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The Role That Microbes May Be Playing in Progressive Brain Disorders

Michael G. Schmidt, Ph.D.

AIOB 23 October 2021

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Do the Bugs From our Mouths Eat Our Memories?

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Disclosures

- *Member of the Scientific Advisory Board of MicroGenDx*
- *Fully vaccinated, 23 February 2021*
- Also unrelated to this talk, I am supported by an Award No.2020-V7-GX-K002 from the Office for Victims of Crime, Office of Justice Programs, US Department of Justice.
- *The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author and do not necessarily reflect the views of the Department of Justice or the Office for Victims of Crime." nor those of my employer, the Medical University of South Carolina*

Learning Objectives

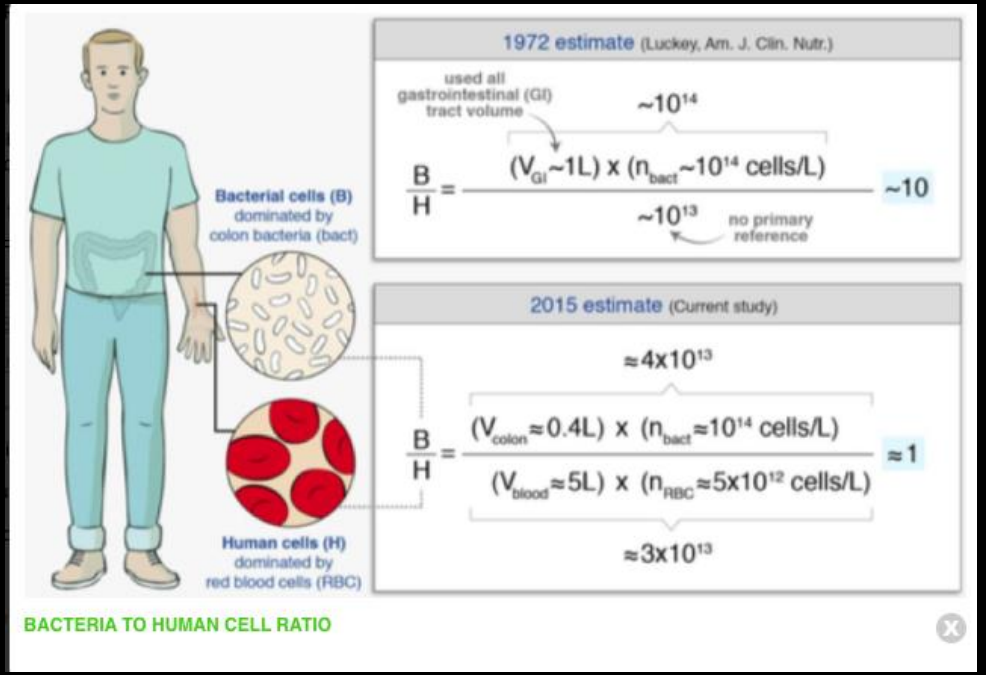
1. Appreciate how perturbations to periodontal health can introduce a member of the host's microbiome to host niche with unintended consequences
2. Evaluate whether periodontal dysbiosis is causally linked to the Alzheimer's Disease pathology
3. Discuss how inhibiting a key virulence factor of a microbe associated with periodontal disease might arrest the development of Alzheimer's Disease Pathology

Review, from yesterday

- The first birthday gift we each received was identical in name and given freely from our mothers
- It was literally delivered precisely at the time of our birth, providing us with an inter-generational hand-off that serves as the foundation for our 13th organ system, our microbiome.
- Today, we will explore how perturbations made to this, our 13th organ system, or ***microbiome***, can profoundly influence our lives.

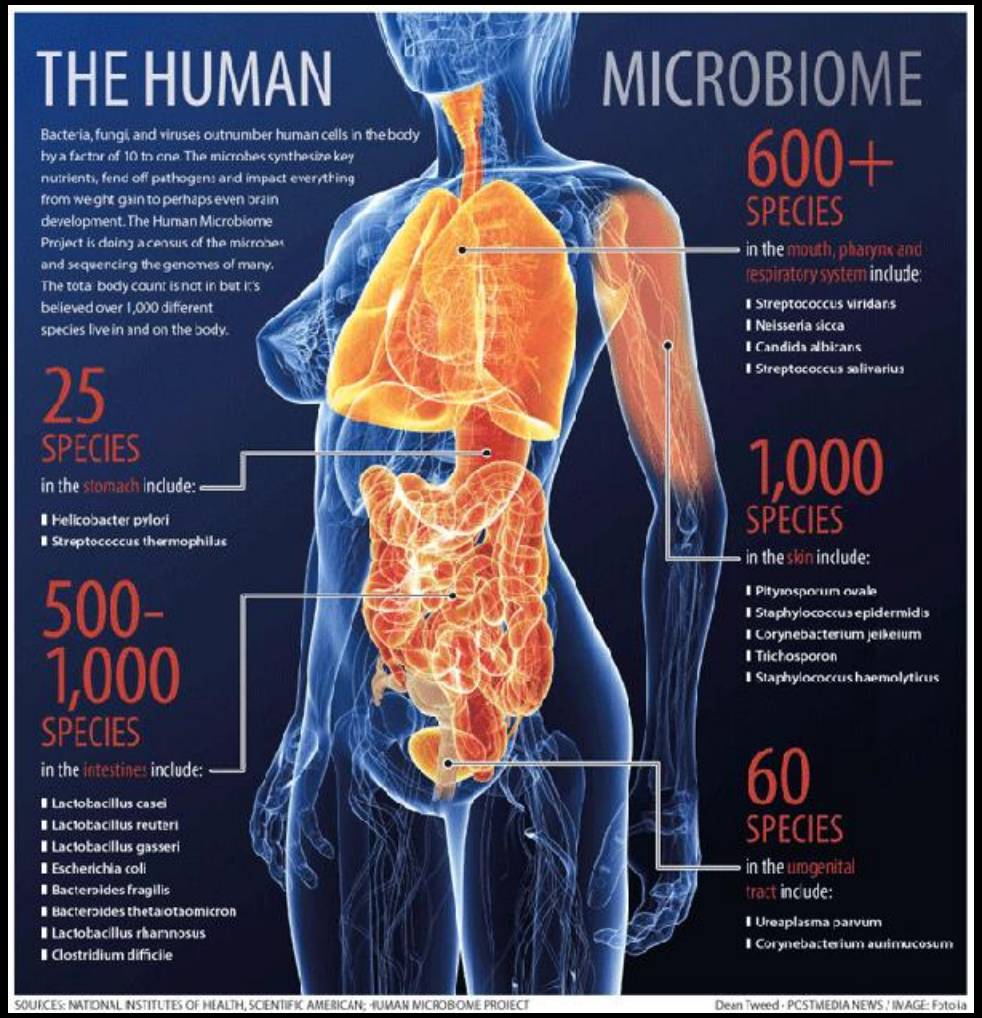
Human Microbiome

- What do we know?
  - You are 1 part human - 1 part bacteria - formerly thought it was 10 parts bacteria::1 part human



Did you know?

- At this moment 2 to 6 pounds of microbes are living in and on you?
  - *Who are they?*
  - *Where are they?*
  - *What do they do?*
  - *Are they good or bad?*
  - *Can they be dragooned for good?*



They are talking among, and with us!

Gut-Microbiota –Brain Axis

It's role in Neurological Diseases

- I'm not an *expert*, I'm an observer and student of our newest organ, the *microbiome, my goal today is to enlist your help in helping healthcare build an approach that will help the human race –*
  - *Understand the disease process and progression so that the medical community can assist us design prevention approaches and treatments that can work in limiting these debilitating diseases*

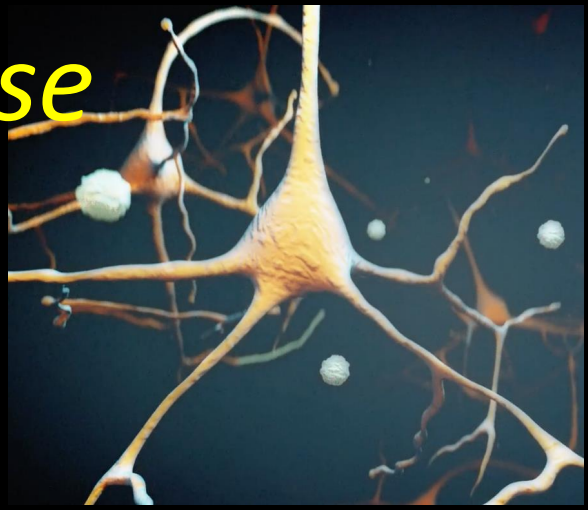
Gut-Microbiota –Brain Axis

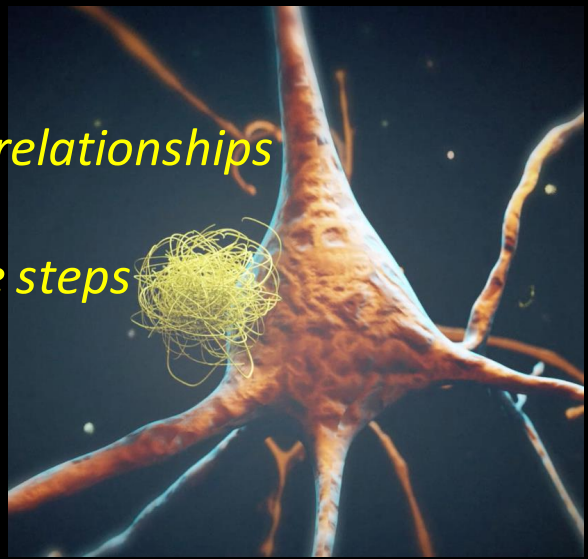
It's role in Neurological Diseases

- We are going to focus on 1 disease
  - Alzheimer's disease (AD)
- *Why only one?*
  - Experiments have helped us build and then test the validity of various hypotheses for the role of microbes
  - Results can inform us of a path forward for how dental medicine may be able to contribute to our understanding and *developing therapeutic strategies.*

Alzheimer's Disease

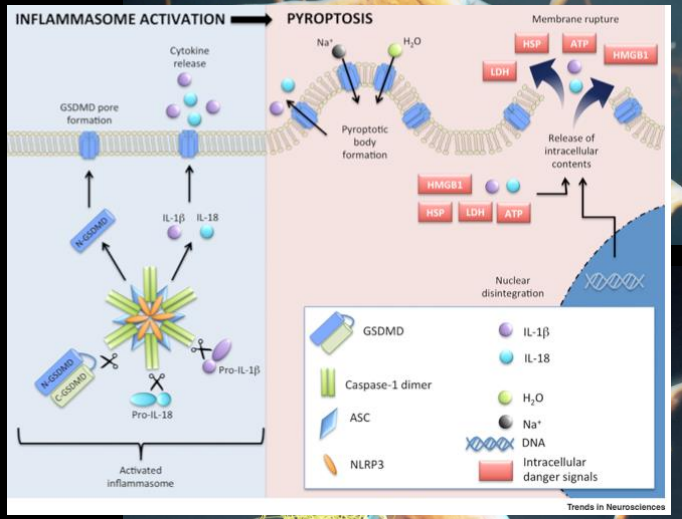
- *Most common form of dementia*
- *IT IS NOT a normal part of aging!*
- *It is a progressive disease*
  1. *Memory loss that disrupts daily life*
  2. *Challenges in planning or solving problems*
  3. *Difficulty completing familiar tasks*
  4. *Confusion with time or place*
  5. *Trouble understanding visual images and spatial relationships*
  6. *New problems with words in speaking or writing*
  7. *Misplacing things and losing the ability to retrace steps*
  8. *Decreased or poor judgement*
  9. *Withdrawal from work or social activities*
  10. *Changes in mood and personality*





Alzheimer's Disease-2

- The pathologists will offer that pathology of *Alzheimer's is very similar to the host's response to a pathogen*
  - *Chronic low-grade inflammation (TNF-alpha)*
  - *Complement activation and dysregulation*
  - *Inflammasome activation*
    - Results in secretion of inflammatory cytokines (IL-1ß and IL-18) and cell death via *pyroptosis (firery cell death -Caspase1-Activation; generally, in macrophages likely triggered by an infection)*



How I got interested?

- ***Porphyromonas gingivalis* in Alzheimer's disease brains: *Evidence for disease causation and treatment with small-molecule inhibitors***
- ***Published 23 January 2019***
- ***BLUF-* microbe implicated in chronic periodontitis appears to be a player in Alzheimer's pathology…**
  - say what? … *flossing may prevent Alzheimer's after we heard that flossing was useless*
  - an oral, a black-pigmented, ***assaccharolytic***, non-motile Gram-negative microbe that requires anaerobic conditions for growth,

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

***Porphyromonas gingivalis* in Alzheimer's disease b… Evidence for disease causation and treatment with small-molecule inhibitors**

Stephen S. Dominy[1*†], Casey Lynch[1*], Florian Ermini[1], Malgorzata Benedyk[2,3], Agata Marczyk[2], Andrei Konradi[1], Mai Nguyen[1], Ursula Haditsch[1], Debasish Raha[1], Christina Griffin[1], Leslie J. Holsinger[1], Shirin Arastu-Kapur[1], Samer Kaba[1], Alexander Lee[1], Mark I. Ryder[4], Barbara Potempa[5], Piotr Mydel[2,6], Annelie Hellvard[3,6], Karina Adamowicz[2], Hatice Hasturk[7,8], Glenn D. Walker[9], Eric C. Reynolds[9], Richard L. M. Faull[10], Maurice A. Curtis[11,12], Mike Dragunow[11,13], Jan Potempa[2,5*]

Porphyromonas gingivalis, the keystone pathogen in chronic periodontitis, was identified in the brain of Alzheimer's disease patients. Toxic proteases from the bacterium called gingipains were also identified in the brain of Alzheimer's patients, and levels correlated with tau and ubiquitin pathology. Oral *P. gingivalis* infection in mice resulted in brain colonization and increased production of $A\beta_{1-42}$, a component of amyloid plaques. Further, gingipains were neurotoxic in vivo and in vitro, exerting detrimental effects on tau, a protein needed for normal neuronal function. To block this neurotoxicity, we designed and synthesized small-molecule inhibitors targeting gingipains. Gingipain inhibition reduced the bacterial load of an established *P. gingivalis* brain infection, blocked $A\beta_{1-42}$ production, reduced neuroinflammation, and rescued neurons in the hippocampus. These data suggest that gingipain inhibitors could be valuable for treating *P. gingivalis* brain colonization and neurodegeneration in Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) patients exhibit neuroinflammation consistent with infection, including microglial activation, inflammasome activation, complement activation, and altered cytokine profiles (*1*, *2*). Infectious agents have been found in the brain and postulated to be involved with AD, but robust evidence of causation has not been established (*3*). The recent characterization of amyloid-β (Aβ) as an antimicrobial peptide has renewed interest in identifying a possible infectious cause of AD (*4*–*6*).

Chronic periodontitis (CP) and infection with *Porphyromonas gingivalis*—a keystone pathogen in the development of CP (*7*)—have been identified as significant risk factors for developing Aβ plaques, dementia, and AD (*8*–*12*). A prospective observational study of AD patients with active CP reported a notable decline in cognition (Alzheimer's Disease Assessment Scale—Cognitive and Mini Mental State Examination scales) over a 6-month period compared to AD patients without active CP, raising questions about possible mechanisms underlying these findings (*13*). In $Apoe^{-/-}$ mice, oral infection with *P. gingivalis*, but not with two other oral bacteria, results in brain infection and activation of the complement pathway (*14*). In transgenic mice overexpressing mutated human amyloid precursor protein (hAPP-J20), oral infection with *P. gingivalis* impairs cognitive function, increases the deposition of AD-like plaques, and results in alveolar bone loss compared to control hAPP-J20 mice (*15*). *P. gingivalis* lipopolysaccharide has been detected in human AD brains (*16*), promoting the hypothesis that *P. gingivalis* infection of the brain plays a role in AD pathogenesis (*17*).

P. gingivalis is mainly found during gingival and periodontal infections; however, it can also be found at low levels in 25% of healthy individuals with no oral disease (*18*). Transient bacteremia of *P. gingivalis* can occur during common activities such as brushing, flossing, and chewing, as well as during dental procedures (*19*), resulting in documented translocation to a variety of tissues including coronary arteries (*20*), placenta (*21*), and liver (*22*). A recent study found that 100% of patients with cardiovascular disease had *P. gingivalis* arterial colonization (*23*).

P. gingivalis is an asaccharolytic Gram-negative anaerobic bacterium that produces major virulence factors known as gingipains, which are cysteine proteases consisting of lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). Gingipains are secreted, transported to outer bacterial membrane surfaces, and partially released into the extracellular milieu in soluble and outer membrane vesicle (OMV)–associated forms (*24*, *25*). Kgp and RgpA/B are essential for *P. gingivalis* survival and pathogenicity, playing critical roles in host colonization, inactivation of host defenses, iron and nutrient acquisition, and tissue destruction (*24*, *26*). Gingipains have been shown to mediate the toxicity of *P. gingivalis*

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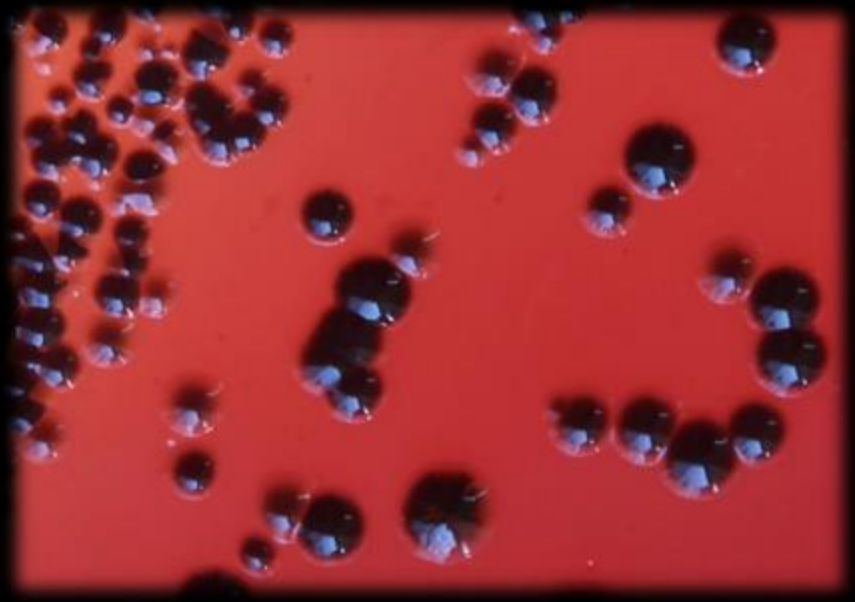
What do we know?

- Pigmentation results from accumulation of Fe-protophorphyrin IX (FePPIX) from erythrocytic hemoglobin
- Lys-X (Lys-gingipain) and Arg-X (Arg-gingipain) cysteine proteases of *P. gingivalis* bind and degrade erythrocytes.
- Blocking gingipain proteolytic activity with short peptide analogs reduces *P. gingivalis* virulence
- See - https://journals.asm.org/doi/epub/10.1128/JB.181.16.4905-4913.1999

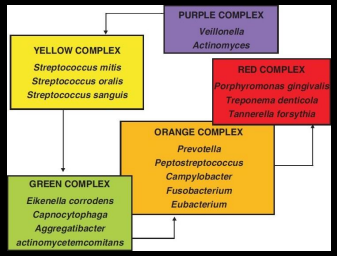


FePPIX on the cell surface producing a characteristic black pigment after about 7 days of anaerobic incubation

What do we know-2?

- ***Microbial Dysbiosis***
  - ***PG has at least 12 virulence factors, like proteases, that allow it to thrive in a polymicrobial community that fosters inflammation through its secretion of virulence factors that subvert leukocytes.***
  - ***Enter the dentist… as our gums recede… this microbe invades and evades***
    - ***The back story… or how to design an experiment that demonstrates causality***

Literature suggests Periodontal Disease bacteria increase risk for AD disease



- ***2007-Tooth loss, dementia and neuropathology in the Nun Study***
- ***Conclusion*:** Participants with the fewest teeth had the highest risk of prevalence and incidence of dementia.
- ***Clinical implications*:** Edentulism or very few (one to nine) teeth may be predictors of dementia late in life.

COVER STORY

Tooth loss, dementia and neuropathology in the Nun Study

Pamela Sparks Stein, DMD; Mark Desrosiers, PhD; Sara Jean Donegan, SSND, DDS; Juan F. Yepes, DDS, MD, MPH; Richard J. Kryscio, PhD

Many cross-sectional studies[1-14] and some longitudinal studies[15,16] have shown that patients with dementia are more likely to have poor oral health. Few investigators,[17-19] however, have attempted to relate oral disease to the subsequent risk of developing cognitive impairments and dementia. Such an association is biologically plausible.[20] Potential mechanisms include inflammatory mediators produced in response to periodontal pathogens,[21-28] which produce chronic systemic inflammation and neuropathology; increased risk of stroke and cerebrovascular injury in those with periodontal disease[29-34]; and dissemination of oral gram-negative bacteria to the brain[35-46] via a transient bacteremia. Oral bacteria also may spread to the brain via neuronal pathways. Riviere and colleagues[47] suggested that oral bacteria may use branches of the trigeminal nerve to reach the brain. In their postmortem examination of brain tissues, they detected antigens of oral treponemes more often in samples from subjects with Alzheimer disease (14 of 16) than in samples from control subjects (four of 18).

ABSTRACT

ARTICLE 1

Background. Numerous studies have linked dementia to the subsequent deterioration of oral health. Few investigators, however, have examined oral disease as a potential risk factor in the development of dementia. The authors conducted a study to investigate a potential association between a history of oral disease and the development of dementia.
Methods. Longitudinal dental records supplemented data collected from 10 annual cognitive assessments of 144 Milwaukee participants in the Nun Study, a longitudinal study of aging and Alzheimer disease, who were 75 to 98 years old. Neuropathologic findings at autopsy were available for 118 participants who died.
Results. A low number of teeth increased the risk of higher prevalence and incidence of dementia.
Conclusion. Participants with the fewest teeth had the highest risk of prevalence and incidence of dementia.
Clinical implications. Edentulism or very few (one to nine) teeth may be predictors of dementia late in life.
Key Words. Epidemiology; periodontal disease; Alzheimer disease.
JADA 2007;138(10):1314-22.

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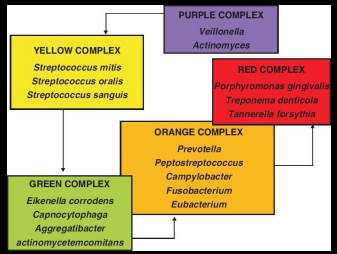
Dr. Kryscio is the director, Biostatistics and Data Management, Alzheimer's Disease Center, Sanders-Brown Center on Aging; the chair, Department of Biostatistics, College of Public Health; and a professor, Department of Statistics, College of Arts and Sciences, University of Kentucky, Lexington.

Literature suggests Periodontal Disease bacteria increase risk for AD disease



- ***2012-Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease***
- **Conclusions**—This study provided initial data that demonstrated elevated antibodies to periodontal disease bacteria *in subjects years prior cognitive impairment*
- Data suggested that periodontal disease could potentially contribute to the risk of AD onset/progression.
- Additional cohort studies profiling oral clinical presentation with systemic response and AD and prospective studies to evaluate any cause-and-effect association was advocated by the authors as warranted.

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Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease

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Abstract

Background—Chronic inflammation in periodontal disease has been suggested as a potential risk factor in Alzheimer's disease. The purpose of this study was to examine serum antibody levels to bacteria of periodontal disease in participants who eventually converted to Alzheimer's disease (AD) compared to the antibody levels in control subjects.

Methods—Serum from 158 participants in the BRAINS (Biologically Resilient Adults in Neurological Studies) research program at the University of Kentucky were analyzed for IgG antibody levels to 7 oral bacteria associated with periodontitis including: *Aggregati-bacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Campylobacter rectus*, *Tre-ponema denticola*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and *Prevotella intermedia*. All 158 participants were cognitively intact at baseline venous blood draw. Eighty one of the participants developed either mild-cognitive impairment (MCI) or Alz-heimer's disease (AD) or both, and 77 controls remained cognitively intact in the years of follow up. Antibody levels were compared between controls and AD subjects at baseline draw and after conversion and controls and MCI subjects at baseline draw and after conversion using the Wilcoxon rank-sum test. AD and MCI participants were not directly compared. Linear regression models were used to adjust for potential confounding.

Results—Antibody levels to *F. nucleatum and P. intermedia*, were significantly increased (α = 0.05) at baseline serum draw in the AD patients compared to controls. These results remained significant when controlling for baseline age, Mini-Mental State Exam (MMSE) score and apolipoprotein epsilon 4 (*APOE e4*) status.

Conclusions—This study provides initial data that demonstrate elevated antibodies to periodontal disease bacteria in subjects years prior cognitive impairment and suggests that periodontal disease could potentially contribute to the risk of AD onset/progression. Additional cohort studies profiling oral clinical presentation with systemic response and AD and prospective studies to evaluate any cause-and-effect association are warranted.

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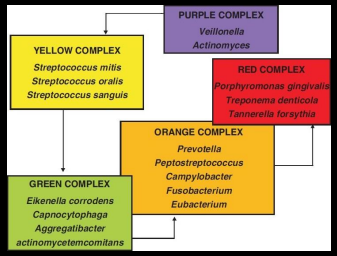
Literature suggests Periodontal Disease bacteria increase risk for AD disease



- **2015-Periodontal disease associates with higher brain amyloid load in normal elderly**
- ***Conclusion*—**Showed for the first time in humans an association between periodontal disease and *brain Aβ* load.
- These data were consistent with prior animal studies showing that peripheral inflammation/infections are sufficient to produce brain Aβ accumulations.

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Periodontal disease associates with higher brain amyloid load in normal elderly

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Abstract

Background—The accumulation of amyloid β plaques (Aβ) is a central feature of Alzheimer's disease (AD). First reported in animal models, it remains uncertain if peripheral inflammatory/infectious conditions in humans can promote Aβ brain accumulation. Periodontal disease, a common chronic infection, has been previously reported to be associated with AD.

Methods—Thirty-eight cognitively normal, healthy, community residing elderly (mean age 61; 68% female) were examined in an Alzheimer's Disease research center and a University-based Dental School. Linear regression models (adjusted for age, ApoE and smoking) were used to test

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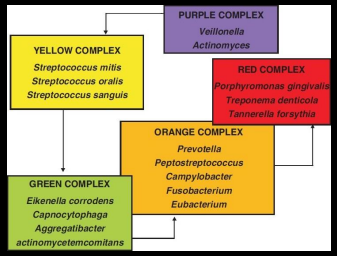
Contributors

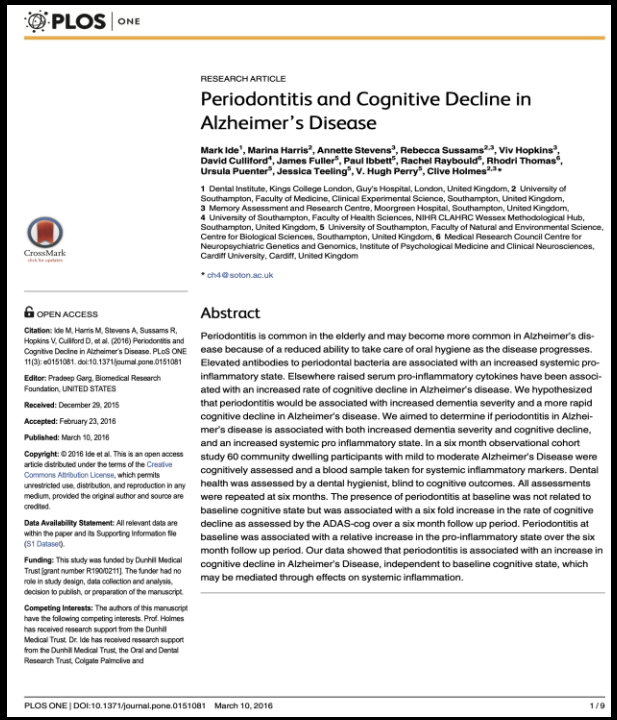
ARK, MJD, RGC, LG and DS designed the study. ARK and EP analyzed the data with assistance from MJD. ARK, MJD and LP interpreted the data. ARK wrote the manuscript with assistance from MJD, LG, RC and LM. PMH and SW performed medical examinations and collected the cognitive data. ARK performed the oral examinations assisted by PC, RL, SS and CS. HR, SV, RL, LM, LY and WT performed image analysis and assisted with data collection and interpretation. All authors reviewed the manuscript for intellectual content and approved the final draft.

Conflict of interest: No conflict of interest is reported for A. Kamer, P. Corby, R. Craig, D. Saxena, H. Rusinek, S. Vallabhajosula, S. Williams, R. Linker, S. Svetco and C. Shi. L. Mosconi, W. Tsui, and M. de Leon have a patent on an image analysis technology that was licensed to Abiant Imaging, Inc, by NYU, and have a financial interest in this license agreement, and NYU holds stock options on the company. Y. Li, L. Mosconi and M. de Leon have received compensation for consulting services from Abiant Imaging. Dr L. Glodzic was a Principal Investigator on an Investigator-Initiated project funded by Forest Laboratories, Inc, and received an honorarium for serving as a consultant to Roche Pharma.

Literature suggests Periodontal Disease bacteria increase risk for AZ disease



- **2016 -Periodontitis and Cognitive Decline in Alzheimer's Disease**
- ***Conclusion***—Data showed that periodontitis is associated with an increase in cognitive decline in Alzheimer's Disease, independent to baseline cognitive state, which may be mediated through effects on *systemic inflammation*



PLOS ONE

RESEARCH ARTICLE

Periodontitis and Cognitive Decline in Alzheimer's Disease

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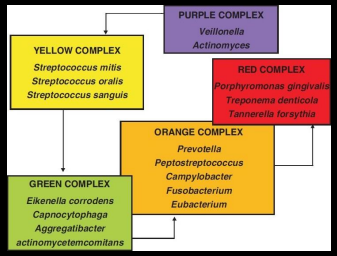
Abstract

Periodontitis is common in the elderly and may become more common in Alzheimer's disease because of a reduced ability to take care of oral hygiene as the disease progresses. Elevated antibodies to periodontal bacteria are associated with an increased systemic pro-inflammatory state. Elsewhere raised serum pro-inflammatory cytokines have been associated with an increased rate of cognitive decline in Alzheimer's disease. We hypothesized that periodontitis would be associated with increased dementia severity and a more rapid cognitive decline in Alzheimer's disease. We aimed to determine if periodontitis in Alzheimer's disease is associated with both increased dementia severity and cognitive decline, and an increased systemic pro inflammatory state. In a six month observational cohort study 60 community dwelling participants with mild to moderate Alzheimer's Disease were cognitively assessed and a blood sample taken for systemic inflammatory markers. Dental health was assessed by a dental hygienist, blind to cognitive outcomes. All assessments were repeated at six months. The presence of periodontitis at baseline was not related to baseline cognitive state but was associated with a six fold increase in the rate of cognitive decline as assessed by the ADAS-cog over a six month follow up period. Periodontitis at baseline was associated with a relative increase in the pro-inflammatory state over the six month follow up period. Our data showed that periodontitis is associated with an increase in cognitive decline in Alzheimer's Disease, independent to baseline cognitive state, which may be mediated through effects on systemic inflammation.

Literature suggests Periodontal Disease bacteria increase risk for AD disease



- **2020-Periodontal Disease and Incident Dementia: The Atherosclerosis Risk in Communities Study (ARIC)**
- ***Objective*** To test the hypothesis that periodontal disease would be associated with increased risk for dementia and mild cognitive impairment (MCI) by assessing dementia/MCI outcomes after a baseline periodontal examination.
- ***Conclusion*** Periodontal disease was modestly associated with incident MCI and dementia in a community-based cohort of black and white participants.

MCI=*mild cognitive impairment*

ARTICLE

Periodontal disease and incident dementia

The Atherosclerosis Risk in Communities Study (ARIC)

Ryan T. Demmer, PhD, Faye L. Norby, MPH, Kamakshi Lakshminarayan, MD, PhD, Keenan A. Walker, PhD, James S. Pankow, PhD, Aaron R. Folsom, MD, Thomas Mosley, PhD, Jim Beck, PhD, and Pamela L. Lutsey, PhD

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Neurology® 2020;95:e1660-e1671. doi:10.1212/WNL.0000000000010312

Abstract

Objective
To test the hypothesis that periodontal disease would be associated with increased risk for dementia and mild cognitive impairment (MCI) by assessing dementia/MCI outcomes after a baseline periodontal examination.

Methods
Participants enrolled in the Atherosclerosis Risk in Communities study with a clinical periodontal examination (or edentulous participants) at visit 4 (1996–1998; mean ± SD age 63 ± 6 years, 55% female, 21% black) and adjudicated dementia outcomes through 2016 were included (n = 8,275). A subgroup of 4,559 participants had adjudicated dementia and MCI assessments at visit 5 (2011–2013). Participants received a full-mouth periodontal examination and were classified into periodontal profile classes (PPCs) based on the severity and extent of gingival inflammation and attachment loss. MCI and dementia were determined via neurocognitive testing, neurological examination and history, informant interviews, and brain MRI in a subset. Cox proportional hazards models regressed incident dementia on PPCs. Relative risk regression models were used for the composite of MCI/dementia.

Results
The cumulative incidence and incidence density of dementia during follow-up (average 18.4 years) were 19% (n = 1,569) and 11.8 cases per 1,000 person-years. Multivariable adjusted hazard ratios for incident dementia among participants with severe PPC or edentulism (vs periodontal healthy) were 1.22 (95% confidence interval [CI] 1.01–1.47) and 1.21 (95% CI 0.99–1.48), respectively. For the combined dementia/MCI outcome, adjusted risk ratios among participants with mild/intermediate PPC, severe PPC, or edentulism (vs periodontal healthy) were 1.22 (95% CI 1.00–1.48), 1.15 (95% CI 0.88–1.51), and 1.90 (95% CI 1.40–2.58). Results were stronger among younger (≤62 years) participants (p for interaction = 0.02).

Conclusion
Periodontal disease was modestly associated with incident MCI and dementia in a community-based cohort of black and white participants.

From the Division of Epidemiology and Community Health (R.T.D., F.L.N., K.L., J.S.P., A.R.F., P.L.L.), School of Public Health, University of Minnesota, Minneapolis; Department of Epidemiology (R.T.D.), Mailman School of Public Health, Columbia University, New York, NY; Department of Neurology (K.A.W.), Johns Hopkins School of Medicine, Baltimore, MD; Department of Medicine (T.M.), University of Mississippi Medical Center, Jackson; and Division of Comprehensive Oral Health–Periodontology (J.B.), Adams School of Dentistry, University of North Carolina at Chapel Hill.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

So far to fulfillment of Koch's

- *Cross sectional* and *longitudinal studies* show that periodontitis is closely associated with cognitive impairment (CI) and AD.
- Animal models of periodontitis and human post-mortem brain tissues from subjects with AD strongly suggest that a Gram-negative periodontal pathogen, *Porphyromonas gingivalis* (Pg) and/or its product gingipain is/are translocated to the brain
- However, *neuropathology* resulting from *Pg* oral application is not known

➢ Will repeated exposure of *wild type C57BL/6 mice* to orally administered *Pg* results in *neuroinflammation*, *neurodegeneration*, *microgliosis*, *astrogliosis* and formation of intra- and extracellular *amyloid plaque* and *neurofibrillary tangles* (NFTs) which are *pathognomonic signs of AD?*

PLOS ONE

RESEARCH ARTICLE

Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice

Vladimir Hovlid[1], Paulina K. Zuchowska[1], Stefan J. Green[2,3], Peter T. Toth[4,5], Michael E. Ragozzino[6], Khuong Le[7], Halder W. Aljewari[1], Nell M. O'Brien-Simpson[8], Eric C. Reynolds[8], Keiko Watanabe[1,7]*

Check for updates

OPEN ACCESS

Abstract

Background

The results from cross sectional and longitudinal studies show that periodontitis is closely associated with cognitive impairment (CI) and Alzheimer's Disease (AD). Further, studies using animal model of periodontitis and human post-mortem brain tissues from subjects with AD strongly suggest that a gram-negative periodontal pathogen, *Porphyromonas gingivalis* (Pg) and/or its product gingipain is/are translocated to the brain. However, neuropathology resulting from Pg oral application is not known. In this work, we tested the hypothesis that repeated exposure of wild type C57BL/6 mice to orally administered Pg results in neuroinflammation, neurodegeneration, astrogliosis and formation of intra- and extracellular amyloid plaque and neurofibrillary tangles (NFTs) which are pathognomonic signs of AD.

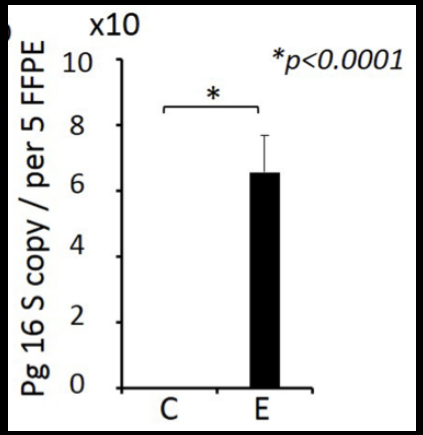
Methods

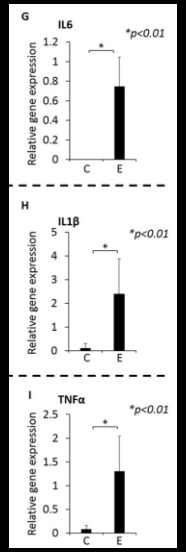
Experimental chronic periodontitis was induced in ten wild type 8-week old C57BL/6 WT mice by repeated oral application (MWF/week) of Pg/gingipain for 22 weeks (experimental group). Another 10 wild type 8-week old C57BL/6 mice received vehicle alone (control group) MWF per week for 22 weeks. Brain tissues were collected and the presence of Pg/gingipain was determined by immunofluorescence (IF) microscopy, confocal microscopy, and quantitative PCR (qPCR). The hippocampi were examined for the signs of

Causation - do we, have it?

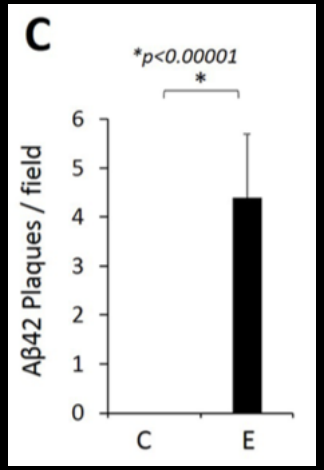
Neuroinflammation

P. gingivalis recovered from the hippocampus

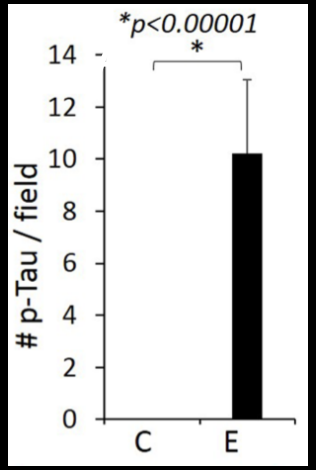




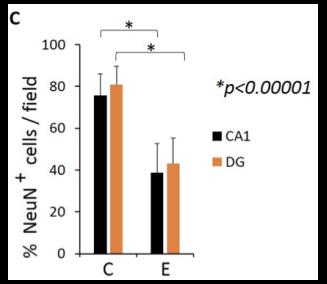
Amyloid Plaque



Tangled Neurons



Neurodegeneration



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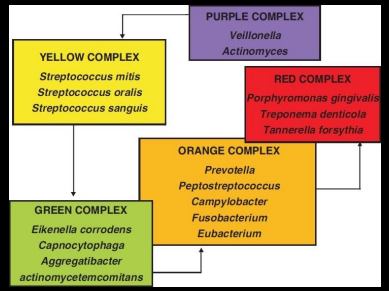
Conclusion -Oral Pg infection of WT mice induces AZ pathology after 22 weeks.

FFPE - formalin fixed paraffin embedded samples, C- Control; E Experimental



P. gingivalis infection

- Perio or GI
- Transits via bloodstream, trauma,
- Genetic Risk
- *apoE4, TLR4, TREM2, CR1, compliment*

Invasion of the brain

- *P. g* is *asaccharolytic*
- Secretes ginipans to liberate protein (food source)

Host Response

Inflammation

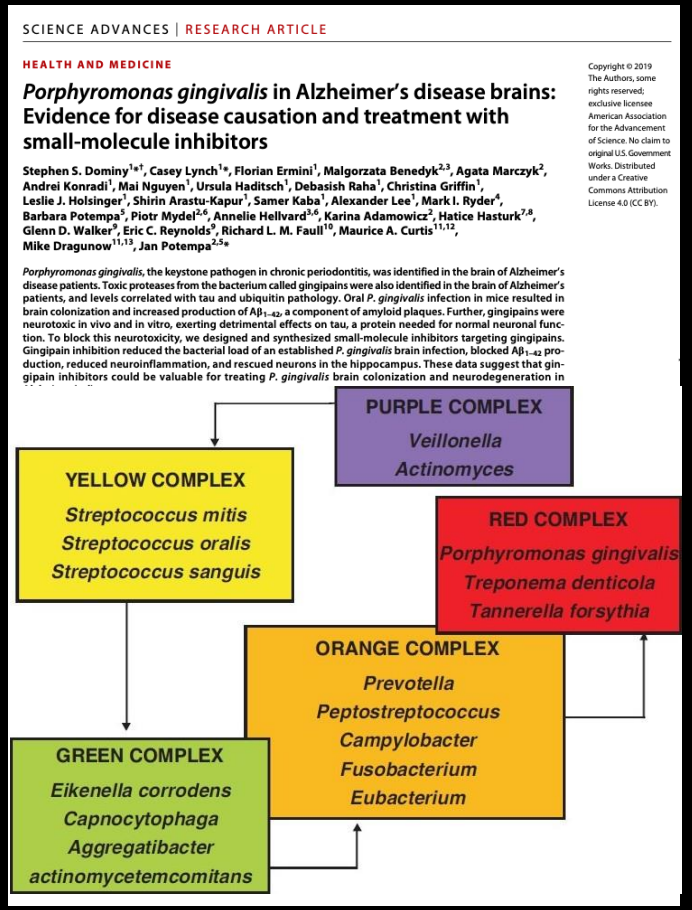
- Train wreck
  - *Microglia activation*
  - *Neuroinflammation*
    Complement induction
    Inflammasome
    Amyloid beta production

Story so far...

What do we need to do to prevent the pathology?

Story so far-2

- *Identified the presence of this keystone pathogen of periodontal disease in the brains of AD subjects.*
- *Gingipain was also recovered from the brains of individuals suffering from AD*
  - *Its concentration correlated with tau and ubiquitin pathology*
  - *Neurotoxic in vitro and in vivo resulting in detrimental effects to tau…*
    - *Tau is key to AD development*



SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors

Stephen S. Dominy[1*†], Casey Lynch[1*], Florian Ermini[1], Malgorzata Benedyk[2,3], Agata Marczyk[2], Andrei Konradi[1], Mai Nguyen[1], Ursula Haditsch[1], Debasish Raha[1], Christina Griffin[1], Leslie J. Holsinger[1], Shirin Arastu-Kapur[1], Samer Kaba[1], Alexander Lee[1], Mark I. Ryder[4], Barbara Potempa[5], Piotr Mydel[2,6], Annelie Hellvard[3,6], Karina Adamowicz[2], Hatice Hasturk[7,8], Glenn D. Walker[9], Eric C. Reynolds[9], Richard L. M. Faull[10], Maurice A. Curtis[11,12], Mike Dragunow[11,13], Jan Potempa[2,5*]

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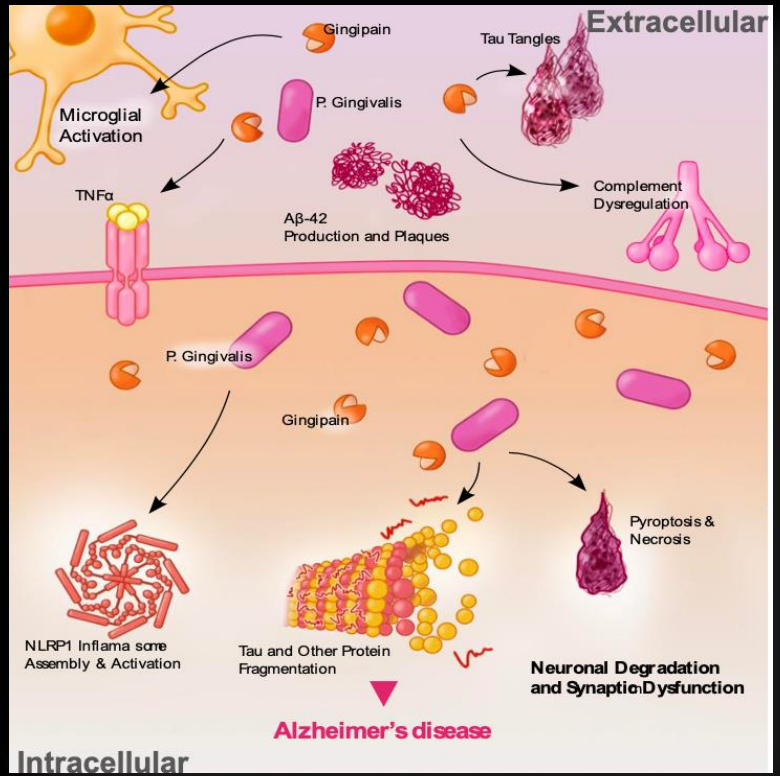
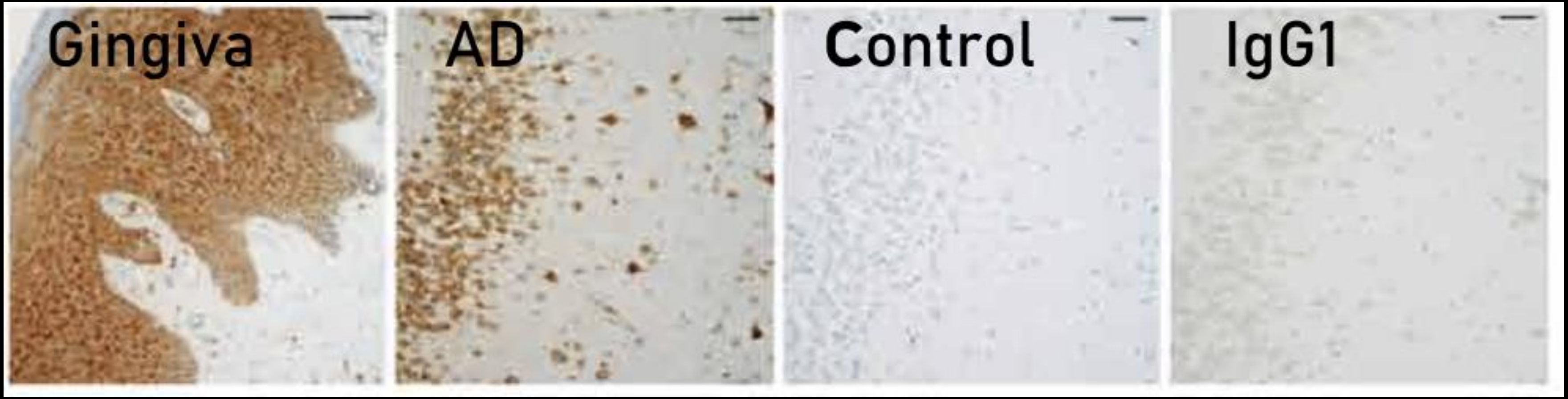
- Hypothesis-

Can inhibition of a key virulence (gp) factor reduce the microbial concentration of Pg and in turn alleviate inflammation->disease progression?

1. ***Tau*** is fragmented and aggregated in the AD brain and by Pg
2. ***ApoE*** is attacked by Gingipans in the AD brain
3. ***Amyloid ß, Microglia,Inflammasomes and Compliment*** are each activated /dysregulated in AD brains 2° to PG infection/ Gingipans
4. ***Neurodegeneration***/pathology is evident in concert with PG gingipans



Detected gingipan (R) in the Brains of AZ subjects



- Immunohistochemistry looking for evidence of the protease in the tissue
  - ***Brown his bad***
- Bottom line… ***Fig. 2. RgpB colocalizes with neurons and pathology in AD hippocampus***

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

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INTRODUCTION

Alzheimer's disease (AD) patients exhibit neuroinflammation consistent with infection, including microglial activation, inflammasome activation, complement activation, and altered cytokine profiles (*1*, *2*). Infectious agents have been found in the brain and postulated to be involved with AD, but robust evidence of causation has not been established (*3*). The recent characterization of amyloid-β (Aβ) as an antimicrobial peptide has renewed interest in identifying a possible infectious cause of AD (*4*–*6*).

Chronic periodontitis (CP) and infection with *Porphyromonas gingivalis*—a keystone pathogen in the development of CP (*7*)—have been identified as significant risk factors for developing Aβ plaques, dementia, and AD (*8*–*12*). A prospective observational study of AD patients with active CP reported a notable decline in cognition (Alzheimer's Disease Assessment Scale—Cognitive and Mini Mental State Examination scales) over a 6-month period compared to AD patients without active CP, raising questions about possible mechanisms underlying these findings (*13*). In $Apoe^{-/-}$ mice, oral infection with *P. gingivalis*, but not with two other oral bacteria, results in brain infection and activation of the complement pathway (*14*). In transgenic mice overexpressing mutated human amyloid precursor protein (hAPP-J20), oral infection with *P. gingivalis* impairs cognitive function, increases the deposition of AD-like plaques, and results in alveolar bone loss compared to control hAPP-J20 mice (*15*). *P. gingivalis* lipopolysaccharide has been detected in human AD brains (*16*), promoting the hypothesis that *P. gingivalis* infection of the brain plays a role in AD pathogenesis (*17*).

P. gingivalis is mainly found during gingival and periodontal infections; however, it can also be found at low levels in 25% of healthy individuals with no oral disease (*18*). Transient bacteremia of *P. gingivalis* can occur during common activities such as brushing, flossing, and chewing, as well as during dental procedures (*19*), resulting in documented translocation to a variety of tissues including coronary arteries (*20*), placenta (*21*), and liver (*22*). A recent study found that 100% of patients with cardiovascular disease had *P. gingivalis* arterial colonization (*23*).

P. gingivalis is an asaccharolytic Gram-negative anaerobic bacterium that produces major virulence factors known as gingipains, which are cysteine proteases consisting of lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). Gingipains are secreted, transported to outer bacterial membrane surfaces, and partially released into the extracellular milieu in soluble and outer membrane vesicle (OMV)–associated forms (*24*, *25*). Kgp and RgpA/B are essential for *P. gingivalis* survival and pathogenicity, playing critical roles in host colonization, inactivation of host defenses, iron and nutrient acquisition, and tissue destruction (*24*, *26*). Gingipains have been shown to mediate the toxicity of *P. gingivalis*

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*These authors contributed equally to this work as co-senior authors.
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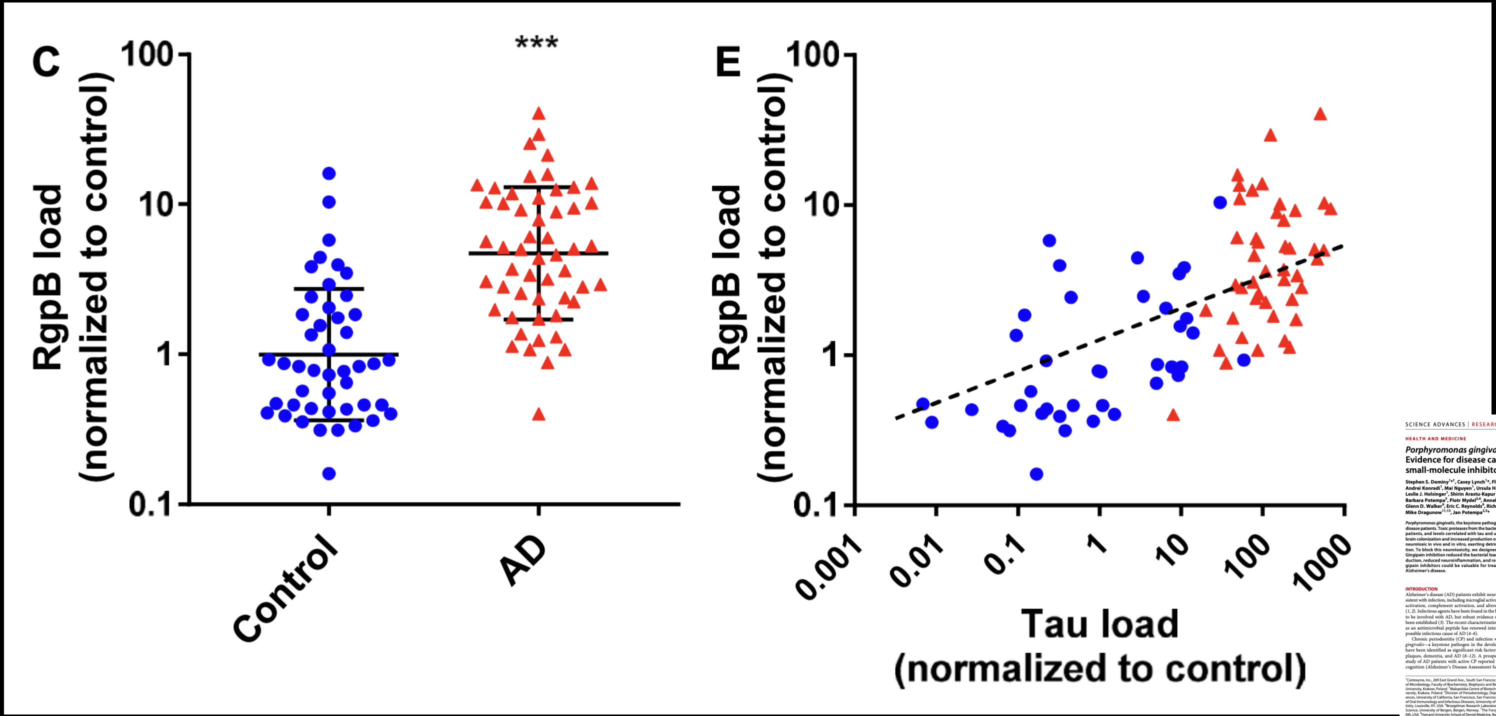
P. gingivalis invades the brain and is correlated with Az symptoms and pathology



SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

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*These authors contributed equally to this work as co-senior authors.
†Corresponding author. Email: sdominy@cortexyme.com

Clinical Trial

- Enter inhibitor of lysine gingipain
- Potent Target IC_{50} <50 pg
- Oral
- Cross Blood Brain Barrier
- ***Atuzaginstat (Cor388) -*** https://www.clinicaltrials.gov/ct2/results?cond=&term=cor388&cntry=&state=&city=&dist=
- ***GAIN (GingipAIN Inhibitor for Treatment of Alzheimer's Disease)***
- ***Results in November 2021!***

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

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Porphyromonas gingivalis, the keystone pathogen in chronic periodontitis, was identified in the brain of Alzheimer's disease patients. Toxic proteases from the bacterium called gingipains were also identified in the brain of Alzheimer's patients, and levels correlated with tau and ubiquitin pathology. Oral *P. gingivalis* infection in mice resulted in brain colonization and increased production of $A\beta_{1-42}$, a component of amyloid plaques. Further, gingipains were neurotoxic in vivo and in vitro, exerting detrimental effects on tau, a protein needed for normal neuronal function. To block this neurotoxicity, we designed and synthesized small-molecule inhibitors targeting gingipains. Gingipain inhibition reduced the bacterial load of an established *P. gingivalis* brain infection, blocked $A\beta_{1-42}$ production, reduced neuroinflammation, and rescued neurons in the hippocampus. These data suggest that gingipain inhibitors could be valuable for treating *P. gingivalis* brain colonization and neurodegeneration in Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) patients exhibit neuroinflammation consistent with infection, including microglial activation, inflammasome activation, complement activation, and altered cytokine profiles (*1*, *2*). Infectious agents have been found in the brain and postulated to be involved with AD, but robust evidence of causation has not been established (*3*). The recent characterization of amyloid-β (Aβ) as an antimicrobial peptide has renewed interest in identifying a possible infectious cause of AD (*4*–*6*).

Chronic periodontitis (CP) and infection with *Porphyromonas gingivalis*—a keystone pathogen in the development of CP (*7*)—have been identified as significant risk factors for developing Aβ plaques, dementia, and AD (*8*–*12*). A prospective observational study of AD patients with active CP reported a notable decline in cognition (Alzheimer's Disease Assessment Scale—Cognitive and Mini Mental State Examination scales) over a 6-month period compared to AD patients without active CP, raising questions about possible mechanisms underlying these findings (*13*). In *Apoe*$^{-/-}$ mice, oral infection with *P. gingivalis*, but not with two other oral bacteria, results in brain infection and activation of the complement pathway (*14*). In transgenic mice overexpressing mutated human amyloid precursor protein (hAPP-220), oral infection with *P. gingivalis* impairs cognitive function, increases the deposition of AD-like plaques, and results in alveolar bone loss compared to control hAPP-220 mice (*15*). *P. gingivalis* lipopolysaccharide has been detected in human AD brains (*16*), promoting the hypothesis that *P. gingivalis* infection of the brain plays a role in AD pathogenesis (*17*).

P. gingivalis is mainly found during gingival and periodontal infections; however, it can also be found at low levels in 25% of healthy individuals with no oral disease (*18*). Transient bacteremia of *P. gingivalis* can occur during common activities such as brushing, flossing, and chewing, as well as during dental procedures (*19*), resulting in documented translocation to a variety of tissues including coronary arteries (*20*), placenta (*21*), and liver (*22*). A recent study found that 100% of patients with cardiovascular disease had *P. gingivalis* arterial colonization (*23*).

P. gingivalis is an asaccharolytic Gram-negative anaerobic bacterium that produces major virulence factors known as gingipains, which are cysteine proteases consisting of lysine-gingipain (Kgp), arginine-gingipain A (RgpA), and arginine-gingipain B (RgpB). Gingipains are secreted, transported to outer bacterial membrane surfaces, and partially released into the extracellular milieu in soluble and outer membrane vesicle (OMV)–associated forms (*24*, *25*). Kgp and RgpA/B are essential for *P. gingivalis* survival and pathogenicity, playing critical roles in host colonization, inactivation of host defenses, iron and nutrient acquisition, and tissue destruction (*24*, *26*). Gingipains have been shown to mediate the toxicity of *P. gingivalis*

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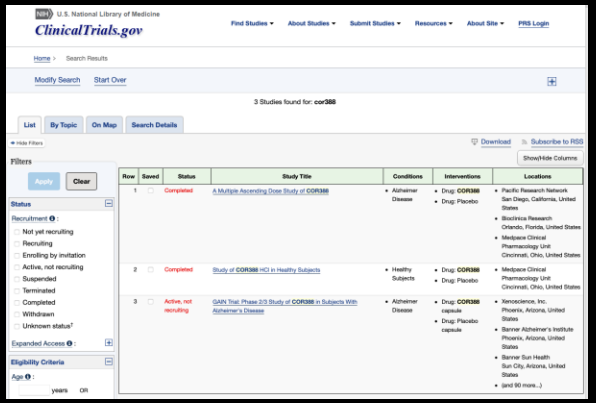


Trial Design- Did it work?

- *3 groups Double Blind*
  - *Third -80 mg orally twice each day*
  - *Third - 40 mg orall twice each day*
  - *Third – placebo*
  - *N=573*
- *Enrollment Completed September 2020*
- Interim Analysis, December 2020, no sample size adjustment (N=643) with periodontal sub-study- 233 subjects assessing pocket depth and clinical attachment at 6 and 12 months
- *Top line Data Next Month!*
- *Outcomes* →

Outcomes

Co-Primary:

ADAS-Cog11 and ADCS-ADL (AD standard scoring systems)

Secondary:

CDR-SB, MMSE and NPI

Exploratory:

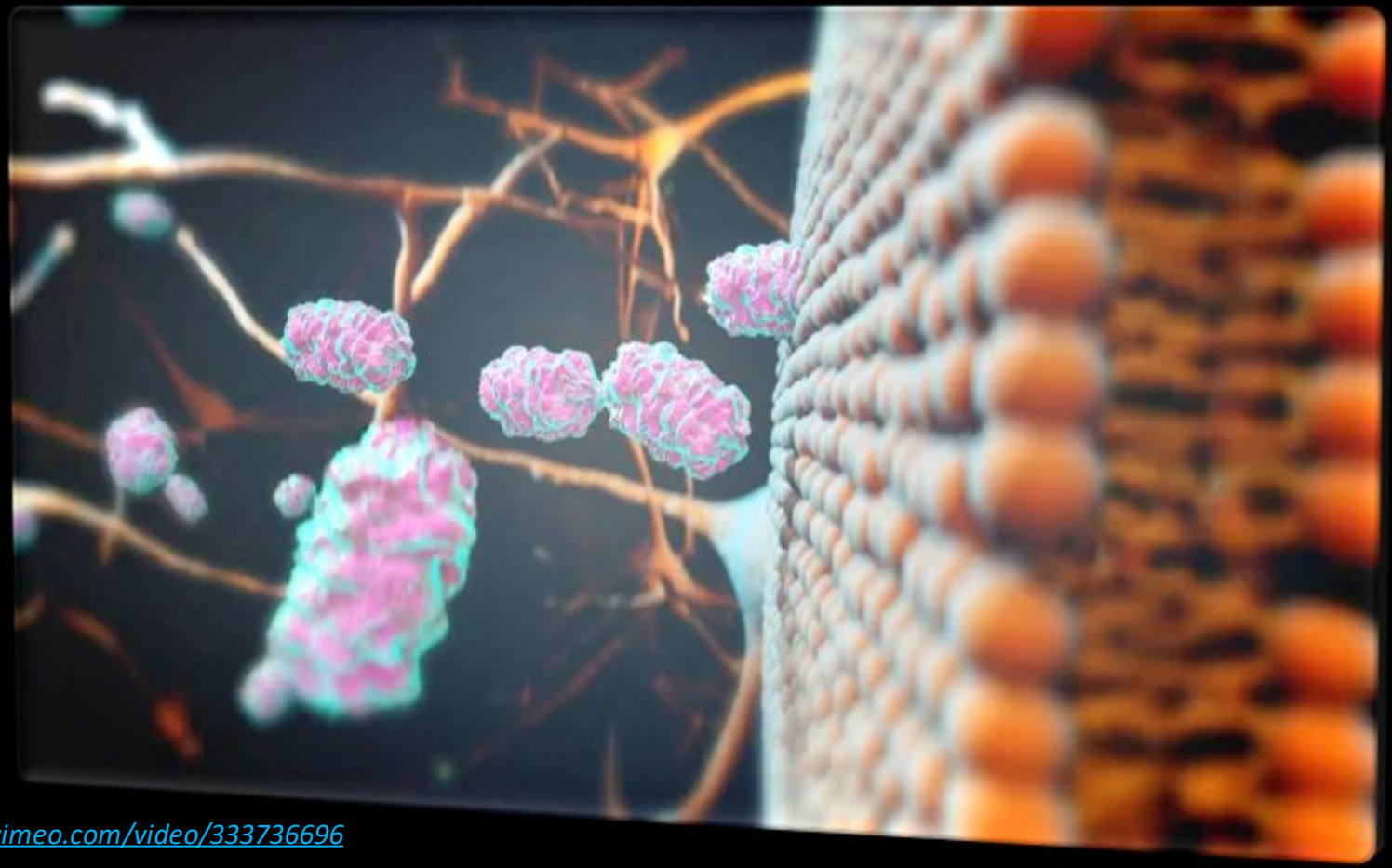
Winterlight, MRI (hippocampal volume and cortical thickness)

Biomarkers of Pg and gingipain activity – Blood saliva, and oral microbiome

Biomarkers of AD CSF, Aß, tau and p-tau

Diagnostic Markers of disease modification- MRI volumetric measures

Putting it all together- Why it might work



https://player.vimeo.com/video/333736696

Story is getting interesting-2021

- *Gingipans* identified in >90% of AZ brains
- Mice and rat studies have shown that *P.g.* invades the brain after oral infection and triggers AD pathology.
- *apoE4 greatest genetic risk factor for sporadic AD and ApoE fragments are recovered from AD patients.*
- Protease responsible unknown… *until now…*
- New data suggest Gingipans directly cleave ApoE *in vivo, specifically ApoE4*
- CSF evaluated from a 28-day phase 1b clinical trial of *atuzaginstat*, a brain-penetrant gingipain inhibitor, in mild-to-moderate AD patients revealed a significant reduction of low-molecular-weight ApoE fragments compared to placebo that *was strongly correlated with a reduction in the pathologic decline* of CSF Aβ 1-42 levels

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors

Stephen S. Dominy[1*†], Casey Lynch[1*], Florian Ermini[1], Malgorzata Benedyk[2,3], Agata Marczyk[2], Andrei Konradi[1], Mai Nguyen[1], Ursula Haditsch[1], Debasish Raha[1], Christina Griffin[1], Leslie J. Holsinger[1], Shirin Arastu-Kapur[1], Samer Kaba[1], Alexander Lee[1], Mark I. Ryder[4], Barbara Potempa[5], Piotr Mydel[2,6], Annelie Hellvard[3,6], Karina Adamowicz[2], Hatice Hasturk[7,8], Glenn D. Walker[9], Eric C. Reynolds[9], Richard L. M. Faull[10], Maurice A. Curtis[11,12], Mike Dragunow[11,13], Jan Potempa[2,5*]

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INTRODUCTION

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CellPress Sneak Peek
A PREVIEW OF PAPERS UNDER REVIEW

Gingipains Identified in Alzheimer's Disease Brains Differentially Fragment ApoE Proteins

Cell Reports Medicine

34 Pages • Posted: 3 May 2021 • Publication Status: **Review Complete**

Debasish Raha
Cortexyme, Inc.

Sean Broce
Cortexyme, Inc.

More...

Abstract

Gingipains are protease virulence factors from the periodontal bacterial pathogen *Porphyromonas gingivalis* and were recently identified in greater than 90% of Alzheimer's disease (AD) brains. Studies in wild-type mice and rats have demonstrated that *P. gingivalis* invades the brain after oral infection and triggers characteristic AD pathology. The *APOE4* gene is the greatest genetic risk factor for sporadic AD, and ApoE protein fragments have been identified in the brain and cerebrospinal fluid of AD patients, but the protease(s) responsible for ApoE fragmentation remain unknown. Here we report that gingipains directly cleave ApoE proteins in vitro, with ApoE4 preferentially cleaved compared to ApoE3 and ApoE2. Cerebrospinal fluid analyzed from a 28-day phase 1b clinical trial of atuzaginstat, a brain-penetrant gingipain inhibitor, in mild-to-moderate AD patients revealed a significant reduction of low-molecular-weight ApoE fragments compared to placebo that was strongly correlated with a reduction in the pathologic decline of CSF Aβ 1-42 levels.

Keywords: Alzheimer's disease, Porphyromonas gingivalis, gingipains, ApoE4

Suggested Citation:

Raha, Debasish and Broce, Sean and Arastu-Kapur, Shirin and Haditsch, Ursula and Nguyen, Mai and Rodriguez, Leo and Ermini, Florian and Wang, Jianhong and Hennings, D. David and Detke, Michael J. and Lynch, Casey and Holsinger, Leslie J. and Dominy, Stephen, Gingipains Identified in Alzheimer's Disease Brains Differentially Fragment ApoE Proteins. Available at SSRN: https://ssrn.com/abstract=3838996 or http://dx.doi.org/10.2139/ssrn.3838996

How can you help?

1. *Appreciate how perturbations to periodontal health can introduce a member of the host's microbiome to host niche with unintended consequences*
2. *Evaluate whether periodontal dysbiosis is causally linked to the Alzheimer's Disease pathology*
3. *Discuss how inhibiting a key virulence factor of a microbe associated with periodontal disease might arrest the development of Alzheimer's Disease Pathology*

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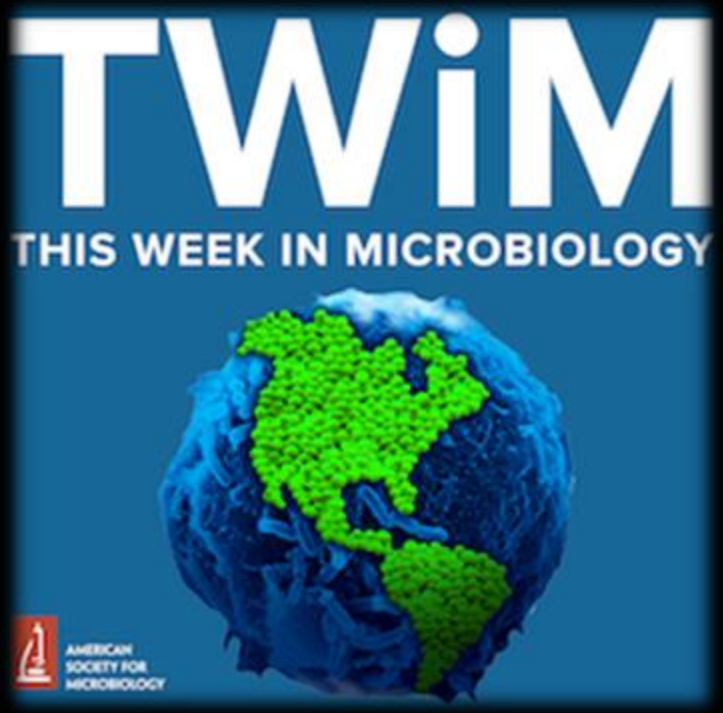
Thank you!



Questions?

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MEDICAL UNIVERSITY of SOUTH CAROLINA
Changing What's Possible

The Role That Microbes May Be Playing in Progressive Brain Disorders

Michael G. Schmidt, Ph.D.

AIOB 23 October 2021