence with significant results for DNA methylation clock Grim Age (aHR=1.46, 95% CI 1.29, 1.64, P <0.001) and Physiology-based Phenotypic Age clock (aHR=1.24, 95%

CI 1.12, 1.38, P<0.001). Similarly, one tertile increase in biological age was associated with almost three times higher risk of new stroke occurrence compared to the lowest tertile as reference as observed on DNA methylation clock Grim Age (aHR=2.64, 95% CI 1.90, 3.66, P < 0.001) and Physiology-based Phenotypic Age clock (aHR=1.75, 95% CI 1.27, 2.42, P=0.001. Telomere length measured in saliva was not associated with stroke (aHR: 1.01, 95% CI 0.93, 1.09, p = 0.73) (Table 2).

Relationship between biological aging and heart disease

In propensity score matched analysis, compared to the lowest tertile, DNA methylation Grim Age (aOR=1.95, 95% 1.53, 2.48, P<0.001) compared with physiology-based biological aging with (aOR= 2.36, 95% CI 1.86, 2.99, P <0.001). Telomere length measured in saliva was not associated with heart disease (aOR 0.99; 95% CI 0.91, 1.08, P = 0.99) (etables 3, 4).

In Cox regression models, Older biological age based on DNA methylation showed similar patterns with heart disease with slightly attenuated magnitudes. Statistically significant results were observed for DNA methylation clocks Levine (aHR=1.17, 95% CI 1.10, 1.24, P<0.001), Grim Age (aHR=1.24, 95% CI 1.16, 1.32, P<0.001) physiology-based Pheno Age (aHR=1.24, 95% CI 1.18, 1.32, P < 0.001). Individuals in the highest biological age tertile had almost two times higher risk of new heart disease occurrence as observed by both DNA methylation clocks (Levine, aHR=1.53, 95% 1.31, 1.79, P < 0.001), Grim Age aHR= 1.77, 95% CI 1.49, 2.11, P<0.001) and Physiology-based clock (Phenotypic Age, aHR=1.61, 95% CI 1.36, 1.90, P <0.001). Telomere length measured in saliva was not associated with heart disease (aHR: 1.00, 95% CI 0.96, 1.05, P = 0.72) (etable 4).

Sensitivity analyses

Similar associations were observed after adjusting for presence of any prevalent disease including prior stroke, high-blood pressure, heart disease, diabetes mellitus, lung disease or cancer (etables 5,6). Repeating the models using Log Telomere length and after excluding and Telomere length T/S ratio greater than 2.0 showed similar associations.

In stratified sensitivity analysis, higher education level (college education and higher) was associated with higher odds of stroke with every one-year increase in biological age based on DNA methylation (OR: 1.5, 95% CI 1.1, 2.0), similarly for less education attainment (OR: 1.3, 95% CI 1.2, 1.5). Physiology-based biological aging showed higher odds of stroke only among those with less education attainment (OR: 1.2, 95% CI:1.1, 1.4) while the association was not statistically significant among those with higher education level (OR: 1.2, 95% CI 0.9, 1.6) suggesting potential impact of social health determinants on aging free of stroke.

DISCUSSION

Age is a major risk factor for stroke and related cerebrovascular and cardiovascular diseases [1]. Risk of stroke and heart disease continues to escalate with aging of populations and remain a leading cause of years of life lost [39]. Aging biology biomarkers may represent novel screening tools for optimal vascular health through early identification of risk and improved precision for timing-specific evaluation and implementation of preventative measures beyond aging based on chronological age [40]. However, the complexity of addressing these research gaps is amplified by the aging process itself, whose biological underpinnings remain poorly understood in the context of health and disease [41].

In the present study, we found strong associations between stroke, heart disease and blood-based aging measures including physiology and some DNA methylation biomarkers, while we found no associations between saliva-based telomere length, stroke and heart disease. Previous investigations reported similar findings on the relationship between telomere length and diabetes in HRS [42].Variations in relation to stroke and heart disease were observed in relation to two biological measures of aging based on physiology (a system-based measure using Phenotypic Age) and DNA methylation (using GrimAge) approaches. These differences in the associations between the biological aging DNA metrics could be potentially attributed to differences in the set of CPGs represented in each clock. We observed that changes in physiology in patients with or on the trajectory of stroke and heart disease progression varied in sensitivity in relation to aging processes compared to DNA methylation [41]. These observations were prominent in propensity-scores matched samples where individuals were matched on chronological age and sex, further reducing confounding effects of chronological age itself. Individuals who were biologically older had 2-to-3-fold increase in likelihood of new stroke or heart disease occurrence. Simultaneously, individuals who already had stroke or heart disease were biologically older compared to the general population, consistent with previous observations on older biological age among those with recurrent stroke [43].

The consistent estimates across models and waves provide reassurance regarding the reliability of biological aging metrics as biomarkers for vascular outcomes. The need for novel aging biomarkers that are specific and sensitive for detecting those at risk of cerebrovascular and cardiovascular disease stems from several key issues. First, the close relatability of aging as a triggering mechanism by itself to vascular outcomes including stroke [43]. This is evident by the increase in absolute numbers of these diseases with aging of the population as well as the increase in associated burden of disease, dementia and cognitive impairment in particular [44]. Considering these observations, the measurement of aging based on chronological age may be imprecise and non-modifiable. Thus, traditional interventions or *"business as usual"* that determine risk or prognosis by calendar years are suboptimal for achieving primary and secondary prevention of these disorders and their associated sequalae [1]. Second, the increased lifespan on the account of health-span, has created a need to revisit our approaches to risk assessments in older individuals [45, 46]. Therefore, novel biomarkers that may capture aging processes or changes with aging prior to manifestation of clinical stroke or cardiovascular disease are increasingly important in healthy aging [3]. Understanding measures of optimal vascular health are essential routes for

investigating opportunities for primary prevention of cerebrovascular and cardiovascular disorders [47]. The role of social determinants of health such as educational attainment on biological versus chronological aging may also contribute to disparities as observed in the present study and in other settings [48]. Third, the recent accumulating evidence on individuals' resilience in aging despite presence of classic risk factors of stroke and heart disease and their ability to withstand such stressors at multi-organ or system levels including the central nervous system may reflect factors that extend beyond measures immediately and readily assessed at time of stroke or heart disease diagnosis [49]. In particular, biomarkers that are able to mirror individual differences in life-time exposures and resiliency are key to understanding the etiology of cerebrovascular-free and cardiovascular disease-free aging to achieve primary prevention and extend health span.

There are several limitations in the present study. First, Stroke subtype specification was not feasible in the present study, however, it is common to analyze stroke outcomes without stratification in the context of epidemiological cohorts [44]. Second, new strokes assessed within each wave were not first-ever strokes, however similar associations were observed after adjustment for presence of prevalent disease, thus further ascertaining our findings. Third, currently there is no gold standard for biological aging measures, therefore comprehensive comparisons provide a rigorous, neutral and reproducible way to capture biological aging effects. Lastly, the limited longitudinal follow-up time both in relation to assessments of new events and biological aging measures hinders our ability to understand the dynamic relationship between aging and disease in the setting of stroke and heart disease. However, the presence of strong associations despite the relatively short follow-up provides a solid foundation for extended investigations. Advances in molecular biology enables the measurement of high-dimensional cellular level changes that would aid the understanding of aetiologic pathways and our ability to develop personalized prevention tools for cerebrovascular and cardiovascular disease and progression to cognitive impairment and dementia as key sequalae in aging populations.

Our data show that biomarkers measured in different tissues may have remarkably different sensitivity to stroke and heart disease. In addition, individuals with similar risk profiles for cardiovascular disease and stroke were shown to have variabilities in aging biology. Such differences may influence their ability to live healthfully or progress to symptomatic disease in advanced age. These aging processes likely reflect rates of aging according to biology [41]. In our study, physiology-based processes and some DNA methylation clocks showed strong associations with both existing and new stroke and heart disease. The consistent relationships observed with physiology-based biomarkers could be related to the standardized nature and established best practices in their method of measurement across laboratories. In addition, a previous investigation by Kraus and colleagues suggested that subclinical changes in physiology are related to cardiovascular disease occurrence in the setting of a randomized controlled trial [50].

The results of our study suggest that blood-based biological aging measures including physiology and some DNA methylation biomarkers are strongly associated with stroke and heart disease and are more precise and sensitive metrics of aging compared to saliva-based biomarkers, telomere length in

particular. Saliva-based telomere length and blood-based DNA methylation and physiology biomarkers of biological aging likely represent different aspects of aging and accordingly vary in their precision as novel biomarkers for prevention of stroke and heart disease. These variations in precision and sensitivity of aging biomarkers provide novel opportunities for primary prevention of age-related vascular disorders that require extension beyond existing diagnostic frameworks [45, 46].

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Figure 1. The relationship between measures of aging biology and stroke per tertile increase in biological age.

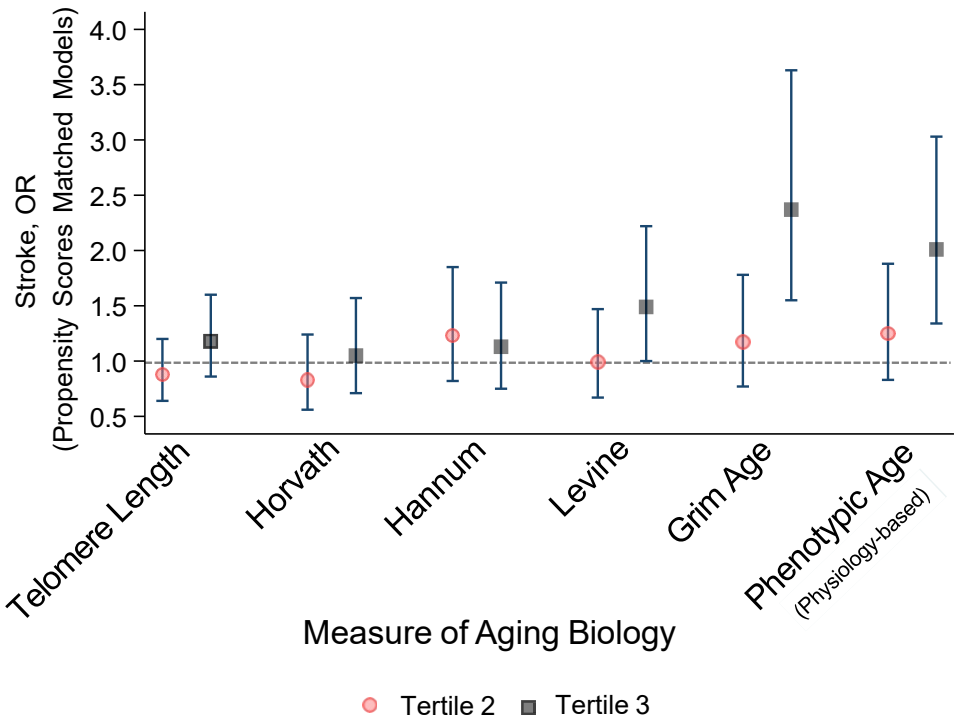

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Table 1. Detailed Sample characteristics of Individuals with Stroke and Comparison

Characteristic	**Stroke Prevalent**		**Stroke Incident**	
	Stroke	Comparison	Stroke	Comparison
	Biological Age Biomarkers			
	Saliva-based			
N	**478**	**5,324**	**395**	**4,818**
	A-Telomeres			
Telomere length, median (IQR)	1.26 (1.07, 1.48)	1.28 (1.12, 1.49)	1.25 (1.09, 1.47)	1.29 (1.12, 1.49)
Chronological age at time of measurement, median (IQR)	76 (68, 83)	68 (60, 76)	73 (66, 80)	68 (60, 75)
Sex, female %	891 (54)	9, 289 (59)	835 (54)	11, 896 (58)
Race, African American %	297 (18)	2, 128 (14)	376 (25)	3, 771 (19)
	Blood-based¶			
	B-DNA Methylation			
N	**310**	**3,342**	**291**	**3,222**
Horvath, median (IQR)	68 (62, 74)	64 (59, 71)	68 (62, 75)	64 (58, 71)
Hannum, median (IQR)	57 (50, 63)	53 (47, 60)	57 (50, 63)	53 (47, 59)
Levine, median (IQR)	60 (54, 67)	56 (50, 63)	60 (54, 67)	56 (50, 63)
GrimAge, median (IQR)	72 (64, 78)	67 (61, 73)	72 (65, 78)	67 (60, 73)
	C-Physiology measures			
Phenotypic Age, median (IQR)	76 (66, 85)	68 (59, 77)	76 (66, 86)	68 (59, 78)
Sample Characteristics				
Chronological age at time of measurement, median (IQR)	72 (64, 80)	68 (61, 76)	72 (65, 89)	68 (61, 76)
Sex, female %	165 (53)	2,001 (60)	160 (55)	1,921 (59)
Race, African American %	69 (23)	526 (16)	66 (23)	507 (16)

Table represents unweighted detailed sample characteristics; Prevalent stroke represents data at the time of biomarker measurement, incident events represent data at the subsequent wave; ¶ Blood-based sample represents individuals alive in 2016 with complete DNA methylation data for the present analysis.

Table 2. The relationship between Biological Aging Biomarkers and Stroke

Biological aging measure	**Propensity Scores' Matched Analysis**						**Cox regression models**					
	Per one year increase in biological age			*Per tertile increase in biological age*			*Per one year increase in biological age*			*Per tertile increase in biological age*		
	aOR[a]	**95% CI**	**P value**	**aOR[a,b]**	**95% CI**	**P value**	**aHR[a]**	**95% CI**	**P value**	**aHR[a,b]**	**95% CI**	**P value**
	Saliva-based											
	A-Telomeres											
Telomere length	1.12	0.94, 1.33	0.19	0.88	0.64, 1.20	0.44	1.01	0.93, 1.09	0.73	0.84	0.66, 1.08	0.18
				1.18	0.86, 1.60	0.28				1.00	0.78, 1.27	0.96
	Blood-based											
	B-DNA methylation											
Horvath	1.12	0.96, 1.31	0.12	0.83	0.56, 1.24	0.37	1.13	1.00, 1.27	0.04	0.98	0.73, 1.32	0.93
				1.05	0.71, 1.57	0.77				1.11	0.83, 1.48	0.47
Hannum	1.11	0.95, 1.29	0.18	1.23	0.82, 1.85	0.30	1.08	0.96, 1.22	0.15	1.14	0.85, 1.53	0.37
				1.13	0.75, 1.71	0.53				1.12	0.83,1.51	0.42
Levine	1.19	1.01, 1.41	0.03	0.99	0.67, 1.47	0.98	1.15	1.03, 1.29	0.01	1.21	0.89, 1.64	0.21
				1.49	1.00, 2.22	0.04				1.62	1.21, 2.17	0.001
GrimAge	1.55	1.29, 1.85	< 0.001	1.17	0.77, 1.78	0.43	1.46	1.29, 1.64	<0.001	1.61	1.14, 2.26	<0.01
				2.37	1.55, 3.63	<0.001				2.64	1.90, 3.66	<0.001
	C-Physiology measures											

Phenotypic Age	1.40	1.17, 1.66	< 0.001	1.25	0.83, 1.88	0.27	1.24	1.12, 1.38	<0.001	1.45	1.03, 2.02	0.03
				2.01	1.34, 3.03	0.001				1.75	1.27, 2.42	0.001
[a] Adjusted for chronological age, sex and race; [b] reference as the lowest tertile; biomarkers calculated as standardized residuals regressed on age at time of measurement.												

Title page: Is Alzheimer's a terminal disease? *A life-limiting disease with a burden of a terminal illness*

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Abstract

Purpose of review. An estimated 6.5 million Americans live with Alzheimer's Dementia. Better understanding of advanced stages of AD and timely monitoring of its preventable complications would translate to improved survival and quality of life in this population.

Recent findings. In the present perspective review, we proposed shifting the narrative to recognizing AD as a chronic life-limiting illness instead of a terminal disease. In support of this view, we discussed: 1) the biochemical, cellular (preclinical) and clinical phases of AD; 2) survival following AD; 3) AD therapeutics and potential implications for the AD population in the advanced stages.

Summary. On the bases of the prolonged preclinical phase in AD, promising advances in AD therapeutics and the varying survival following AD, we proposed a new classification for AD and more broadly neurodegenerative disorders to be recognized as chronic '*life-limiting illnesses*' rather than '*terminal diseases'* with important implications for AD patients in the advanced stages given the challenges that are specific to this population.

Summary Box

- An estimated 6.5 million Americans live with Alzheimer's Dementia.
- Better understanding of advanced stages of AD and timely monitoring of its preventable complications are necessary to improve survival and quality of life in the AD population.
- AD and more broadly neurodegenerative disorders should be recognized as chronic '*life-limiting illnesses*' rather than '*terminal diseases'* in light of the phases of disease progression, the varying survival following AD and promising advances in AD therapeutics.

An estimated 6.5 million Americans live with Alzheimer's Dementia[51, 52]. Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of Beta-amyloid in the form of extracellular plaques and neurofibrillary tangles with progression to neurodegeneration as a result, leading to progression to AD dementia [53, 54]. In the present perspective we propose shifting the narrative to recognizing AD as a chronic life-limiting illness instead of a terminal disease. In support of this view we: 1) Discussed the biochemical, cellular (preclinical) and clinical phases of AD; 2) Systematically reviewed recent literature on survival following AD and lastly, we; 3) Discussed AD therapeutics and potential implications for the AD population in the advanced stages. On the bases of the prolonged preclinical phase in AD, the promising advances in AD therapeutics and the varying survival following AD, we proposed a new classification for AD and more broadly neurodegenerative disorders to be recognized as chronic '*life-limiting illnesses*' rather than '*terminal diseases'* with important implications for AD patients in the advanced stages given the challenges that are specific to this population.

Biochemical, Cellular (preclinical) and Clinical phases of AD

AD has been largely considered a pathological disease. However, in recent years there has been a greater understanding of the biological and cellular aspects of AD. The shift towards a biological definition is supported by the ATN framework that underscores amyloid-beta and tau as defining characteristics of AD with support of a biological definition of AD that is based on biomarkers in addition to the clinical syndrome[55, 56]. A current gap exists between the definition of AD in the clinic versus the biological definition of AD that is commonly used in clinical trials of AD therapeutics [57-59] . We illustrate the biochemical and cellular phases of AD that are hypothesized to precede the clinical phase of AD. Tau and beta-amyloid are main components of the biochemical phase[57]. Changes during the biochemical phase lead to accumulation of proteopathic stress that triggers the cellular phase of AD[60]. It constitutes a prodromal stage of AD prior to symptomatic manifestation of AD owing to the ability of the brain to maintain homeostasis and survive the initial reversible effects of proteopathy for many years[60, 61]. Alterations in astroglia, microglia, neurons and vessels constitute the landscape of the cellular phase[62-65]. The cellular phase triggers progression to the clinical phase, that is likely independent of the initial beta-amyloid and tau accumulation, when a threshold of disturbance is reached beyond the cellular capacity to maintain homeostasis[66, 67 68]. Stressors' effects including aging, ischemia and blood brain barrier leakage contribute throughout the process of progression from the biochemical phase to the advanced stages of clinical AD[61, 69-71] (Supplementary Figure 1). Lastly, NIA/AA recent staging system emphasizes the preclinical and asymptomatic phases of AD [72]. Altogether, alongside the increasing public awareness about brain health more individuals are expected to be diagnosed in the very early phases of AD in the near future and therefore shifting AD towards a chronic non-terminal disease framework [73].

Survival following AD

In a similar manner to the refractory nature of complex disorders such as cancers, there is currently no cure for AD. In advanced stages of the disease, AD progresses to involve diffuse cortical functions leading to the inability to manage activities of daily living [74]. Therefore, this

lack of independence is a main differentiating sign between mild cognitive impairment and clinical Alzheimer's disease[75]. AD also causes a number of psychological and behavioural changes which can cause significant distress to both patients and care-givers[74, 76]. Unlike other complex disorders, the prognostic duration of AD is more challenging to determine.[77] Existing evidence supports several underlying causes of death beyond dementia including pneumonia, cerebrovascular disease, cardiovascular disease and cancers [78, 79]A majority of AD patients die from potentially preventable complications such as bronchopneumonia or cerebrovascular disorders[80, 81]. To assess average survival following AD we conducted a systematic review of recent literature published between 2013 and 2023. A total of 16 studies provided data on survival following AD and were included in a random effects meta-analysis[82-94]. The combined meta-analysed average survival following AD diagnosis was 54.43 months (95% CI 54.31, 64.54) with significant heterogeneity and variations overall and according to race (Supplementary Figure 2). Jointly, better understanding of the natural history of AD and targeting preventable causes could contribute to significant improvements in quality of life and survival in this population.

Recent advances in AD therapeutics

While to date there no cure for AD, recent advances in AD therapeutics have opened new avenues for improving cognition, particularly in the early stages of AD[95]. AD therapeutics can be broadly divided to monoclonal antibodies, vaccines, antisense oligonucleotides and gene therapy[57]. The *first-in-class* anti-amyloid monoclonal antibody to be approved by FDA for treatment of AD was Aducanumab[96, 97]. It showed significant slowing in disease progression on the Clinical Dementia Rating–Sum of Boxes (CDR-SB) scale compared to placebo[98]. Major side effects of Aducanumab included amyloid-related imaging abnormalities [ARIA][96, 98]. Lecanemab, a humanized IgG1 monoclonal antibody in persons with early Alzheimer's disease, has also been recently granted accelerated FDA approval. It showed favorable outcomes on the Alzheimer's Disease Composite Score (ADCOMS) in the treatment arm with adjusted least-square mean change from baseline at 18 months equals 1.21 with Lecanemab and 1.66 with placebo (95% CI -0.67 to -0.23, P <0.001). Lecanemab has high affinity to amyloid-beta (Aβ) soluble protofibrils that are suggested to be more toxic to neurons than monomers or insoluble fibrils[99-101]. Reduction in the burden of brain amyloid was also observed with Lecanemab than with placebo[102]. Adverse effects in the treatment arm included transfusion reactions (26.4%) and amyloid-related imaging abnormalities with edema or effusions (12.6%). Although longer trials are required to confirm the safety and efficacy of Lecanemab, its favorable outcomes on cognition in early disease phases concur with the need to recognize AD as a non-terminal illness.

Potential implications for the AD population in the advanced stages

There is currently no evidence to support clinical use of existing monoclonal antibodies in patients with AD in the advanced stages and there has been no clinical evidence to support reversing late-stage degeneration in AD, likely due to the complex nature of degeneration of the AD brain in the advanced stages that goes beyond amyloid[55, 60], thus furthering the gap in potentially applying such therapies in this population[96]. An important difference between early-

stage AD and advanced stage AD is the presence of comorbidities in advanced AD. Delineating this aspect between early stage and advanced stage AD would have important implications for clinical management and prognosis. Best practices to address this disparity in the advanced AD population remain unclear. Amyloid beta vaccines are in progress in AD trials and are considered promising routes in AD therapeutics[57, 103, 104]. Other mechanistic therapeutic targets being pursued in AD trials include tau, neurotransmitter receptors, neurogenesis, epigenetics, oxidative stress, vasculature, proteostasis, metabolism, synaptic plasticity and inflammation[51]. The ongoing progress in the development of new therapeutic targets and drug discovery for AD is anticipated to unravel better treatments in the near future. As newer treatments become available it becomes critical to invent precise diagnostic and prognostic biomarkers that allow early detection and diagnosis of AD for timely treatment initiation, to assess responses to treatment and for successful implementation of therapies[55, 105]. Acknowledging AD as a non-terminal illness would likely result in important practical ramifications. This classification would support wider inclusion of patients across the AD spectrum, would help emphasize the importance of consistent follow-up and monitoring and potentially result in better and wider insurance coverage for prevention of reversible AD complications in this population [79].

Conclusions

Better understanding of advanced stages of AD and timely monitoring of its preventable complications would translate to improved survival in this population and potentially further opportunities for slowing disease progression. A majority of AD patients die from potentially preventable complications such as bronchopneumonia or cerebrovascular disorders. In the present manuscript we discussed the biochemical, cellular (preclinical) and clinical phases of AD, survival following AD and lastly, we highlighted recently approved AD therapeutics and potential implications for the AD population in the advanced stages. On the bases of the prolonged preclinical phase in AD, the promising advances in AD therapeutics and the varying survival following AD, we proposed a new classification for AD and more broadly neurodegenerative disorders to be recognized as chronic '*life-limiting illnesses*' rather than '*terminal diseases'* given the challenges that are specific to this population[106, 107].

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Contributions: Conception, funding, data acquisition & approvals, statistical analysis and drafting the article: RW; Review of the manuscript and critical appraisal: RW and OW.

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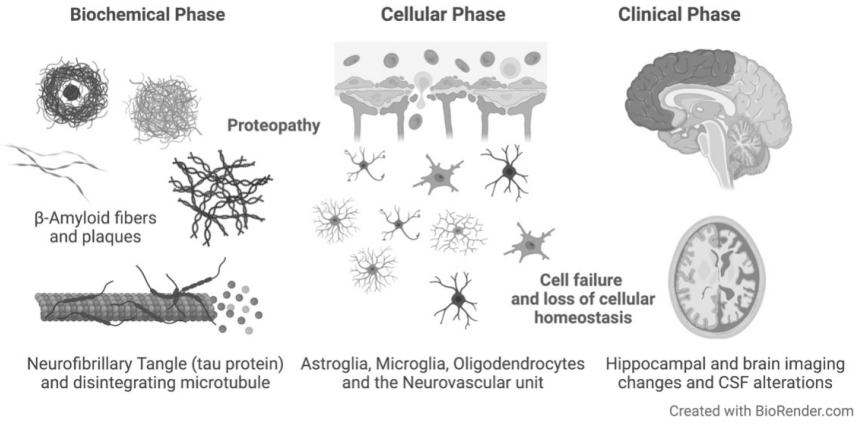
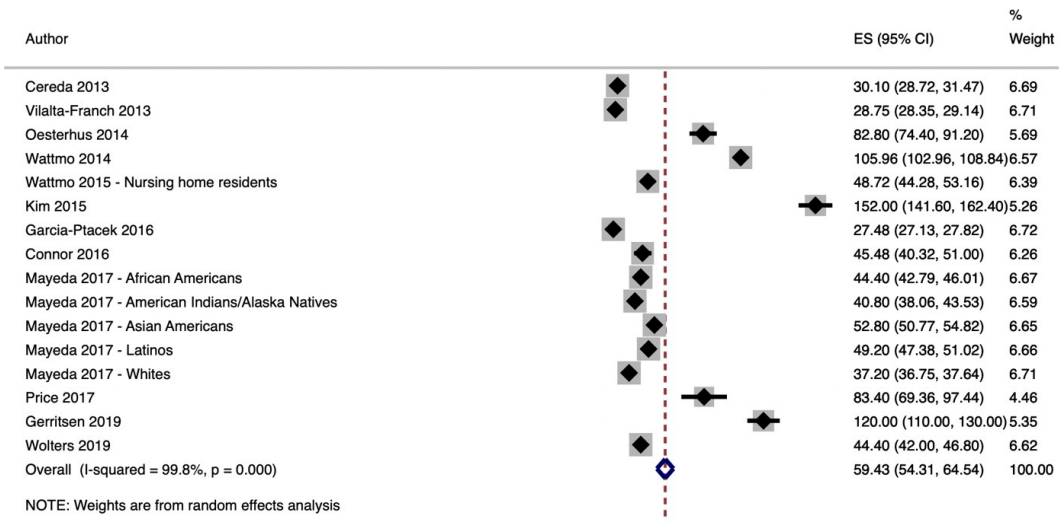
Supplementary Figure 1. Biochemical, Cellular (preclinical) and Clinical phases of AD



Supplementary Figure 2. Random effects meta-analysis of average survival in months following diagnosis of Alzheimer's Disease



Title page: Dementia Risk Following Ischemic Stroke: A Systematic Review and Meta-Analysis of Factors Collected at Time of Stroke Diagnosis

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Title: 18
References: 53
Figures: 4
Tables: 3
Supplementary figures: 9
Supplementary tables: 3

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ABSTRACT

Background: The majority of stroke cases are ischemic in origin and ischemic stroke survivors represent a high-risk population for progression to dementia. **Objective:** To determine incidence rates and predictors of dementia after ischemic stroke. **Methods:** A systematic review and meta-analysis compliant with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA). **Results:** 5,843 studies were screened for title and abstract. 292 eligible studies were screened for full text. A total of 22 studies met the inclusion criteria and were included, representing 55,929 ischemic stroke survivors. Cumulative incidence of dementia after stroke was 20% at 5 years, 30% at 15 years and 48% at 25 years of follow-up. Dementia incidence rates were 1.5 times higher among patients with recurrent ischemic stroke compared to patients with first-time stroke. Predictors of dementia after ischemic stroke included female gender (OR 1.2, 95% CI (1.1, 1.4)), hypertension (1.4, (1.1, 2.0)), diabetes mellitus (1.6, (1.3, 2.1)), atrial fibrillation (1.9, (1.2, 3.0)), previous stroke (2.0, (1.6, 2.6)), presence of stroke lesion in dominant hemisphere (2.4, (1.3, 4.5)), brain stem or cerebellum (OR 0.5, (0.3, 0.9)) or frontal lobe (3.7, (1.2, 12.0)), presence of aphasia (OR 7.9, (2.4, 26.0)), dysphasia (5.8, (3.0, 11.3)), gait impairment (1.7, (1.1, 2.7)), presence of white matter hyperintensities (3.2, (2.0, 5.3)) and medial temporal lobe atrophy (3.9, (1.9, 8.3)). **Conclusion:** Factors routinely collected for stroke patients are a useful resource for monitoring dementia progression in this population. In the present meta-analysis, cardiovascular factors, stroke location, stroke-related disability and chronic brain changes were predictors of dementia after ischemic stroke.

Keywords: dementia, ischemic stroke, systematic review, meta-analysis

INTRODUCTION

On average, every 40 seconds someone in the United States suffers a stroke [108]. Stroke survivors represent a high-risk population for progression to cognitive impairment with one in three patients developing dementia after any stroke [109-112].

The majority of stroke cases are ischemic in origin. There have been continuing advances in ischemic stroke treatment through timely treatment and counselling. Meta-analyses on dementia after ischemic stroke as the major driver of stroke burden remain to be scarce. Over the past decade, there has been a lot of emphasis on risk of dementia, but the majority of studies in both meta-analyses and individual studies focused on stroke in general rather than stroke subtypes [44, 113]. This limitation in the literature, together with the continuous developments in clinical diagnoses of stroke subtypes created a need for a dedicated investigation with a focus on ischemic stroke. Ischemic stroke remains to be a major driver of morbidity among stroke survivors [114]. Stroke subtypes vary substantially in severity, management, and prognosis, yet combined evidence in this area remains to be very limited and inconsistent.

In the present report, we conducted a systematic review and meta-analysis to assess incidence rates and predictors of dementia occurrence after ischemic stroke. We aimed to determine the most useful factors that can be used in clinical research for monitoring incident dementia in this population.

METHODS

Search strategy and Selection Criteria

A comprehensive literature search was conducted on MEDLINE and EMBASE (as of May 1st, 2022) using a previously validated search strategy [44]. Searches were limited to studies with human participants. The reference lists of relevant reports and books of abstracts from major recent international stroke and dementia conferences (European Stroke Conference 2018; Alzheimer Association International Conference 2018; International Stroke Conference 2019) were scrutinized for additional studies. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and preregistered on Prospero [115], registration number CRD42022316904 [supplementary table 2].

Inclusion and exclusion criteria were determined a priori. Eligible study designs included retrospective or prospective observational cohort studies, randomized controlled trials and interventional studies. Studies were included if they: 1) assessed dementia incidence among patients with ischemic stroke diagnosis and 2) excluded patients with prevalent dementia at baseline. Studies that included other stroke subtypes without data on ischemic stroke were excluded. In the case of multiple eligible studies from the same population, the most complete or the most recent study respectively were included in the meta-analysis. Incidence rates were assessed per 1000 person-months.

Data extraction and management

Data extraction was completed using a standardized form designed in advance. Factors extracted included patient-level predictors (demographics, cardiovascular disease (CVD) history and major cardiac events, previous stroke and transient ischemic attacks (TIA), stroke location, post-stroke disability, chronic brain changes (including infarctions, brain atrophy and stroke mechanism). Additional predictors assessed included study setting (clinical or registry-based), method of dementia diagnosis (Diagnostic and Statistical Manual of Mental Disorders (DSM), National Institute of Neurological Disorders and Stroke (NINDS) or both) and inclusion of patients with first-ever or recurrent stroke. In addition, study demographic, clinical and epidemiological

variables were extracted (Table 1). A separate dataset was set-up for each predictor to calculate the measures of association with dementia.

Risk of bias assessment

Risk of bias in observational studies was assessed using a modified version of the Newcastle-Ottawa scale that assesses study quality on the basis of selection, comparability and outcome and ranked as low, high or unclear bias in a similar manner to the Cochrane Risk of Bias tool [116, 117].

Data synthesis and analysis

Characteristics of individual studies were summarized as averages for continuous variables and proportions for categorical variables. The following analyses for incident dementia after ischemic stroke were assessed: 1) Proportions; 2) incidence rates and 3) patient-level predictors (demographics, CVD history and major cardiac events, previous stroke and TIA, stroke location, disability post-stroke, chronic brain change and stroke mechanism). Additional predictors assessed included study setting (clinic or registry), method of diagnosis (DSM, NINDS or both) and inclusion of patients with recurrent or first-ever stroke. Incidence rates calculation was restricted to studies with more than 3 months of follow-up after stroke to minimize reverse causation (i.e. counting dementia cases that were likely present before or at the time of ischemic stroke diagnosis). All rates are expressed per 1000 person-months with the exception of figure 2 for the purpose of comparison. Incidence rates were calculated using log transformed rates and inverse variance of rates and average number of person-months following standard approaches for meta-analyses [44, 118-120]. Odds ratios were calculated using the metan command and a combined estimate was calculated for each predictor using a random effects model [120] .

Assessment of heterogeneity and publication bias

Metaregression analysis was performed to assess the impact of hypothesized factors as sources of heterogeneity including assessment of average age, average follow-up, study design, setting and inclusion of recurrent strokes [121]. Begg's and Egger's tests were used to assess publication bias and small-study effects as traditional funnel plot assessments can yield false-positive rates that are higher than the nominal level in the setting of binary outcomes [122, 123]. The I^2 statistic was used to assess heterogeneity between studies [124].

Sensitivity analysis

Sensitivity analyses were performed to assess the influence of including studies with less than 3 months of follow-up. Analyses were performed using Stata v14.0 (StataCorp, College Station, Texas).

RESULTS

Characteristics of included studies

5,843 citations were retrieved from the electronic search. After removing duplicates (n=1,331), 4,512 abstracts of citations were reviewed, and 292 citations were eligible for full text screening. A total of 22 studies matched the eligibility criteria and were included in the meta-analysis [125-145] [Figure 1-PRISMA flow diagram].

The included studies comprised 55,929 patients with ischemic stroke, among which 11,739 dementia cases occurred. The average follow-up was 28.7 months and average age at baseline was 70 years. The majority of included studies were in hospital-based setting (n=18/22), published between 1996 up to 2022 in US, Europe, Asia and Australia. [Figures 1,2, Table 1].

Cumulative incidence of dementia after ischemic stroke

Cumulative incidence of dementia after ischemic stroke was 20% at 5 years, 30% at 15 years and 48% at 25 years of follow-up according to data from three studies from which this data was available [126, 135, 143]. When stratified on study settings, cumulative incidence (expressed as

percentage) of dementia after ischemic stroke for hospital-based setting was: 9.4% at 1 year; 21.4% at 3 years; 26.0% at 5 years and 39.5% at 7 years, while estimates from two registry-based studies (Kokmen [126] et al and Kim [143] et al respectively) were at 1 year 7.0%, 10.4%; at 3 years 10.0% ,15.0%; at 5 years 15.0%, 19.5% and at 7 years (Kim et al) 22.9% [Figure 2].

Incidence rates of dementia after ischemic stroke

The overall proportion of dementia after ischemic stroke was 25% (95% CI 18%, 32%). Reported estimates from hospital-based studies were ~5% higher than those reported from registries [Figures 2b-4].

All rates are expressed per 1000 person-months. The overall rate of dementia after ischemic stroke was 8.6 (95% CI 3.8, 19.6). In stratified analyses the incidence rates of dementia post-ischemic stroke from hospital-based studies were 11.1 (95% CI 4.6, 26.4) compared to 1.8 (95% CI 0.7, 4.2) from registry-based studies. The overall rate among those with previous strokes was 9.4 (95% CI 3.2, 27.5) compared to 7.4 (95% CI 3.4, 16.5) among those with first stroke. The incidence rates in the first decade between 1996-2006 was 33% higher compared to 2007-2019, [Figure 4c]. A sensitivity analysis was conducted to assess the influence of including studies with less than 3 months of follow-up, with overall estimate of 19.67 among those with previous strokes compared to 12.43 among those with first stroke and 16.77 in hospital-based studies compared to 1.80 in registry-based studies [Supplementary figure 9].

Predictors of post-ischemic stroke dementia

Demographics and other general factors

Among 55,929 patients included, the estimated proportion of female cases was 50% and dementia cases among females per study ranged between 35% and 77%. The pooled odds ratio for female versus male gender was 1.2 (95% CI 1.0, 1.4) [Tables 2, 3, Figure 5, Supplementary figure 1].

Relevant clinical history

Molad et al. showed a non-significant odds ratio for post-ischemic stroke dementia by APOE-ε4 carriers versus non carriers of APOE-ε4 (OR 1.1; 95% CI 0.6, 2.1). *Among those with hypertension,* the overall estimated proportion of dementia cases was 69% and ranged between 47% and 88% [table 2]. The pooled odds ratio was (OR 1.4; 95% CI 11.0, 2.0). *Among patients with history of diabetes mellitus,* the overall estimated proportion of cases was 43% and ranged between 17% and 76%. The pooled odds ratio was (OR 1.6; 95% CI 1.3, 2.1). *Among individuals with smoking history,* the overall estimated proportion of cases was 35% and ranged between 16% and 55%. The pooled odds ratio was (OR 0.8; 95% CI 0.7, 0.9). *Among individuals with hypercholesterolemia,* the overall estimated proportion of cases was 41% and ranged between 20% and 68% [table 2]. The pooled odds ratio was (OR 0.8; 95% CI 0.5, 1.3). *Among persons who reported drinking alcohol,* the overall estimated proportion of cases was 18% and ranged between 0% and 44% [table 2]. The pooled odds ratio was (OR 0.9; 95% CI 0.5, 1.7) [Tables 2,3, Figure 5, Supplementary figure 2].

Major cardiac events

The overall estimated proportion of cases among those with *ischemic heart disease* was 24% and ranged between 12% and 33%. The pooled odds ratio was (OR 1.6; 95% CI 0.8, 2.8). *Among patients with atrial fibrillation,* the overall estimated proportion of cases was 25% and ranged between 10% and 40%. The pooled odds ratio was (OR 1.8; 95% CI 1.2, 3.0). *Among patients with history of myocardial infarction,* the overall estimated proportion of cases was 16% and ranged between 13% and 22%. The pooled odds ratio was (OR 0.8; 95% CI 0.5, 1.3). *Among patients with angina pectoris,* the overall estimated proportion of cases was 21% and ranged between 19% and 23%. The pooled odds ratio was (OR 1.2; 95% CI 0.7, 1.8). The overall estimated proportion of cases among patients with *history of heart failure* was 21% and ranged between 3% and 59%. The pooled odds ratio was (OR 1.3; 95% CI 0.6, 2.6) [Tables 2,3, Figure 5, Supplementary figure 3].

Previous stroke or history transient ischemic attack (TIA)

Previous stroke was associated with increased odds of dementia after ischemic stroke (OR 2.0; 95% CI 1.6, 2.6, I^2 5.1%, p=0.4), *history of TI*A was not associated with higher odds of post-

stroke dementia (OR 1.0; 95% CI; 0.7, 1.6, I-squared 13%, p=0.3) [Table 3, Figure 5, Supplementary figure 4].

Stroke location

Among patients with stroke in *the dominant hemisphere*, the estimated proportion of cases was 46% and ranged between 12% and 80%. The pooled odds ratio was 2.4 (95% CI 1.3, 4.5). *Among those with hemispheric lesions,* the estimated proportion of cases with hemispheric lesion was 87% and ranged between 81% and 93%. The pooled odds ratio was 1.2 (95% CI 0.1, 19.4). The estimated proportion of cases among those with a *middle cerebral artery (MCA) lesion* was 73% and ranged between 53% and 93%. The pooled odds ratio was 2.6 (95% CI 0.4, 18.3). The estimated proportion of cases among those with *internal carotid artery lesion,* was 5.0 % compared to 3.8% for non-cases. The pooled odds ratio was 1.3 (95% CI 0.5, 3.5). This in contrast to *cerebellar or brain stem stroke location, where* the estimated proportion of cases was 18.5% compared to 32.9% in patients with no lesion in the cerebellum. The pooled odds ratio was 0.5 (95% CI 0.3, 0.9). Among patients with *frontal lobe stroke,* the estimated proportion of cases was 66.7% and 35% for cases without frontal lobe stroke. The pooled odds ratio was 3.7 (95% CI 1.2, 11.9) [Tables 2, 3, Figure 5, Supplementary figure 5].

Disability post-stroke

Censori et al. showed higher odds of post stroke dementia for *aphasia* (OR 7.8, 95% CI 2.4, 25.9). Zhou et al. demonstrated a higher risk for post-ischemic stroke dementia according to *presence of dysphasia* (OR 5.8, 95% CI 3.0, 11.3) and among those who developed *gait impairment* after stroke, (OR1.7, 95% CI 1.1, 2.3). The study by Zhou et al showed a non-significant association with post-ischemic stroke dementia according to *presence of dyskinesia* (OR 1.1, 95% CI 0.7, 1.7) and *sensory disturbance* (OR 0.9, 95% CI 0.5, 1.6) (Table 3)

Chronic brain changes

One study by Censori et al showed non-significant associations between post-ischemic stroke dementia risk and *presence of cortical atrophy* (OR 1.2, 95% CI 0.4, 3.7) or *subcortical atrophy* (OR 2.1, 95% CI 0.7, 6.5). Censori et al showed a non-significant association between *leukoaraiosis* and post-ischemic stroke dementia risk (OR 2.5, 95% CI 0.7, 8.3). Among patients

with *white matter hyperintensities (WMH)*, the estimated proportion of cases was 9.5% for cases and 6.4% for non-WMH cases [table 2]. The pooled odds ratio was 3.2 (95% CI 1.9, 5.3). The estimated proportion of cases among persons with *medial temporal lobe atrophy (MTLA)* was 60% and range between 50% and 69%. The pooled odds ratio was 3.9 (95% CI 1.8, 8.3). Among patients with *global atrophy*, the estimated proportion of cases was 42% and range between 28% and 56% [table 2]. The pooled odds ratio was 2.5 (95% CI 0.8, 8.1) [Tables 2, 3, Figure 5, Supplementary figure 6].

Stroke mechanism

Among stroke cases with *atherosclerosis* as the main stroke mechanism, the estimated proportion of cases was 46% and ranged between 23% and 81% [table 2]. The pooled odds ratio was 0.7 (95% CI 0.4, 1.3). *Among those with embolic stroke,* the estimated proportion of cases was 20% and ranged between 15% and 24%. The pooled odds ratio was 1.7 (95% CI 0.9, 2.9). Among those with *lacunar stroke,* the estimated proportion of cases was 33% and ranged between 22% and 41%. The pooled odds ratio was 0.9 (95% CI 0.7, 1.4) [Table 3, Supplementary figure 7]. Lastly, among studies that reported 'unknown' as stroke etiology the proportion of cases was 13% and ranged between 5% and 24%. The pooled odds ratio was 1.02; 95% CI 0.68, 1.54 [Tables 2, 3, Figure 5, Supplementary figure 7].

Sources of heterogeneity and publication bias.

Factors that contribute to heterogeneity based on the literature were assessed including average age, follow-up, study design, setting and inclusion of recurrent strokes. Study setting was a significant factor and a potential contributor to heterogeneity with P=0.014 [Supplementary table 2]. Publication bias was not significant with Begg's test P=0.29, P=0.30 (corrected for continuity). Furthermore, egger's test for small-study effects was not significant (P = 0.71).

DISCUSSION

In the present report, we synthesized the literature to summarize incidence rates and predictors of post-ischemic stroke dementia. Our analysis included 22 studies representing 55,929 patients from studies published between 1996 up to 2019 in the Americas, Europe, Asia and Australia. We analyzed 40 predictors of dementia after ischemic stroke. Predictors that were associated with dementia occurrence after ischemic stroke were female gender, hypertension, diabetes mellitus, atrial fibrillation, previous stroke, presence of stroke lesion in (dominant hemisphere, brain stem and cerebellum or frontal lobe), presence of aphasia, dysphasia, gait impairment, presence of WMH and medial temporal lobe atrophy.

Incidence rates of dementia were much higher in studies with very short follow-up (i.e. smaller denominator) and those studies were excluded from the analysis. Studies conducted in a clinical or hospital setting reported higher rates compared to population-based studies. Lastly, rates were higher in settings where DSM alone was the main method of diagnosis compared to NINDS alone or combined with DSM. There was almost 33% difference in incidence rates of dementia in the later study period (2007 – 2019) compared to earlier (1996 – 2006). Incidence rates of dementia after ischemic stroke were 1.5 times higher in the presence of stroke history as opposed to first-time stroke.

Progression to dementia among stroke survivors is multifactorial and could be explained within three main categories, 1) patient-related factors including demographics, 3) severity and location of the presenting ischemic stroke and 3) presence of relevant comorbidities, vascular risk factors, and diffuse cerebrovascular disease. Ischemic infarcts frequently occur alongside arteriosclerotic small vessels disease, which may further contribute to cerebral atrophy and cognitive decline [146-148]. Ischemic lesions of the left hemisphere correlate with intellectual deterioration. It has been suggested that the left hemisphere is responsible for both language and generalized cognitive function [128, 149, 150]. Further, large lesions in the dominant hemisphere in the middle cerebral artery and left carotid artery territories are strongly associated with dementia [128, 149-151]. Evidence also suggests the involvement of limbic structures among stroke patients and this points to the

importance of the medial frontal and medial temporal lobes in memory [128]. Other important factors include presence of a previous stroke that has been independently associated with risk of dementia [152]. In our meta-analysis, studies that included patients with recurrent stroke generally reported higher incidence rates of dementia.

Individual characteristics are key in understanding progression to dementia after ischemic stroke. Older age, years of education and non-white race have been associated with higher occurrence of dementia among stroke patients in general. In our study, age ranged between 65 and 80, likely representing a population at higher risk compared to the general population. Previous studies were inconsistent regarding the role of sex [152-154]. Our results suggest women to have higher odds of developing dementia after ischemic stroke.

Chronic hypoperfusion is an important underlying mechanism through which cardiovascular risk factors could contribute to cognitive decline and dementia in the setting of ischemia. Chronic hypoperfusion results in leukoaraiosis with subsequent functional consequences and impairment of emboli clearance from the brain [155-157]. Our results confirm that patients with atrial fibrillation represent a very high-risk group for dementia after ischemic stroke. This is consistent with the literature on increased risk of dementia after any stroke [152, 158]. Similarly, the presence of diabetes mellitus has been associated with high risk of dementia through indirect effects on cerebral blood flow that are possibly linked to disruption in autoregulation [159].

Our study has several limitations. First, pooled analyses for all factors of interest were hampered by data reporting limitations according to stroke subtype in individual studies. However, to account for this limitation we leveraged data from studies that reported case counts to calculate the odds ratios for predictors of interest. Further, the included studies were heterogeneous in terms of follow-up duration after stroke onset. Therefore, we restricted the combined analysis to studies with more than 3 months of follow-up to minimize reverse causality. Additionally, while I^2 analyses were assessed for each predictor, these estimates remain to be limited to statistical heterogeneity and do not necessarily reflect clinical heterogeneity. To assess heterogeneity further we have conducted an independent meta-regression analysis. Lastly, the predictors

assessed in our analysis are not exclusive factors and are limited by data reported in the individual studies.

Through the present systematic review and meta-analysis, we assessed frequency, patient-level predictors (demographics, CVD history and major cardiac events, previous stroke and TIA, stroke location, disability post-stroke, chronic brain change and stroke mechanism) and study-level predictors of dementia after ischemic stroke (setting; hospital or registry), (methods of dementia diagnosis; DSM, NINDS or both), (first-ever stroke or recurrent stroke).

Given the variations in estimates on post-stroke dementia in the ischemic stroke population according to study-level predictors, comparing evidence from various data sources and study settings is essential. Factors routinely collected for stroke patients are a useful resource for monitoring dementia progression in this population. In the present meta-analysis, cardiovascular factors, stroke location, stroke-related disability and chronic brain changes after stroke were associated with progression to dementia after acute ischemic stroke.

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Table 1. Summary of included studies (N=55,929 ischemic stroke patients)

Author	Year	Country	Design	Setting	N	Average age (years)	Average follow-up (months)(1)	First-ever stroke	Dementia cases (N)	Dementia diagnosis
Censori [125]	1996	Italy	Prospective	Hospital	104	65.0	3.0	Y	15	NINDS-AIREN
Kokmen [126]	1996	USA	Retrospective	Registry	971	NA	84.0	Y	196	Medical records
Bornstein [127]	1996	Israel	Prospective	Hospital	157	72.3	60.0	Y	56	DSM-IIIR
Desmond [128]	2000	USA	Prospective	Hospital	453	70.4	21.0	N	119	DSM-IIIR
Pohjasvaara[129]	2000	Finland	Prospective	Hospital	337	70.2	3.0	N	87	DSM-III
Mok [130]	2004	China	Prospective	Hospital	75	71.0	3.0	N	10	CDR
Rasquin [131]	2004	Netherlands	Prospective	Hospital	176	68.0	12.0	Y	17	DSM-IV
Zhou [132]	2004	China	Prospective	Hospital	434	69.5	3.0	N	118	DSM-IV
Sachdev [133]	2006	Australia	Prospective	Hospital	104	72.2	4.0	N	36	By consensus
Gur [134]	2010	Israel, Turkey	Prospective	Hospital	37	76.0	4.5	Y	17	DSM-IV/ NINDS-AIREN
Allan [135]	2011	UK	Prospective	Hospital	50	80.0	45.5	N	23	DSM IIIR
Melkas [135]	2012	Finland	Prospective	Hospital	263	70.8	90.0	N	47	DSM-IV
Brucki [136]	2012	Brazil	Prospective	Hospital	172	67.7	12.0	N	21	NINDS-AIREN
Sibolt [137]	2013	Finland	Prospective	Hospital	388	72.0	144.0	Y	115	DSM-III
Tu [138]	2014	China	Cross-sectional	Registry	689	68.6	3.0	N	67	NINDS-AIREN
Yang [139]	2015	China	Prospective	Hospital	1013	69.2	4.5	N	88	DSM-IV
Mok [140]	2016	China	Prospective	Hospital	919	67.6	36.0	N	40	DSM-IV
Makin [141]	2018	UK	Prospective	Registry	264	67.0	12.0	N	3	Clinically diagnosed
Surwan [142]	2018	Thailand	Prospective	Hospital	401	63.0	6.0	N	227	DSM-V
Kim [143](2)	2019	Korea	Retrospective	Registry	47779	NA	NA	N	10357	Medical records
Molad [144]	2019	Israel	Prospective	Hospital	397	69.0	24.0	Y	80	MoCA/NeuroTax
Kim [145] (3)	2021	South Korea, Philippines, Hong Cong and China	Prospective	Hospital	746	64	31.2	N	NA	NA

NINDS: National Institute of Neurological Disorders; DSM: Diagnostic and Statistical Manual of Mental Disorders; Association Internationale pour la Recherche et l'Enseignement en Neurosciences; MoCA: Montreal Cognitive Assessment; (1) Studies with follow-up ::3 months were excluded from the rates analysis; (2) Kim 2019 was based on data presented in abstract; (3) Kim 2021[145] was not included in the analysis due to assessment only of cognition outcomes without dementia ascertainment.

Table 2. Summary characteristics of individuals with and without dementia after ischemic stroke

Characteristic		Dementia			No dementia		
	Study, n	mean	minimum	maximum	mean	minimum	maximum
Demographics							
Female	14	50	35	77	42	31	55
Education, years	5	7	3	11	6	4	11
Clinical History							
APOE-ε4 carriers	2	13	7	19	55	17	93
Hypertension	9	69	47	88	57	24	74
Diabetes mellitus	10	43	17	76	33	12	88
Smoking	11	35	16	55	40	16	60
Ischemic heart disease	3	24	12	33	13	8	18
Atrial fibrillation	7	25	10	40	14	8	17
Myocardial infarction	3	16	13	22	18	17	19
Angina	2	21	19	23	19	17	21
Heart failure	5	21	3	59	24	3	86
Hypercholesterolemia	7	41	20	68	47	24	73
Alcohol	4	18	0	44	20	6	50
Previous stroke	5	28	17	38	17	13	21
Previous transient ischemic attack (TIA)	5	12	2	25	10	3	17
Location							
Dominant hemisphere	2	46	12	80	28	6	51
Hemisphere lesion	2	87	81	93	68	67	70
Middle cerebral artery	2	73	53	93	54	48	60
Veretebrobasilar	2	13	8	18	23	13	32
Chronic brain changes							
Number of old infarcts, mean	2	5	3	8	3	2	6
Presence of medial temporal lobe atrophy	2	60	50	69	28.5	28	29
Presence of global atrophy	2	42	28	56	22	22	22
Stroke mechanism							
Atherosclerosis	5	46	23	81	62	18	90
Embolism	5	20	15	24	13	6	20
Lacunar	3	33	22	41	36	32	38
Unknown/other	3	13	5	24	12	3	24

*Represent the maximum and minimum of the factor specified at the study level. All numbers are percentages unless otherwise specified; This table represent covariates that were available in two or more studies.

Table 3. Predictors of post-ischemic stroke dementia (OR, 95% CI)

Determinant	OR and 95% CI
Demographics	
1. Gender*	OR 1.22; 95% CI 1.04, 1.43
Relevant Clinical History	
2. APOE-ε4[I]	OR 1.10; 95% CI 0.58, 2.07
3. Hypertension *	OR 1.43; 95% CI 1.02, 2.01
4. Diabetes mellitus *	OR 1.61; 95% CI 1.26, 2.05
5. Smoking*	OR 0.79; 95% CI 0.66, 0.95
6. Hypercholesterolemia	OR 0.81; 95% CI 0.51, 1.28
7. Alcohol use	OR 0.95; 95% CI 0.54, 1.70
Major Cardiac Events and CVD history	
8. Ischemic heart disease	OR 1.56; 95% CI 0.86, 2.83
9. Atrial fibrillation *	OR 1.88; 95% CI 1.16, 3.04
10. Myocardial infarction	OR 0.96; 95% CI 0.66, 1.39
11. Angina pectoris	OR 1.15; 95% CI 0.74, 1.81
12. Heart Failure	OR 1.26; 95% CI 0.60, 2.63
Previous Stroke or Transient Ischemic Attack	
13. Previous Stroke*	OR 2.03; 95% CI 1.57, 2.64
14. Previous transient ischemic attack	OR 1.05; 95% CI 0.69, 1.59
Stroke Location	
15. Dominant hemisphere*	OR 2.41; 95% CI 1.28, 4.53
16. Hemispheric lesion	OR 1.21; 95% CI 0.07, 19.44
17. Brain stem/Cerebellar*[I]	OR 0.51; 95% CI 0.28, 0.92
18. Frontal lobe*[I]	OR 3.74; 95% CI 1.17, 11.92
19. Middle cerebral artery	OR 2.63; 95% CI 0.38, 18.33
20. Internal carotid artery[I]	OR 1.31; 95% CI 0.49, 3.53
Disability Post-Stroke	
21. Aphasia*	OR 7.89, 95% CI 2.40, 25.97
22. Dysphasia*[I]	OR 5.83, 95% CI 3.02, 11.26
23. Dyskinesia[I]	OR 1.09, 95% CI 0.71, 1.67
24. Gait impairment*[I]	OR 1.74; 95% CI 1.13, 2.267
25. Sensory disturbance[I]	OR 0.93; 95% CI 0.53, 1.65
Chronic Brain Changes	
26. Cortical atrophy[I]	OR 1.15; 95% CI 0.36, 3.68
27. Subcortical atrophy[I]	OR 2.12; 95% CI 0.70, 6.45
28. Total white matter hyperintensities*[I]	OR 3.24; 95% CI 1.98, 5.28
29. Presence of medial temporal lobe atrophy	OR 3.92; 95% CI 1.85, 8.34
30. Global Atrophy	OR 2.54; 95% CI 0.80, 8.10
31. Leukoaraiosis[I]	OR 2.47; 95% CI 0.74, 8.26
Stroke Mechanism	
32. Atherosclerosis	OR 0.74; 95% CI 0.42, 1.31
33. Embolism	OR 1.70; 95% CI 0.97, 2.99
34. Lacunar	OR 0.99; 95% CI 0.73, 1.35
35. Unknown/Other	OR 1.02; 95% CI 0.68, 1.54

**Indicates a statistically significant association with post-ischemic stroke dementia;*! represent predictors for which data were available in single studies for OR calculations.

Title page: First-ever hemorrhagic or ischemic stroke, *APOE* ε4 genotype carriership and risk of dementia in the general population: A three-part modelling approach applied to prospective cohorts in Europe and USA

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Background. Dementia syndromes are among the most complex disorders of mental health in aging populations. Stroke continues to be a major driver of complex mental health disorders including dementia and depression. Previous evidence is limited by inconsistent designs and methods for accurate inference in the general population. The effect of *APOE* ε4 carriership as a risk factor gene for dementia according to stroke subtype remains to be unknown. We assessed the 5-year risk of dementia following first-ever hemorrhagic or ischemic stroke and effect of *APOE* ε4 allele carriership in the general population.

Methods. Meta-analysis of nested cohort studies conducted in three prospective population-based studies in Europe and USA (Three City: cohort baseline 1999; Framingham Heart Study and the Rotterdam Study: cohorts' baseline 1990). We conducted a three-step modelling approach in each cohort. A nested cohort approach was implemented using incident hemorrhagic and ischemic strokes and randomly assigned stroke-free individuals matched 1:2 on age and sex to each individual with stroke. Second, we conducted risk of dementia analyses starting from stroke index date. Last, we applied a random effect meta-analyses of combined 5-year hazard ratios across the three cohorts. We assessed effects of *APOE* ε4 allele carriership and differences in impact of early dementia after excluding individuals with less than 90 days of follow-up. Methods were harmonized and reproduced in identical manner across the three cohorts.

Results. The study included 4,308 individuals (198 individuals with hemorrhagic stroke and 396 stroke-free matched individuals, 1,238 individuals with ischemic strokes and 2,476 stroke-free matched individuals). In the hemorrhagic stroke group compared to stroke-free individuals, the combined hazard ratio was 2.60 (95% CI 0.97, 6.97) with some attenuation after *APOE* ε4 adjustment (2.37, 95% CI 0.95, 5.88). In the ischemic stroke group compared to stroke-free individuals, the combined hazard ratio was 2.21 (95% CI 1.32, 3.69) with no difference after *APOE* ε4 adjustment (2.26, 95% CI 1.28, 3.98). After excluding individuals with less than 90 days of follow-up, the risk of dementia in the hemorrhagic stroke group was 2.51 (95% CI 0.84, 7.50) and 1.47 (95% CI 1.07, 2.04) in the ischemic stroke group.

Interpretation. Risk of dementia syndromes is increased following hemorrhagic stroke as well as Ischemic stroke. The risk of dementia following hemorrhagic stroke but not Ischemic stroke, is slightly decreased after *APOE* ε4 adjustment. Risk of dementia following ischemic stroke is not affected by *APOE* ε4 carriership as a risk factor gene. These findings provide evidence support to guide eligibility of patients for inclusion in clinical trials on Alzheimer's and Related Dementias among stroke survivors.

Funding. The Three-City Study is conducted under a partner- ship agreement among INSERM, Bordeaux School of Public Health (ISPED) of the University of Bordeaux, and Sanofi- Aventis. The “Fondation pour la Recherche Me´dicale” funded the preparation and initiation of the study. The Three-City Study is also supported by the “Caisse Nationale Maladie des Travailleurs Salarie´s,” “Direction Ge´ne´rale de la Sante´,” “Mutuelle Ge´ne´rale de l’Education Nationale,” “Institut de la Longe´vite´,” “Conseils Re´gionaux of Aquitaine and Bourgogne,” “Fondation de France,” and Ministry of Research-INSERM Programme “Cohortes et collections de donne´es biologiques,” French National Research Agency COGINUT ANR-06- PNRA-005 and COGICARE ANR Longvie (LVIE-003-01), the “Fondation Plan Alzheimer” (FCS 2009-2012), and the “Caisse Nationale pour la Solidarite´ et l’Autonomie”. The Framingham Heart Study is supported by contracts HHSN 26820150001I and 75N9019D00031, NIA R01 NS107950 R01 AG054076 P30066546. The Rotterdam Study is sponsored by the Erasmus Medical Centre and Erasmus University Rotterdam, The Netherlands Organization for Scientific Research (NWO), The Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), The Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG 259 XII), and the Municipality of Rotterdam. Further support was obtained from the Netherlands Consortium for Healthy Ageing. Grant K99 AG075196 from the National Institute of Aging, and a Clinical Translational Scholarship in Cognitive Aging and Age-Related Memory Loss funded by the McKnight Brain Research Foundation through the American Brain Foundation, in collaboration with the American Academy of Neurology, a Rose Travelling Fellowship Rose traveling fellowship Program in Chronic Disease Epidemiology and Biostatistics from Harvard T.H.Chan School of Public Health at Harvard University and a pilot grant from the Gertrude H. Sergievsky Center at the Department of Neurology of Vagelos College of Physicians and Surgeons at Columbia University.

INTRODUCTION

Dementia syndromes are among the most complex disorders of mental health in aging populations. Stroke is the most common cerebrovascular disorder in old age and the second leading cause of disability in aging populations [109-111, 160, 161]. Up to 20% of patients develop dementia after major stroke of either ischemic or hemorrhagic origin, and the risk is much higher after recurrent stroke [44, 109-111, 161]. Over the past decade, there has been emphasis on risk of dementia after stroke with limited data on risk according to subtype, with most evidence drawn from clinical cohorts and inconsistent designs and follow-up durations [44, 113, 162].

Stroke subtypes vary substantially in severity, management and prognosis. Ischemic strokes represent ~85% of cases, while hemorrhagic strokes are associated with worse prognosis and high mortality rates. Patients with stroke and their caregivers often are anxious about the future risk of dementia [163-165]. Delineating profiles of dementia risk according to stroke subtype can help to provide patients with more precise prognostic estimates. Moreover, it has direct public health implications through improving clinical monitoring of dementia risk by stroke subtype and to better guide inclusion in clinical trials through enabling interventions for prevention of progression to cognitive impairment and dementia according to stroke subtype and *APOE* ε4 allele carriership, given the role the latter plays in driving both hemorrhage and dementia. Yet data remains limited in the context of population-based cohorts with lack of consistent approaches for reproducibility and replication [109, 111, 166-171].

Hemorrhagic strokes represent 10-15% of cases and are often more severe than ischemic strokes with greater lesion volume, higher mortality and less effective treatment strategies [172, 173]. Despite its rarer occurrence, prognostically, hemorrhagic stroke accounts for 50% of stroke-related disability including triggering subsequent ischemic stroke, rebleeding and progressive cognitive impairment and mortality [174, 175]. However, the long-term risk of dementia after hemorrhagic stroke compared to ischemic stroke and the effect of *APOE* ε4 carriership as a risk factor gene for dementia according to stroke subtype remain unknown. We aimed to assess the five-year risk of dementia after first-ever hemorrhagic and ischemic stroke in the general population and effect of *APOE4* genotype carriership.

METHODS

Study population

The present study is a combined meta-analysis that included individuals with stroke and matched stroke-free individuals from three population-based cohort studies, the Three City Study, the Framingham Heart Study and the Rotterdam Study (table 1).

Three City is a longitudinal population-based study of the relation between vascular diseases and dementia in persons aged 65 years and older [176]. Between 1999 and 2001 a total of 9,294 noninstitutionalized persons recruited and examinations were performed at home or in a dedicated research center every 2 years after the baseline assessment, comprising standardized questionnaires, clinical examinations and detailed cognitive assessment. **Framingham Heart Study** began in 1948 with the recruitment of an original cohort of 5,209 men and women who were 28–62 years of age at entry [177] In 1971, a second generation of study participants, including 5,124 children and spouses of children of the original cohort, were enrolled [178] The original and second generation cohort participants return for clinic examination (or are examined at home or nursing home) every 2 and 4-8 years, respectively. All participants are under continuous surveillance for stroke and dementia. **Rotterdam Study** is a prospective population-based cohort study comprising 14, 926 participants aged 45 years or older[179, 180]. Baseline data of 7,893 participants were collected between 1990 and 1993 (response rate 78%) with subsequent cohort expansions in 2000 (3,011 individuals, 67%) and 2006 (3,236 individuals, 65%). Participants are interviewed at home and reexamined at a dedicated research center once every 4 years. The entire cohort is continuously under surveillance for disease outcomes, including stroke and dementia, through linkage of electronic medical records with the study database.

Ascertainment of stroke

The Three City Study: Diagnosis of stroke was determined during the face-to-face interviews performed by trained psychologists or nurses at each follow-up visit or by a self-questionnaire sent to participants [176, 181]. Written indications given to the interviewers suggested that description of symptoms like hemiplegia, sudden difficulties of speech, sudden paralysis should be employed. If someone declared having had a stroke, that persons was then asked regarding hospitalization for the declared stroke. With this information, a procedure of collecting all available information was started, including hospital records, letters from treating practitioners, and communication

with the general practitioner and the family or next of kin in case few information available. When the information was deemed as complete, the file was then submitted to a stroke validation committee composed of stroke neurologists who adjudicated the case in: stroke confirmed yes/no; type of stroke: brain infarct or intracerebral hemorrhage or unknown; and, in case of brain infarct, its subtype according to the TOAST classification: atherosclerosis, lacunar, cardioembolic, other cause, unclassified. **The Framingham Heart Study:** Diagnosis of stroke and stroke subtype was determined by a panel of 3 investigators (at least 2 neurologists) using ongoing clinic and hospital surveillance data: medical records, imaging, and, when available, examination by a FHS neurologist. Clinical stroke was defined as rapidly developing focal neurologic symptoms and signs of presumed vascular etiology, lasting more than 24 hours [182, 183]. Ischemic stroke was diagnosed if brain imaging showed no hemorrhage, if imaging showed an infarct that correlated with the clinical deficit, or if an ischemic infarction was documented at autopsy. **The Rotterdam Study:** Stroke was defined according to the World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin [180, 184, 185]. History of stroke at baseline was assessed during baseline interview and verified by reviewing medical records. After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners. Nursing home physicians' and general practitioner files of participants who moved out of the district were checked on a regular basis as well. Additional information was obtained from hospital records. Potential strokes were reviewed by research physicians and verified by an experienced neurologist. Stroke subtype (hemorrhagic or ischemic), location, cause and other stroke specific characteristics were based on neuroimaging reports and/or hospital discharge letters. If these were absent, then the stroke subtype was classified as unspecified. This classification corresponded with ICD-10 codes I61, I63 and I64. Participants were followed until the date of stroke, date of death or date of the last contact in case of follow up or 1 January 2016 (end of the study period), whichever came first. Follow-up was virtually complete (95.8%).

Ascertainment of dementia

The Three City Study: Diagnosis of dementia was based on a three-step procedure. Trained psychologists administered a battery of neuropsychological tests. All the participants in Bordeaux

and Montpellier were then examined by a neurologist at baseline, whereas in Dijon only those who screened positive underwent further examination because of the many participants. At follow-up, the participants who were suspected of incident dementia on the basis of their neuropsychological performance were examined by a neurologist in the three study centers. Finally, an independent committee of neurologists reviewed all potential prevalent and incident cases of dementia to obtain a consensus on its diagnosis and etiology according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition. Cases of AD were classified as probable or possible AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria [186]. (Figure 1). **The Framingham Heart Study:** Attendees of clinic examinations undergo an initial screen for dementia with the Mini Mental State Examination (MMSE). Those whose score is lower than education-based cutoffs, or who are referred by clinic staff, caregivers, or self-report of memory problems at the examination or at between examination health status updates, are administered a full neuropsychological battery. Based on those results, the participants may subsequently undergo a neurological examination and then dementia review. The dementia review panel, which includes a neurologist and a neuropsychologist, has reviewed every case of possible cognitive decline and dementia ever documented in the Framingham Heart Study. For cases that were detected before 2001, a repeat review was completed after 2001 so that up-to-date diagnostic criteria could be applied. The panel determines whether a person had dementia, as well as the dementia subtype and the date of onset, using data from previously performed serial neurologic and neuropsychological assessments, telephone interviews with caregivers, medical records, neuroimaging studies, and, when applicable and available, autopsies. After a participant dies, the panel reviews medical and nursing records up to the date of death to assess whether the participant might have had cognitive decline since his or her last examination. The diagnosis of dementia is based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). The diagnosis of Alzheimer's disease is based on criteria for possible, probable, or definite Alzheimer's disease from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA)[186]. (Figure 1). **The Rotterdam Study:** in the baseline and follow-up examinations participants undergo an initial screen for dementia with the Mini Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS), followed by an

examination and informant interview with the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX) in screen positives (MMSE<26 or GMS>0). All participants routinely undergo neuropsychological examinations. For the detection of interval cases in between visits, information is obtained from the GPs and the regional institute for outpatient mental health care. A consensus panel headed by a consultant neurologist makes the final diagnoses in accordance with standard criteria (DSM-III-R criteria; NINCDS-ADRDA; NINDS-AIREN) [187]. (Figure 1)

Statistical analysis

Population characteristics were summarized as mean (Standard Deviations) for continuous variables and frequencies and percentages for categorical variables. The analysis was done in three steps (Figure 2). First the matching in each cohort, second risk of dementia analysis using matched samples and third random effect meta-analyses (Figure 2). Comparisons were conducted on individuals with hemorrhagic strokes and their matched stroke-free comparison and ischemic strokes and their matched stroke-free comparison.

Step one: Matching and follow-up

We used a matched design to create a sample of dementia-free participants with and without stroke in each cohort [188-190]. We performed the matching as described by Matthews and Brill [191]. After excluding participants with prevalent dementia at the time of stroke and those with no dementia follow-up after the stroke, each participant with ischemic or hemorrhagic stroke was matched to 2 randomly selected dementia- and stroke-free individuals with dementia follow-up after the date of their corresponding individual with stroke. From the Three City Study, Framingham Heart Study and the Rotterdam Study (table 1). Incidence density sampling was used to sample stroke-free individuals from the larger population in each cohort [192]. Matching was done on age (+/-1 year) and sex. Follow-up starts for both individuals with stroke and stroke-free individuals at stroke index date. Stroke-free individuals are assigned random ID numbers, assembled in one dataset. The program enables looping through the stroke dataset, one individual at a time and comparing each individual with stroke to the potential stroke-free set to match on age at stroke-subtype diagnosis, that is unique and randomly assigned to each individual with stroke. The iterative loop cycles through the first observation to the last observation among individuals with stroke, retrieving only one observation at a time from the

stroke data set. It then iterates over all the stroke-free data set observations to select the matched stroke-free individuals according to each incident stroke subtype specific age and sex. Individuals with stroke cannot be their own matched comparison, and stroke-free individuals were chosen without replacement. Individuals with stroke and stroke-free individuals were followed up prospectively for occurrence of dementia or death whichever occurs first. Individuals with unspecified stroke diagnosis were excluded. Follow-up time was limited to five years following stroke.

Step two: Risk of dementia analyses

Cox proportional hazards regression analysis was conducted to assess risk of dementia following first-ever hemorrhagic or ischemic stroke [193]. Models were adjusted for sex, age and *APOE* ε4. In the models adjusting for *APOE* ε4, stroke and stroke-free sets in which all three members had available *APOE* ε4 data were included. A sensitivity analysis was conducted to assess the impact of ascertained early dementia after excluding individuals with less than 90 days of follow-up. This analysis was restricted to the Rotterdam study as it had enough participants with hemorrhagic stroke.

Step three: Meta-analysis

The inverse variance weighted random effects model was used to pool the log transformed effect estimates from primary studies. The I^2 statistic was used to assess heterogeneity between studies [124]. Analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC) and Stata software version 15.1 (StataCorp, TX).

RESULTS

Baseline characteristics

The study included a total of 4,308 individuals (198 individuals with hemorrhagic stroke and 396 stroke-free matched individuals, 1,238 individuals with ischemic stroke and 2,476 stroke-free matched individuals). Mean matching ages for individuals with hemorrhagic stroke ranged between 74 and 80. Mean matching ages for individuals with ischemic stroke ranged between 77

and 79. The majority of individuals with hemorrhagic and ischemic strokes were women. Detailed population characteristics are described in table 1.

Risk of dementia among individuals with hemorrhagic stroke versus stroke-free comparison

The combined hazard ratio was 2.15 (95% CI 0.80, 5.57, I^2 =27.8%, P value=0.25). After adjusting for age and sex, the HR is 2.60 (95% CI 0.97, 6.97, I^2 25.0%, P value= 0.26), Figure 3, supplementary figure 1. Among the hemorrhagic group, the combined age, sex and *APOE* ε4 adjusted hazard ratio was 2.37 (95% CI 0.95, 5.88). $I^2$for this analysis was 5.3%, P value=0.35 (Figure 3). After excluding individuals with less than 90 days of follow-up in the RS, the risk of dementia after hemorrhagic stroke was 2.51 (95% CI 0.84, 7.50) and the age and sex adjusted HR was 2.65 (95% CI 0.88, 7.98).

Risk of dementia among individuals with ischemic stroke versus stroke-free comparison

The combined hazard ratio was 1.98 (95% CI 1.09, 3.62, I^2=84.0%, P value=0.002). After adjusting for age and sex, the HR was 2.21 (95% CI 1.32, 3.69, $I^2$77.9%, P value= 0.01) Figure 4, supplementary figure 1. In the ischemic stroke group, the combined age, sex and *APOE* ε4 adjusted hazard ratio was 2.26 (95% CI 1.28, 3.98). The I^2 for this analysis was 79.9%, P value=0.007 (Figure 4). After excluding individuals with less than 90 days of follow-up in the RS, the risk of dementia after ischemic stroke was 1.47 (95% CI 1.07, 2.04) and the age and sex adjusted was 1.53 (95% CI 1.10, 2.11).

DISCUSSION

Risk of dementia as a complex mental health disorder is increased following both, ischemic and haemorrhagic stroke. The risk of dementia following hemorrhagic stroke is slightly attenuated after *APOE* ε4 adjustment while the risk of dementia following ischemic stroke is not affected by *APOE* ε4 carriership as a risk factor gene.

Findings of the present study extend observations in previous reports in selected high-risk groups in longitudinal samples [109, 111, 112, 126, 194-196]. The overall 2.2 to 2.6 fold increase in 5-year stroke

subtype-specific risk of dementia observed in our study is comparable with earlier reports that have shown 2-fold increase in dementia risk within 10 years of follow-up [109, 111]. Two previous registry-based studies also reported similar patterns for the comparison of *stroke cases and stroke-free controls* from Taiwan and Denmark, however the associations were of much higher magnitude, given that these observations were drawn from samples of patients who are likely at higher risk [197]. Another contributing factor is the greater burden of cerebral small vessel disease, atherosclerosis, and higher rates of vascular dementia in Asian populations [198-201].

These observations are supported by the natural history of both stroke subtypes, with high death rates reaching almost 60% in the first 30 days after hemorrhagic stroke and higher survival rates after ischemic strokes, that results in step-wise decline in cognition and more gradual progression to dementia with aging [202, 203]. The immediate effect of stroke on dementia risk is suggested to occur through circuit disruption, diaschisis, inflammatory mediators from the acute vascular lesion or its systemic complications [204-208] In contrast, long-term elevated risk in the ischemic stroke group is likely attributed to longitudinal aging effects over time [159].

The contribution of a high vascular and cerebrovascular disease risk profile is manifested in the incident stroke event itself, and that carries forward the risk of post-event dementia [190, 203, 209]. The lack of association between vascular risk factors and post-event dementia has been documented since early 1990s [203] . Such observations are also supported by evidence from negative clinical trials on the effect of intensive management of vascular risk factors on dementia risk after stroke [210-212]

The frequencies of *APOE* ε4 allele in our study were similar to other stroke populations [44, 213]. The presence of the APOE alleles and APOE e4 allele in particular, are key genetic risk factors for dementia onset, may accelerate the process that leads to CAA-related hemorrhagic stroke [198, 214]. In the present analysis, the risk of dementia following hemorrhagic stroke was slightly attenuated following *APOE* ε4 adjustment. Other potential risk factors for dementia include more extensive acute and chronic inflammatory changes following the stroke event [215, 216]. In contrast the risk of dementia in ischemic strokes compared to stroke-free individuals showed no difference after *APOE* ε4 adjustment. Furthermore, *APOE* ε4 allele effects on risk of dementia

are less pronounced after hemorrhagic or ischemic stroke compared to the rates observed in the general population among stroke-free individuals [217-219].

Strengths and limitations

Our study has several limitations. First the lack of information on stroke etiology and severity across the three cohorts hindered further sensitivity analyses regarding these factors. Previous reports from patients' samples showed persistent patterns of risk following adjustment for these factors [190]. Adding to the limited specificity of small vessel disease in general that would add little beyond a well-confirmed overt stroke event diagnosis. Second, our sample included a proportion of unspecified strokes on which stroke subtype data were not available and thus were not included in the analysis. This could have resulted in underestimation of dementia rates [202, 220]. Third, our sample is restricted to middle-aged and elderly populations, thus may not be readily extrapolated to younger stroke survivors who likely would have different outcomes according to advances in treatment and higher education attainment.

Our study has several strengths. Alongside the consistent dementia follow-up across the cohorts and state-of-the-art clinical examinations, a key strength of our study includes the rigorous design, the use of population-based and randomly assigned stroke-free samples who were followed up prospectively, thus mitigating common selection biases and minimizing bias from misbalance in background covariates and self-selection that are common in studies comparing disease and non-disease groups [221].

Our results have public health significance. Although it is commonly accepted that reducing stroke incidence reduces dementia risk, stroke-subtypes vary greatly in their course of dementia progression, management in both the acute and subacute settings as well as preventive strategies [222-224] . Efforts to understand potential mechanisms related to ischemic stroke remain to be of paramount importance and potentially higher impact on the total burden of dementia, given that it represents the majority of cases and its associated dementia risk exponentially increases with aging.

Future studies should focus on the differences in aging-related factors that trigger progression to Alzheimer's and related-dementias in this vulnerable aging population to accelerate discoveries

of targeted therapeutics and to promote cognitive health[225]. Such dedicated investigations would extend existing knowledge and open new avenues to effectively reduce the burden of dementia and Alzheimer's disease in our aging society.

Conclusion

Risk of dementia is increased following both, ischemic and haemorrhagic stroke. The risk of dementia following hemorrhagic stroke is slightly attenuated after *APOE* ε4 adjustment while the risk of dementia following ischemic stroke group is not affected by *APOE* ε4 carriership as a risk factor gene. These findings provide evidence support to guide eligibility of patients for inclusion in clinical trials on Alzheimer's and Related Dementias among stroke survivors.

Contributors. RW: designed and conceptualized the study, analyzed the data and drafted the manuscript; AB, CD, FJW: analyzed and interpreted the data, revised the manuscript for intellectual content; MAI supervised the study and revised the manuscript for intellectual content; AH, MKI, SD, LF, JRM, HA, LV, AV, KS, JG, SS: Interpreted the data and revised the manuscript for intellectual content. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Declaration of interests: the authors declare no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Data and Code availability: Data and code can be made available to qualified researchers upon request. Framingham Study data are available through BioLINCC, where qualified researchers can apply for authorization to access (biolincc.nhlbi.nih.gov/studies/framcohort/?q=Framingham). Data of European cohorts are available upon request, after approval by the relevant institutional review boards, in keeping with informed consent and the national and EU data protection regulations. Requests can be directed to the following contacts: for RS, data manager Frank J.A. van Rooij (f.vanrooij@erasmusmc.nl), for 3C, the coordinating center (E3C.CoordinatingCenter@gmail.com).

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PNRA-005 and COGICARE ANR Longvie (LVIE-003-01), the “Fondation Plan Alzheimer” (FCS 2009-2012), and the “Caisse Nationale pour la Solidarite´ et l’Autonomie”. The Framingham Heart Study is supported by contracts HHSN 26820150001I and 75N9019D00031, NIA R01 NS107950 R01 AG054076 P30066546. The Rotterdam Study is sponsored by the Erasmus Medical Centre and Erasmus University Rotterdam, The Netherlands Organization for Scientific Research (NWO), The Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), The Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG 259 XII), and the Municipality of Rotterdam. Further support was obtained from the Netherlands Consortium for Healthy Ageing. Grant K99 AG075196 from the National Institute of Aging, Clinical Translational Scholarship in Cognitive Aging and Age-Related Memory Loss funded by the McKnight Brain Research Foundation through the American Brain Foundation, in collaboration with the American Academy of Neurology, a Rose Travelling Fellowship Rose traveling fellowship Program in Chronic Disease Epidemiology and Biostatistics from Harvard T.H.Chan School of Public Health at Harvard University and a pilot grant from the Gertrude H. Sergievsky Center at the Department of Neurology of Vagelos College of Physicians and Surgeons at Columbia University.

Ethics approval: All the participating studies were approved by their respective institutional review committees, and all participants provided written informed consent.

TABLES & FIGURES

Table 1. Description of the cohorts and Population characteristics

Characteristic	Three City		Framingham Study		Rotterdam Study	
Country	France		USA		Netherlands	
Study baseline	1999		1990		1990	
Study sites	3		1		1	
Dementia follow-up, years	16		25		25	
Diagnosis of dementia	DSM-IV		DSM-IV		DSM-IIIR	
	Hemorrhagic	**Ischemic**	**Hemorrhagic**	**Ischemic**	**Hemorrhagic**	**Ischemic**
Total N, strokes	56	244	40	304	102	690
Dementia among stroke survivors, n	2	32	1	31	7	72
N, stroke-free	112	488	80	608	204	1380
Dementia among stroke-free individuals, n	6	21	6	73	11	101
Mean follow-up stroke (years), mean (SD)	2.4 (1.9)	2.6 (1.8)	1.7 (2.2)	2.9 (2.1)	1.4 (1.9)	3.1 (1.9)
Mean follow-up stroke-free (years), mean (SD)	4.6 (1.0)	4.6 (1.0)	4.1 (1.5)	4.1 (1.5)	4.1 (1.3)	4.07 (1.5)
Matching age stroke (years), mean (SD)	80.7 (5.0)	79.3 (5.0)	74.2 (13.1)	77.1 (10.6)	80.0 (7.7)	78.0 (7.4)
Matching age stroke-free (years), mean (SD)	80.7 (5.0)	79.2 (5.2)	74.1 (13.0)	77.0 (10.6)	80.0 (7.6)	78.0 (7.3)
Female stroke, N (%)	32 (57)	132 (54)	23 (57)	166 (54)	65 (63)	385 (55)
Female stroke-free, N (%)	64 (57)	264 (54)	46 (57)	332 (54)	130 (63)	770 (55)
APOE ε4 stroke, N (%)	12 (21)	47 (19)	10 (25)	73 (24)	20 (20)	156 (23)
APOE ε4 stroke-free, N (%)	15 (13)	96 (20)	17 (21)	119 (20)	48 (23)	329 (24)

Figure 1. Steps of Dementia ascertainment in each cohort

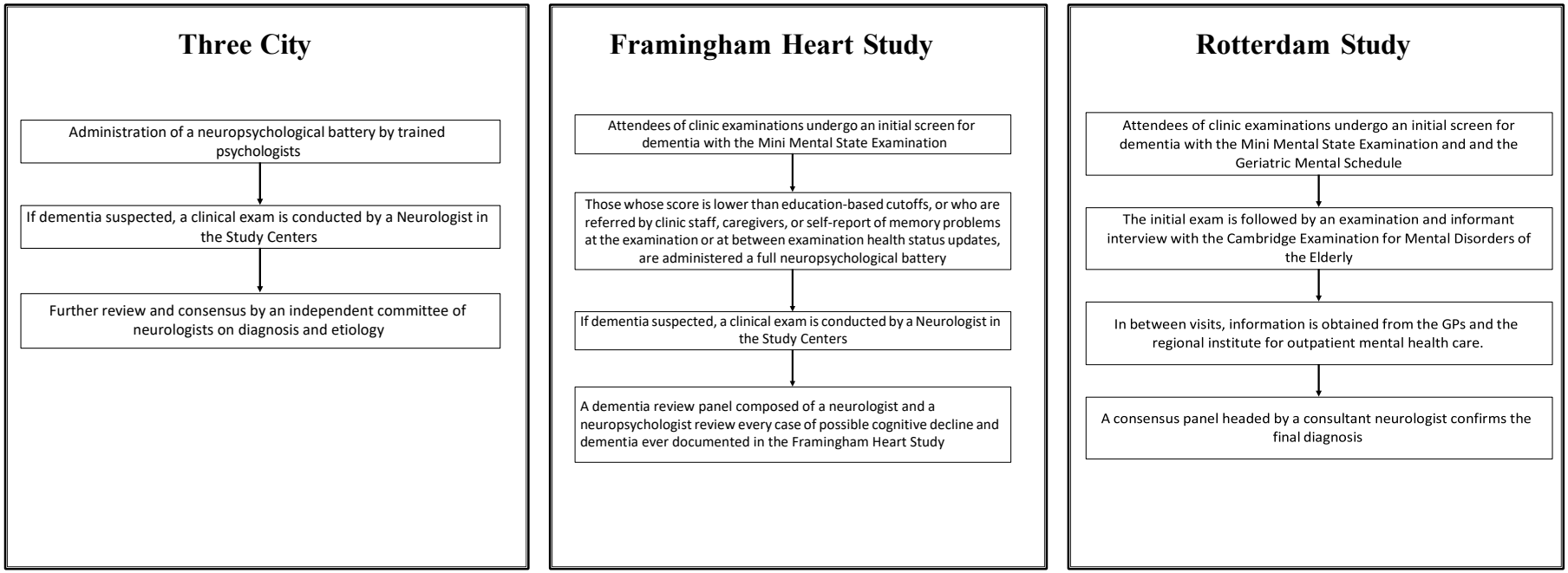


Figure 2. Diagram illustrating the 3-step modelling approach across the cohorts as follows: 1- At the individual level in each cohort (step 1); 2-At the stroke-subtype level in each cohort on matched samples (step 2); 3-At the study-level combining the three cohorts (step 3)

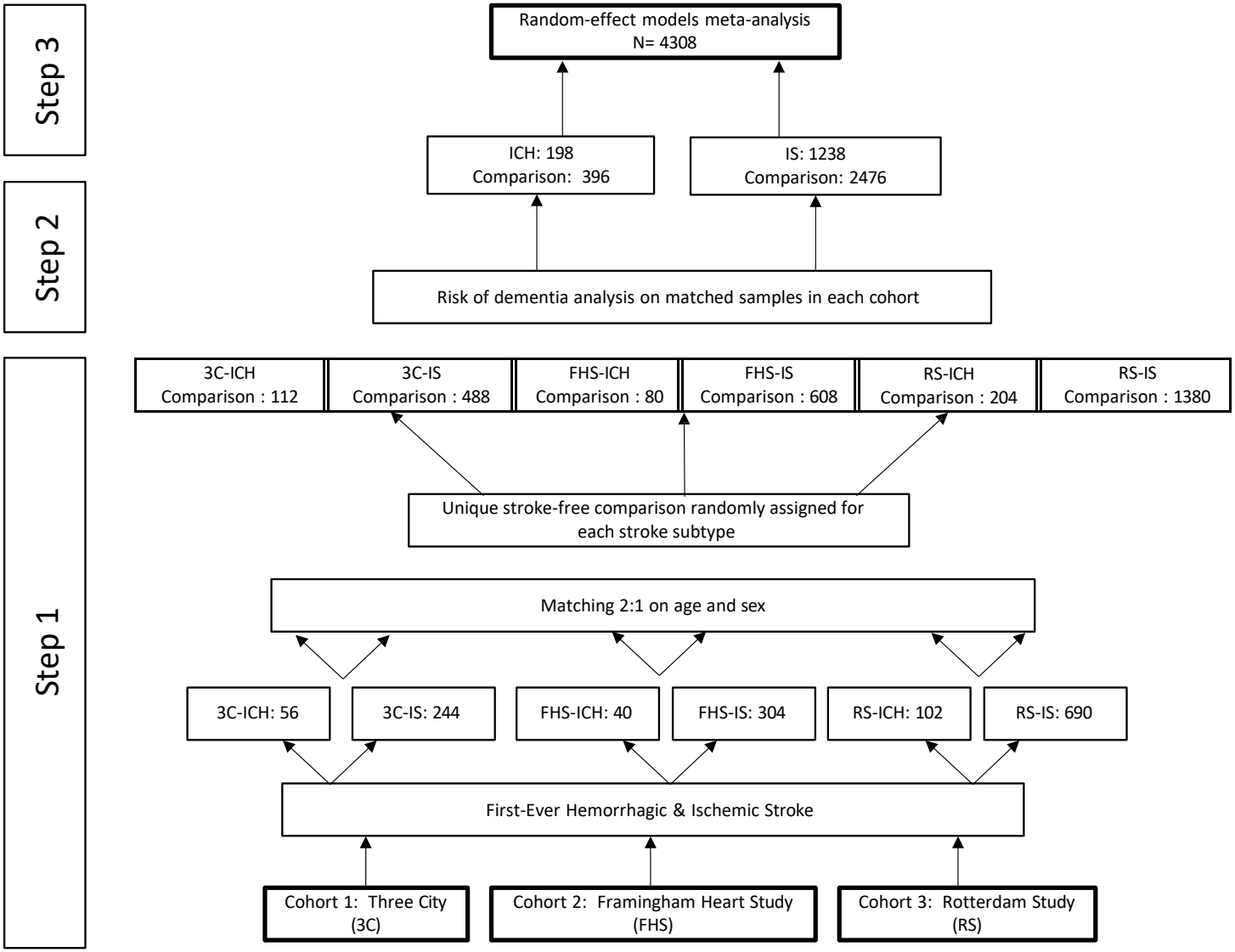


Figure 3. Risk of incident dementia following first-ever hemorrhagic stroke compared to stroke-free individuals in the general population.

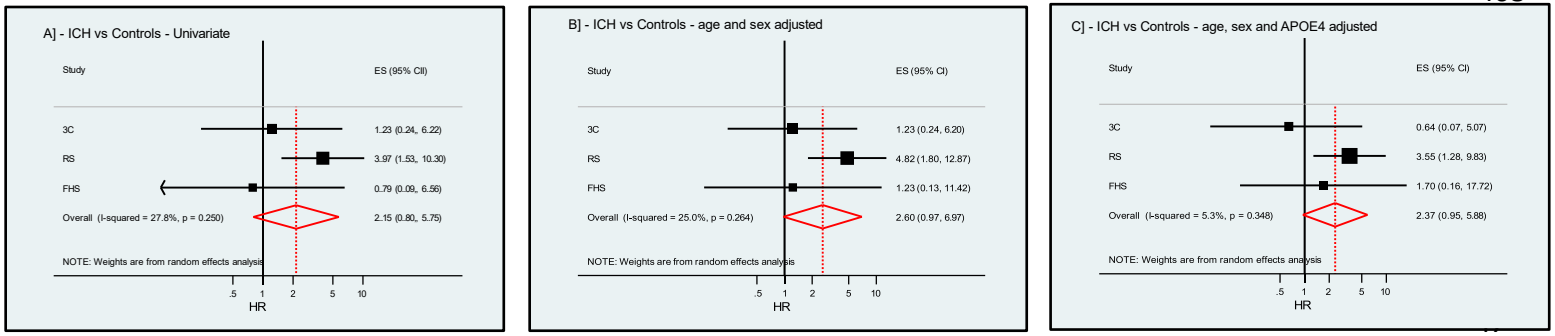
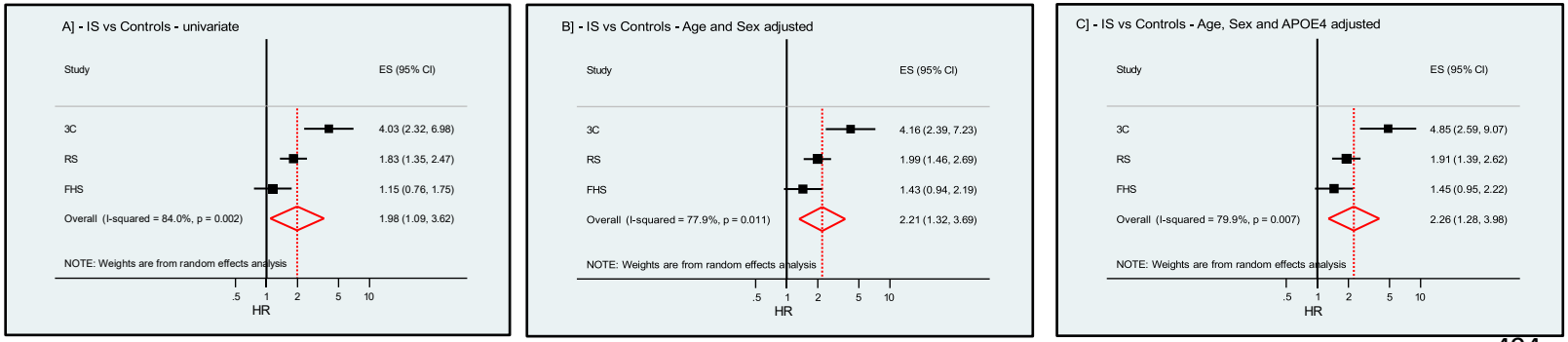


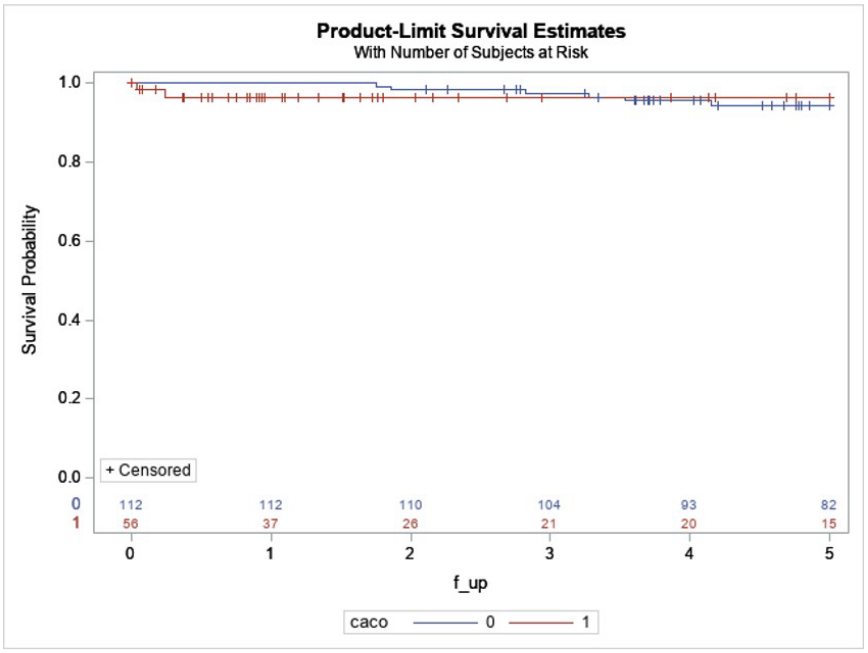
Figure 4. Risk of incident dementia following first-ever ischemic stroke compared to stroke-free individuals in the general population.



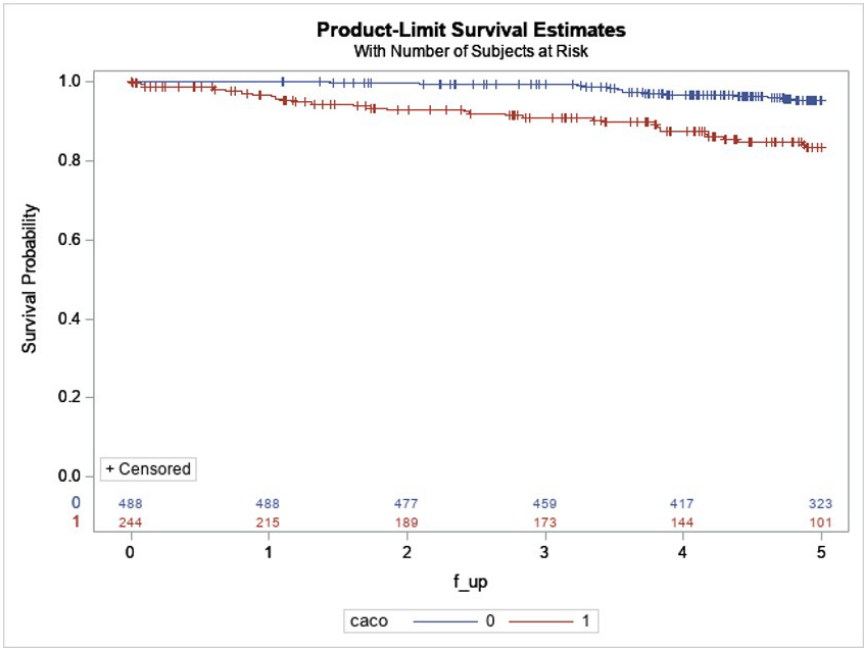
Supplementary figure 1. Kaplan Meier graphs for hemorrhagic and ischemic strokes compared to stroke-free individuals.

Supplementary Figure 1-A: Three City

A] -Three City – Hemorrhagic vs stroke-free

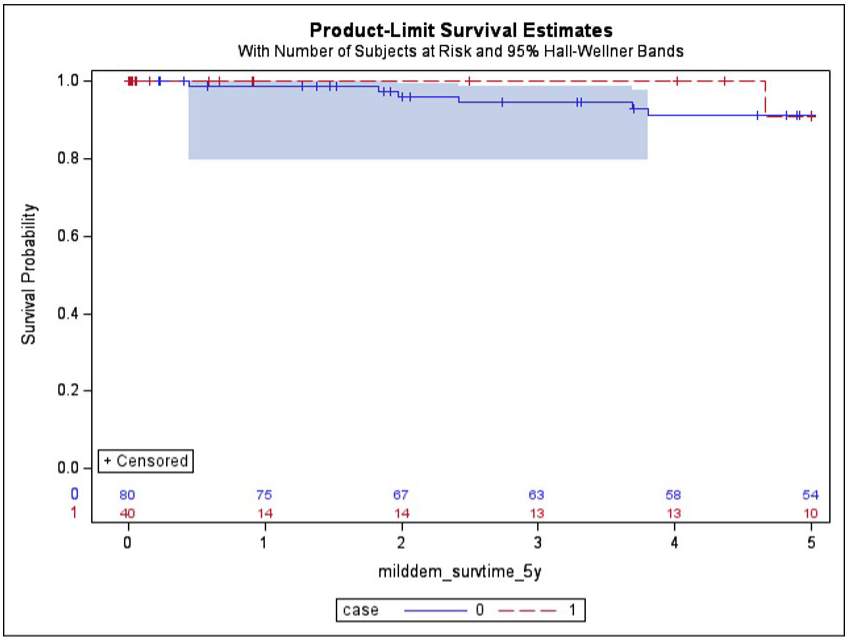


B] -Three City – Ischemic vs stroke-free

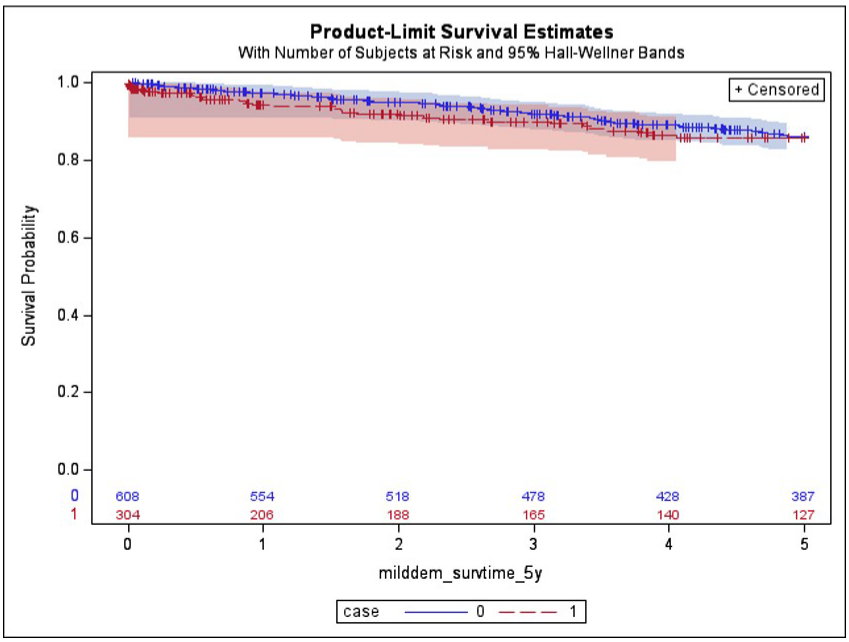


Supplementary Figure 1-B: Framingham Study

A] -Framingham– Hemorrhagic vs stroke-free

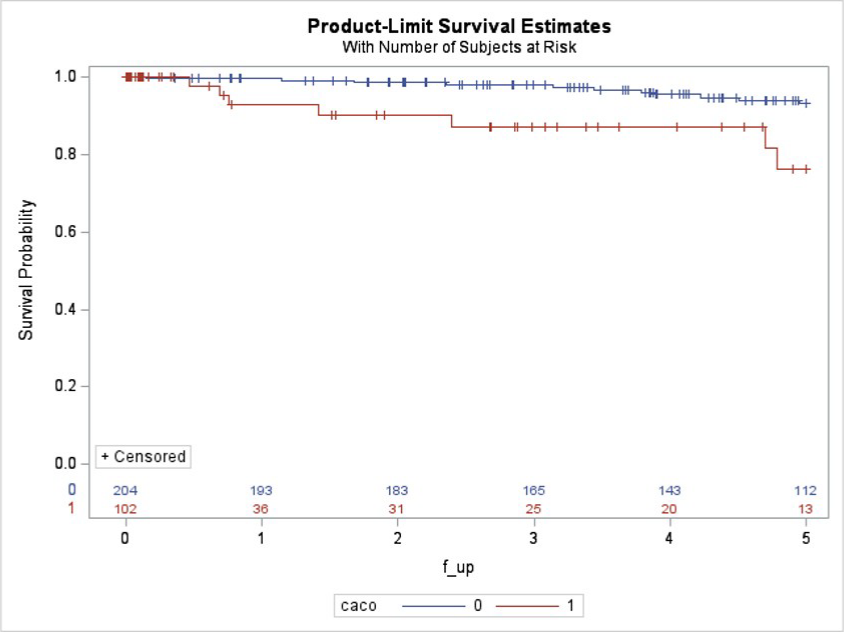


B] -Framingham – Ischemic vs stroke-free

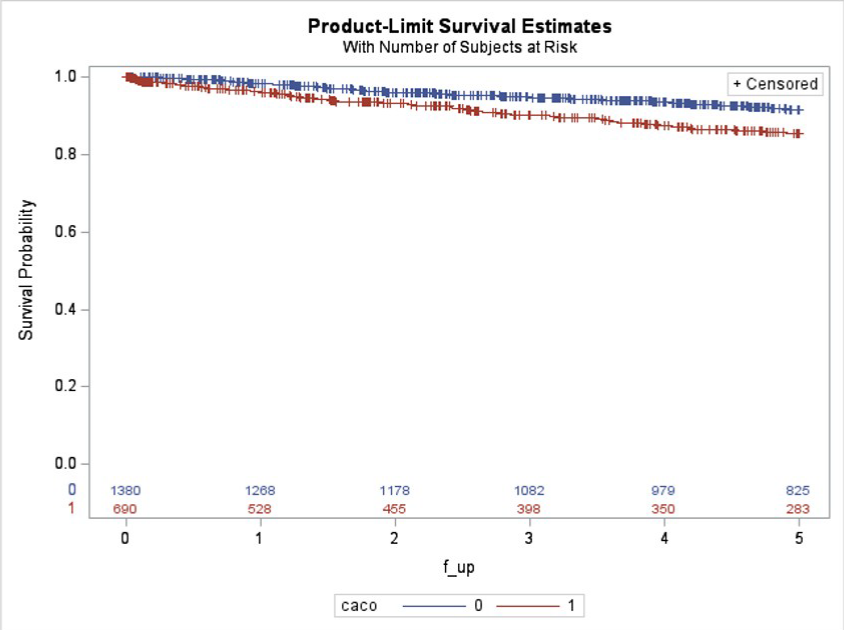


Supplementary Figure 1-C: Rotterdam Study

A] -Rotterdam – Hemorrhagic vs stroke-free



B] -Rotterdam – Ischemic vs stroke-free



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Title page: Biological Aging for Risk Prediction of First-Ever Intracerebral Hemorrhage and Cerebral Infarction in Advanced Age

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Keywords: biological aging; intracerebral hemorrhage; cerebral infarction

Short title: biological aging and risk of intracerebral hemorrhage and cerebral infarction

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ABSTRACT

Background and objectives: successful interventions to prevent cerebrovascular disease and stroke require early identification of persons at risk before clinical manifestation of disease. We assessed the predictive value of biological age (BA) as an early indicator for cerebrovascular disease and risk of first-ever intracerebral hemorrhage (ICH) and cerebral infarction (CI) in advanced age and compared these relationships with commonly used biomarkers including tau and Aβ40 and Aβ42.

Methods: The study included Individuals who consented for blood draw and follow-up. We computed biological age using structural equation modelling. The algorithm integrates biomarkers that represent six body systems involved in overall cerebrovascular health including metabolic function, cardiac function, lung function, kidney function, liver function, immunity and inflammation. Time to event analysis was conducted using Cox-regression models. Prediction analysis was conducted using Harrel's C and Area under the receiver operating characteristic curve.

Results: the sample included a total of 1699 individuals at baseline followed up over a median of 11 years. During a period of 15, 780 and 16, 172 person-years a total of 17 first-ever intracerebral hemorrhage and 83 cerebral infarction cases occurred. In time-to-event analysis, BA showed higher magnitude of associations with ICH compared to CA ($HR_{BA\text{-}ICH:}$ 2.30, 95% CI: 1.20, 4.30; $HR_{CA\text{-}ICH:}$ 1.40, 95% CI: 0.76, 2.53) and higher precision with CI ($HR_{BA\text{-}CI:}$ 1.30, 95% CI: 1.01,1.75; $HR_{CA\text{-}CI:}$1.90, 95% CI: 1.48, 2.66). BA outperformed CA for prediction of ICH (AUC: 0.68 vs 0.53; Harrel's C: 0.72 vs 0.53) and for CI (AUC:0.63 vs 0.62; Harrel's C: 0.68 vs 0.67).

Conclusions: Biological aging based on integrated physiology biomarkers provides a novel tool for monitoring and identification of persons at highest risk of cerebrovascular disease in advanced age. Wider applicability requires extension of such findings from the general population to the clinical and primary care settings.

INTRODUCTION

The risk of intracerebral hemorrhage (ICH) and cerebral infarction (CI) double with every decade of life and continue to escalate with the aging of populations [1, 39]. Chronological age is not a specific indicator for risk prediction of cerebrovascular disease. Data from autopsy studies have shown wide variability between individuals in their ability to tolerate clinically occult pathological changes and physiological degradation before manifestation of disease [226, 227]. Traditional approaches that account for aging through chronological age overlook within and between individual variations in aging, that are hypothesized to be root causes of these devastating disorders [41]. These observations collectively suggest that variations between individuals might be linked to individual specific factors such as life-time exposures and resiliency [228, 229].

The relationship between biological aging using integrated physiology approaches and risk of incident (new-onset) age-related cerebrovascular disorders has not been assessed before. Previous studies that assessed relationship between aging biomarkers and health outcomes were limited in design and suffered temporality in assessment of disease endpoints. The especial emphasis on physiology-based biomarker panels is informed by previous studies that observed weak to moderate associations of other biological aging metrics with health outcomes beyond what is explained by chronological age [230-232].

In the present study, we assessed the relationship between biological age and risk of first occurrence of intracerebral hemorrhage and cerebral infarction in advanced age and compared these relationships with commonly used biomarkers including tau and Aβ40 and Aβ 42.

METHODS

The Rotterdam Study

The present report included participants from the Rotterdam Study, a prospective population-based cohort [179, 180]. In 1990, residents aged 55 years and older residing in Ommoord, a district of Rotterdam, the Netherlands, were invited to participate. Of 10,215 invited inhabitants, 7,983 agreed to participate in the baseline examinations. In 2000, 3,011 participants (of 4,472 invitees) who had reached 55 years of age or moved into the study district since the start of the study were added to the cohort. Follow-up examinations take place every 3–4 years. For the purpose of this study, 2000 individuals were randomly selected from the fourth visit of Rotterdam Study-I (RS-I-4) and the second visit of Rotterdam Study-II (RS-II-2). The inclusion criteria included the availability of informed consent and valid serum samples collected between 2002 and 2005 in these visits of Rotterdam study I and II (Figure 1, Supplementary Figure 1).

Informed Consent and Ethics Approval

The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). When visiting the study center, participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

NHANES

The reference population included participants from the Third National Health and Nutrition Examination Survey (NHANES III), a nationally representative, cross-sectional study conducted

by the National Center for Health Statistics conducted between 1988 and 1994. Data collection was done through at-home interviews and examinations. Further details on study population and design are available through the Centers for Disease Control and Prevention [233]. This external independent population was used to train and calibrate the biological aging algorithm to be assessed for risk prediction of our outcomes of interest in the Rotterdam Study population.

Stroke ascertainment in the Rotterdam study population

Stroke was defined according to the World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin [179, 184, 234]. History of stroke at baseline was assessed during baseline interview and verified by reviewing medical records. After enrollment, participants were continuously monitored for incident stroke through automated linkage of the study database with files from general practitioners. Nursing home physicians' and general practitioner files of participants who moved out of the district were checked on a regular basis as well. Additional information was obtained from hospital records. The information collected on potential strokes was reviewed and classified by research physicians and verified in a consensus panel led by two experienced stroke neurologists. Final stroke diagnosis was adjudicated in accordance with the above mentioned standardized diagnostic WHO criteria, which were held constant over the entire follow-time. Strokes were further classified as ischemic or primary intracerebral haemorrhage based on neuroimaging reports or hospital discharge letters. If neuroimaging was not conducted or reported, a stroke was classified as unspecified. This classification corresponds with ICD-10 codes I61, I63 and I64. In the stroke analysis, participants could contribute to follow-up until

first-ever stroke, death, loss to follow-up or last health status update when they were known to be free of stroke, whichever came first, until January 2016. Follow-up was virtually complete (97.8%).

Quantification of biological age

A detailed description of the biological aging calculation is provided in a previous report [3]. Briefly, nine biomarkers were selected based on: 1) their independent relationship with age; 2) their availability in the Rotterdam Study, and; 3) validation of the algorithms in previous studies [235, 236]. The biomarkers represent six systems as follows: total cholesterol (metabolic function); systolic blood pressure (cardiac function); forced expiratory volume (lung function); serum creatinine and serum urea nitrogen (kidney function); serum alkaline phosphatase and serum albumin (liver function) and C-reactive protein, cytomegalovirus optical density (immune function and inflammation) [3]. These biomarkers were measured using serum. (Table 1, Figure 1, Supplementary Figures 1, Supplementary Tables 1-3).

Biological age was calculated as the weighted mean of these 9 parameters using structural equation modelling (Supplementary Figure 5). Two separate models were used for men and women. The calculations included three main steps in SAS software. First, we used the function PROC CALIS to generate a set of weights (i.e., coefficients) using biomarker data from NHANES for multiplication with chronological age and the biomarkers in the structural equation models [237]. These weights are the product of the variance covariance matrix [238]. Second, we multiplied the biomarker weights by chronological age and the biomarker values through PROC SCORE. Finally, we scaled the calculated biological age to the chronological age scale. This is

an alternative to normalizing chronological age and biomarkers using the mean and standard deviation. Given biomarkers of whole blood samples (as HBA1c) were not available in our data, we compared nine versus ten biomarkers algorithm in NHANES and the results showed similar results of hazard ratios and model performance based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Supplementary Table 3).

Measurement of biomarkers of total-tau, Aβ 40 and Aβ 42

EDTA plasma was sampled, aliquoted and frozen at -80ºC according to standard procedures. Measurements were obtained from a random selection of 1000 participants from sub-cohort RS-I-4 and 1000 from RS-II-2. All measurements were performed at Quanterix (Lexington, MA, USA) on a Single Molecule Array (Simoa) HD-1 analyzer platform. Samples were tested in duplicate. Two quality control (QC) samples were run on each plate for each analyte. The Simoa Human Neurology 3-Plex A assay (N3PA) was used for measuring the concentration of total-tau, Aβ 40 and Aβ 42. When duplicates or single measurements were missing or in the case the concentration coefficient of variation (CV) exceeded 20% or control samples were out of range, participant's data were excluded from the analyses. A dedicated investigation of biomarkers of neurodegeneration in relation to stroke has been conducted in a larger sample of the Rotterdam Study[27].

Statistical analysis

Delta biological age was calculated as the difference between biological age and chronological age for each individual in the Rotterdam Study population. Delta biological age in this case represents the absolute difference between predicted biological age and observed chronological

age. We assessed onset of two clinical endpoints: 1) Intracerebral hemorrhage; and 2) Cerebral infarction. Follow-up was defined as time from visit date (date of biological age measurement) at which biological age was calculated to stroke date, death or end of follow-up on January 1st, 2016, whichever occurred first. In the time to event analysis using Cox model, individuals who had stroke date equals death date or end of study follow-up, contributed one day of follow-up in the analysis [202]. Those with prevalent stroke or unspecified stroke diagnosis were excluded. Models were adjusted for age, sex and BMI. Biomarkers were categorized in tertiles and assessed for risk prediction of intracerebral hemorrhage and cerebral infarction in separate models. Area under the receiver operating characteristic curve (AUC) analysis was conducted to assess the sensitivity (true positive rate) and specificity (true false rate) of delta biological age for prediction of intracerebral hemorrhage and cerebral infarction.

RESULTS

Population characteristics

The sample included a total of 1699 individuals at baseline followed up over a median of 11 years. During a total of 15, 780 person-years a total of 17 intracerebral hemorrhage cases occurred, among which 47% were females, 52% were overweight and 41% were APOE4 carriers. During 16, 172 person-years a total of 83 cerebral infarction cases occurred, among which 73% were females, 79% were overweight and 37% were APOE4 carriers (Table 1).

Biological age as a predictor of risk of ICH and IC

In time-to-event analysis risk of intracerebral hemorrhage (compared to the lowest tertile) was 2.30 (95% CI 1.20, 4.30) per tertile increase of delta biological age. We compared this in the same sample for which our biological aging biomarkers are computed to per tertile change in chronological age 1.40 (95% 0.76, 2.53). BA exceeded CA for prediction of ICH (AUC: 0.68 vs 0.53; Harrel's C: 0.72 vs 0.53) (Tables 1-2, Figure 1, Supplementary figures 2-4, Supplementary table 1).

In time-to-event analysis, risk of cerebral infarction (compared to the lowest tertile) was 1.30 (95% CI 1.01, 1.75) per tertile increase of delta biological age. We compared this in the same sample for which our biological aging biomarkers are computed to per tertile change in chronological age 1.90 (95% 1.48, 2.66). BA exceeded CA for prediction of IC (AUC:0.63 vs 0.62; Harrel's C: 0.68 vs 0.67) (Tables 1-2, Figure 1, Supplementary figures 2-4, Supplementary table 1).

DISCUSSION

Biological age measured before clinical disease manifestation was able to identify individuals at risk of developing first-ever onset of intracerebral hemorrhage and cerebral infarction with varying magnitude and precision.

The ability of delta biological age, calculated for stroke-free individuals at baseline, to predict risk of intracerebral haemorrhage and cerebral infarction many years before onset could be discussed in several ways. First, the algorithm is based on physiological parameters that reflect the various body systems and the overall underlying individual specific physiological integrity, the various pathophysiological processes and susceptibility to ischemia [239]. Second, the algorithm was free of brain age biomarkers that are usually significantly higher in later stages along the brain aging continuum and thus offer less opportunities for reversing the progression of cerebrovascular diseases. Furthermore, in our analysis biological aging differences showed varying degrees for risk prediction of cerebral infarction compared to Tau and Beta-amyloid biomarkers[240]. Third, the long follow-up and rigorous clinical examinations allowed precise monitoring of individuals in healthy states prior to clinical manifestation of cerebrovascular disease including strokes. Lastly, the use of the delta biological age measured as the difference between biological age and chronological age reflects within individual variation in aging that cannot be captured by variables measured at time of assessment that mostly reflect cross-sectional data.

Our study has several limitations. First, repeatedly measured biomarker data was not available to calculate that pace of aging that could further confirm the rate of change in biological age.

Second, biomarkers of whole blood samples (as HBA1c) were not available; however, the results are comparable as we validated nine versus ten biomarkers in NHANES. Lastly, despite the limited power of the outcome events in the intracerebral hemorrhage group, our sample at baseline was sufficiently powered to detect effect estimates in both groups.

Biological aging is rarely addressed in neurological investigations and its relation to stroke subtypes has not been assessed before. Biological aging is quantified through algorithmic combinations of biological parameters that are sensitive to aging [18, 235]. Among these approaches, combinations of physiological biomarkers are accessible from blood tests and show minimum biases in relation to method of measurement [241]. Therefore, the present investigation has important clinical implications. Given the frequency of stroke occurrence and the multi-factorial nature of the disease, development of tools that are accessible and easy to administer in the general practice setting, would accelerate primary prevention efforts of cerebrovascular disorders, through identifying individuals at high risk many years before disease onset. This would offer opportunities for early interventions for those at most risk and potentially reverse risk profiles before clinical manifestation has occurred [242]. The discovery of minimally invasive and cost-effective biomarkers for cerebrovascular disorders that are extracted from accessible tissue such as serum and sensitive to within individual differences in aging, have the potential to transform clinical research and practice by permitting widespread low-cost screening, risk prediction, and efficient identification of persons with the greatest disease risk for inclusion in clinical trials.

Biological age predicts risk of risk of first-ever ICH and CI in advanced age. The time lag between degradation at the physiological level and onset of cerebrovascular disorders observed in our analysis is an ideal time for intervention in populations at risk. Future studies should confirm our observations and further characterize aging mechanisms that contribute towards brain reserve and resilience among individuals with similar risk profiles.

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CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

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Table 1. Population characteristics

	All	Intracerebral hemorrhage	Cerebral infarction
N	1699	17	83
Age, baseline	70 (65, 76)	71 (66, 76)	74 (70, 79)
Female	967 (57)	8 (47)	61 (73)
BMI, mean (SD)	27 (25, 30)	26 (24, 27)	27 (25, 29)
BMI categories[a]			
≤25	464 (27)	8 (47)	29 (35)
25-30	806 (48)	9 (52)	66 (79)
>30	421 (25)	3 (17)	24 (29)
APOE4%	430 (25)	7 (41)	31 (37)
Individual biomarkers[b]			
C-reactive protein (mg/dL)	0.30 (0.10–15)	0.21 (0.10–0.40)	0.21 (0.10–0.50)
Creatinine (mg/dL)	0.90 (0.50–40)	0.96 (0.91–1.04)	0.98 (0.84–1.14)
Albumin (g/dL)	4.00 (3.00–5.00)	4.60 (4.40–4.70)	4.50 (4.30–4.60)
Total cholesterol (mg/dL)	217 (86–360)	207 (177–241)	209 (178–235)
Cytomegalovirus optical density	87 (5–180)	1.80 (1.20–2.10)	1.90 (0.70–2.10)
Urea nitrogen (mg/dL)	15 (4–66)	15.90 (12.70–19.60)	15.90 (13.40–19.80)
Alkaline phosphatase SI (U/L)	78 (22–437)	77.5 (72.50–95.50)	79 (65–95)
Forced expiratory volume (mL)	2 (0.20–7.20)	2.30 (1.80–3.10)	2.20 (1.80–2.90)
Systolic blood pressure	146 (92–225)	161.5 (139.5–172.7)	150.5 (136.5–164.5)

BMI was categorized according to the CDC definition of normal (18.5-25), overweight; 25-30; obese 30 or higher. b Individual biomarkers values rounded to first decimal place

Table 2. Chronological age, delta biological age, Tau, Aβ 42/Aβ 40 ratio and risk of intracerebral hemorrhage or cerebral infarction

	Intracerebral hemorrhage			Cerebral infarction		
Biomarker (per tertile)	**HR**	**95% CI**	***P value***	**HR**	**95% CI**	***P value***
Chronological age	1.40	0.76, 2.53	0.20	1.90	1.48, 2.66	<0.001
Biological age, delta	2.30	1.20, 4.30	0.01	1.30	1.01, 1.75	0.04
Total tau	1.30	0.75, 2.46	0.30	1.30	1.00, 1.66	0.10
Aβ 42/Ab40	1.20	0.69, 2.26	0.40	1.40	1.05, 1.83	0.02

Figure 1. Study flowchart

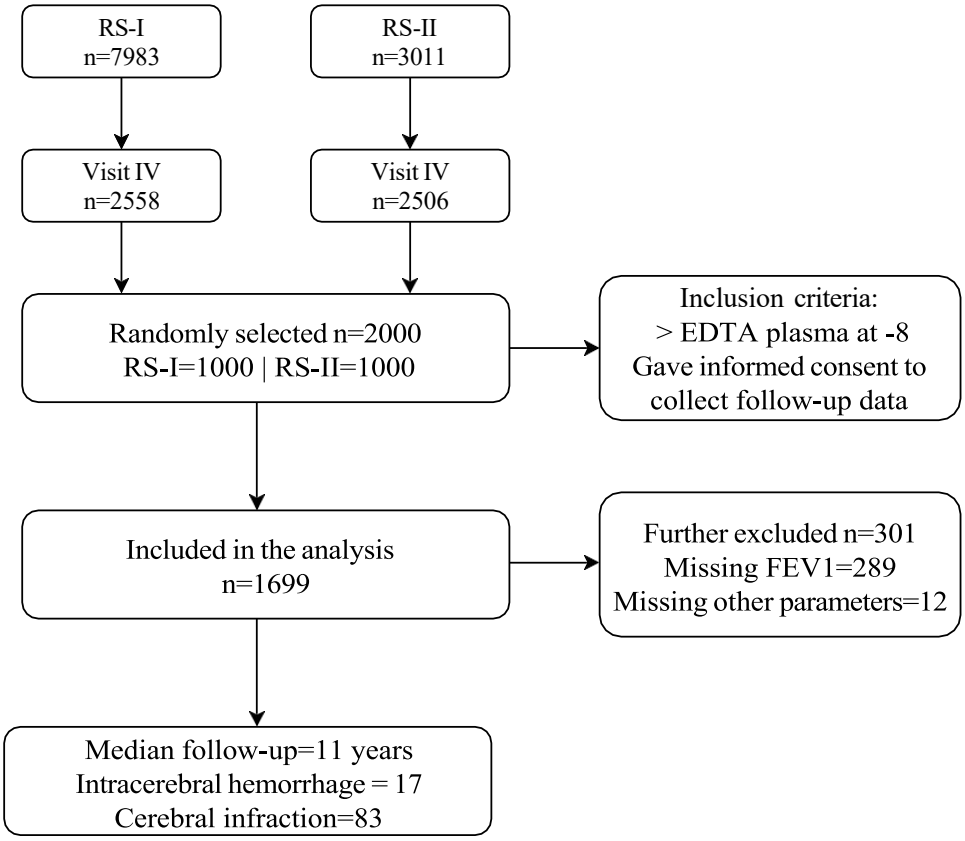
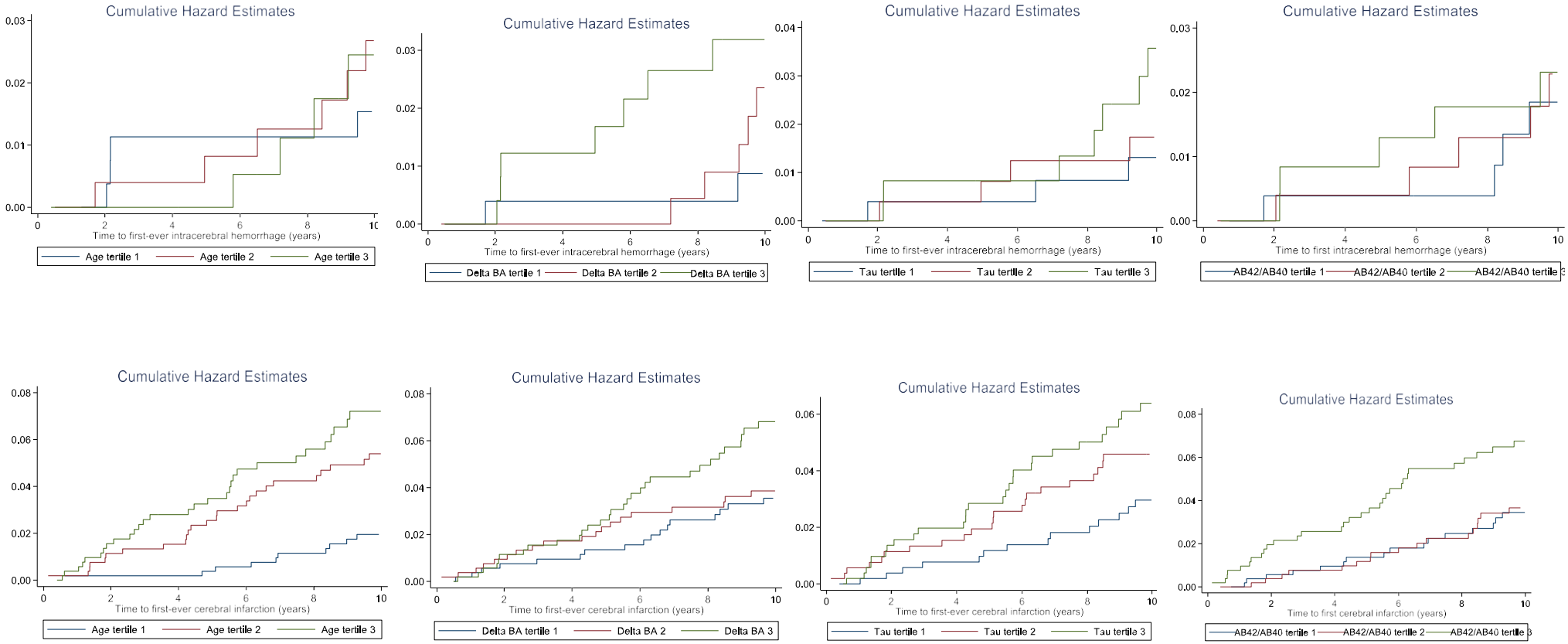


Figure 2. Cumulative hazards of time to intracerebral hemorrhage (upper row) or cerebral infarction (lower row) by tertiles of biomarkers



Title page: The Role of Inflammation and Biological Aging in the Relationship between Stroke and Cognition in Older Adults.

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ABSTRACT

Background. Previous investigations have focused on the direct effects of stroke and vascular disease on cognition and ADRD, the role of indirect pathways that influence cognition and ADRD in this population remain unclear and would yield important insights on mechanistic and pathophysiological processes that link vascular aging effects and ADRD.

Objective. To delineate the effects of inflammation, biological aging and genetics on the pathway between stroke and cognition

Methods. The present study included individuals who consented for blood draw in the Health and Retirement 2016 Venous blood study. Four-way decomposition models were conducted to assess the direct and indirect relationship between stroke and cognition. Biomarkers assessed included: biological aging-physiology, DNA methylation, Mitochondrial DNA Copy number, C-reactive protein, interlukein-6, interlukein-1RA, interlukein-10, tumor necrosis factor-alpha and tumor necrosis factor-receptor I and transforming growth factor-beta activated form (TGF-beta)

Results. Cytokines showed synergistic mediated interaction effect on the relationship between stroke and cognition as follows: mediated interaction coefficient = 0.14 (95% CI 0.09, 0.27, P value < 0.0001), while physiological and DNA methylation-based biological aging showed antagonistic mediation effects on the relationship between stroke and cognition as follows: mediation coefficient = -1.22 (95% -1.33, -1.112, P value < 0.0001).

Conclusions. In the relationship between stroke and cognition, inflammatory pathways including cytokines play a synergistic interaction role. In contrast, biological aging mediates the relationship between stroke and cognition. These results indicate different mechanistic roles of aging and vascular disease on cognition.

INTRODUCTION

Aging is hypothesized to be a root cause of morbidity in elderly including cerebrovascular disease and stroke [3, 41, 225]. Since calendar time passes at the same rate for everyone, aging biology but not chronological age, is a more accurate and precise estimate to account for aging effects in humans in relation to both healthy aging and vascular aging [243].

Stroke by itself, is an established risk factor for cognitive fluctuations and progression to cognitive impairment and ADRD in older adults such that individuals with stroke have 2 to 3 times higher risk of developing deficits in cognitive function compared to the general population [109, 159, 244]. Biologically older individuals are at higher risk of developing first-time stroke and have higher burden of cerebrovascular and cardiovascular disease [225]. In addition, inflammation and key genetic factors such as APOE allele carriership are suggested to play key roles in the susceptibility of an affected individual to age with intact cognition or progression to cognitive impairment and dementia. Since previous investigations have focused on the direct effects of stroke and vascular disease on cognition and ADRD, the role of indirect pathways that influence cognition and ADRD in this population remain unclear and would yield important insights on mechanistic and pathophysiological processes that link vascular aging effects and ADRD.

In the present investigation we aimed to delineate the effects of inflammation, biological aging and genetics on the pathway between stroke and cognition.

METHODS

Study population

The Health and Retirement Study (HRS) is a nationally representative longitudinal survey that recruited more than 37,000 individuals aged 50 and older in the U.S. The survey has been conducted every two years since 1992 with a focus on issues related to changes in health and economic circumstances in aging at both the individual and population levels [4]. HRS data are linked to records from Social Security, Medicare, Veteran's Administration, the National Death Index and employer-provided pension plan information. Genetic ancestry in HRS is identified through PC analysis on genome-wide SNPs [4]. HRS is coordinated by the Institute for Social Research at the University of Michigan.

Standard Protocol Approvals, Registrations, and Patient Consents

The present study included individuals who consented for blood draw in the Health and Retirement 2016 Venous blood study [4, 5]. The HRS (Health and Retirement Study) is sponsored by the National Institute on Aging (NIA U01AG009740) and is conducted by the University of Michigan. The Health and Retirement study is reviewed and approved by the University of Michigan's Health Sciences IRB. The present study has been conducted in accordance with STROBE guidelines for observational studies [7].

Blood sample collection

Blood collection in HRS VBS was done by a premiere organization for collection of biological samples (Hooper Homes Health and Wellness). Contact information of participants who consented for blood draw was sent to the phlebotomists' homes in advance of the scheduled appointment. All blood draw appointments were scheduled within four weeks of the HRS core interview. Participants were recommended but not required to be fasting. 50.5 mL blood was collected in six tubes. 82.9% of participants who consented for blood draw had a complete blood collection.

Biological aging biomarkers

We used two blood-based biological aging clocks: a) Phenotypic-age which is a measure of physiological-level aging, b) and GrimAge which is a measure of aging at the DNA methylation level. Phenotypic Age is described in detail by Liu et al [18]. This clock uses the following 9 parameters: albumin, creatinine, glucose, [log] C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count. These parameters were previously validated as predictors of aging in comparable settings [18]. GrimAge is available through the HRS epigenetic clocks release [8, 12]. GrimAge was constructed as a composite marker calculated from epigenetic surrogate markers for 12 plasma proteins ((adrenomedullin, β-2-microglobulin, CD56, ceruloplasmin, cystatin C, epidermal growth factor (EGF) fibulin-like extra-cellular matrix (ECM) protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor 1, serum paraoxonase/arylesterase 1, and tissue inhibitor metalloproteinases 1) and smoking-pack years [12]

Inflammatory biomarkers

We used cytokines as the main biomarker set for assessment of inflammation. The Cytokine panel assays were measured on the whole sample of the HRS VBS and included: interlukein-6

(IL-6), interlukein-1RA (IL-1RA), interlukein-10 (IL-10), tumor necrosis factor-alpha and tumor necrosis factor-receptor I (TNF-alpha, sTNFR-I) and transforming growth factor-beta activated form (TGF-beta). In addition, we conducted additional models after inclusion of C-reactive protein (CRP). CRP was measured as part of the HRS DBS [245].

Genetic biomarkers

We included two main genetic biomarkers, namely: APOE genetic allele carriership and mitochondrial DNA copy number. APOE was measured for HRS participants who consented and completed DNA collection in phases 1-4 [246]. Mitochondrial DNA Copy number was measured on a subsample of the HRS VBS sample [247].

Exposure and outcome ascertainment

Stroke was assessed through self or proxy report. Although imperfect, the high correlation between self-reported strokes and hospital records is well documented [2, 19-22]. Stroke subtype specification is not available in HRS. Stroke events were ascertained as first reported event that is nonfatal or fatal based on self or proxy-report (for fatal events) of a doctor's diagnosis (e.g., "Has the doctor ever told you that you had a stroke or transient ischemic attack?" [23]. New stroke cases were ascertained using the same question but specific to the wave ("e.g., reports stroke this wave"). Participants were also asked about the stroke month and year and when such information was not available, the median stroke month for events reported by other participants in the same interview wave. Individuals who reported a possible stroke/TIA were not included. Cognition was assessed using the total cognition score that sums the total recall and mental status indices at time of examination in visit 13 [23].

Statistical analysis

Participants who consented for blood draw in HRS VBS were included [4-6]. Summary statistics were assessed as median (IQR) for continuous variables and percentages for categorical variables. To calculate the natural direct and indirect effects in the relationship between stroke and cognitive outcomes we adopted a four-way decomposition modelling approach that calculates the controlled direct effect, pure indirect effect, reference interaction and mediated interaction as proposed by Tyler Vanderweele [248]. The package CMA Verse was used to calculate the following estimates [249]: (controlled direct effect (cde); pure natural direct effect (pnde); total natural direct effect (tnde); pure natural indirect effect (pnie); total natural indirect effect (tnie); total effect (te); reference interaction (intref); mediated interaction (intmed); proportion controlled direct effect (cde prop); proportion reference interaction (intref prop); proportion mediated interaction (intmed prop); proportion pure natural indirect effect (pnie prop); overall proportion mediated (pm); overall proportion attributable to interaction (int); overall proportion eliminated (pe). Standard errors were calculated using 20 bootstrapped samples. Missing data was handled using multiple imputation [250]. The total direct effects (TDE) can be summarized by the formula: TDE = Controlled Direct Effect + Reference Interaction + Mediated Interaction and The total indirect effects (TIE) can be summarized by the formula: TIE = Pure Indirect Effects + Mediated Interaction.

Sensitivity analyses were conducted to assess compound effects of genetic biomarkers with inflammatory biomarkers on the relationship between stroke and cognition.

RESULTS

Population characteristics

The study population included 1, 853 individuals with stroke at visit 13 and 19, 035 individuals without stroke, among which 13, 685 had complete cognitive assessment details, 9, 819 had inflammatory biomarkers data, 9, 344 had physiology biomarkers data and 4, 013 had DNA methylation data. APOE data was available for 13, 215 individuals and mitochondrial DNA copy number was available for 3, 766 individuals.

Inflammatory Cytokines on the Pathway Between Stroke and Cognition

Cytokines showed synergistic mediated interaction effect on the relationship between stroke and cognition as follows: mediated interaction coefficient = 0.14 (95% CI 0.09, 0.27, P value < 0.0001). Proportion of mediated interaction = -0.053 (95% -0.10, -0.035, P value < 0.0001) and proportion of mediation = 0.09 (95% CI 0.048, 0.11, P value < 0.0001). The overall proportion of interaction were not statistically significant. The controlled direct effect coefficient = -3.19 (95% CI -4.10, -2.38, P value < 0.0001) compared to total effect coefficient = -2.69 (95% CI -3.07, -2.57, P value < 0.0001) denoting antagonistic mediated interaction effects of inflammatory cytokines on the pathway between stroke and cognition.

Inflammatory Cytokines and C-Reactive Protein on the Pathway Between Stroke and Cognition

Cytokines combined with C-reactive proteins showed both reference interaction and mediated interaction effects on the relationship between stroke and cognition as follows: mediated interaction coefficient = 0.14 (95% CI 0.09, 0.28, P value < 0.0001), reference interaction coefficient = 0.48 (95% CI 0.089, 0.74, P value < 0.0001). Proportion of mediated interaction = -0.053 (95% -0.09, -0.035, P value < 0.0001), proportion of reference interaction = -0.17 (95% CI -0.26, -0.037, P value < 0.0001) and proportion of mediation = 0.03 (95% CI 0.016, 0.071, P value < 0.0001). The controlled direct effect coefficient = -3.14 (95% CI -3.52, -2.51, P value < 0.0001) compared to total effect coefficient = -2.77 (95% CI -3.01, -2.42, P value < 0.0001) denoting synergistic combined effects of inflammatory cytokines and CRP on the pathway between stroke and cognition.

Physiological and DNA Methylation-based Biological Aging on the Pathway Between Stroke and Cognition

Physiological and DNA Methylation-based Biological Aging showed antagonistic mediation effects on the relationship between stroke and cognition as follows: mediation coefficient = -1.22 (95% -1.33, -1.112, P value < 0.0001). Overall proportion of mediation = 0.46 (95% CI 0.38, 0.49, P value < 0.0001) and proportion of pure natural indirect effect = 0.42 (95% CI 0.36, 0.47, P value < 0.0001). The controlled direct effect coefficient = -0.73 (95% CI -2.26, 0.028, P value=0.1) compared to total effect coefficient = -2.85 (95% CI -3.19, -2.58, P value < 0.0001) denoting antagonistic combined mediation effects of biological aging on the pathway between stroke and cognition.

Sensitivity analysis

Inflammatory Cytokines and Genetic biomarkers (APOE and Mitochondrial DNA Copy Number) on the Pathway Between Stroke and Cognition

Cytokines combined with genetic biomarkers showed synergistic mediated interaction effect on the relationship between stroke and cognition as follows: mediated interaction coefficient = 0.15 (95% CI 0.05, 0.30, P value < 0.0001). Proportion of mediated interaction = -0.057 (95% -0.10, -0.018, P value < 0.0001) and proportion of mediation = 0.08 (95% CI 0.046, 0.12, P value < 0.0001). The overall proportion of interaction = -0.21 (95% CI -0.51, 0.054, P value < 0.0001) and therefore showed statistically significant interaction effects. The controlled direct effect coefficient = -2.95 (95% CI -4.18, -2.40, P value < 0.0001) compared to total effect coefficient = -2.76 (95% CI -3.11, -2.44, P value < 0.0001) denoting antagonistic mediated interaction effects of inflammatory cytokines on the pathway between stroke and cognition and general attenuation of cytokine effects after adding APOE as a genetic biomarker (Supplementary Table 1). Mediated interaction effects remained significant after adding Mitochondrial DNA copy number with coefficient = 0.26 (95% CI 0.06, 0.37, P value < 0.0001), however overall proportion of interaction was not statistically significant.

Physiology-based or DNA methylation-based biological Aging Combined with Chronological Aging on the Pathway Between Stroke and Cognition

Overall mediation effects of biological aging remained statistically significant after adding chronological age to physiology-based biological aging = 0.43 (95% CI 0.37, 0.51, P value < 0.0001) or DNA methylation-based biological aging = 0.38 (95% CI 0.28, 0.49, P value < 0.0001) denoting intact mediation effects of biological aging on the pathway between stroke and cognition.

DISCUSSION

In the present study we conducted a decomposition analysis to delineate the precise differences between effects of biological aging and inflammation in the relationship between stroke and cognition. We evaluated biological aging in terms of DNA methylation and physiological aging clocks. We assessed the role of inflammation with a focus on cytokines and c-reactive protein. Our results indicate an antagonistic mediative role of biological aging, in contrast to inflammation that showed an interactive effect including both pure interaction role and mediated interaction. These results remained consistent after inclusion of key genetic risk factors including APOE allele carriership and mitochondrial DNA copy number.

Part of the effects of stroke on cognition is suggested to be driven by inflammation [251-254]. Inflammatory biomarkers vary in their specific roles, particularly in the context of aging [254]. Previous evidence suggested that inflammation due to aging or what is commonly termed inflamm-aging to play a distinct role in age-related morbidity [255-257]. Cytokines serve as important inflammatory biomarkers given their distinct cellular functions [258-262]. Cytokines also have been commonly used as targets in development of therapeutics [259, 263, 264]. Their precise roles have not been defined on the pathway between stroke and cognition. Our results indicate a consistently interactive role of cytokines both as reference interaction and mediated interaction that is amplified when adding c-reactive protein, rather than a pure mediation effect. That suggests that inflammation indeed contributes to cognitive decline and ADRD even in the absence of stroke or cerebrovascular disease as the main trigger.

While aging itself is not necessarily considered a pathological process, accumulating evidence regards biological aging as a root cause of morbidity in aging [41]. Therefore, the traditional framework of single risk factor and single outcome has resulted in longer lives rather than healthier lives. In previous work we established cerebrovascular events including stroke as diseases of aging that result from programmatic phenomena rather than accidents [265, 266]. It is also known that cognition and ADRD are largely rooted in cumulative aging effects [267]. Therefore, the decomposition of biological aging effects in the relationship between stroke and cognition is of special importance to understand the mechanistic nature of these relationships. Stroke in particular serve as an ideal population to understand effects of aging on cognition, given its clear diagnosis and ascertainment [268]. Our results have shown consistent mediation rather than interactive role of biological aging on cognition among stroke survivors. Therefore, interventions and therapeutics that target stroke solely are less likely achieve the target of prevention of cognitive deficits and progression to ADRD that are common in this population. The closeness of the connections between cerebrovascular events, biological aging and cognition clearly highlights the role of vascular aging as a key component to achieve healthy aging and potentially reverse cognitive deficits, especially after the acute effects of stroke has waned [252].

There are several limitations in the present study. Data on stroke subtype were not available. However, subtype specification is unlikely to change the direction of the main results given that the vast majority of stroke cases are ischemic in nature [202]. Follow-up time was limited in relation to cognition assessments and that hindered incorporation of time to event analysis. However, the presence of strong associations despite the relatively short follow-up confirms the nature and magnitude of the direct and indirect relationships.

There are several strengths in the present study. First, the comprehensive blood-based biomarkers in the HRS venous blood study allowed for detailed analyses on the role of key inflammatory and biological aging biomarkers. Second, the large population that is representative of the general population in the U.S. allows for generalizability to other settings of older adults. Lastly, the use of powerful analytical approaches designed specifically to evaluate indirect and possibly implicit relationships provided a validated means to delineate the role of key pathways of interest related to cognition among stroke survivors.

Inflammatory pathways including cytokines plays a synergistic interaction role in the relationship between stroke and cognition. In contrast, biological aging mediates the relationship between stroke and cognition. These results indicate different mechanistic roles of aging and vascular disease on cognition.

Title page: Connections between Cross-Tissue and Intra-Tissue Biomarkers of Aging Biology in Older Adults

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ABSTRACT

Background: Saliva measures are generally more accessible than blood, especially in vulnerable populations. However, connections between aging biology biomarkers in different body tissues remain unknown.

Methods: The present study included individuals (N=2, 406) who consented for blood and saliva draw in the Health and Retirement Venous blood study and Telomere length study who had complete data for both tissues. We assessed biological aging based on Telomere length in saliva, DNA methylation and physiology measures in blood. DNA methylation clocks combine information from CpGs to produce the aging measures representative of epigenetic aging in humans. We analyzed DNA methylation clocks proposed by Horvath (353 CpG sites), Hannum (71 CpG sites), Levine (referred to as phenoAge, (513 CpG sites)), GrimAge, (epigenetic surrogate markers for select plasma proteins), Horvath Skin and Blood (391 CpG sites), Lin (99 CpG sites), Weidner (3 CpG sites), and VidalBralo (8 CpG sites). Physiology measures (referred to as Phenotypic age) included albumin, creatinine, glucose, [log] C-reactive protein, lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count. Phenotypic Age algorithm is based on parametrization of Gompertz proportional hazard models. Average telomere length was assayed using quantitative PCR (qPCR) by comparing telomere sequence copy number in each patient's sample (T) to a single-copy gene copy number (S). The resulting T/S ratio was proportional to telomere length, mean. Within individual relationships between aging biology measures in blood and saliva and variations according to sex were assessed.

Results: Both physiology-based and DNA methylation-based aging biology biomarkers in blood showed inverse relationships with saliva-based telomere length. Longer saliva-based telomere length was associated with 1 to 4 years slower biological aging based on blood-based biomarkers with the highest magnitude being Weidner (β = -3.97, P = 0.005), GrimAge (β = -3.33, P <0.001), and Lin (β = - 3.45, P = 0.008) biomarkers of DNA methylation.

Conclusions: There are strong connections between aging biology biomarkers in saliva and blood in older adults. Changes in DNA methylation and physiology biomarkers of aging biology vary with changes in telomere length. We observed variations in the relationship between each body system represented by physiology biomarkers and biological aging, particularly at the DNA methylation level. These observations provide novel opportunities for integration of both blood-based and saliva-based biomarkers in clinical care of vulnerable and clinically difficult to reach populations where either or both tissues would be accessible for clinical monitoring purposes.

INTRODUCTION

Aging is hypothesized to be a key driver cause of major age-related pathologies and vascular disease[45, 239, 269]. Measures of aging biology have been proposed as a proxy to the global aging status of an individual[41]. Different tissues are commonly used, including saliva and blood derivatives, to measure biological aging. Blood tissues used include venous versus capillary, and whole blood versus point of care and other blood products such as plasma[270]. Saliva is considered one of the most accessible body tissues and have been effectively used in clinically difficult populations, among whom obtaining blood access is not feasible[271]. Clinical uses of saliva include monitoring drug doses of cardiovascular and neurological disorders such as diabetes mellitus, multiple sclerosis and epilepsy[271]. It has proven economic efficacy due to its easier access and the need for less materials and time to obtain, ship and store samples[272, 273].

There are variations in concentration and abundance of molecular analytes between saliva and blood[274]. These established differences would prompt the use of either tissue according to the targeted investigation, age group or endpoint being assessed[275]. Differences in abundance may therefore directly affect the precision of what is measured and may translate in differences in predicting risk of complications[275-277]. Similarly, differences between blood and saliva include variations according to age, sex, type and size of salivary gland, blood type and physiological status, which are more likely to influence salivary tissue content[278, 279].

The precise differences between those measures would provide opportunities for more effective diagnostic and prognostic tools in clinical medicine and would open avenues for interventions at a whole population scale as cost-effective screening and monitoring tools become available in the near future[279]. In the present study we aimed to assess connections between cross-tissue (i.e. saliva - blood) and intra-tissue (i.e. blood – blood) aging biomarkers to evaluate whether saliva-based telomere length biomarkers of aging biology reflect changes in blood-based DNA methylation and physiology biomarkers in older adults.

METHODS

Study population

The Health and Retirement Study (HRS) is a nationally representative longitudinal survey that recruited more than 37,000 individuals aged 50 and older in the U.S. The survey has been conducted every two years since 1992 with a focus on issues related to changes in health and economic circumstances in aging at both the individual and population levels [4]. HRS data are linked to records from Social Security, Medicare, Veteran's Administration, the National Death Index and employer-provided pension plan information. Genetic ancestry in HRS is identified through PC analysis on genome-wide SNPs [4]. HRS is coordinated by the Institute for Social Research at the University of Michigan. The present study included individuals who consented for blood and saliva draw in the Health and Retirement 2016 Venous blood study and 2008 Telomere length study respectively and who had complete data for both tissues (N=2, 406) [4-6]. Individuals without data on telomere length, physiology and DNA methylation measures were not included in the present investigation to allow for direct individual comparisons using blood-based biomarkers and saliva-based biomarkers. The included sample had similar overall age and sex distribution to the full sample with weighted mean age equals 67 and 54% females. The Health and Retirement study is reviewed and approved by the University of Michigan's Health Sciences IRB. The present study has been conducted in accordance with STROBE guidelines for observational studies (https://www.strobe-statement.org/).

Biological aging biomarkers

We assessed biological aging based on A-Telomere length, B-DNA Methylation and C-Physiology measures.

A- Telomere Length measurement

Telomere length data were available from 5808 HRS respondents who consented for saliva sample draw during the 2008 interview wave. Assays were performed by Telome Health (Telomere Diagnostics, http://www.telomehealth.com/). Average telomere length was assayed using quantitative PCR (qPCR) by comparing telomere sequence copy number in each patient's sample (T) to a single-copy gene copy number (S). The resulting T/S ratio was proportional to telomere length, mean. The HRS *2008 Telomere* data set is sponsored by the National Institute on Aging (NIH NIAU01AG009740 and RC4 AG039029) and was conducted by the University of Michigan[6].

The Venous blood substudy (VBS) and assay protocol in 2016 Health and Retirement Study

All respondents, with the exception of proxy and nursing home respondents, who completed the HRS interview in 2016 (visit 13) were asked to consent for blood draw (78.5% of participants interviewed through September 5 2017) [5]. Physiology based biomarkers were assessed on the whole sample of participants who consented for blood draw, while DNA-methylation was assessed in a subsample randomly selected and fully representative of the whole sample [5]. Blood samples were centrifuged and shipped overnight to CLIA-certified Advanced Research and Diagnostic Laboratory at the University of Minnesota. Tube processing was done within 24 hours of arrival at the lab (within 48 hours of collection). All assays were done at the University of MN Advanced Research and Diagnostic Laboratory (ARDL) under the direction of Bharat Thyagarajan.

B-DNA Methylation

DNA methylation assays were done by Infinium Methylation EPIC BeadChip at the University of Minnesota following the manufacturer's instruction. The minfi package in R was used in data processing and DNA methylation measures were provided through HRS to the research community[8, 280]. Epigenetic clocks are commonly based on portions of the genome where methylation changes are related to chronological age, and more recently, health outcomes[8]. The clocks combine information from CpGs to produce the aging measure that represents an indicator of epigenetic aging[8]. DNA methylation assessment included the following clocks: Horvath [9, 17] and Hannum et al.[10], Levine et al (referred to as phenoAge) [15], Lu et al (referred to as GrimAge) [12], Horvath Skin and Blood [281], Lin[282-284], Weidner[284], and VidalBralo[285]. The Horvath epigenetic clock predicts age using 353 CpG sites in the DNA methylation profile and incorporates 51 healthy tissues and cell types[9, 17]. The Hannum et al. clock comprises 71 CpG sites selected from the Illumina 450k array that capture changes in chronological age. It was developed in whole blood of humans at ages 19 to 101[10]. DNAm PhenoAge was developed in whole blood using composite clinical biomarkers combined into a multisystem measure of biological age (phenotypic age). DNAm PhenoAge based on 513 CPGs predicted Phenotypic age in whole blood from the same sample [15]. GrimAge is a mortality predictor and was constructed based on surrogate markers for select plasma proteins ((adrenomedullin, β-2-microglobulin, CD56, ceruloplasmin, cystatin C, EGF fibulin-like ECM protein 1, growth differentiation factor 15, leptin, myoglobin, plasminogen activator inhibitor 1, serum paraoxonase/arylesterase 1, and tissue inhibitor metalloproteinases 1) and smoking-pack years in a two-stage procedure [12].GrimAge is suggested to have predictive ability for time to death, coronary heart disease, cancer and age-related conditions [12]. Skin and Blood was developed as a novel and highly robust DNAm age estimator (based on 391 CpGs) for human fibroblasts, keratinocytes, buccal cells, endothelial cells, lymphoblastoid cells, skin, blood, and saliva samples [281]. The clock is suggested to have high age correlations in sorted neurons, glia, brain, liver and bone samples [281]. The Skin & Blood shares 45 CpGs with blood-based Hannum and 60 CpGs with Horvath pan-tissue clock[9, 10]. Lin was developed using a 99-CpG aging model derived in DNAm profiles of normal blood samples and trained on life expectancy [282-284].Weidner was developed based on three age-related CpGs located in the genes ITGA2B, ASPA and PDE4C to estimate epigenetic aging in blood[284]. VidalBralo was developed in whole blood of 8 CpG sites that were selected as the most informative CpGs in a training dataset of 390 healthy subjects and validated in three datasets[285].

The chronological classification of the clocks can be summarized as follows: the first-generation clocks were developed using machine-learning to predict chronological age. These clocks demonstrated two important proofs of concept: they recorded increases in clock-age within individuals as they grew older[13, 14]; and more advanced clock-age estimates (i.e. clock ages older than chronological age) were associated with increased mortality risk among individuals of the same chronological age[15]. Second generation DNAm clocks were developed from analysis of mortality risk, incorporating information from DNAm prediction of physiological parameters [12, 15]. These second-generation clocks are more predictive of morbidity and mortality[11] and are proposed to have improved potential for testing impacts of interventions to slow aging [16].

C-Physiology measures

Among available and validated measures of biological aging based on physiologic parameters, we opted to use physiology-based Phenotypic Age described in detail by Liu et al [18]. This clock uses the following nine biomarkers assessed in blood representing the different body systems: albumin, creatinine, glucose,

[log] C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count[286]. These biomarkers were selected using a Cox proportional hazard elastic net model for mortality. Phenotypic Age algorithm is based on parametrization of 2 Gompertz proportional hazard models, one including the 10 selected variables and the other including only chronological age [18, 287].

Statistical analysis

Summary statistics were assessed as median (IQR) for continuous variables and percentages for categorical variables. Survey weights for variables included were used in descriptive analysis [31].Correlation coefficients (Pearson) between cross-tissue (saliva – blood) and intra-tissue (blood – blood) measures were assessed for magnitude, direction, statistical significance with correction for multiple comparison [288, 289]. We have also assessed correlations between each physiological biomarker with telomere length and DNA methylation biological aging measures. Linear Regression models were conducted with DNA methylation or physiology-based biological aging measure as the dependent variable. Telomere length was logged and included in the models as tertiles to allow for interpretable change per unit increase in telomere length. In all the analyses we used the raw form of the measures to allow direct assessment of changes across-tissues[290]. Models were adjusted for sex, sex and telomere length interaction and chronological age difference in years between time at saliva and blood draws. Covariates were determined a priori with emphasis on sex and chronological age for their established biological relevance and data completeness[286]. The relationship between blood-based biological aging and saliva-based telomere length stratified by sex was also assessed. Sensitivity analysis were conducted to assess the models after exclusion of telomere length T/S ratio greater than 2.0 since greater values are more likely to be artificial in salivary samples [38]. All analyses were conducted using Stata SE V.16.0.

RESULTS

Population Characteristics

A total of 2,406 individuals had complete data on salivary telomere length and physiology-based measurements and 1,029 had complete data on salivary telomere length, physiology-based and DNA-based measurements. Median age for the study sample at the time when saliva was drawn was 66 years (IQR 59, 72), median age at time of blood draw was 74 (IQR 67, 80), the majority were females (60%) and African Americans comprised 11% of the sample while the majority were Whites (77%). In terms of self-reported health, respondents who reported ‘very good’ and ‘good’ represented 34% and 31% respectively. Median time difference between saliva and blood draw was 8 years (IQR 8, 8.3) (table 1, supplementary table 1). For saliva-based telomere length median telomere length was 1.30 (IQR 1.14 and 1.50). For blood-based measures of DNA methylation, median biological ages were as follows: Horvath, 69.00, Hannum, 57.80, Levine 60.70, Skin & Blood 73.54, Lin 61.60, Weidner 67.43, VidalBralo 65.15 and Grim Age 71.30. Median age for physiology-based biological aging, Phenotypic Age, was 74.41 (table 1). Weighted summary for variables included is described in supplementary table 1.

Connections between cross-tissue biomarkers of biological aging
All blood-based measures showed an inverse relationship with saliva-based telomere length, such that younger biological aging reflect longer telomeres. Measures that showed statistically significant correlation coefficients included Lin (-0.067, P=0.031) and Weidner (-0.064, P=0.037) (table 2).

In tertile analysis of saliva-based telomere length, compared to the lowest tertile, longer saliva-based telomere length was associated with 1 to 4 years slower biological aging based on blood-based biomarkers with the highest magnitude being Weidner (Ω = -3.97, P = 0.005), GrimAge (Ω = -3.33, P <0.001), and Lin (Ω = -3.45, P = 0.008) biomarkers of DNA methylation. Models were adjusted for sex, sex and telomere length interaction and time difference between saliva and blood draw. Similar results were observed after exclusion of telomere length T/S ratio greater than 2 (table 3, Supplementary table 2). Sex stratified adjusted models showed variations in effect estimates across measures of biological aging in blood between males and females with every tertile increase in telomere length (Figure 1).

Estimates varied widely across the nine physiological biomarkers with DNA methylation measures with the highest correlation observed with creatinine and lymphocyte percent (supplementary tables 3, 4).

Connections between intra-tissue (blood - blood) biomarkers of biological aging
In a secondary analysis, all blood-based biomarkers of DNA methylation showed directly proportional and strong statistically significant relationships with blood-based physiology measures of biological aging. Among blood-based DNA methylation biomarkers, GrimAge showed the strongest correlation with blood-based physiological measure, Phenotypic Age (correlation coefficient = 0.75, P <0.001) followed by Hannum (0.68, P <0.001) and Horvath skin & blood measure (0.66, P<0.001) (table 2).

DISCUSSION

In the present study we found that increases in telomere length measured in saliva was reflected in younger biological age based on DNA methylation and physiology measured in blood ($P<0.001$) eight years later. Such that one tertile increase in telomere length in saliva translated to ~ 4 years younger biological age in blood measures, with some variations between males and females, in a representative population of older adults, despite relatively weak correlations between cross-tissue measures compared to intra-tissue measures in blood. We also observed correlations between physiological biomarkers, creatinine and lymphocyte percent in particular, with DNA methylation-based biological aging, suggesting potentially prominent role of kidney and immunity functions on accelerating biological aging. In contrast, alkaline phosphatase showed little correlation with biological aging measures.

Common biomarkers used in aging investigations include telomere length, physiological measures and epigenetic clocks[41, 291, 292]. At the molecular level biological aging changes have been translated to several domains including telomere attrition, epigenetic alterations, genomic instability, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication[41]. The goal of measuring aging is to identify both modifiable targets and informative surrogate endpoints that can be used to track effects of aging interventions in humans [293, 294]. There are several traits that have to be recognized in aging measures to allow optimal function and replication[293]. Reliability, sensitivity to changes in aging and feasibility in terms of access and costs are among the most important traits that make an aging metric widely implemented and accordingly optimize its development and generalizability[293, 295]. Existing evidence supports associations between telomere length and global DNA methylation in youth, which are suggested to affect genome stability and disease risk susceptability[296]. In addition, DNA methylation-based estimator of telomere length is suggested to be more strongly related to age compared to measured telomere length[297].

Saliva-based biomarkers are likely to provide real-time reflections of the individual's health at the time of collection [279]. In addition to their highly economic advantages and accessibility, saliva is generally a preferred means for populations and age groups among whom a blood draw is not accessible and in clinically difficult situations [271]. The ease of access however should be considered alongside factors that directly affect the composition and molecular abundance in saliva tissue. Saliva is secreted in response to sympathetic and parasympathetic stimuli, and nervous stimuli variations directly affect saliva characteristics in terms of volume, viscosity, protein and mucin concentrations. Previous observations suggest that telomere length is likely not the ultimate biological aging measure, in addition qPCR is suggested to be less optimal compared to other methods [239, 298, 299]. In addition, recent evidence suggests DNA methylation variations across cell types compared to within a cell type likely due to variations in immune cell contamination [16, 300]. In addition, saliva composition could be affected by medications use, age, circadian rhythms among other individual-specific exposures and conditions such as DM, multiple sclerosis, liver conditions and infection diseases[271, 301, 302]. It is critical to also consider the method of measurement to allow for meaningful comparisons. In addition, recent data have shown utility of DNA methylation measured in saliva and buccal cells considering 10 ng of genomic DNA for reproducibility [303, 304] .

In terms of blood-based biomarkers, in the present study we used DNA methylation and physiology-based measures of aging biology. DNA methylation is the mechanism by which a methyl (CH3) group is added to DNA resulting in modification of genetic function without changes to DNA sequence. This

process regulates gene expression and therefore plays an important role in human development and disease[305]. However, DNA methylation remains to be challenged by cost-related barriers and limited consistency in the measurements as there is no gold standard to define the optimal genome sites and methods of measurements in humans[306, 307]. Therefore, comprehensive comparisons provide a rigorous and reproducible way to capture biological aging effects. A significant proportion of thousands of data points do not yield the equivalent value when quantified twice from the same DNA sample, furthermore repeated measures are crucial to uncover consistent replicable signals of DNA methylation dynamics across important variables including time, between populations and between exposures[308]. That said, cross-tissue variability in DNA methylation profiles have been suggested to be more concordant across tissues than gene expression changes across tissues with age[9, 284]. Physiology-based measures largely represent composites of blood-based analytes that represent the various body domains that change with aging[45]. In the present study, we adopted phenotypic age, representing parameters that have been validated as predictors of aging in comparable settings including albumin, creatinine, glucose, [log] C-reactive protein [CRP], lymphocyte percent, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count [18]. A core motivation to using phenotypes of physiological biomarkers is that one biomarker might not be sufficient to delineate the underlying pathogenesis, therefore combinations of biomarkers provide an added value over single biomarkers and hence more powerful diagnostic and prognostic tools [3, 225].

The wide variations in the relationships between each physiology biomarker individually with DNA methylation-based biological aging measures suggest differences in how each body system interacts with aging at the physiological level. For example, creatinine, a biomarker of kidney function, and lymphocyte percent, a blood and immunity biomarker, showed strong positive and negative correlations respectively with some of the DNA methylation-based biological aging measures that exceeded their correlation with chronological age in the same sample[309-313]. These strong correlations suggest potent role of the renal and immunity systems in accelerating biological aging.

There are several limitations in the present study. The biomarkers were measured at two different time points. This could have impacted the aging profiles across time; however, time differences were adjusted for across all the models. In addition, measures of biological aging other than telomere length were not available in saliva, however the sample was restricted to individuals with complete information for the three measures and therefore allowed for within individual comparisons. In addition, accumulating evidence suggests that biological aging measures are likely complimentary to each other rather than alternative exclusive tools since they capture different aspects of the aging process that are likely independent of the tissue type [303]. Our study was also limited in the number of measurements on each individual; therefore, we were not able to assess changes in biomarkers overtime. Repeated measures however would mainly serve as a confirmatory step and might not be feasible to assess over long durations for the same population. Lastly, median age in the present investigation is 66 years (IQR 59, 72) and therefore the results cannot be extrapolated to other age groups and younger individuals who joined HRS after 2008[4].

Collectively, differences in methods of measurement, cost, accessibility and generalizability between saliva and blood-based measures determine their applicability in large scale investigations and the feasibility of their integration in clinical practice[279, 314, 315]. It is also important to consider that blood and saliva secretions are interconnected in many ways, presumably a drug circulating in blood passes through capillary wall, the basement membrane and glandular epithelial cells prior to being secreted into

the salivary duct. This plasma-salivary interchange is bound by active and passive processes and an ultrafiltration step. Drug or compound specific characteristics also play a role in this process and determine according to their abundance in salivary fluid[271, 279]. Therefore, uncovering connections between both tissues represent an important step towards optimization of accessibility for integration in clinical practice and wide scale investigations.

Conclusions

Results of the present investigation suggest strong connections between aging biology biomarkers in saliva and blood in older adults. We observed variations in the relationship between each body system represented by physiology biomarkers and biological aging, particularly at the DNA methylation level. These observations provide novel opportunities for integration of both blood-based and saliva-based biomarkers in clinical care of vulnerable and clinically difficult to reach populations where either or both tissues would be accessible for clinical monitoring purposes.

TABLES & FIGURES

TABLE 1. Study Sample Characteristics

Characteristic	Summary (unweighted)
N	2, 406
Chronological age (years), median (IQR) [1]	66 (59, 72)
Female, %	1, 449 (60)
Time difference, median (IQR)[2]	8 (8, 8.3)
Race	
African American, N (%)	270 (11)
Self-reported health	
Excellent	350 (15)
Very good	815 (34)
Good	732 (31)
Fair	359 (15)
Poor	98 (4)
Biomarkers[3]	
Saliva-based	
Telomere length, median (IQR)	1.30 (1.14, 1.50)
Blood-based Physiological measure	
Phenotypic Age, median (IQR)	74.41 (65.6, 83.55)
Blood-based DNA methylation measures[4]	
Horvath, median (IQR)	69.00 (63.00, 75.00)
Hannum, median (IQR)	57.80 (52.06, 63.55)
Levine, median (IQR)	60.70 (54.41, 66.84)
Skin & blood, median (IQR)	73.54 (67.70, 78.25)
Lin, median (IQR)	61.61 (55.09, 68.85)
Weidner, median (IQR)	67.43 (60.90, 76.82)
VidalBralo, median (IQR)	65.15 (61.67, 69.00)
GrimAge, median (IQR)	71.30 (65.64, 77.25)

1 Chronological age at time of Telomere assessment; Median chronological age at time of blood draw assessment 74 (IQR=67, 80); 2 Time difference represents time years between saliva and blood draw; 3 Biological aging measures are scaled per one year change; 4 n=1, 029 had complete data on salivary telomere length, physiology-based and DNA methylation measures.

TABLE 2. Correlation coefficients of the relationship between 1] cross-tissue biomarkers of biological aging, and 2] intra-tissue biomarkers of biological aging.

	Cross-tissue biomarkers		**Intra-tissue biomarkers**	
	Telomere Length (saliva-based)		**Phenotypic Age (blood-based)**	
Biomarkers (blood-based)	Correlation Coefficient	P value	Correlation Coefficient	P value
Phenotypic Age	-0.0264	0.1959	1.00	--
Horvath	-0.0452	0.1478	0.5776	<0.001*
Hannum	-0.0318	0.3082	0.6896	<0.001*
Levine	-0.0320	0.3056	0.6525	<0.001*
Skin & blood	-0.0367	0.2391	0.6640	<0.001*
Lin	-0.0670	0.0317	0.5839	<0.001*
Weidner	-0.0647	0.0379	0.3229	<0.001*
VidalBralo	-0.0529	0.0897	0.5204	<0.001*
Grim Age	-0.0438	0.1608	0.7498	<0.001*

**Denotes a significant P value with Bonferroni correction[289]*

TABLE 3. Cross-tissue comparisons between blood-based biomarkers of biological aging (outcome variable) and (log) saliva-based telomere length (with lowest tertile as reference).

Biomarkers [a]		95% CI	P value
Physiological measure, Phenotypic Age	--		
2nd tertile	-2.30	-4.30, -0.30	0.024
3rd tertile	-2.38	-4.37, -0.38	0.019
Horvath			
2nd tertile	-1.10	-3.27, 1.07	0.320
3rd tertile	-1.55	-3.74, 0.63	0.163
Hannum			
2nd tertile	-1.61	-3.59, 0.36	0.109
3rd tertile	-2.40	-4.39, -0.40	0.018
Levine			
2nd tertile	-1.10	-3.35, 1.14	0.335
3rd tertile	-2.45	-4.71, -0.19	0.033
Skin & blood			
2nd tertile	-0.99	-2.88, 0.88	0.298
3rd tertile	-1.91	-3.81, -0.02	0.047
Lin			
2nd tertile	0.12	-2.40, 2.65	0.921
3rd tertile	-3.45	-6.00, -0.91	0.008
Weidner			
2nd tertile	-1.27	-4.05, 1.50	0.369
3rd tertile	-3.97	-6.76, -1.17	0.005
VidalBralo			
2nd tertile	-1.49	-2.93, -0.06	0.040
3rd tertile	-2.11	-3.55, -0.67	0.004
Grim Age			
2nd tertile	-1.75	-3.56, 0.05	0.057
3rd tertile	-3.33	-5.15, -1.51	<0.001

a Measures per tertile increase in telomere length with lowest tertile as reference; Models were adjusted for sex, sex and telomere length interaction and time difference between saliva and blood draw.

Ethics approval: The Health and Retirement study is reviewed and approved by the University of Michigan's Health Sciences IRB. The present study has been conducted in accordance with STROBE guidelines for observational studies (https://www.strobe-statement.org/).

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Consent for publication: NA

Competing interests: the authors declare no conflicts of interest in relation to this work.

Data availability: Data was extracted from the Health Retirement Study. More details in https://hrs.isr.umich.edu/data-products/access-to-public-data.

Authors' contributions: RW: Conception, funding, data acquisition & approvals, statistical analysis and drafting the article; YG, OW and SH: Review of the final drafts and critical appraisal.

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Title page: Causes of Death following Alzheimer's Disease and Variations According to Calendar Period

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ABSTRACT

Background: Characterizing the leading causes of death in the AD population is critical to determine reversible causes that guide prevention efforts among those with AD diagnosis early in the disease course.

Objective: To quantify and rank the combined estimates of causes of death following AD and to assess potential differences according to calendar period based on year of study publication.

Methods: A systematic review and Meta-analysis conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Results: A total of 232 studies were screened in title and abstract, 30 studies were included in full text screening and 9 studies were included representing 5995 patients with Alzheimer's disease from Asia, Europe and USA. In combined meta-analysis, dementia contributed only 26% (95% CI 14% - 39%) of underlying causes of death as recorded on death certificates. 16% (95% CI 8% - 23%) of deaths were due to unknown causes, 13% (95% CI 6% - 21%) due to cardiovascular disease and 6% due to infectious disease (95% CI 4% - 7%). Pneumonia, cerebrovascular disease or cancer, each contributing ~ 8%, accounted equally as underlying causes of death among AD patients.

Conclusions: In the present study our results have highlighted several underlying causes of death in the advanced AD population that could potentially be reversible and necessitate close and consistent monitoring among those with AD diagnosis. These findings are further supported by the potentially varying trends in causes of death in the AD population in the recent decade compared to earlier time periods.

INTRODUCTION

Alzheimer's disease is a neurodegenerative progressive disorder that is hypothesized to start with an initial pathological phase of Tau and Beta-amyloid accumulation[316, 317]. The cellular phase of AD that is suggested to be triggered by accumulation of neurodegenerative pathology, is a preclinical phase with a relatively varying speed of progression before the symptomatic manifestation of clinical AD[60]. The varying speed of progression of AD, particularly in the cellular phase, is a promising time window for interventions aiming at slowing disease progression and potentially prevention of reversible causes of death.

Survival following AD is relatively variable and recent evidence suggests varying underlying non-dementia factors that contribute as underlying causes of death including infectious diseases, pneumonia, cerebrovascular and cardiovascular events [79]. Characterizing the leading causes of death in the AD population is critical to determine reversible causes that guide prevention efforts among those with AD diagnosis early in the disease course.

In the present meta-analysis, we aimed to quantify and rank the combined estimates of causes of death following AD and to assess potential differences according to calendar period.

METHODS –

Search strategy

The main search was conducted on PubMed as of March 6th, 2023, using keyword combinations including [Alzheimer's Disease] and [Cause of Death] or [Death Certificates] or [Complications] or [Prognosis]. To avoid potential heterogeneity in AD diagnosis and clinical care we first restricted the PubMed search to studies published in last decade between 2013 and 2023. Then we hand-searched reference lists of included papers and similar research articles using the related research articles feature on PubMed. We repeated the search using google scholar for additional highly cited articles that may have not picked up by PubMed without restricting time period for comparison. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Selection Criteria

Inclusion and exclusion criteria were determined a priori. Eligible study designs were epidemiological studies including randomized clinical trials. Studies were included if they reported causes of death in patients with a diagnosis of Alzheimer's disease. Studies that included rates of death without specification of underlying causes of death were not included. In case of multiple studies from the same population published in different time periods, the most recent and complete studies were included.

Data extraction and data management

The main parameters extracted included causes of death including pneumonia, cerebrovascular disease, cardiovascular disease, dementia, cancer, other infectious disease, unknown cause of death and year of study publication, country and population size [Table1].

Data analysis and risk of bias assessment

The main causes that were reported consistently in the literature and were included in the meta-analysis included: pneumonia, cerebrovascular disease, cardiovascular disease, dementia, cancer, other infectious disease and unknown causes. We calculated proportions using the [METAPROP] command of Stata and then reproduced the analysis on R using the [META] package for comparability[318]. The standard errors were calculated using the [METAPROP] and replicated for comparisons for each outcome from the 95% confidence intervals [CI] divided by 3.92[319]. To assess time effects, we calculated time intervals in three periods according to year of study publication. All meta-analyses were conducted using random effects models.

We assessed risk of bias in relation to selection, comparability and outcome using a modified version of the Newcastle-Ottawa scale [320, 321]. Heterogeneity between studies and publication bias were assessed using I^2 and egger's test respectively [322]. In addition, Influence of each study on the combined estimates were assessed using [MATAINF][323, 324]. All analyses were conducted using STATA version [Stata/SE 16.1].

Sensitivity analysis

Sensitivity analyses across calendar periods between 1986 – 1993, 1994 – 2005 and 2013 – 2023 were conducted to assess variations in causes of death according to calendar year period for each outcome as a cause of death. In addition, we repeated the analysis to assess impact of removing Zuo et al that included dementia with Lewy bodies[325].

RESULTS

Characteristics of included studies

A total of 234 studies were identified through PubMed search. After duplicates removal, a total of 232 studies were screened in title and abstract screening, 30 studies included in full text screening and 9 studies were included in the meta-analysis representing 5995 patients with Alzheimer's disease from Asia, Europe and USA. The main causes of death assessed included pneumonia, cerebrovascular disease, CVD, dementia, cancer, other infectious disease and unknown causes of death. Given that we conducted the search on two phases, the calendar period in the primary search ranged between 1993 and 2023; in the subsequent search phase, additional key studies were added and represented older time periods ranging between 1986 to 2005 for which we conducted sensitivity analysis to assess variations according to each time period [figure 1].

Underlying causes of death in patients with Alzheimer's disease

In combined meta-analysis, dementia contributed only 26% (95% CI 14% - 39%) of underlying causes of death as recorded on death certificates. 16% (95% CI 8% - 23%) of deaths were due to unknown causes, 13% (95% CI 6% - 21%) due to cardiovascular disease and 6% due to infectious disease (95% CI 4% - 7%). Pneumonia, cerebrovascular disease or cancer, each contributing ~ 8%, accounted equally as underlying causes of death among AD patients [figures 2 – 8].

Variations in cause of death according to calendar period

In a sensitivity analysis we then assessed variations across calendar periods between 1986 – 1993, 1994 – 2005 and 2013 – 2023. Variations were observed as follows: causes of death that increased in the most recent calendar period included cancer, cardiovascular disease and dementia; causes that remained relatively stable across time were cerebrovascular death, pneumonia and other infectious disease, while other or unknown causes of death showed substantial decline between 2013 and 2023 (9%, CI 95% 5 -13) compared to the earlier time

periods (1994 – 2005 (48%, 95% 44% - 53%)); (1986 – 1993 (17%, 95% 12% - 23%)) [Supplementary figures 1 – 7].

Risk of bias, heterogeneity, publication bias and individual study effects

Given the nature of the AD diagnosis and the death as the main outcome in the present study, risk of bias in relation to selection, comparability and outcome ascertainment was generally low across the studies. Egger's test for publication bias assessment through small study effects showed no statistically significant difference with P value =0.97[326]. The effect of each individual study was assessed through omitting one study at a time for each cause of death. Repeating the analysis without the most influential studies did not result in significant change in the combined effect estimates.

DISCUSSION

In the present meta-analysis across 9 studies representing 5995 patients with Alzheimer's disease from Asia, Europe and USA, our results have shown that dementia contributed only 26% as the underlying cause of death recorded on death certificates in the AD population. While unknown causes contributed about 16% of deaths followed by cardiovascular disease, infectious disease and pneumonia, cerebrovascular disease and cancer that accounted for similar estimates as underlying causes of death in the AD population.

Studies were inconsistent in reporting information on race with inconsistent effect estimates. Some studies suggest potential confounding effect of race on AD-mortality relationship in unadjusted compared to age, gender and race adjusted model, while other studies suggest little difference in time to clinical events according to race [327, 328].

Varying trends in underlying causes of death in the AD population were observed for time interval analyses between 1986 and 2023, with some causes showing an increase and others declined in the most recent decade between 2013 and 2023 compared to previous time periods. Causes of death that showed an uptrend include cancer, cardiovascular disease and dementia as compared to other causes that remained relatively stable including cerebrovascular death, pneumonia and other infectious disease. Interestingly, unknown or other causes of death showed substantial decline between 2013 and 2023 compared to the earlier time periods. Differences in causes of death across time in the AD population, confirm that dementia is not essentially the main underlying cause of death, especially in patients with comorbidities. Such comorbidities vary in nature according to their onset in relation to AD diagnosis and it is critical to delineate conditions that are prevalent in the AD population from events and complications that occur in the advanced stages of AD[60, 79].

In the COVID-19 era, the AD population and their caregivers faced unique challenges [329]. Data has shown increased risk of infection and death in AD patients living in care home facilities compared to patients with AD

living at home and those with other types of dementia such as vascular dementia and frontotemporal dementia [330-332]. Conversely, the pandemic could have resulted in undiagnosed AD cases thus likely underestimating COVID-19 related mortality in this population [333].

The present study has several limitations. Information on demographics and medical history was not consistently available across the studies and hence were not included in the analysis. However, the majority of included samples represent populations of advanced AD in older adults that likely carry some degree of homogeneity given the natural history of the AD progression[57, 334]. In addition, given the study-level nature of the present analysis, random error is inevitable, however the majority of causes of death showed fairly consistent estimates thus providing further reassurance on clinical homogeneity in patients with AD in the advanced stages.

Our study has key strengths. The sufficient power and inclusion of samples from wide geographic variation strengthens the generalizability of the results. The main search was focused on the most recent period, thus allowing to indirectly control for heterogeneity related to clinical management and diagnosis across time in the AD population and in aging populations in general. Such differences could have impact on the course of AD progression as well as onset of comorbidities that are age- rather than AD-related[335, 336].

The findings of the present investigation have important public health implications for the AD population. Most recent therapies are tailored towards early-stage AD and mild MCI who are less likely to have age-related and AD-related comorbidities compared to advanced-stage AD[102, 336]. Therefore, in the light of lack of effective therapies in the advanced stages of AD, characterizing and targeting reversible comorbidities that could be age-related or AD-related could serve as an important route to slow progression of AD and potentially improving survival in this population.

Conclusions

In the present study our results have highlighted several underlying causes of death in the advanced AD population that could potentially be reversible and necessitate close and consistent monitoring among those with AD diagnosis and in patients at high risk of developing age-related comorbidities or AD. These findings are further supported by the potentially varying trends in causes of death in the AD population in the recent decade compared to earlier time periods.

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Conflicts of Interest: RW is an editorial Board Member of Journal of Alzheimer's Disease but was not involved in the peer-review process nor had access to any information regarding its peer-review.'

Contributions: Conception, funding, data acquisition & approvals, statistical analysis and drafting the article: RW; Review of the manuscript and critical appraisal: RW and OW.

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Table and Figures

Figure 1. Study flow chart

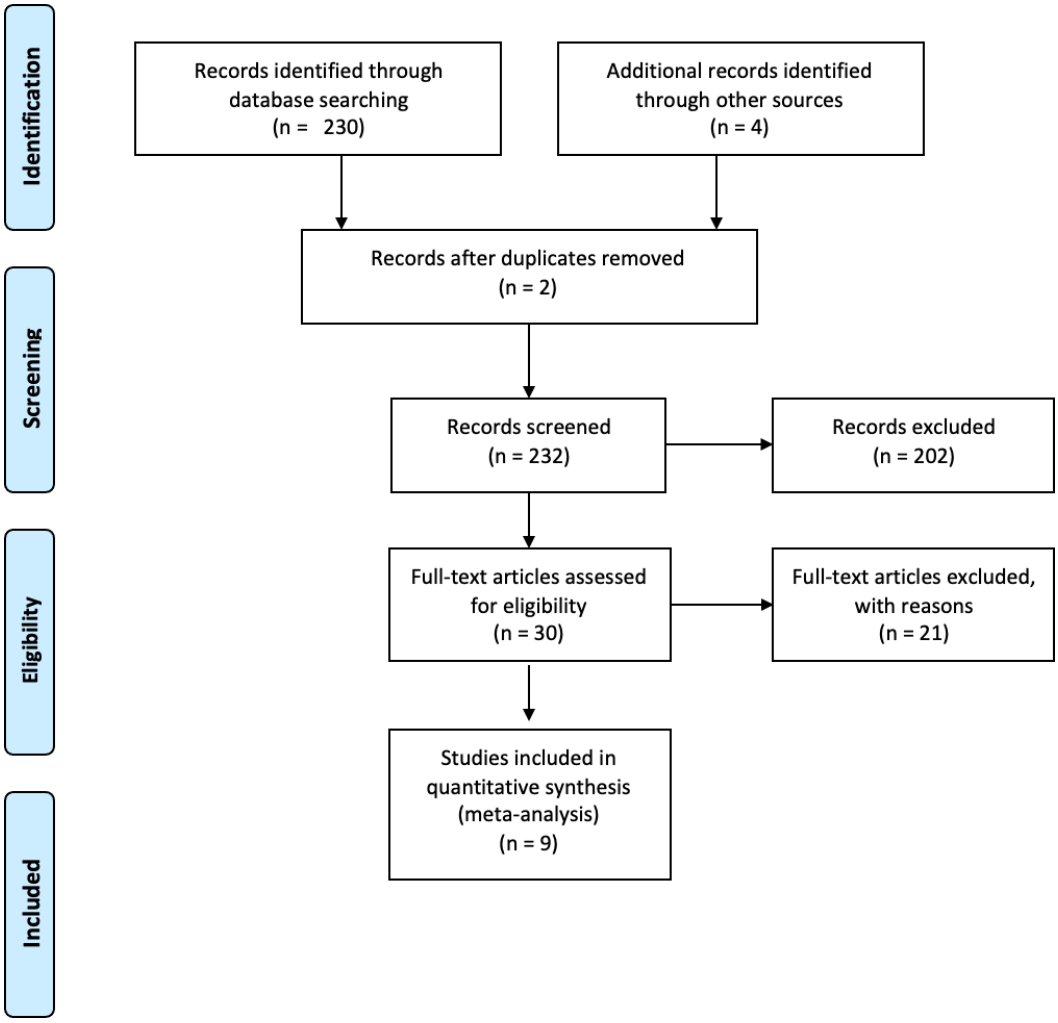


Table 1. Characteristics of included studies.

Study	N	Year	Country	Setting	Causes of death among patients with Alzheimer's disease (%)						
					Pneumonia	Cerebrovascular disease	CVD	Dementia	Cancer	Other infectious disease	Unknown cause of death
Molsa	172	1986	Finland	Survey	--	9.3	3.5	68.0	2.3	--	16.8
Kukull	104	1994	USA	Registry	12.3	11.0	15.3	20.0	7.6	7.6	1.9
Thomas	398	1997	Scotland	Registry	--	7.3	10.8	20.4	1.2	--	58.5
Fitzpatrick	79	2005	USA	Prospective cohort	7.6	8.8	24.0	19.0	17.7	--	17.7
Todd	85	2013	Ireland	Prospective cohort	17.6	11.8	10.6	23.5	16.4	2.3	4.7
Kim	364	2015	South Korea	Registry	1.9	10.7	6.0	36.8	9.0	6.8	6.0
Kuller	271	2016	USA	Prospective cohort	4.1	6.3	28.4	24.4	14.0	--	13.2
Golüke	4498	2019	Netherlands	Registry	9.9	8.0	23.7	7.5	8.6	5.9	12.1
Zuo	24	2022	China	Memory clinics	29.2	12.5	4.2	--	4.1	--	8.3

Figure 2. Combined effects meta-analysis of [cancer] as a cause of death following AD.

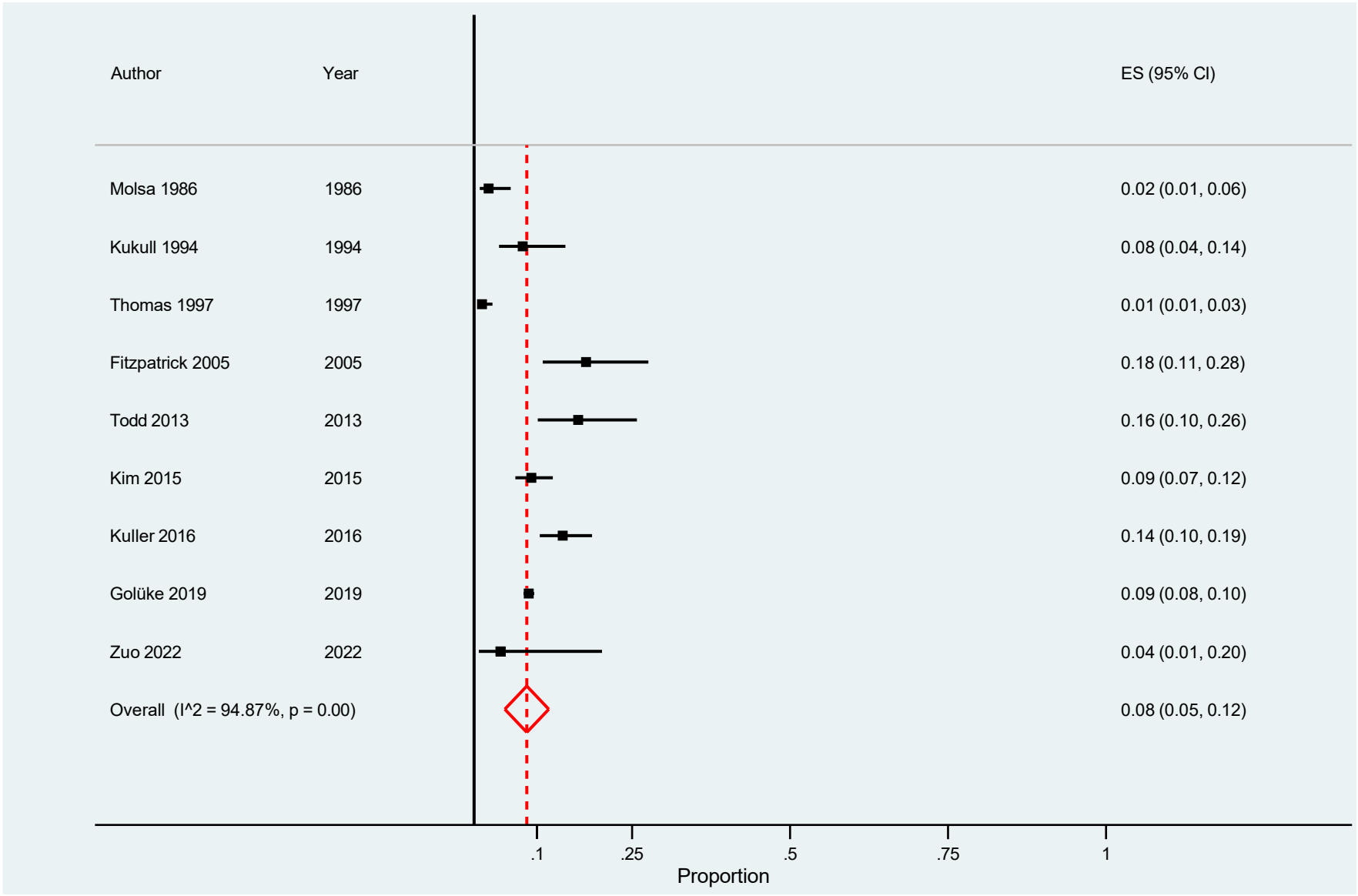


Figure 3. Combined effects meta-analysis of [cerebrovascular disease] as a cause of death following AD.

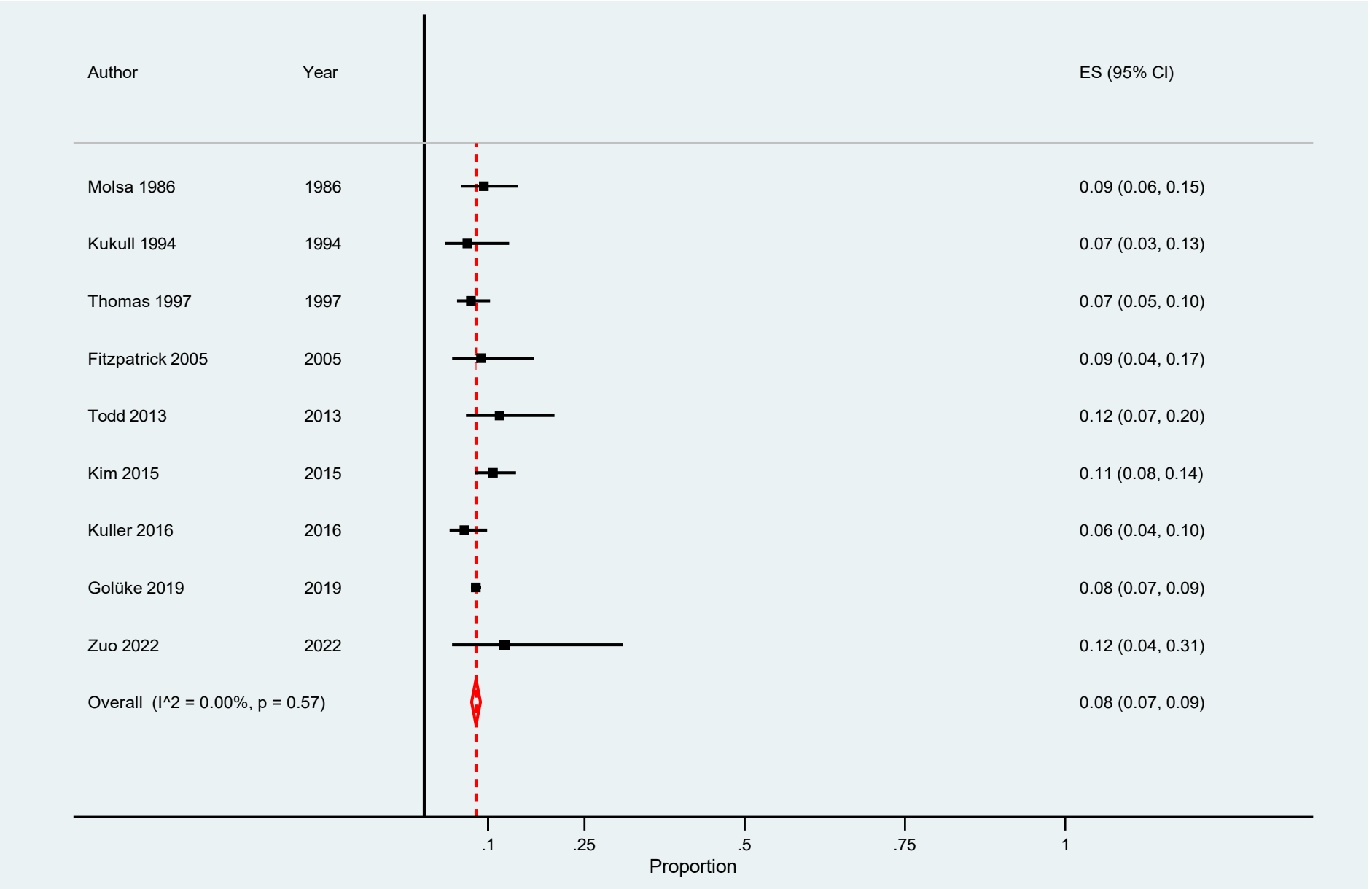


Figure 4. Combined effects meta-analysis of [cardiovascular disease] as a cause of death following AD.

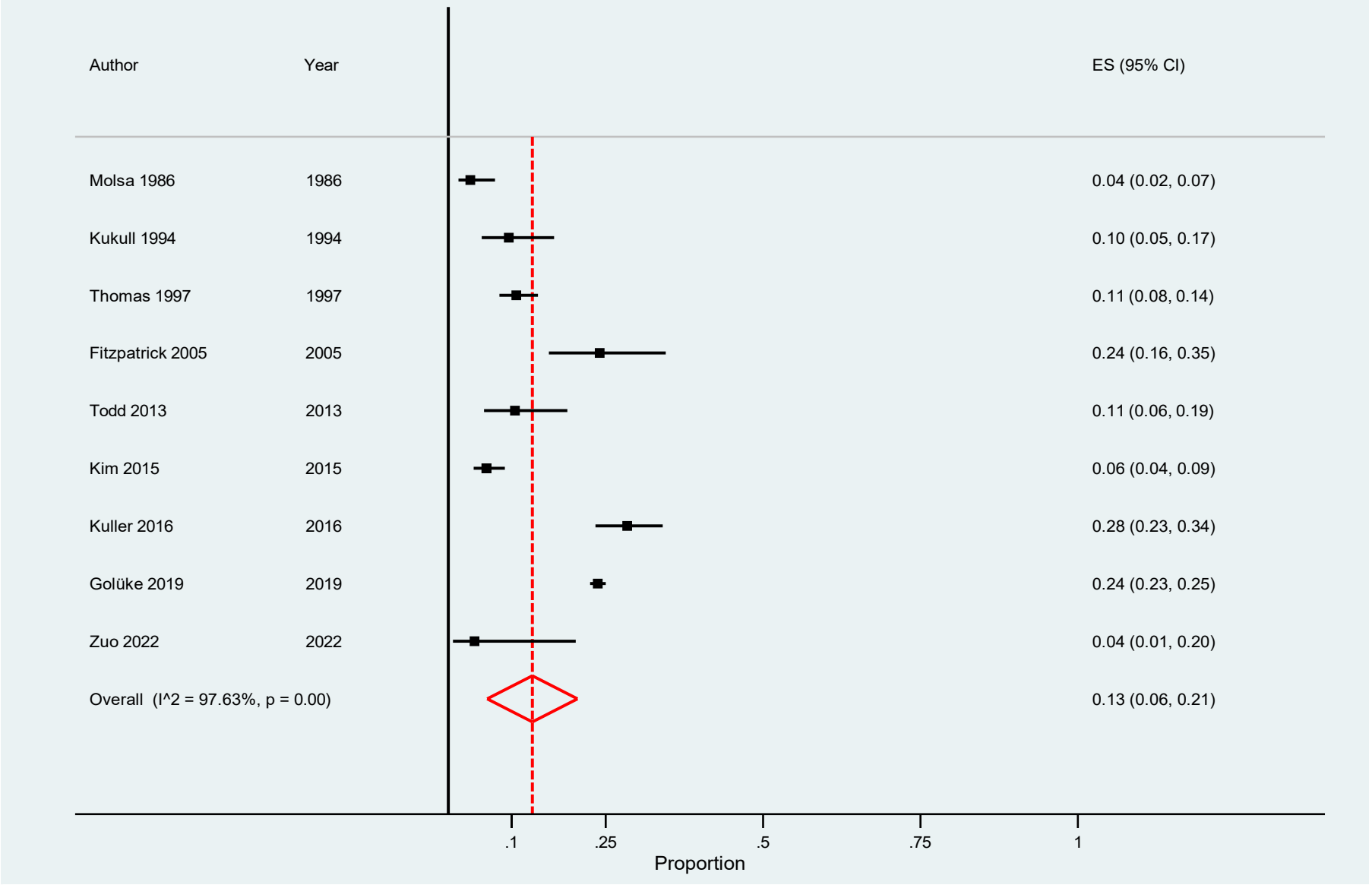


Figure 5. Combined effects meta-analysis of [dementia] as a cause of death following AD.

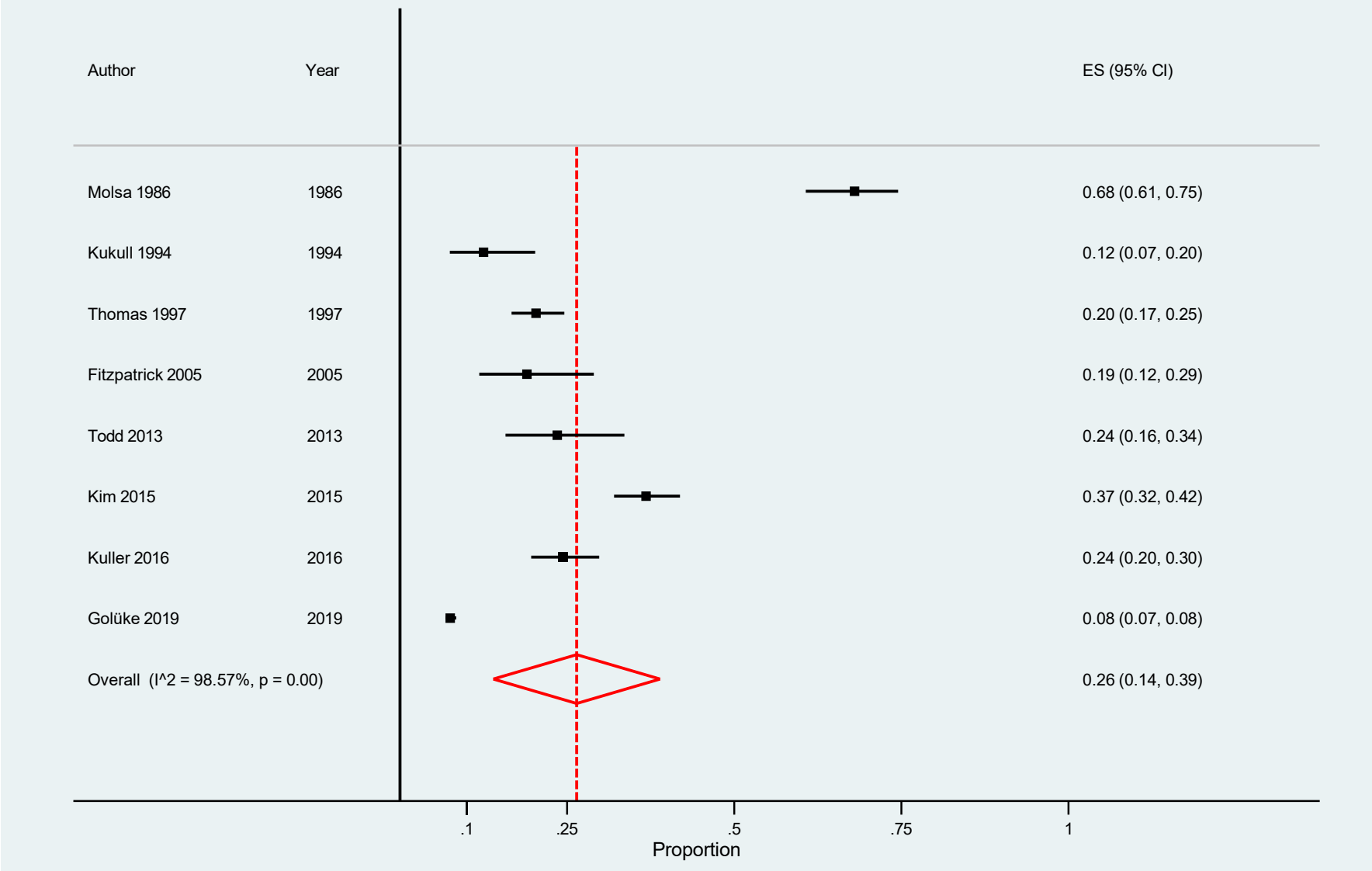


Figure 6. Combined effects meta-analysis of [pneumonia] as a cause of death following AD.

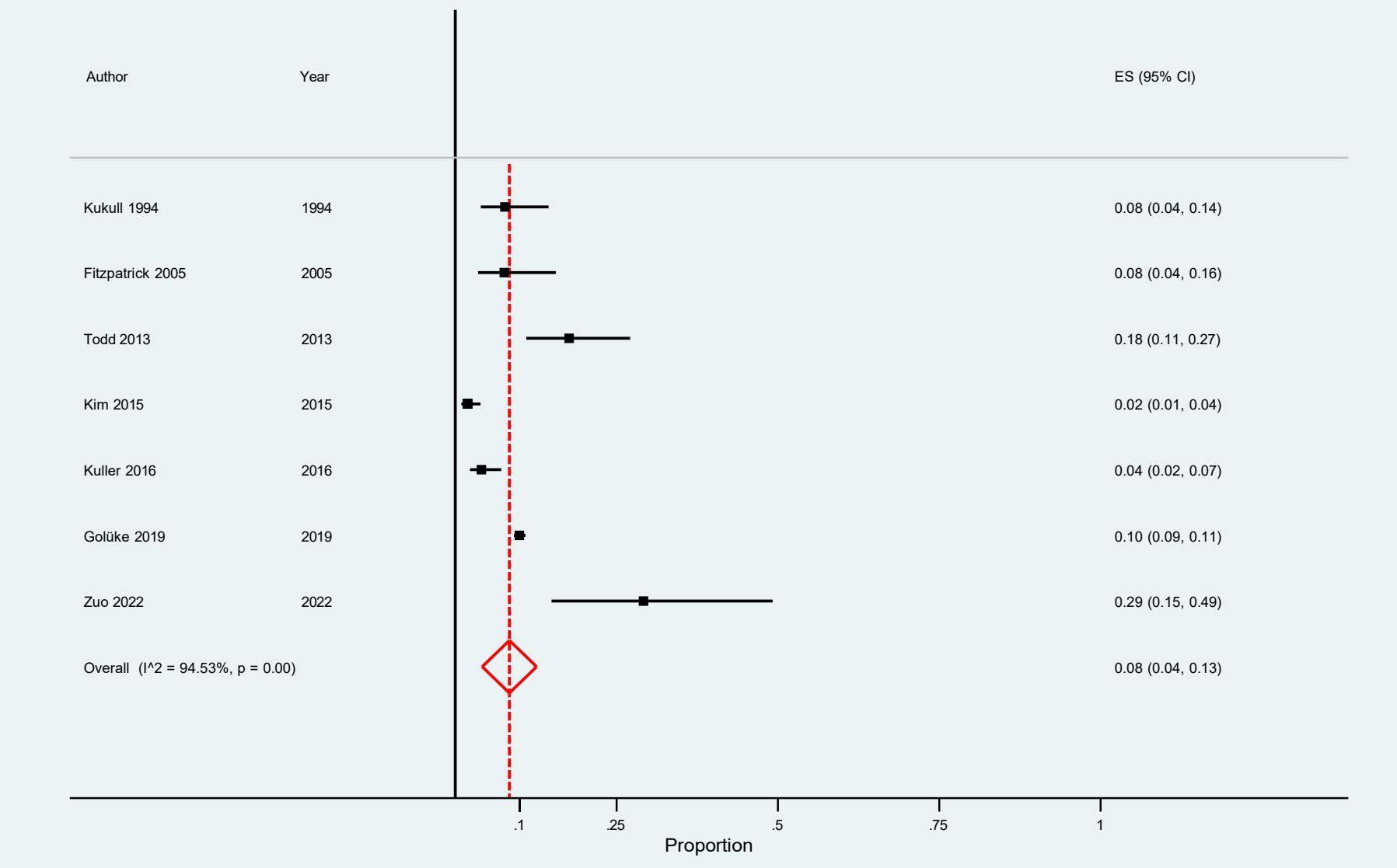


Figure 7. Combined effects meta-analysis of [other infectious disease] as a cause of death following AD.

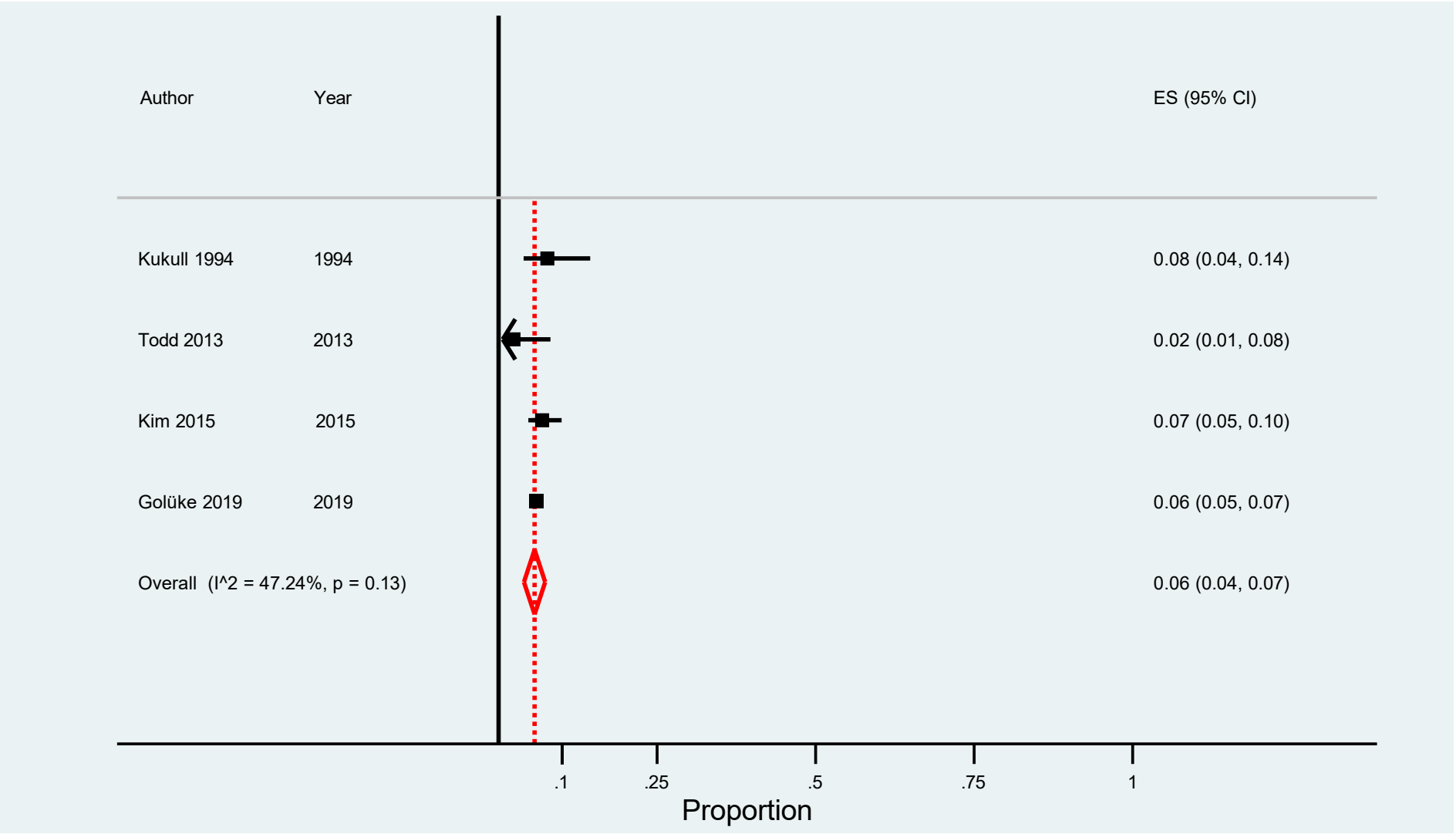
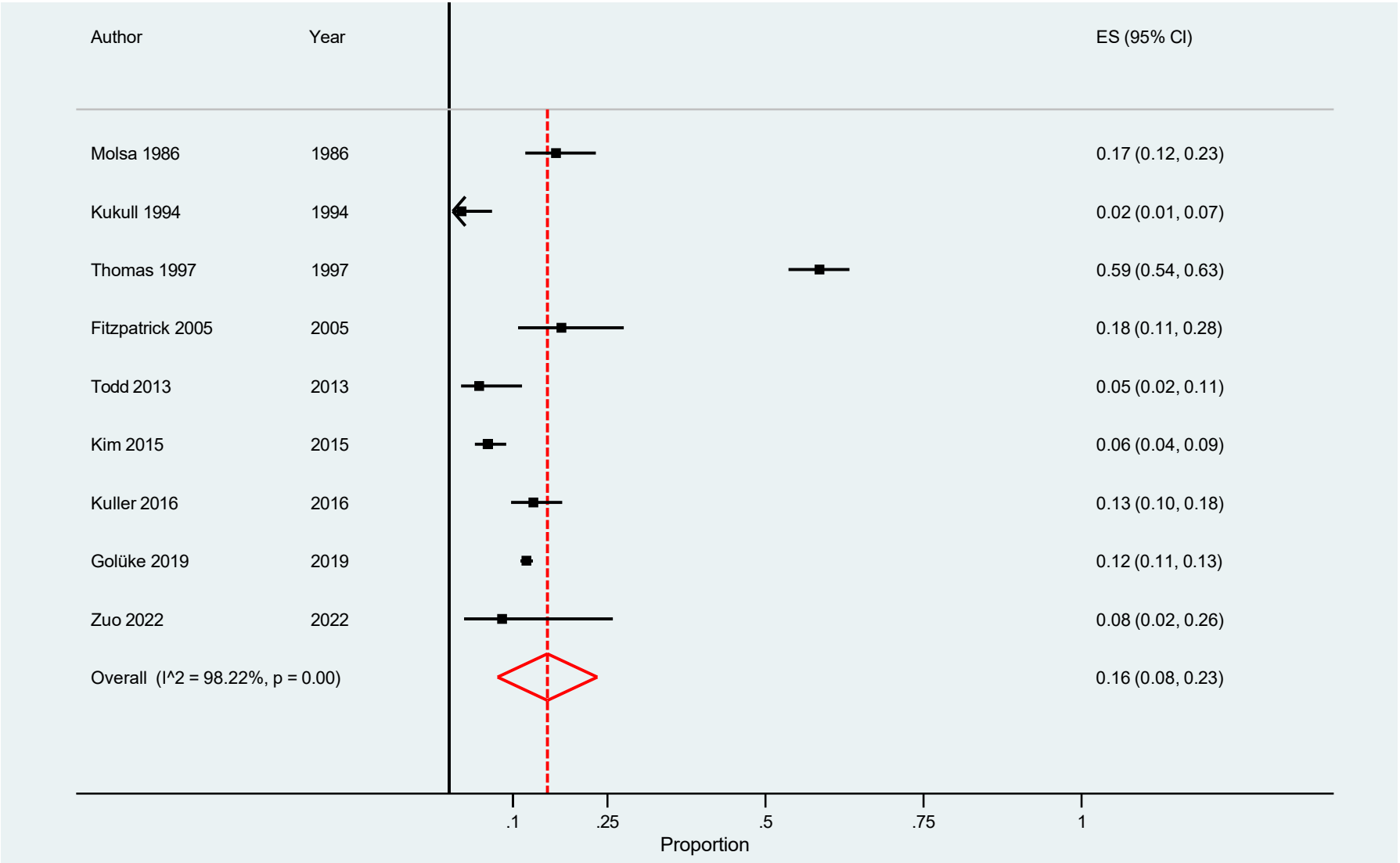
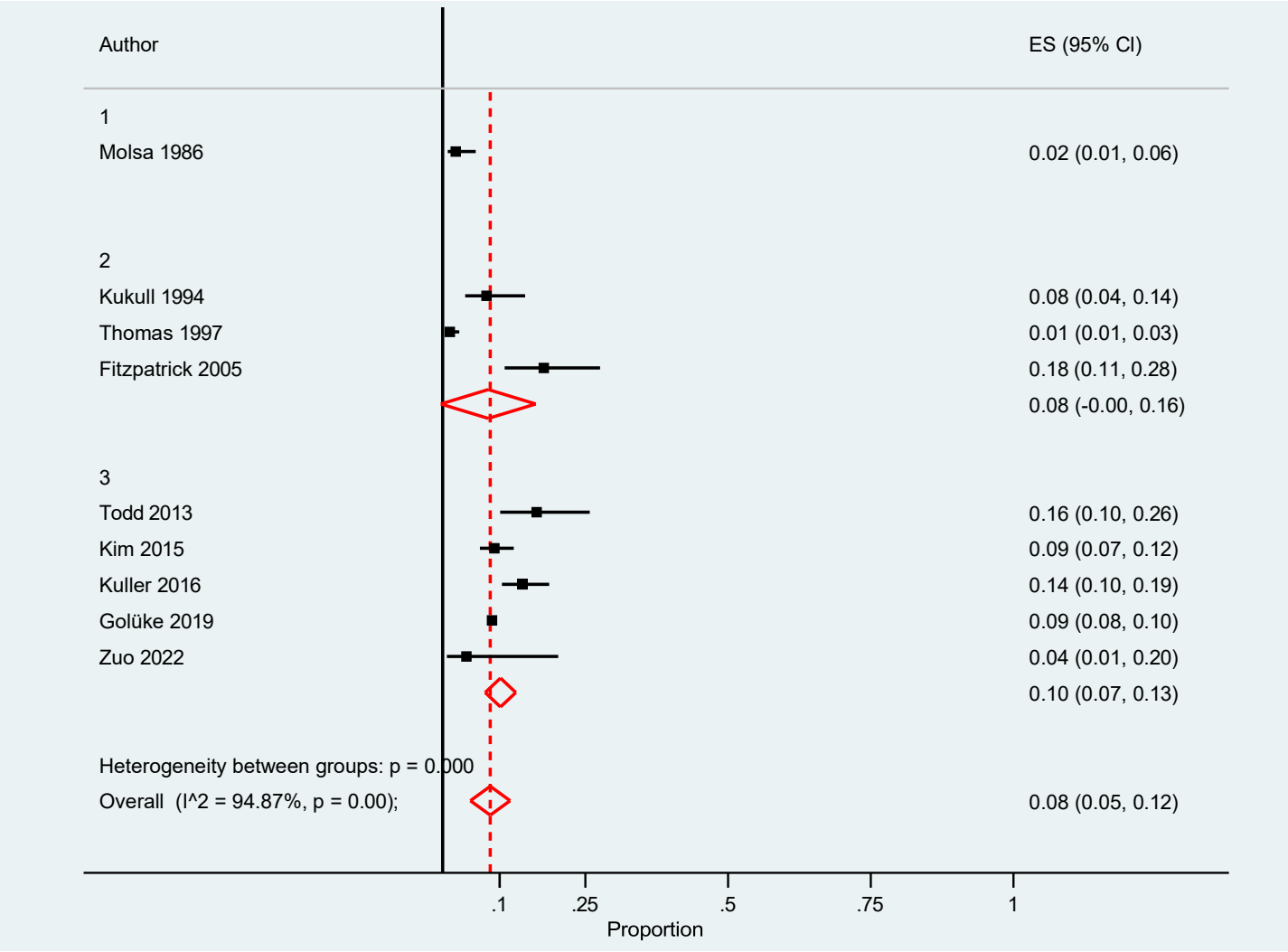


Figure 8. Combined effects meta-analysis of [unknown or other cause of death] as a cause of death following AD.

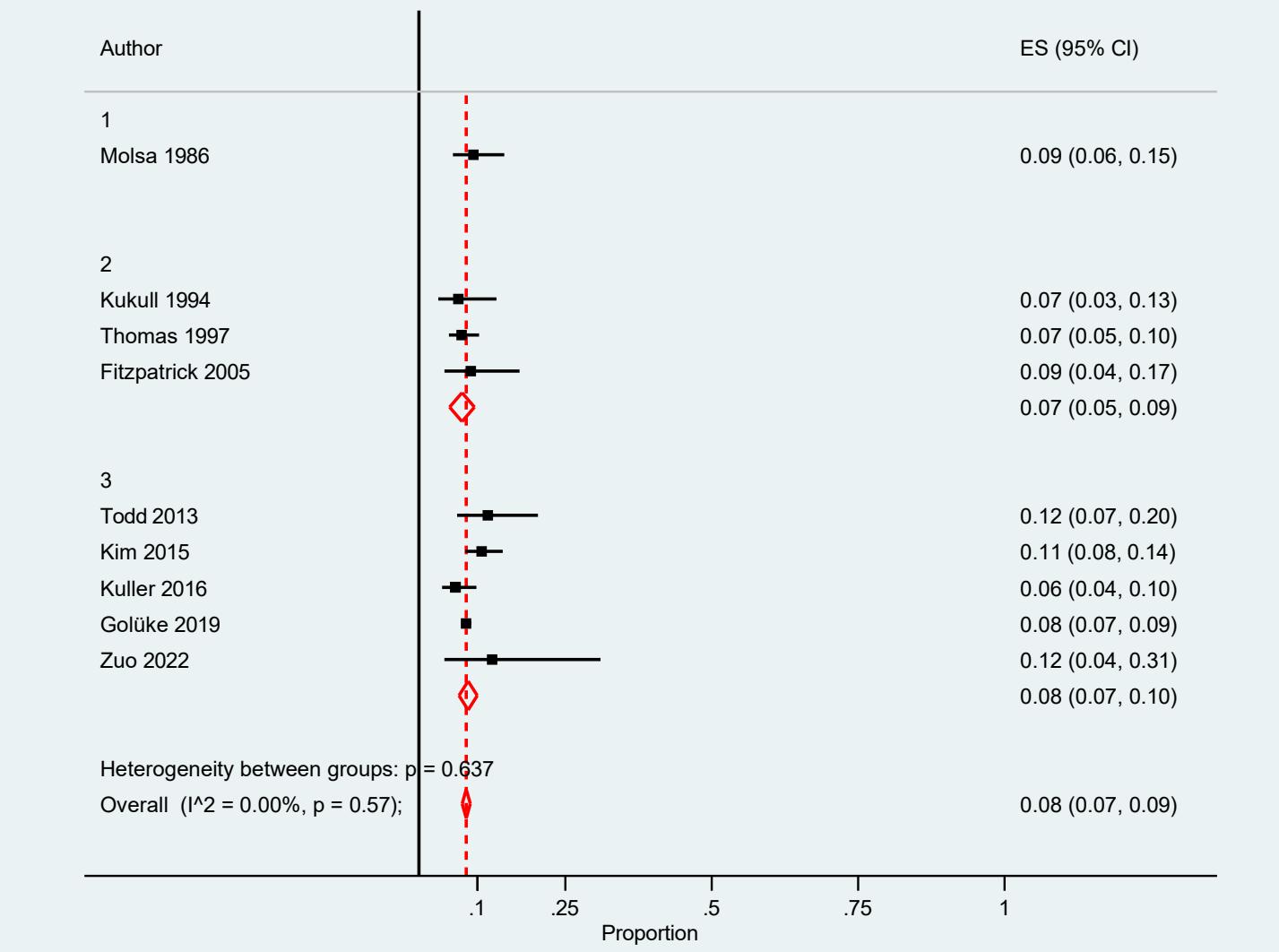


Supplementary material

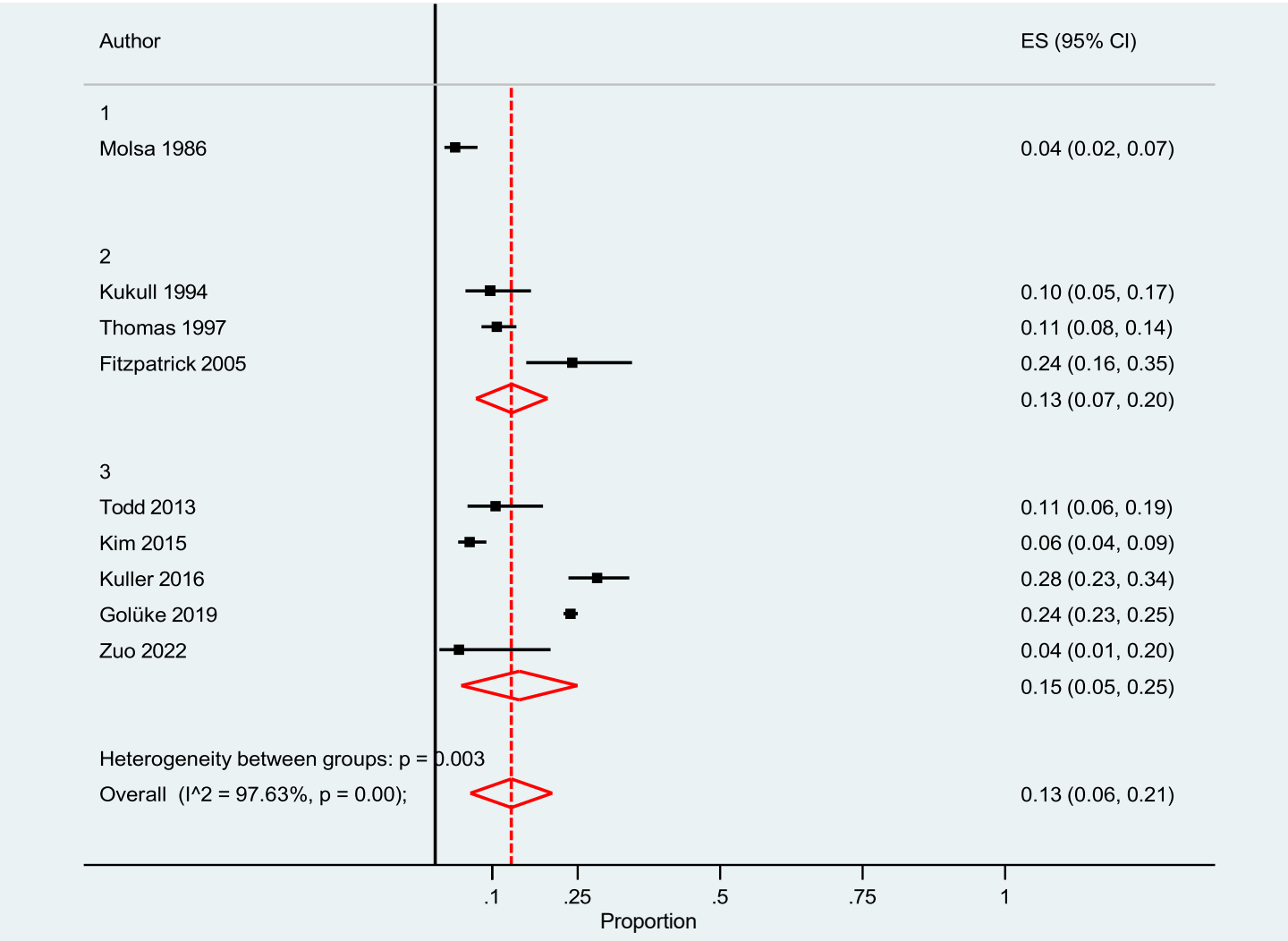
Supplementary figure 1. Combined effects meta-analysis of [cancer] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023)



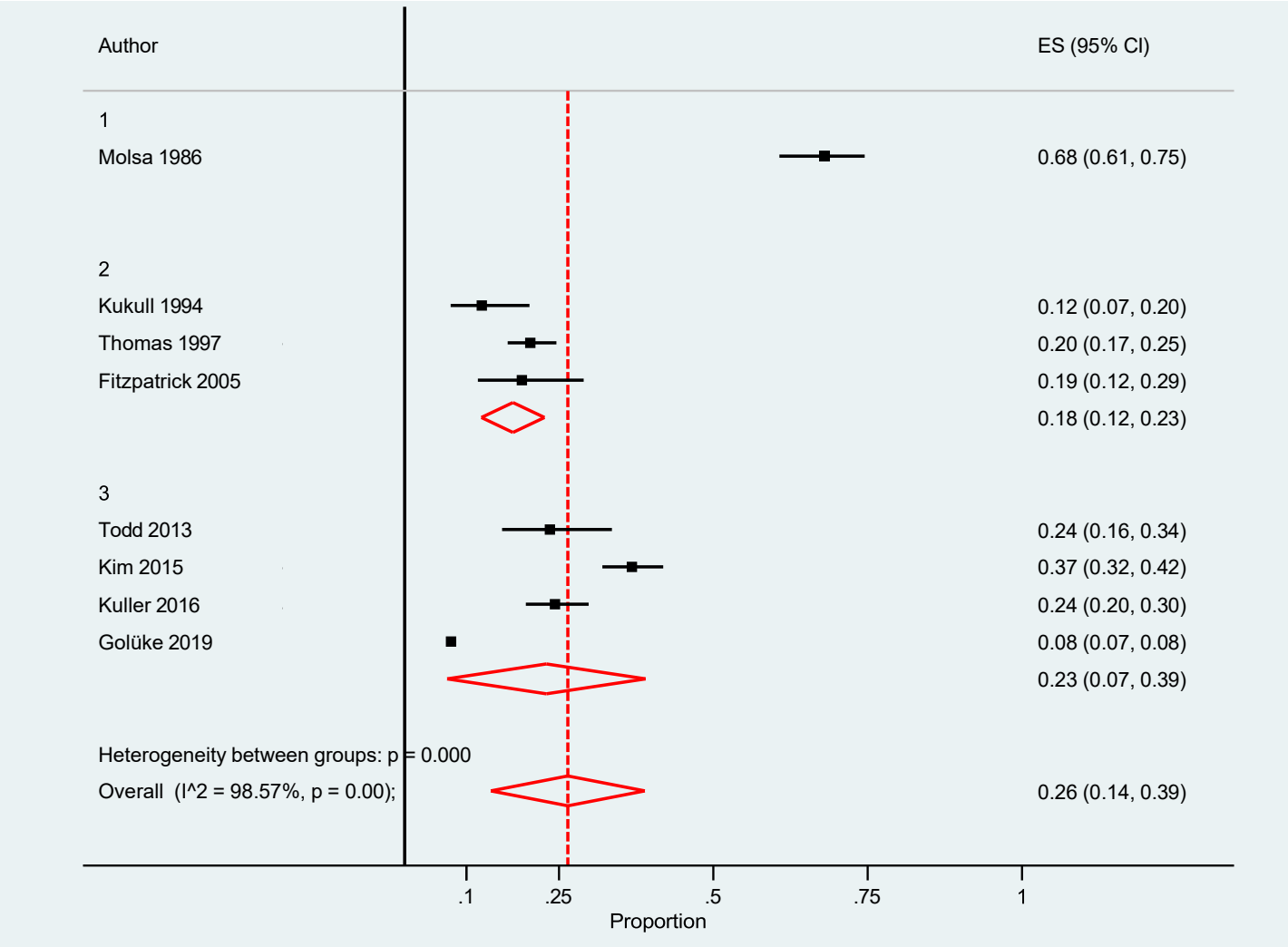
Supplementary figure 2. Combined effects meta-analysis of [cerebrovascular disease] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



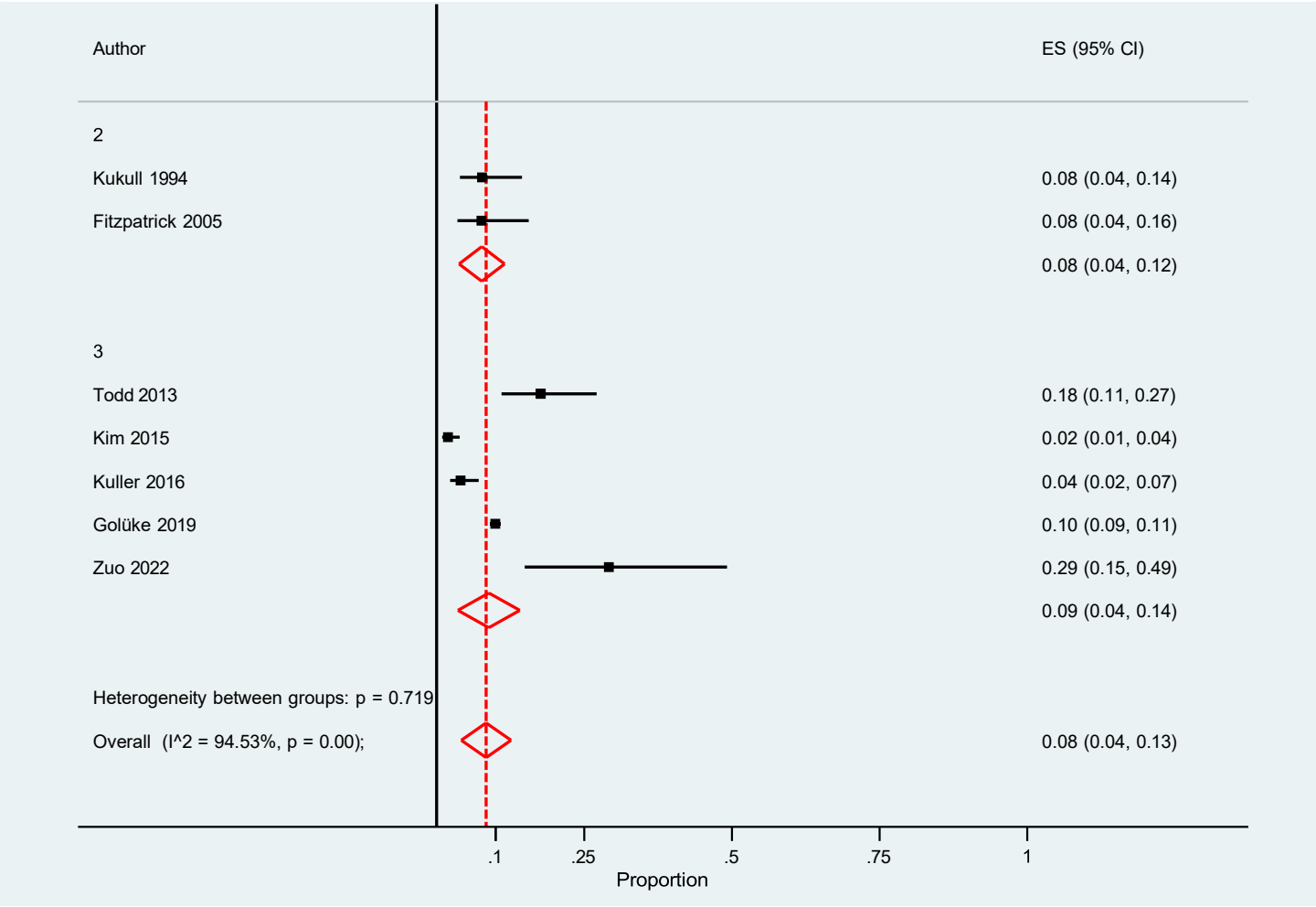
Supplementary figure 3. Combined effects meta-analysis of [cardiovascular disease] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



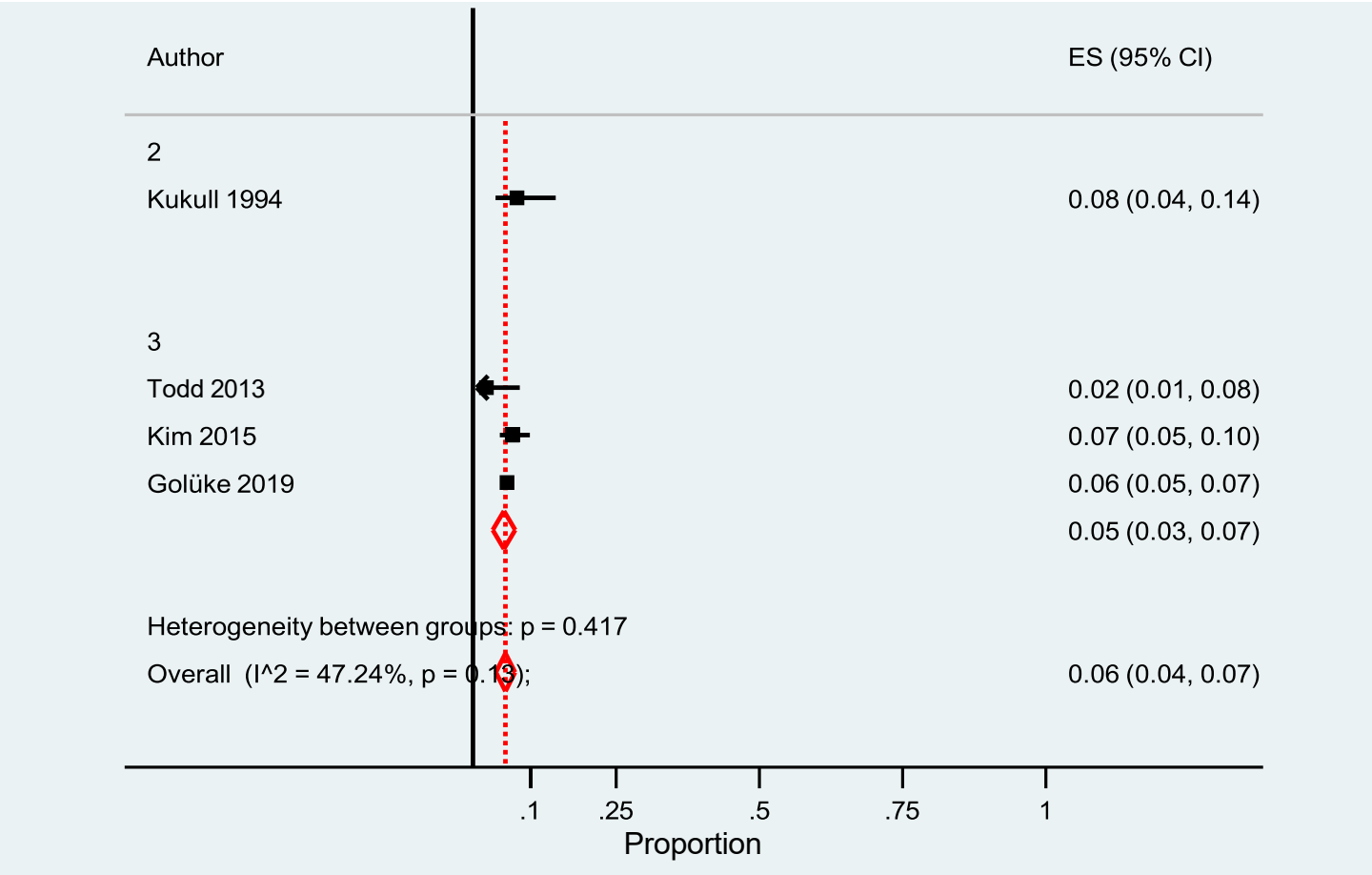
Supplementary figure 4. Combined effects meta-analysis of [dementia] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



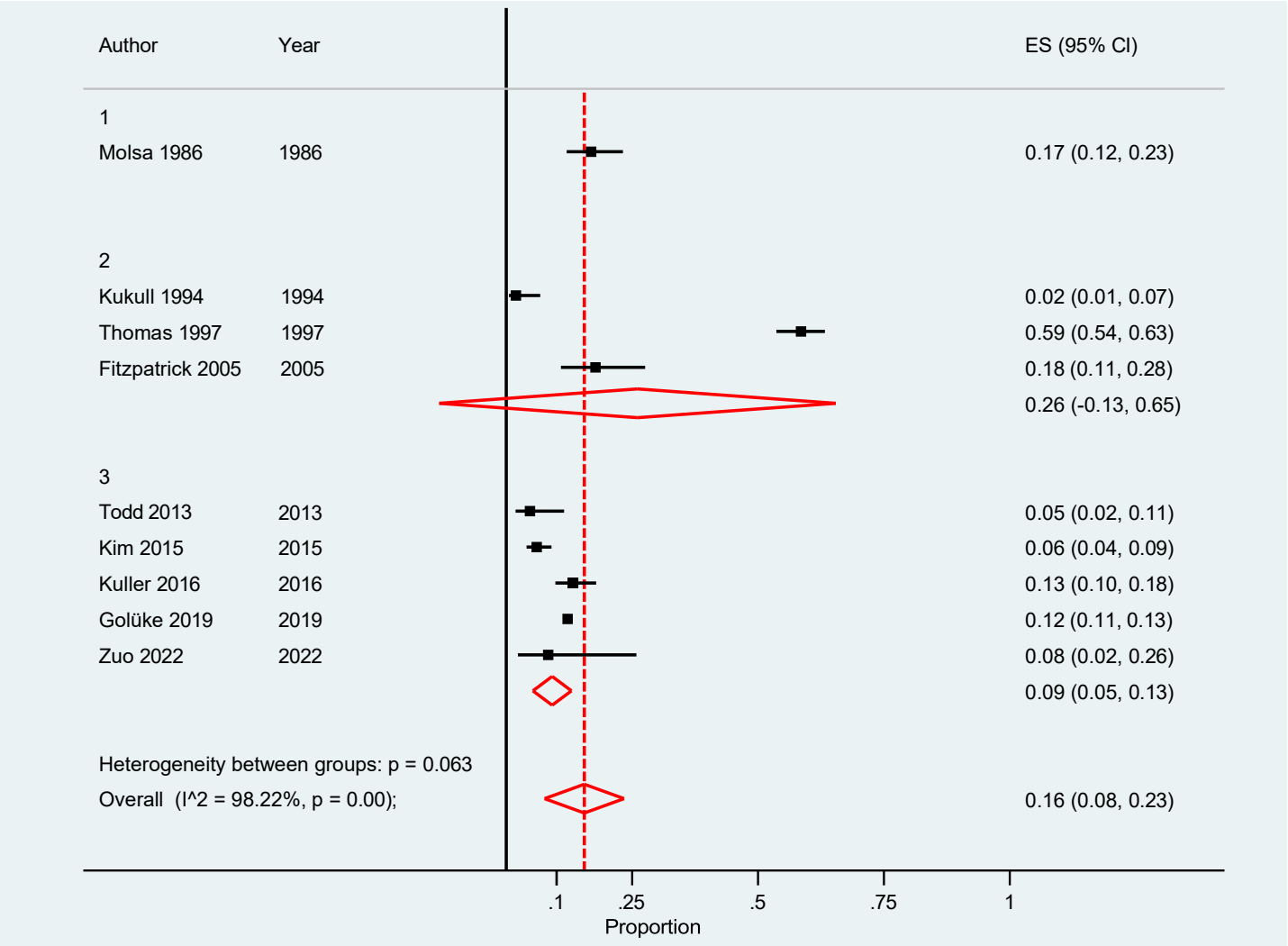
Supplementary figure 5. Combined effects meta-analysis of [pneumonia] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



Supplementary figure 6. Combined effects meta-analysis of [other infectious disease] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



Supplementary figure 7. Combined effects meta-analysis of [unknown or other cause of death] as a cause of death following AD according to calendar period (period 1: 1986 – 1993; **period 2:** 1994 – 2005, and **period 3:** 2013 – 2023).



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PROJECT TITLE

“Non-invasive neuromodulation to enhance targeted cognitive remediation in older adults with depression.”

EFFORT

I have 80% protected time for career development and mentored research training and this effort is supported by my primary mentor (Dr. Warren Taylor)’s grants, this McKnight award, and departmental funds. The other 20% of my time is spent conducting clinical neuropsychological evaluations.

GOALS OF THE PROJECT

In older adults, depression is associated with difficulties in multiple cognitive domains, with deficits in executive functioning being most prominent and corresponding with higher risk of cognitive and functional decline and transition to dementia. Executive deficits in older adults with depression (OAD) are associated with underlying structural and functional brain alterations in the cognitive control network (CCN), which contribute to accelerated cognitive and brain aging. Currently, there are no FDA-approved interventions to treat cognitive deficits in OAD. Finding treatments that target and promote plasticity of the underlying CCN neural circuitry may improve executive functions and other outcomes, thereby counteracting age- and depression-related cognitive changes.

Recent development of a novel computerized cognitive remediation (nCCR) intervention that specifically targets depression-related executive difficulties in OAD shows efficacy, as well as transfer effects to non-trained cognitive domains (i.e., memory). In non-depressed older adults, accumulating evidence shows that transcranial direct current stimulation (tDCS) applied bilaterally over the prefrontal cortex augments working memory performance when also paired with cognitive training – via the mechanism of enhancing working-memory related brain activity. tDCS primes brain networks to better engage with cognitive training; thus, allowing for more cognitive benefit than observed with either intervention alone. Working memory, a component of executive functioning that is subserved by the CCN, is impaired in OAD, making it a target of engagement relevant for both aging and depression. To date, no studies have investigated the benefit of combining tDCS with nCCR in OAD.

The primary goal of this study is to determine whether the addition of active tDCS to nCCR enhances brain activity and cognitive functions in OAD to a greater degree than nCCR with sham stimulation. We will randomize 20 elderly depressed outpatients to either double-blinded active or sham bifrontal tDCS plus daily nCCR over 4-weeks. Multimodal MRI (focused on the CCN) and psychiatric and neuropsychological evaluations will be obtained at baseline and following intervention completion. Long-term CCN cognitive effects will be explored 3-months post-intervention via cognitive assessments.

Primary Aim: To determine whether bifrontal tDCS augments the effects of targeted cognitive remediation on CCN brain and cognitive functions (via neuroimaging and cognitive assessments).

Exploratory Aims: To examine treatment-related differences in functional connectivity between CCN regions pre-and post-intervention; and test for longer-term (3-months post-intervention) cognitive performance benefit.

The goals have not changed since the proposal was awarded. Progress towards the aims are outlined in the section below.

PROGRESS SINCE RECEIVING NOTICE OF AWARD

Study Start-Up: I have submitted the IRB and received approval to conduct the study, added the study to clinicaltrials.gov, obtained the Soterix clinical trials tDCS device and associated materials (e.g., rechargeable batteries, head straps, sponges, saline) and 2 Samsung tablets for the nCCR, have weekly meetings with Dr. Taylor, and regular meetings with my off-site mentors. I have obtained randomization codes and identified lab (but not study) personnel who will give me these codes at the start of the intervention and will maintain the blinding until interim analysis (detailed below). I piloted and finalized the neuroimaging sequence in our scanner and am in the process of finalizing the Manual of Procedures and REDCap database. I have also been training staff on study procedures (mainly the intervention but their role on other aspects of the study will increase as the study progresses).

Obstacles: The primary obstacle I have encountered thus far was getting the fMRI task to work in the VUMC 3T scanner. I obtained the task from Dr. Adam Woods at the University of Florida (co-mentor on this project) and the task kept freezing after the scanner sent the trigger to the experimental laptop. After troubleshooting various things and multiple trips to the scanner, I eventually got the task to work. The problem was that the task was created in Eprime 2, which was not syncing with our scanner equipment for some reason. I was able to get the task to work on other lab laptops, but not the study laptop. I eventually upgraded the study laptop to Eprime 3 and this has fixed the issue. Unfortunately, this obstacle took 3 to 4 months to resolve, which delayed the starting of recruitment.

Recruitment: I began study recruitment in Mar 2023 by turning on the study in Research Match and sending out an email blast to Vanderbilt's email research recruitment tool. As of the time of this writing, I have had 15 people complete the prescreening questionnaires, but unfortunately no one has been eligible to come in for study procedures (mainly due to minimal depressive symptoms or conflicting schedules). I also recently put flyers up around the medical center in high traffic areas to increase awareness of the study.

Formal Trainings: In Aug 2022, I went to the University of Florida for a 1-week hands-on refresher course of tDCS, under the guidance of Dr. Woods. I also took the online Martinos CONN course (November to December 2022). I was unable to audit the biostatistics course in Spring 2023 due to scheduling conflicts. I have also attended several Clinical Research Center: Research Skills Workshops on topics relevant to the conduct of clinical research.

Employment: I was promoted from Research Instructor to the rank of Assistant Professor in Sept 2022.

Publications / Presentations (since submission of McKnight application): Over the past year, I have published 7 papers (with 3 more under review, 1 as first author), including a paper using some of Dr. Woods' pilot data on the benefits of tDCS plus cognitive training on subclinical depressive symptoms in older adults without clinical depression (which is directly related to this award). Aspects of this work have also been presented at national and local meetings this past year.

- Taylor, W. D., Zald, D. H., Felger, J. C., Christman, S., Claassen, D. O., Horga, G., Miller, J. M., Gifford, K., Rogers, B., **Szymkowicz, S. M.,** & Rutherford, B. R. (2022). Influences of dopaminergic system dysfunction on late-life depression. *Molecular Psychiatry, 27*, 180-191. doi: 10.1038/s41380-021-01265-0.
- **Szymkowicz, S. M**., Jones, J. D., Timblin, H., Ryczek, C. A., Taylor, W. D., & May, P. E. (2022). Apathy as a within-person mediator of depressive symptoms and cognition in Parkinson's disease: Longitudinal mediation analyses. *American Journal of Geriatric Psychiatry, 30*(6), 664-674. doi: 10.1016/j.jagp.2021.11.007.
- **Szymkowicz, S. M.,** Taylor, W. D., & Woods, A. J. (2022). Augmenting cognitive training with bifrontal tDCS decreases subclinical depressive symptoms in older adults: Preliminary findings. *Brain Stimulation, 15*, 1037-1039. doi: 10.1016/j.brs.2022.07.055.
    - **Szymkowicz, S. M**., Taylor, W. D., & Woods, A. J. (Feb 2023). Cognitive training paired with bifrontal tDCS decreases depressive symptoms in a non-clinical sample of older adults: Preliminary evidence. Poster presented at the 51st annual International Neuropsychological Society (INS) meeting, San Diego, CA.
    - **Szymkowicz, S. M**., Taylor, W. D., & Woods, A. J. (Mar 2023). Cognitive training paired with bifrontal tDCS decreases depressive symptoms in a non-clinical sample of older adults: Preliminary evidence. Poster presented at the 4th annual Vanderbilt Memory and Alzheimer's Center (VMAC) Research Day, Vanderbilt University Medical Center, Nashville, TN.
    - **Szymkowicz, S. M**., Taylor, W. D., & Woods, A. J. (Jun 2023). Cognitive training paired with bifrontal tDCS decreases depressive symptoms in a non-clinical sample of older adults: Preliminary evidence. Poster to be presented at the 4th annual Academic Psychiatric Symposium, Vanderbilt University Medical Center, Nashville, TN.
- Ahmed, R., Ryan, C., Christman, S., Elson, D., Bermudez, C., Landman, B. A., **Szymkowicz, S. M.**, Boyd, B. D., Kang, H., & Taylor, W. D. (2022). Structural MRI-based measures of accelerated brain aging do not moderate the acute antidepressant response in late-life depression. *American Journal of Geriatric Psychiatry, 30*(9), 1015-1025. doi: 10.1016/j.jagp.2021.11.011.
- **Szymkowicz, S. M.,** Ryan, C., Elson, D. M., Kang, H., & Taylor, W. D. (2023). Cognitive phenotypes in late-life depression. *International Psychogeriatrics, 35*(4), 193-205. doi: 10.1017/S1041610222000515.
- Hemphill, L., Valenzuela, Y., Luna, K., **Szymkowicz, S. M.**, & Jones, J. D. (2023). Synergistic associations of depressive symptoms and aging on cognitive decline in

early Parkinson's disease. *Clinical Parkinsonism & Related Disorders, 8*, 100192. doi: 10.1016/j.prdoa.2023.100192.

- Ahmed, R., Boyd, B. D., Elson, D., Albert, K., Begnoche, J. P., Kang, H., Landman, B. A., **Szymkowicz, S. M.**, Andrews, P., Vega, J., & Taylor, W. D. (In press). Influences of resting-state intrinsic functional brain connectivity on the antidepressant treatment response in late-life depression. *Psychological Medicine*. doi: 10.1017/S0033291722003579.

GOALS OF NEXT CYCLE

Recruitment: The primary focus over the next reporting period is to increase enrollment, which will allow for progress towards the study aims. As mentioned above, I am actively recruiting with a combination of clinical referrals, referrals from other studies in our research group, and community outreach (e.g., Research Match, flyers). However, if the in-person time commitment for the intervention portion of this study (i.e., daily sessions over 4 weeks) ends up being a limiting factor for recruitment, we will explore alternate strategies (such as at-home tDCS paired with nCCR and virtual visits) to improve recruitment, retention, and study completion.

Grant Preparation: Following discussions with my mentors, I will conduct interim analyses after 8 participants (4 active, 4 sham) have completed the post-intervention cognitive testing and MRI procedures. This will provide pilot data to support submission to an internal Vanderbilt K12 research scholars program and an NIH K23 award (funding would transition from the K12 to the K23 should that be received).

Formal Trainings: I will be attending a full-day Responsible Conduct of Research Training in May 2023, as well as attending the didactic sessions of the Advanced Psychometric Methods for Cognitive Aging Research in Aug 2023 (topic: Bayesian approaches to modeling cognition). I will again see if I am able to audit the biostatistics course in Spring 2024. I plan to attend relevant Clinical Research Center: Research Skills Workshops and will participate in Vanderbilt Grantsmanship offerings as I get closer to grant submission (i.e., Specific Aims and / or Grant Review Studios and Grant Pacing Workshop).

Publications / Presentations (relevant to application): I will be attending the AAN conference virtually this year (Apr 2023) and plan to attend in-person in 2024 (ideally to present some of this work). I will also submit findings from the completed study to a reputable psychiatry, neuropsychology, or aging journal**.**

MENTOR STATEMENT OF PROGRESS

Dr. Szymkowicz has been doing quite well. She is making good progress on this specific project and developing her research career more broadly as demonstrated by her presentation record, publication record, and career training.

She has approached the project funded under this award with careful consideration. I am impressed with the rigor in how she approached startup procedures. She has been

diligent in training Research Assistants who are supporting her. She is clearly ready to proceed with enrollment and has been actively recruiting participants. I am working to support these efforts in my laboratory, with my research coordinators screening participants for potential interest and eligibility. This work, combining noninvasive neuromodulation techniques with computerized cognitive training, continues to be novel. I am committed to continuing supporting both her project and her career development.

I continue to be impressed by her work and have absolutely no concerns about her progress. She remains highly committed to developing a career as a clinician-scientist focused on interventions to improve cognitive function. I fully support her continuing this award.

Warren D Taylor, MD
James G Blakemore Professor of Psychiatry
Vanderbilt University Medical Center 04/14/2023

American Academy of Neurology - American Brain Foundation - McKnight Brain Research Foundation
Research Scholarship in Cognitive Aging and Age-Related Memory Loss / PI: Sarah Szymkowicz / Interim Financial Status Report
4-04-527-6132
7/1/2022 - 6/30/2024

		BUDGET		EXPENSES		
CONTRACTOR INFORMATION: Institution Name: Vanderbilt University Medical Center Address: 3319 West End Avenue, Suite 700 City, State, Zip: Nashville, TN 37203		(COLUMN B) CURRENT REPORTING PERIOD	(COLUMN C) ACTUAL PREVIOUSLY REPORTED	(COLUMN D) CURRENT REPORTING PERIOD	(COLUMN E) (COL. C + D) CUMULATIVE EXPENSE	(COLUMN F) (COL. A + B - E) BUDGET BALANCES
		From: 7/1/2022 To: 3/31/2023	From: To:	From: 7/1/2022 To: 3/31/2023		
Salary + Fringe = PI (Szymkowicz; 10% effort)		7,975.00	-	4,354.47	**4,354.47**	**3,620.53**
Salary + Fringe = RA (Renfro; 25% effort)		9,510.00	-	3,873.52	**3,873.52**	**5,636.48**
Subtotal Personnel Costs		**17,485.00**	**-**	**8,227.99**	**8,227.99**	**9,257.01**
Research Supplies		2,939.00	-	2,333.02	**2,333.02**	**605.98**
Research Costs		19,525.00				
Education and Travel		4,476.00	-	425.00	**425.00**	**4,051.00**
Subcontracts < $25k		30,575.00	-	5,617.74	**5,617.74**	**24,957.26**
		-	-	-	**-**	**-**
		-	-	-	**-**	**-**
Subtotal Direct Cost:Non-Personnel		**57,515.00**	**-**	**8,375.76**	**8,375.76**	**49,139.24**
Subtotal Direct Cost		**75,000.00**	**-**	**16,603.75**	**16,603.75**	**58,396.25**
Indirect Cost (0%)		-	-	-	**-**	**-**
TOTALS:		**75,000.00**	**-**	**16,603.75**	**16,603.75**	**58,396.25**
Less: Payments Received To Date					**75,000.00**	
Cash On Hand -Positive Balance / Cash Deficit (Negative Balance)					**58,396.25**	

CERTIFICATION: I hereby certify that the costs incurred are identified on the Project Budget, within the budgeted amount estimated for such costs, have been properly recorded in the accounting records of the Project, and are supported by written documentation.

Jennifer Nixon Digitally signed by Jennifer Nixon Date: 2023.04.28 14:09:37 -05'00' / **Jennifer Nixon** / **Sr. Accounting Manager**

Financial Officer Signature / Date **Printed Name** **Title**

Final Report - 2501 - Quantifying Vascular Contributions to Cognitive Aging Mediated by White Matter Injury and Tau

Dr. Wai-Ying Yau, MD

Massachusetts General Hospital, Brigham, Harvard

Restatement of Specific Aims and progress achieved for each Aim
Aim 1: Determine the impact of systemic vascular risk on longitudinal white matter injury and tau accumulation.

We have completed the sub-aim investigating the interactive relationship between systemic vascular risk and beta-amyloid (Aβ) burden on longitudinal tau accumulation. Consistent with our hypothesis, we found a synergetic association between higher baseline Framingham cardiovascular risk score and elevated Aβ burden with greater inferior temporal tau accumulation. We further determined that of the individual vascular risk factors, elevated systolic blood pressure and body mass index were significant and independent drivers of this interaction with Aβ on tau. This work was selected for an oral presentation at the Alzheimer's Association International Conference (AAIC) 2022. A first-author manuscript was subsequently published in Annals of Neurology (Yau WW et al. 2022).

We have made good progress towards completing the white matter injury sub-aim. We have processed the first two longitudinal MRI scans using an improved method for quantifying white matter hyperintensity volume, and a newer diffusion tensor imaging method of measuring white matter microstructure. The processing of the third and fourth longitudinal MRI scans is ongoing. Once complete, we will pursue the proposed analyses to determine whether systemic vascular risk and amyloid burden have independent or synergistic effects on promoting white matte injury over time.

Aim 2: Define the relationship between novel vascular plasma markers, longitudinal WM injury and tau.

We have completed the sub-aim investigating the interactive effects of baseline angiogenesis plasma markers with Aβ burden on longitudinal tau accumulation. Our findings demonstrated that low levels of vascular endothelial growth factor A (VEGF A) – a marker that promotes canonical angiogenesis, and high levels of placental growth factor

(PlGF) – a marker implicated in pathological angiogenesis and inflammation, were synergistic with elevated Aβ in accelerating longitudinal inferior temporal tau accumulation. This work was selected for an oral presentation at the recent AAIC 2023 conference. A co-first-author manuscript is currently under peer review in Brain.

Once we have completed the longitudinal processing of white matter measurements as described above, we will examine the independent and synergistic effects of vascular plasma markers and amyloid burden on longitudinal progression of white matter injury.

Aim 3: Quantify mediating mechanisms of vascular contributions to cognitive decline in aging.

We have made significant progress towards completing this aim. We have conducted moderated mediation analyses which revealed that inferior temporal tau accumulation significantly mediated the effects of systemic vascular risk and plasma angiogenesis measures on prospective decline in individuals who have high baseline Aβ burden (i.e. individuals with preclinical Alzheimer's disease). Our findings indicated that tau accumulation mediated 33% of the systemic vascular risk effect on cognitive decline in setting of elevated Aβ burden, with even a greater percentage of effects mediated by tau (over 60%) when examining systolic blood pressure and BMI specifically. Similarly, more rapid tau accumulation fully mediated the effects of low VEGF-A and partially mediated the effects of high PlGF on accelerated cognitive decline in individuals with elevated Aβ. Once the longitudinal white matter injury measurements are completed, we will use structural equation modeling to examine the relative contributions of white matter injury and tau in mediating these vascular contributions to cognitive decline.

Describe obstacles, if any, in achieving each Specific Aim
Our Positron Emission Tomography (PET) imaging facility hasve experienced a number of technical difficulties which have led to closures and delays in acquiring longitudinal tau PET scans. This has impacted the number of participants with longitudinal tau PET data (currently around 200), and the acquisition of the third and fourth tau scans for these individuals.

Plans for Next Year
Building on the work supported by the AAN scholarship, I submitted a K23 proposal in February 2023 to examine the critical time window during mid- to late-life when hypertension and obesity interacts with emerging Aβ pathology to promote tau pathology. I received a score of 25, which is within NIA's AD/ADRD career development award pay line (35). I anticipate that I will begin this project in fall of 2023.

Coursework and other educational opportunities and activities

Conferences: I attended the AAN 2022 and 2023 Annual Meeting. I attended AAIC 2022 and 2023 and was selected for an oral presentation at both meetings. I was invited to be a symposium speaker at the Brain & Brain PET 2023 meeting on a session titled “Neurodegeneration and dementia - should the vasculature be our main priority?”.

Course work: I attended the PET Pharmacokinetics Course and the Summer Course on Causal Inference at the Harvard T.H. Chan School of Public Health in 2022. I also completed courses in grant writing including Conquering the K and the Center for Faculty Development Grant Writing Course 2022.

Publications, presentations, awards, resulting from current American Academy of Neurology support
Publications:
A first-author publication highlighting results from Aims 1 and 3 was published in Annals of Neurology.
Yau WW et al. Tau mediates synergistic influence of vascular risk and Aβ on cognitive decline. Ann Neurol. 2022 Nov;92(5):745-755.

A co-first-author manuscript highlighting results from Aims 2 and 3 is currently under peer review in Brain.

Other co-authored manuscripts:
1. Shirzadi Z, Schultz SA, Yau WW, et al. Etiology of white matter hyperintensities in autosomal dominant and sporadic Alzheimer's disease. Accepted, JAMA Neurology.
2. Coughlan GT, Betthauser TJ, Boyle R, Koscik RL, Klinger HM, Chibnik LB, Jonaitis EM, Yau WW, et al. Association of Age at Menopause and Hormone Therapy Use With Tau and β-Amyloid Positron Emission Tomography. JAMA Neurol. 2023 Apr 3. [Epub ahead of print]
3. Shirzadi Z, Yau WW, et al. Progressive White Matter Injury in Preclinical Dutch Cerebral Amyloid Angiopathy. Ann Neurol. 2022 Sep;92(3):358-363.

Oral presentations:
1. AAIC 2022: Longitudinal tau burden partially mediates synergistic influence of vascular risk and amyloid-beta on cognitive decline in clinically normal older adults
2. Mass General Brigham and MGH Memory Disorders Unit & Movement Disorders Unit Conference Memory Disorders Unit & Movement Disorders Unit Conference (2022): The Role of Tau in the Synergistic Influence of Vascular Factors and Amyloid on Cognitive Decline in Preclinical Alzheimer’s disease
3. Brain & Brain PET 2023 symposium “Neurodegeneration and dementia - should the vasculature be our main priority?”: Vascular Risks Accelerate Tau Accumulation and Cognitive Decline in Preclinical Alzheimer’s Disease

4. AAIC 2023: High VEGF-A and low PlGF in plasma are associated with reduced longitudinal tau PET burden and slower cognitive decline in preclinical Alzheimer's disease

Other co-authored abstracts:
1. Shirzadi Z, Yau WW et al. Cerebrovascular injury markers explain the effect of systemic vascular risk on cognitive decline in older adults with lower amyloid burden. AAIC 2022.
2. Shirzadi Z, Schultz SA, Yau WW et al. Progressive white matter injury in autosomal dominant Alzheimer's disease is strongly associated with cerebral microbleeds and neurodegeneration. AAIC 2022.
3. Riphagen JM, Chhatwal JP, Becker A, Kwong K, Engels N, Yau WW et al. Vascular health is associated with locus coeruleus-related entorhinal tau deposition. AAIC 2022.
4. Shirzadi Z, Yau WW et al. Systemic vascular risk, white matter injury, and relative cerebral blood flow independently contribute to cognitive decline beyond amyloid and tau burden. AAIC 2023.
5. Townsend D, Properzi MJ, Betthauser TJ, Klinger HM, Boyle R, Coughlan GT, Hanseeuw BJ, Yang HS, Amariglio RE, Farrell ME, Jacobs HIL, Shirzadi Z, Yau WW et al. Estimating a clinically normal individual's position along a preclinical Alzheimer's disease continuum using cognitive and amyloid trajectories. AAIC 2023.

Awards:
Alzheimer's Imaging Consortium (AIC) Best Oral Presentation Award at AAIC 2022
Massachusetts Alzheimer's Disease Research Center Research Education Core (REC) Scholar (2022)

Please include a one page research summary written for a lay audience (minimum of 150 words). Please gear the summary to an audience with a 2nd grade reading level
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Studies show that systemic vascular risk factors and vascular disease increase people's risk for memory and thinking problems as they grow older. This is especially true for people who also have Alzheimer's pathologies in their brain (abnormal proteins such as amyloid plaques and tau tangles) and are already at risk for developing Alzheimer's dementia. Even though there are new treatments for Alzheimer's disease that can clean up the amyloid proteins in the brain, they only have a small effect in helping memory and thinking. There is still a lot of work to do to find better ways to treat Alzheimer's disease and other causes of memory and thinking problems in old age. One approach to achieving this is by understanding how vascular diseases and Alzheimer's proteins work together and by themselves to cause memory and thinking problems. Understanding these relationships may help us find ways to combine the treatments we currently have for Alzheimer's and vascular diseases so that they can be even more effective together, or to come up with new treatments to keep our brains healthy as we get older.

To answer these questions, we studied a group of healthy older people without memory and thinking problems and followed them for up to 12 years with brain scans, blood markers, medical history, and cognitive tests. We use all this information to find out how vascular disease and amyloid proteins cause brain abnormalities and memory problems as people get older. What we have found so far is that systemic vascular risk factors, especially high blood pressure and being overweight, work together with amyloid proteins in the brain to increase the amount of tau tangles and make memory and thinking worse over time. This suggests that targeting high blood pressure and obesity, either by themselves or together with medications that clear amyloid proteins, may be an effective way to reduce brain damage and cognitive problems in people who have early Alzheimer's disease. We also identified two blood proteins that are important for the growing blood vessels and keeping them healthy, that either slowed down the development of tau tangles and helped memory and thinking (vascular endothelial growth factor A) or made things worse (placental growth factor) in people who have too much amyloid proteins in the brain. What we have found helps us better understand the biology of how vascular problems work with Alzheimer's proteins to causes brain disease in old age. We can use this knowledge to find new treatments for very early Alzheimer's disease, before people develop memory and thinking problems, so we have a better chance of slowing or stopping the disease and keeping our brains healthy.

Mentor Comments

Thank you for being a mentor for Wai-Ying Yau on their American Academy of Neurology award. Please comment on your mentee's progress in achieving their specific research aims and their overall progress on the project.

Dr. Yau has done a superb job in accomplishing the goals of this AAN McKnight Fellowship. Her work during this fellowship has substantially added to our knowledge about the role of vascular risk in cognitive decline associated with aging and preclinical Alzheimer's disease. Her first authored publication in Annals of Neurology is an important contribution to the literature and served as critical preliminary data for her NIH K23 career development award application. She received excellent reviews on this K23 application and it is anticipated that the K23 will be awarded this fall. In addition, Wendy was promoted to Instructor in Neurology at Harvard Medical School earlier this year, and we are thrilled that she will remain as faculty at MassGeneralBrigham working with our group. Wendy has a very bright future ahead of her working in this important field, and her academic trajectory has been greatly accelerated with this AAN fellowship.

Tau Mediates Synergistic Influence of Vascular Risk and Aβ on Cognitive Decline

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Objective: Elevated vascular risk and beta-amyloid (Aβ) burden have been synergistically associated with cognitive decline in preclinical Alzheimer's disease (AD), although the underlying mechanisms remain unclear. We examined whether accelerated longitudinal tau accumulation mediates the vascular risk-Aβ interaction on cognitive decline.
Methods: We included 175 cognitively unimpaired older adults (age 70.5 ± 8.0 years). Baseline vascular risk was quantified using the office-based Framingham Heart Study general cardiovascular disease risk score (FHS-CVD). Baseline Aβ burden was measured with Pittsburgh Compound-B positron emission tomography (PET). Tau burden was measured longitudinally (3.6 ± 1.5 years) with Flortaucipir PET, focusing on inferior temporal cortex (ITC). Cognition was assessed longitudinally (7.0 ± 2.0 years) using the Preclinical Alzheimer's Cognitive Composite. Linear mixed effects models examined the interactive effects of baseline vascular risk and Aβ on longitudinal ITC tau. Additionally, moderated mediation was used to determine whether tau accumulation mediated the FHS-CVD*Aβ effect on cognitive decline.
Results: We observed a significant interaction between elevated baseline FHS-CVD and Aβ on greater ITC tau accumulation ($p = 0.004$), even in individuals with Aβ burden below the conventional threshold for amyloid positivity. Examining individual vascular risk factors, we found elevated systolic blood pressure and body mass index showed independent interactions with Aβ on longitudinal tau (both $p < 0.0001$). ITC tau accumulation mediated 33% of the interactive association of FHS-CVD and Aβ on cognitive decline.
Interpretation: Vascular risks interact with subthreshold levels of Aβ to promote cognitive decline, partially by accelerating early neocortical tau accumulation. Our findings support vascular risk reduction, especially treating hypertension and obesity, to attenuate Aβ-related tau pathology and reduce late-life cognitive decline.

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Background

Despite recent progress in anti-amyloid therapy,[1] there remains an urgent need for safe and effective treatments that can meaningfully alter the course of Alzheimer's disease (AD). There is growing consensus that elevated beta-amyloid (Aβ) burden is necessary but insufficient to cause AD.[2] It is therefore critical to identify, characterize, and target factors that modify the emergence of cognitive decline in concert with Aβ pathology.

Elevated vascular risk and cerebrovascular disease are very common with aging, often co-exist with AD pathology,[3] and are increasingly recognized as significant contributors to AD-related cognitive decline.[4–8] Elevated systemic vascular risk, particularly when combined with

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elevated Aβ burden, is associated with accelerated cognitive decline and increased dementia risk in cognitively unimpaired (CU) individuals.[7,9] This suggests that interventions to reduce vascular risk may be effective in delaying the onset or progression of cognitive decline. However, the mechanisms underlying this clinically important interaction between vascular risk and Aβ remain to be elucidated.

A previous study demonstrated that higher baseline vascular risk and Aβ were synergistically associated with greater cross-sectional inferior temporal cortex (ITC) tau burden in older CU adults.[10] These findings implicate vascular risk related promotion of tau pathology as a potential mechanism linking vascular risk to AD pathology and early AD-related cognitive decline. Better elucidating longitudinal relationships between vascular risk and AD pathology is critical to confirm these cross-sectional findings. If vascular risks are indeed synergistic with Aβ in promoting longitudinal tau accumulation, it would strongly support targeting vascular risk reduction – alone or in combination with AD-pathology focused therapies – as a possible disease-modifying treatment strategy in AD. Additionally, it would support the need to consider vascular risk factors in ongoing and future AD clinical trials, particularly those that use tau positron emission tomography (PET) or cognitive decline as outcomes.

In this context, the present study tested the hypothesis that elevated baseline vascular risk and Aβ burden would be synergistically associated with greater longitudinal ITC tau accumulation, in a deeply phenotyped group of older CU adults participating in the Harvard Aging Brain Study (HABS). We further examined if, and to what extent, tau accumulation mediated the impact of elevated vascular risk on prospective cognitive decline in individuals with elevated Aβ burden.

Methods

Participants

One hundred seventy-five older CU adults were recruited from HABS. We included all participants who had baseline Aβ PET imaging, at least 2 tau PET and cognitive assessments, and sufficient baseline medical information to calculate an algorithmic vascular risk score. At study entry, all participants had global Clinical Dementia Rating[11] of 0, education-adjusted Mini-Mental State Examination (MMSE)[12] score of 27 or greater, and normal Logical Memory IIa delayed recall performance.[13] Exclusion criteria included a modified Hachinski ischemic score greater than 4, and a history of stroke or evidence of infarcts with persistent neurological deficits.[14] Data were collected from April 2010 through August 2021. The Mass General Brigham Institutional Review Board approved HABS protocol and procedures, and all participants signed a written informed consent prior to the completion of any study procedures.

Cardiovascular Disease Risk

Our primary measure of cardiovascular disease risk was the office-based Framingham Heart Study cardiovascular disease risk score (FHS-CVD),[15] which represents a sex-specific weighted sum of age, antihypertensive treatment (dichotomous), systolic blood pressure (SBP; millimeters of mercury), body mass index (BMI), diabetes status (dichotomous), and cigarette smoking status (dichotomous). The FHS-CVD score provides a 10-year probability of future cardiovascular events, including coronary death, myocardial infarction, coronary insufficiency, angina, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease, and heart failure. For sensitivity analyses, we calculated the lipid-based FHS-CVD score[15] and the atherosclerotic cardiovascular disease (ASCVD) risk score[16] for the subset of participants with available serum lipid profiles (n = 155).

PET Imaging

Brain Aβ burden was measured at baseline using ^{11}C-Pittsburgh Compound-B (PiB) PET at the Massachusetts General Hospital using the ECAT EXACT HR+ scanner (Siemens). ^{18}F-Flortaucipir (FTP) PET was introduced into HABS mid-study to measure tau burden, with participants undergoing their first FTP PET at 2.1 ± 1.5 years after baseline visit. Longitudinal tau PET measurements were obtained at year 1 (for those enrolled after FTP PET was introduced), 4, 6, and 9 visits. Detailed Aβ and tau PET protocols have been previously described.[17] Aβ PET measurements were represented as a distribution volume ratio (DVR) across a composite of frontal, lateral temporal and parietal, and retrosplenial regions (FLR), defined using FreeSurfer (version 6.0). Tau PET measurements were computed a standardized uptake value ratio (SUVR) within the ITC, an early site of neocortical tau accumulation associated with clinical impairment[17] and a region in which cross-sectional associations with vascular risk and Aβ were previously observed.[10] Both Aβ and tau PET data used cerebellar gray matter as the reference region and used the geometric transfer matrix method for partial volume correction (PVC).[18]

Cognitive Measures

Cognition was assessed annually using the Preclinical Alzheimer's Cognitive Composite-5 (PACC5).[19] The PACC5 is a composite that includes the MMSE,[12] Wechsler Adult Intelligence Scale–Revised Digit Symbol

Coding,[20] Wechsler Memory Scale–Revised Logical Memory delayed recall,[13] Free and Cued Selective Reminding Test (free recall plus total recall),[21] and Category Fluency Test.[22]

Statistical Analyses

We used R version 4.0.5 for statistical analyses. Linear mixed effects model ("nlme" package) was used to examine the interactive effects of baseline FHS-CVD scores and Aβ on ITC tau burden over time. Time was operationalized as years from the baseline Aβ PET scan. We adjusted for age, sex, APOE ε4 carrier status, their interactions with time, and included random intercepts and slopes. Continuous variables were z-transformed prior to model entry. We performed sensitivity analyses using PiB and FTP PET data without PVC, as well as using the lipid-based FHS-CVD and ASCVD risk scores. For post hoc analyses, we further examined the interactive effects between individual component measures of FHS-CVD and Aβ on longitudinal ITC tau. We applied family-wise error (FWE) correction for multiple comparisons (6 component measures of FHS-CVD), with $p < 0.008$ for significance.

Next, we examined the FHS-CVD by Aβ interaction using floodlight/Johnson-Neyman analysis ("interactions" package) to determine the level of Aβ at which effects on ITC tau accumulation became significant ($p < 0.05$). For floodlight analysis, we extracted individual slopes of ITC tau change from unadjusted linear mixed effects models with random slopes and intercepts that have time as the only predictor of ITC tau.

Additionally, we conducted whole-brain, region of interest (ROI)-based exploratory analyses to examine whether the interaction between baseline FHS-CVD risk and Aβ burden on longitudinal tau accumulation extended beyond the ITC. We used FreeSurfer-defined cortical and subcortical ROIs, averaged across both hemispheres. Models were adjusted for age, sex, APOE ε4 carrier status, their interactions with time, and included random intercepts and slopes.

Last, we used moderated mediation analysis ("mediation" package) to examine whether tau accumulation mediated the interactive effects of FHS-CVD and Aβ on PACC5 decline. Individual PACC5 slopes were extracted from unadjusted linear mixed effects model. We conducted moderated mediation analysis with PACC5 slope as outcome, ITC tau slope as mediator, baseline Aβ burden as moderator, and adjusted for age, sex, APOE ε4 carrier status, education (years), and time interval between baseline Aβ and first tau scan. Mediation models were run at both low and high levels of baseline Aβ burden, defined respectively by the mean Aβ burden of amyloid-negative (PiB PVC-DVR = 1.17) and amyloid-positive (PiB PVC-DVR = 1.85) participants, dichotomized using the conventional amyloid-positivity threshold (PiB PVC-DVR of 1.32 in HABS).

Results

The baseline characteristics of the participants and lengths of follow-up are summarized in Table 1. The individual trajectories for ITC tau burden are shown in Figure 1. To more clearly visualize the individual tau trajectories, the data were plotted according to baseline levels of Aβ burden (divided by median split) and FHS-CVD scores (divided by tertiles).

There was no significant association between baseline FHS-CVD scores and Aβ burden, adjusting for age and sex ($r_{partial} = -0.0013$, $p = 0.99$; $r = 0.098$, $p = 0.20$ without age/sex adjustment). The primary goal of the study was to investigate whether baseline FHS-CVD and Aβ have an interactive effect on longitudinal ITC tau burden. We found a significant interaction between higher baseline FHS-CVD and elevated Aβ in association with greater tau burden over time ($\beta = 0.05$ [0.02–0.09], $t = 2.88$, $p = 0.004$; Fig 2A, Table 2). This interaction remained significant in sensitivity analyses using the lipid profile-based FHS-CVD ($\beta = 0.07$ [0.03–0.12], $t = 3.54$, $p < 0.001$) and ASCVD risk scores ($\beta = 0.06$ [0.01–0.10], $t = 2.64$, $p = 0.009$). Similarly, the interaction remained significant using Aβ and tau PET data without PVC ($\beta = 0.002$ [0.0005–0.004], $t = 2.49$, $p = 0.01$).

To explore whether certain vascular risk factors were driving this interaction with Aβ on longitudinal tau, we conducted post hoc analyses to investigate the interactive effects between individual component measures of FHS-CVD and Aβ on longitudinal ITC tau. Of the 6 FHS-CVD components, only SBP (adjusted for antihypertensive use) and BMI showed significant interactions with Aβ on ITC tau burden over time ($p < 0.008$ for significance for FWE correction), with higher SBP and BMI predicting greater longitudinal ITC tau in setting of elevated baseline Aβ (Table 3; Fig 2B, C). Importantly, there was no significant correlation between baseline SBP and BMI in our sample ($r = 0.09$, $p = 0.23$). We further included SBP and BMI in the same linear mixed effects model to test whether their interactions with Aβ and time have independent effects on ITC tau accumulation. Interactions with both measures remained significant, with effect sizes similar to results from their individual models (SBP*Aβ*Time: $t = 5.28$, $p < 0.001$; BMI*Aβ*Time: $t = 5.08$, $p = < 0.001$). Whereas higher age and having diabetes showed no significant interactive or independent

TABLE 1. Participant Characteristics

Characteristic	All participants, N = 175
Age at baseline, yr, mean (SD)	70.5 (8.0)
Females, n (%)	110 (62.9)
White race, n (%)	150 (85.7)
Education, yr, mean (SD)	16.2 (2.9)
APOE ε4 carriers, n (%)	52 (29.7)
Baseline FHS-CVD risk score, mean (SD)	27.3 (18.5)
Baseline systolic blood pressure, mean (SD), mmHg	137.1 (18.7)
Baseline body mass index, mean (SD)	26.8 (4.4)
Positive diabetes status at baseline, n (%)	13 (7.4)
Positive smoking status at baseline, n (%)	7 (4.0)
Baseline PiB PET PVC-DVR in FLR regions, mean (SD)	1.33 (0.4)
β-Amyloid positive, n (%)	43 (24.6)
Time interval between baseline and first tau PET scan, mean (SD), yr	2.1 (1.5)
Time interval between baseline and last tau PET scan, mean (SD), yr	5.6 (2.1)
First ITC tau PET SUVR, mean (SD)	1.45 (0.2)
# Longitudinal tau PET scans, mean (SD)	2.4 (0.6)
Duration of tau PET follow-up, mean (SD), yr	3.6 (1.5)
# Longitudinal PACC5 assessments, mean (SD)	7.9 (2.5)
Duration of cognitive follow-up, mean (SD), yr	7.0 (2.0)

APOE ε4 = apolipoprotein E ε4 allele; DVR = distribution volume ratio; FHS-CVD = Framingham Heart Study cardiovascular disease risk score; FLR = frontal, lateral temporal and parietal, and retrosplenial regional uptake; ITC = inferior temporal cortex; mm Hg = millimeters of mercury; PACC5 = Preclinical Alzheimer's Cognitive Composite--5; PET = positron emission tomography; PiB = Pittsburgh Compound--B; PVC = partial volume correction; SUVR = standardized uptake value ratio.

effects on ITC tau burden over time, both were associated with greater baseline ITC tau burden (age: t = 3.769, $p < 0.001$; diabetes: t = 2.656, $p = 0.009$).

We next conducted floodlight analysis to determine the level of baseline Aβ burden at which interactions with FHS-CVD on ITC tau accumulation became significant ($p < 0.05$). Results revealed that the FHS-CVD effects on longitudinal ITC tau became significant when baseline Aβ exceeded PiB PVC-DVR of 1.23 (corresponding to 11.8 Centiloids[23] in the HABS cohort), which is substantially lower than the conventional threshold for amyloid positivity in this sample (PiB PVC-DVR >1.32 or 18.2 Centiloids).

Additionally, we explored whether the interactive effects between FHS-CVD and Aβ on longitudinal tau burden are limited to the ITC by performing exploratory whole brain analyses using FreeSurfer-based ROIs. Results revealed additional regions with nominally significant synergistic interactions, predominantly in medial temporal and other areas implicated in early neocortical tau spread: amygdala (t = 3.13, $p = 0.002$), fusiform (t = 2.81, $p = 0.005$), middle temporal (t = 2.31, p = 0.02), supramarginal (t = 2.72, $p = 0.007$), and pars orbitalis (t = 2.48, $p = 0.01$; Fig 3).

Last, we determined whether ITC tau accumulation may mediate the synergistic association between FHS-CVD and Aβ burden with cognitive decline. Similar to prior work from HABS,[7] we observed a significant FHS-CVD*Aβ*Time interaction on longitudinal PACC5 decline (β = −0.04 [−0.06 to −0.02], t = −3.48, $p < 0.001$). Then, using moderated mediation analysis, in models assuming high baseline Aβ burden (PiB PVC-DVR = 1.85), the slopes of ITC tau change partially mediated the effects of higher FHS-CVD on faster PACC5 decline (mediated effects: β = −0.527 [−0.86 to −0.25], $p < 0.001$), accounting for 33% of the total effects (Fig 4). In contrast, in the setting of low baseline Aβ (PiB PVC-DVR = 1.17), whereas FHS-CVD was associated with PACC5 decline (β = −0.50, $p = 0.03$), there was no significant mediation of this effect through ITC tau slopes (β = −0.15, $p = 0.11$).

Discussion

In a well-characterized cohort of 175 older CU adults with longitudinal tau PET imaging and cognitive follow-up, we observed that higher baseline vascular risk was synergistic with elevated Aβ in predicting greater longitudinal tau burden in the ITC, an area of early neocortical tau spread associated with imminent clinical impairment.[24] This interaction was significant at a baseline Aβ level considerably lower than the conventional threshold for amyloid positivity, consistent with growing literature on the deleterious effects of even modestly elevated Aβ.[25,26] Additionally, we examined individual components of FHS-CVD

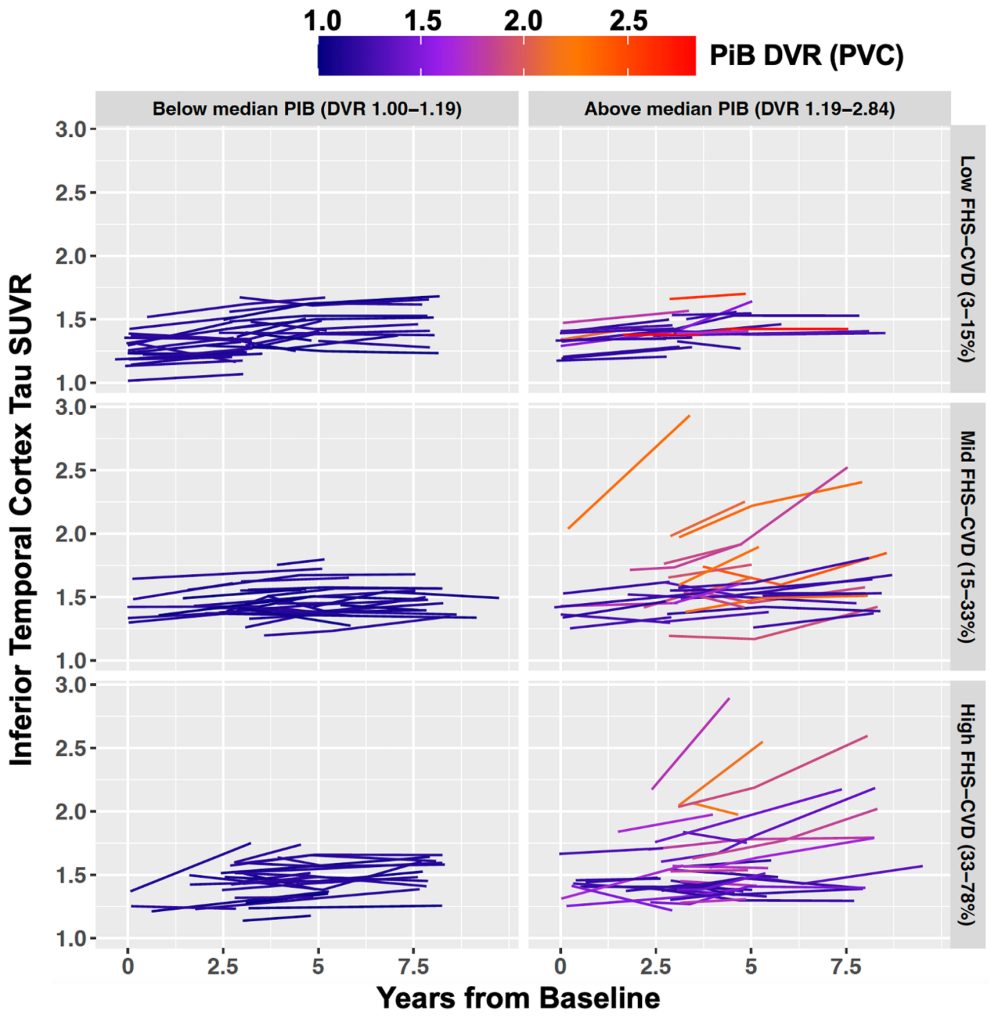

FIGURE 1: Accumulation of tau pathology as a function of baseline Aβ burden and level of systemic vascular risk. Individual ITC tau burden trajectories from all participants are shown. To allow clear visualization of individual tau trajectories, participants were divided according to baseline Aβ burden (by median split) and the FHS-CVD (by tertiles) using cutoffs shown in the facet labels for the columns and rows, respectively. The trajectories are color coded by baseline Aβ burden according to the color bar, with each line representing one participant. The time of baseline PiB PET was used as the study baseline (time = 0). Timing of the first tau PET scan varied across participants (2.1 ± 1.5 years), as tau PET was introduced mid-study in HABS. Aβ = β-amyloid; FHS-CVD = Framingham Heart Study cardiovascular disease risk score; DVR = distribution volume ratio; HABS = Harvard Aging Brain Study; ITC = inferior temporal cortex; PVC = partial volume correction; PET = positron emission tomography; PiB = Pittsburgh Compound--B; SUVR = standardized uptake value ratio.

risk score and observed that elevated SBP and BMI were significant and independent drivers of this interaction. Last, using moderated mediation analysis, we demonstrated that longitudinal tau accumulation significantly mediated one third of the synergistic effects between baseline vascular risk and Aβ on prospective cognitive decline. Together, our findings suggest that accelerating early neocortical tau accumulation may be one mechanism by which vascular risk interacts with even relatively low levels of Aβ to promote cognitive decline in preclinical AD. This provides strong support for targeting vascular risk reduction as a prevention/treatment strategy to modify the trajectory of AD, and the importance of considering vascular risk in the design, execution, and analysis of data from AD clinical trials.

The current results are consistent with previous findings from HABS demonstrating a significant interaction between baseline FHS-CVD score and Aβ burden on cross-sectional ITC tau.[10] The results here further elucidated the temporal ordering of these associations using longitudinal tau PET spanning up to 9.7 years from study baseline. Similar to prior work from HABS, we did not observe an association between baseline Aβ burden and FHS-CVD scores,[7,10] consistent with prior work demonstrating late-life Aβ burden was associated with mid-life but not late-life vascular risk factors.[27] The synergistic effect of vascular risk and Aβ burden on longitudinal tau was robust and remained significant across sensitivity analyses using the lipid profile-based FHS-CVD[15] and

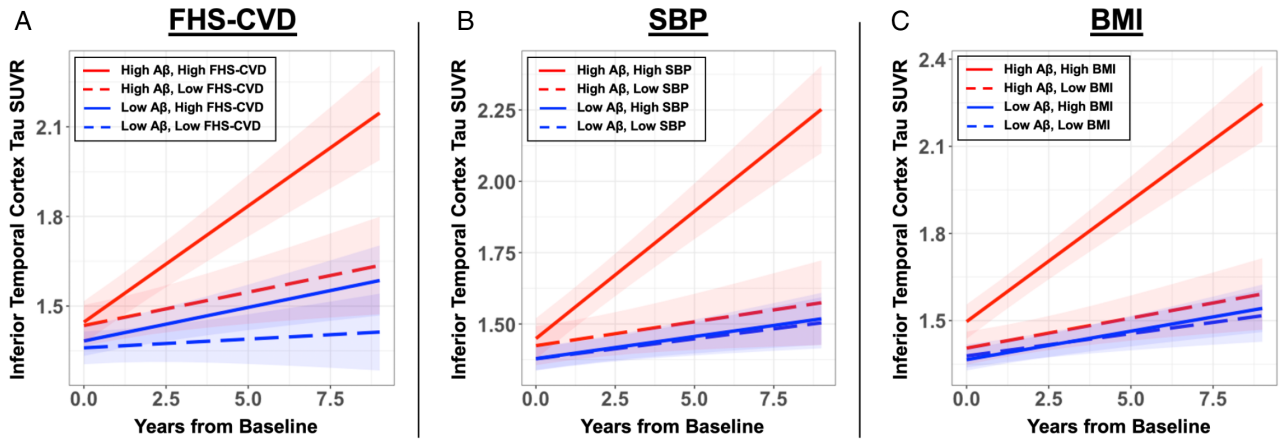

FIGURE 2: Elevated baseline cardiovascular risks and Aβ are synergistically associated with accelerated inferior temporal cortex tau accumulation. The FHS-CVD and its component vascular risk factors were modelled separately in linear mixed effects models. There was significant vascular risk*Aβ*time interaction on longitudinal ITC tau burden for (A) FHS-CVD score (β = 0.05 [0.02–0.09], t = 2.88, $p = 0.004$), (B) SBP (β = 0.09 [0.06–0.12], t = 5.37, $p < 0.001$), and (C) BMI (β = 0.08 [0.05–0.11], t = 5.43, $p < 0.001$). To visualize the model results, estimated ITC tau trajectory based on different levels of baseline vascular risk and Aβ are presented. Low and high vascular risk are represented by −1 SD and +1 SD from mean baseline values: FHS-CVD low 8.8%, high 45.8%; SBP low 118.5 mmHg, high 155.8 mmHg; BMI low 22.4, high 31.2. Low and high Aβ levels are represented by the mean partial volume corrected Pittsburgh Compound-B distribution volume ratio (PiB PVC-DVR) of amyloid-negative (1.17) and amyloid-positive (1.85) participants, respectively, dichotomized using the conventional amyloid-positivity threshold (PiB PVC-DVR of 1.32 in our cohort). Aβ = β-amyloid; BMI = body mass index; FHS-CVD = Framingham Heart Study cardiovascular disease risk score; ITC = inferior temporal cortex; SBP = systolic blood pressure; SUVR = standardized uptake value ratio.

TABLE 2. Summary of Linear Mixed Effects Model Examining the Interactive Effects of Baseline Vascular Risk and Amyloid Burden on Longitudinal Inferior Temporal Tau Burden

Model: ITC tau ~ FHS-CVD*Aβ*Time + Age*Time + Sex*Time + APOE ε4*Time

	β Estimate (95% CI)	Standard Error	*t* Value	*p*
FHS-CVD*Aβ*Time	0.05 [0.2 to 0.9]	0.02	2.88	0.004
FHS-CVD*Aβ	−0.02 [−0.16 to 1.32]	0.07	−0.21	0.84
FHS-CVD*Time	0.07 [0.02 to 0.11]	0.02	2.77	0.01
Aβ*Time	0.10 [0.06 to 0.12]	0.02	5.78	<0.001
FHS-CVD	0.05 [−0.14 to 0.25]	0.10	0.52	0.60
Aβ	0.18 [0.04 to 0.32]	0.07	2.59	0.01
Age*Time	−0.02 [−0.06 to 0.02]	0.02	−1.08	0.28
Sex (F)*Time	0.12 [0.04 to 0.20]	0.04	2.94	0.004
APOE (ε4+)*Time	0.05 [−0.01 to 0.12]	0.04	1.56	0.12
Age	0.24 [0.08 to 0.40]	0.08	2.95	0.004
Sex (F)	0.02 [−0.30 to 0.34]	0.16	0.14	0.89
APOE (ε4+)	−0.06 [−0.34 to 0.22]	0.14	−0.41	0.68
Time	0.03 [−0.02 to 0.09]	0.03	1.15	0.25

All continuous variables were z-transformed prior to model entry.
Aβ = amyloid-beta; APOE ε4 = apolipoprotein E ε4 allele; CI = confidence interval; F= female; FHS-CVD = Framingham Heart Study cardiovascular disease risk score; ITC = inferior temporal cortex.

TABLE 3. Summary of Linear Mixed Effects Models Examining the Interactive Effects of Baseline Framingham Heart Study Cardiovascular Disease Risk Score Components and Amyloid Burden on Longitudinal Inferior Temporal Tau Burden

	β Estimate (95% CI)	Standard Error	*t* Value	*p*
Age*Aβ*Time	0.02 [−0.02 to 0.06]	0.02	1.01	0.31
Sex (F)*Aβ*Time	0.02 [−0.05 to 0.08]	0.03	0.47	0.64
SBP*Aβ*Time	0.09 [0.06 to 0.12]	0.02	5.37	<0.001[a]
BMI*Aβ*Time	0.08 [0.05 to 0.11]	0.01	5.43	<0.001[a]
Smoking*Aβ*Time	−0.03 [−0.19 to 0.12]	0.08	−0.40	0.69
Diabetes*Aβ*Time	0.10 [−0.07 to 0.26]	0.09	1.14	0.25

Individual vascular risk factors that make up the FHS-CVD score were examined separately for interactive effects with Aβ on ITC tau burden across time. Each vascular risk factor (age, sex, SBP, BMI, smoking, or diabetes) was included in a separate linear mixed effects model and the model results for each vascular risk factor's interaction with Aβ and time are shown. Models are specified as follows for each vascular risk factor: ITC tau ~ Vascular Risk Factor*Aβ*Time + Age*Time + Sex*Time + APOE ε4*Time. The model for SBP was also adjusted for antihypertensive medication use and its interaction with time

[a]Significant at FWE-corrected threshold of <0.0083.

Aβ = β-amyloid; APOE ε4 = apolipoprotein E ε4 allele; BMI = body mass index; CI = confidence interval; FHS-CVD = Framingham Heart Study cardiovascular disease risk; FWE = family-wise error; ITC = inferior temporal cortex; SBP = systolic blood pressure.

ASCVD[16] risk scores. To our knowledge, this is the first study to examine the synergistic impact of systemic vascular risk and Aβ burden on longitudinal tau PET in CU older adults. Our results are consistent with a study of longitudinal CSF tau burden in older CU adults,[28] which showed higher FHS-CVD scores were associated with greater increase in CSF tau only in those with baseline CSF Aβ and tau pathology, supporting a synergistic interaction between vascular risk and Aβ on longitudinal tau. In contrast, another study of late mid-life CU adults did not find associations between vascular risk and rate of change in CSF tau.[29] Several methodological differences may explain the discordant findings. First, a potential moderating effect of Aβ was not explored in that study. Additionally, vascular risk was assessed as a summed composite score of the presence or history (versus absence) of 5 vascular risk factors. This resulted in a limited score range in the study sample (0 to 3), which was then dichotomized prior to analysis. The greater dynamic range of the FHS-CVD scores in our current study and its use as a continuous variable may have enabled greater power to detect significant associations.

Growing evidence indicates that conventional approaches to categorizing individuals as "amyloid positive" or "amyloid negative" may underestimate the deleterious effects of subthreshold levels of Aβ on brain health, tau accumulation, and cognitive decline.[25,26] Using floodlight analysis, we demonstrated that the FHS-CVD by Aβ

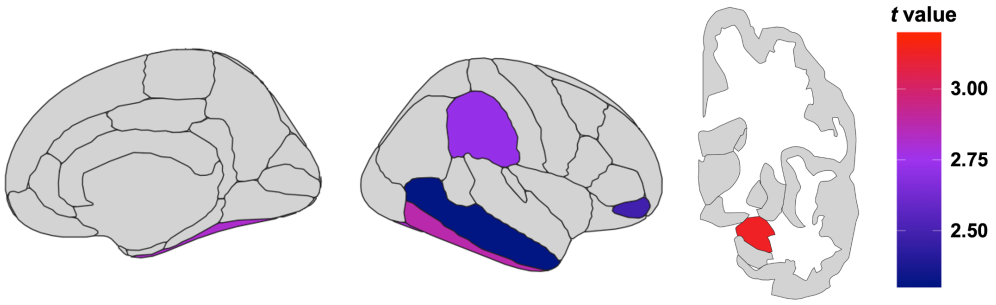

FIGURE 3: Greater systemic vascular risk and baseline Aβ burden are synergistically associated with greater tau accumulation in multiple FreeSurfer-defined regions of interest. We performed an exploratory analysis to assess whether regions beyond the inferior temporal cortex showed greater tau accumulation in the setting of greater vascular risk, as measured by the FHS-CVD, and Aβ burden. Linear mixed effects models revealed significant FHS-CVD*Aβ*time interactions on longitudinal tau burden in the amygdala (t = 3.13, *p* = 0.002), fusiform (t = 2.81, *p* = 0.005), middle temporal (t = 2.31, *p* = 0.02), inferior temporal (t = 2.88, *p* = 0.004), supramarginal (t = 2.72, *p* = 0.007), and pars orbitalis (t = 2.48, *p* = 0.01) regions. Aβ = β-amyloid; FHS-CVD = Framingham Heart Study cardiovascular disease risk score.

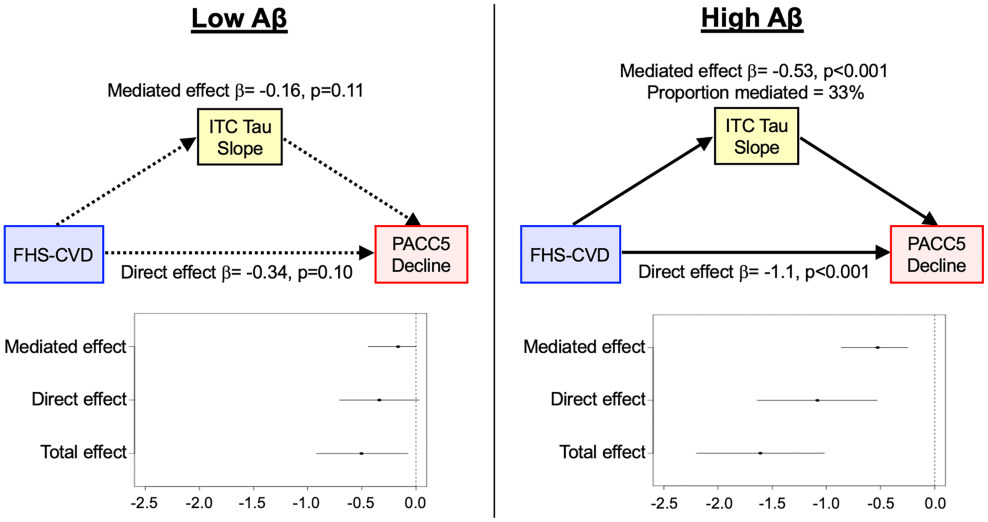

FIGURE 4: Inferior temporal tau accumulation partially mediates elevated systemic vascular risk effects on prospective cognitive decline in individuals with high baseline amyloid burden. Individual ITC tau and PACC5 slopes were extracted from linear mixed effects models for moderated mediation analysis. We modeled FHS-CVD as predictor, ITC tau slope as mediator, and PACC5 decline as outcome, adjusting for age, sex, *APOE* ε4 status, years of education, and time between study baseline and first tau PET. High and low levels of Aβ burden were represented by the mean partial volume corrected Pittsburgh Compound-B distribution volume ratio (PiB PVC-DVR) of amyloid-positive (1.85) and amyloid-negative (1.17) participants, respectively, dichotomized using the conventional amyloid-positivity threshold (PiB PVC-DVR of 1.32 in the HABS cohort). Results indicate partial mediation of FHS-CVD effects on cognitive decline by ITC tau slope in individuals with elevated baseline Aβ burden (right panel; β = −0.53 [−0.86 to −0.25], $p < 0.001$), accounting for 33% of the total effects. No statistically significant mediation was observed in the setting of low baseline Aβ burden (left panel; β = −0.16 [−0.38 to 0.03], $p = 0.11$), though greater baseline FHS-CVD alone continued to be associated with decreasing cognitive performance (total effects: β = −0.50, $p = 0.03$). Aβ = β-amyloid; FHS-CVD = Framingham Heart Study cardiovascular disease risk score; HABS = Harvard Aging Brain Study; ITC = inferior temporal cortex; PACC5 = Preclinical Alzheimer's Cognitive Composite-5; PET = positron emission tomography.

interaction on tau accumulation was significant at an Aβ burden of PiB PVC-DVR >1.23 (corresponding to 11.8 Centiloids), which is substantially lower than the conventional amyloid positivity threshold of 1.32 (18.2 Centiloids) in HABS. This result reinforces the importance of considering subthreshold levels of Aβ in preclinical AD,[25,26] and strongly supports the use of Aβ burden as a continuous variable in analyses, rather than dichotomizing into amyloid positive versus negative subgroups using conventional cutoff points.

To better understand the mechanisms through which vascular risk may interact with Aβ to accelerate tau accumulation, we examined individual components of the FHS-CVD score and demonstrated that only SBP and BMI showed significant interactions with Aβ in predicting greater longitudinal ITC tau. This is consistent with evidence linking hypertension and obesity, particularly during midlife, with increased risk of cognitive impairment and dementia, including AD.[30–32] A study of mid- to early late-life CU adults showed that hypertension and obesity status were both synergistic with elevated Aβ in predicting faster decline in memory performance.[33] In addition, clinical-pathological studies have linked midlife obesity and mid- and late-life hypertension with increased neurofibrillary tangle pathology,[34–36] consistent with our findings that these vascular risk factors can moderate AD trajectory through tau. Importantly, our results showed that when included in the same model, the effects of both SBP and BMI remained significant, with essentially unchanged effect sizes. These findings suggest that hypertension and elevated BMI may accelerate tau accumulation independently and through different mechanisms, and support strategies to target these vascular risk factors in AD prevention trials, both individually and in combination.

Mechanistically, Aβ is known to be vasoactive, and therefore may be synergistic with hypertension in promoting vascular dysfunction, as both have been linked to endothelial dysfunction, oxidative stress, neuroinflammation, impaired neuro-vascular coupling, and reduced cerebral blood flow.[37–41] In particular, hypoperfusion has been shown to promote tau phosphorylation and aggregation/accumulation in animal studies,[42] and has been associated with increased cross-sectional tau PET burden in the temporal lobe.[43,44] Obesity, on the other hand, is associated with chronic systemic inflammation, which may act synergistically with Aβ on microglial priming and activation,[45,46] which, in turn, has been shown to promote tau phosphorylation and accumulation.[47] However, more studies are required to explore this hypothesis,

as growing evidence indicates that the relationship between inflammation and AD pathogenesis may be complex and may depend on the disease stage and the specific inflammatory markers examined.[48,49]

We observed that longitudinal ITC tau change mediated 33% of the FHS-CVD effect on cognitive decline in individuals with elevated Aβ burden. In contrast, in the setting of low baseline Aβ, whereas FHS-CVD was related to cognitive decline, there was no significant mediation by tau change. This is likely due to the relatively low levels of tau in individuals with low levels of Aβ burden. Together, our findings highlight early neocortical tau as one effector mechanism through which Aβ and vascular risk exert their synergistic effects in accelerating cognitive decline. However, given that two thirds of the total FHS-CVD effect was not mediated through ITC tau, future studies investigating additional mechanisms (eg, reduced cerebral blood blow, white matter injury, and cerebral atrophy) are needed to better understand this clinically significant interaction.

The current study has several limitations and the results should be interpreted in context of the cohort studied. HABS excluded participants with baseline uncontrolled hypertension, diabetes, symptomatic stroke, or intracranial hemorrhage. Therefore, individuals with the highest vascular risks are likely under-represented in the current study. In particular, only 7.4% of our participants had diabetes, a risk factor for cognitive impairment and dementia, including AD,[50] which likely reduced our power to detect a significant diabetes by Aβ interaction on longitudinal tau. In addition, the HABS sample is overall highly educated and mostly of non-Hispanic European ancestry, which may also limit the generalizability of our findings to other populations.

In summary, our results suggest that elevated systemic vascular risk, particularly higher SBP and BMI, interacts with even subthreshold levels of Aβ to accelerate ITC tau accumulation, which, in turn, partially mediates the synergistic effect between vascular risk and Aβ on prospective cognitive decline. Together with existing literature, these findings strongly support targeting vascular risk factors, especially the treatment of hypertension and obesity in mid- to early late-life, as a strategy to attenuate Aβ-related tau pathology and potentially modify the trajectory of AD. Additionally, treatment strategies targeting both vascular and Aβ pathologies simultaneously may have the greatest impact on those at highest risk for cognitive decline. Future AD clinical trials should assess for vascular risk, given its modifying effect on tau and cognition. Last, AD trials targeting Aβ and tau mechanisms may want to reconsider excluding participants with significant vascular risks/disease, as this may reduce diversity and decrease the synergy between AD and vascular factors, which may be a critical aspect of modulating tau burden and demonstrating clinical benefit.

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Author Contributions

Conception and design of the study: W.W.Y., R.A.S., and J.P.C. Acquisition and analysis of data: Z.S., H.-S.Y., A.P.I., J.S.R., M.J.P., D.R.K., A.P.S., D.M.R., R.A.S., K.A.J., and J.P.C. Drafting a significant portion of the manuscript or figures: W.W.Y., R.A.S., and J.P.C.

Potential Conflicts of Interests

The authors declare no relevant potential conflicts of interest.

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