



Periodontal disease associates with higher brain amyloid load in normal elderly



Angela R. Kamer^{a,b,*}, Elizabeth Pirraglia^b, Wai Tsui^b, Henry Rusinek^{b,c}, Shankar Vallabhajosula^d, Lisa Mosconi^b, Li Yi^b, Pauline McHugh^b, Ronald G. Craig^{a,e}, Spencer Svetcov^a, Ross Linker^a, Chen Shi^a, Lidia Glodzik^b, Schantel Williams^b, Patricia Corby^{a,f}, Deepak Saxena^e, Mony J. de Leon^b

^a Department of Periodontology and Implant Dentistry, College of Dentistry, New York University, New York, NY, USA

^b School of Medicine, Department of Psychiatry, Center for Brain Health, New York, NY, USA

^c School of Medicine, Department of Radiology, New York, NY, USA

^d Weill Medical Center, Department of Radiology, Cornell University, New York, NY, USA

^e Department of Basic Sciences and Craniofacial Biology, College of Dentistry, New York University, New York, NY, USA

^f College of Dentistry, Bluestone Center for Clinical Research, New York University, New York, NY, USA

ARTICLE INFO

Article history:

Received 30 May 2014

Received in revised form 26 October 2014

Accepted 30 October 2014

Available online 5 November 2014

Keywords:

Alzheimer's disease

Infection

Inflammation

Periodontal disease

Brain amyloid

PIB-PET

Cognition

ABSTRACT

The accumulation of amyloid- β (A β) plaques is a central feature of Alzheimer's disease (AD). First reported in animal models, it remains uncertain if peripheral inflammatory and/or infectious conditions in humans can promote A β brain accumulation. Periodontal disease, a common chronic infection, has been previously reported to be associated with AD. Thirty-eight cognitively normal, healthy, and community-residing elderly (mean age, 61 and 68% female) were examined in an Alzheimer's Disease Research Center and a University-Based Dental School. Linear regression models (adjusted for age, apolipoprotein E, and smoking) were used to test the hypothesis that periodontal disease assessed by clinical attachment loss was associated with brain A β load using ¹¹C-Pittsburgh compound B (PIB) positron emission tomography imaging. After adjusting for confounders, clinical attachment loss (≥ 3 mm), representing a history of periodontal inflammatory/infectious burden, was associated with increased PIB uptake in A β vulnerable brain regions ($p = 0.002$). We show for the first time in humans an association between periodontal disease and brain A β load. These data are consistent with the previous animal studies showing that peripheral inflammation/infections are sufficient to produce brain A β accumulations.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Worldwide, >35 million persons suffer from dementia among which 50%–60% are diagnosed with Alzheimer's disease (AD) (Alzheimer's Association, 2014). It is estimated these numbers will double by 2030 and double again by 2050. These statistics underline the enormous public health importance of identifying modifiable risk factors.

The accumulation of amyloid- β (A β) plaques is a central feature of AD whose cause is poorly understood. Postmortem studies have shown that amyloid accumulation can start as early as 30 years of age and increases with age (Braak and Braak, 1997; Kok et al., 2009).

These findings have been confirmed by the imaging studies (Jack et al., 2009; Klunk et al., 2004; Landau et al., 2012). The results of clinical trials designed to remove brain amyloid from impaired individuals have been largely unsuccessful possibly because of the late intervention (Holmes et al., 2008; Morgan, 2011; Ozudogru and Lippa, 2012). This has placed a great emphasis on identifying factors and mechanisms that promote brain amyloid deposition in advance of symptoms.

Both animal models and clinical evidence show that inflammation is involved in the pathogenesis of AD (Akiyama et al., 2000; Griffin et al., 1998; Holmes and Butchart, 2011; McGeer et al., 2006; Tanzi, 2012), but it remains unknown which peripheral inflammatory and infectious conditions play a role and at which stage of AD development (Kamer et al., 2008a, 2008b; Miklossy, 2011a, 2011b). We examined human periodontal disease as a model for testing the relationship between peripheral inflammation/infections and brain A β . Periodontal disease is a chronic, peripheral, polymicrobial

* Corresponding author at: Department of Periodontology and Implant Dentistry, College of Dentistry, New York University, 345 E 24th St, New York, NY 10010, USA. Tel.: +1 212 998 9868; fax: +1 212 995 4603.

E-mail address: ark5@nyu.edu (A.R. Kamer).

infection (Socransky and Haffajee, 1997) characterized by local and systemic inflammations. Periodontal disease is defined by the loss of the tissues surrounding the teeth, clinically defined by clinical attachment loss (CAL) (Demmer et al., 2008).

The present cross-sectional study used positron emission tomography (PET) amyloid imaging and clinical periodontal examinations to test the hypothesis that in cognitively normal subjects, the magnitude of periodontal disease burden is associated with the brain amyloid load.

2. Methods

2.1. Study subjects and design

Thirty-eight cognitively normal healthy subjects were included in this study. All subjects were participants in the National Institutes of Health (NIH)–supported AD studies at the New York University (NYU) School of Medicine. Subjects were recruited from a random community sampling of voter registration lists. Among the 250 elderly individuals who were contacted and invited to participate, 70 subjects agreed to participate. Of these, 40 subjects had standardized medical and cognitive examinations consistent with the National Alzheimer Coordinating Center guidelines (Beekly et al., 2007). The standardized diagnostic evaluation at the NYU School of Medicine consisted of medical, psychiatric, neuropsychological, apolipoprotein E (ApoE) genotyping, magnetic resonance imaging (MRI) examinations, and standardized periodontal examinations. Thirty-eight subjects also participated in ^{11}C -Pittsburgh compound B (PIB)-PET amyloid brain imaging performed at the Cornell Medical Center. All subjects provided written informed consent to participate in this institutional review board–approved study. The average interval between the PET scan and the periodontal examination was 1.29 ± 0.89 years. All research measures were performed blinded to the clinical data.

2.1.1. Inclusion criteria

All included subjects had at least 12 years of education and were fluent English speakers. Subjects were defined as cognitively normal if they had Clinical Dementia Rating = 0 (Berg, 1984), Global Deterioration Scale ≤ 2 (Reisberg et al., 1982), and Mini-Mental State Examination ≥ 28 (Cockrell and Folstein, 1988).

All subjects were required to have a minimum of 10 evaluable teeth (Stein et al., 2007) and to have the physical capacity to manage their personal dental hygiene.

2.1.2. Exclusion criteria

Individuals were excluded if they had history/medical conditions that could affect brain structure or function, such as clinical or MRI evidence of cortical stroke, uncontrolled hypertension, diabetes, head trauma with loss of consciousness, any manifest neurodegenerative disease, chronic depression, MRI evidence of hydrocephalus, or intracranial mass. Subjects taking anti-inflammatory medications for chronic conditions (i.e., nonsteroidal anti-inflammatory drugs, anti-tumor-necrosis factor α) or antibiotics or having periodontal treatment 3 months before the periodontal evaluation were also excluded.

2.2. Clinical evaluations

2.2.1. Measures of periodontal disease

The assessment for periodontal disease was conducted as follows: teeth were counted, and the presence of dental plaque on 6 surfaces of all teeth was recorded (Silness and Loe, 1964). CAL, the primary dependent variable, was measured using a Michigan probe (Demmer et al., 2008) and recorded in millimeters at 6 sites per

tooth. CAL defined the long-term periodontal inflammatory/infectious condition. CAL was obtained by adding the probing depth (PD) to the distance from the free gingival margin to the cemento-enamel junction (positive if the gingival margin is apical to the cemento-enamel junction and negative if it is coronal). The PD was measured as the linear distance in millimeters from the gingival margin to the base of the periodontal pocket. Bleeding on probing (BOP) was assessed at each probing site.

The primary periodontal exposure was defined as the cumulative number of sites with CAL ≥ 3 mm (CAL3) and provided a measure of periodontal disease burden (Tonetti et al., 2005). The use of CAL3 was based on our a priori hypothesis proposing a linear relationship between the magnitude of periodontal destruction because of the history of periodontal inflammation and brain amyloid accumulation, a chronic process. The threshold of 3 mm also included milder forms of the periodontal disease. These measures were used previously to define relationships between periodontal and cardiovascular diseases and cognitive dysfunction (Beck et al., 2001; Elter et al., 2004). An additional consideration came from the evidence showing that CAL associated better with a chronic systemic process (Demmer et al., 2008) rather than an acute one. CAL are accepted measures of cumulative lifetime experience of periodontitis, and using these measures, the fifth European Workshop in Periodontology proposed the following case definition for periodontitis: the presence of proximal attachment loss of ≥ 3 mm in at least 2 nonadjacent teeth. All our subjects had CAL3 on multiple teeth; thus, they all fell within this case definition. To show consistency in the relationship between measures of periodontal disease and brain amyloid accumulation, other measures of periodontal disease were evaluated: CAL ≥ 4 mm (CAL4) (Page and Eke, 2007), PD ≥ 3 mm (PD3), and BOP. By comparison with CAL, PD and BOP measure the present disease and inflammation. To further define periodontal disease, we combined historical with current measures of periodontal disease: the presence of CAL3 at $\geq 66\%$ sites and concomitant PD ≥ 5 mm (PD5) was defined as Perio1 (Jonsson et al., 2014).

2.2.2. PET outcome variables

2.2.2.1. Acquisition and preprocessing. Subjects received a PIB-PET scan acquired in 3-dimensional mode on an LS Discovery PET scanner (GE Medical Systems, Milwaukee, WI, USA) (Li et al., 2008; Mosconi et al., 2010, 2013a). Briefly, as previously reported, subjects were injected with 15 mCi (550 MBq) of N -methyl[^{11}C]2-(4'-methylaminophenyl)-6-hydroxy-benzothiazole, PIB, followed by a 90-minute PET data acquisition (Mosconi et al., 2010). Image analysis was carried out at NYU, blind to the clinical data. For each subject, summed PET images corresponding to the 60- to 90-minute PIB data were coregistered to the subject's T1 MRI scan using Statistical Parametric Mapping (SPM). Both the summed 60- to 90-minute PIB image and SPM2-segmented MRI gray-matter (GM) and white-matter images were reformatted into SPM's standard template space. In the standard space, regions of interest (ROIs) were intersected with the GM to exclude all non-GM pixels. HIPMASK was used for accurate ROI sampling (Li et al., 2008; Mosconi et al., 2005). A correction for partial volume effects was done using the 2-tissue method of Muller-Gartner, which corrects for both cerebrospinal fluid and white-matter tracer uptake (Muller-Gartner et al., 1992).

2.2.2.2. PET ROIs. The average PIB intensity in each ROI was normalized by the average intensity from a cerebellar GM reference ROI, to create the standard uptake value ratio (SUVR). From our previous work (Li et al., 2008; Mosconi et al., 2013b), 5 bilateral ROIs known to be vulnerable to amyloid depositions were sampled to create a composite neocortical PIB_{AD} mask (Mask_{AD}), which was the primary outcome measure. The regions included in the AD mask were prefrontal cortex, middle frontal gyrus, lateral temporal

lobe, inferior parietal lobule, and posterior cingulate cortex/pre-cuneus (Mosconi et al., 2010).

2.3. Statistical methods

To determine whether measures of periodontal disease were associated with amyloid load in MaskAD (shown to be vulnerable to amyloid accumulation), hierarchical regression analyses were performed in which MaskAD SUVR was the dependent variable and measures of periodontal disease (i.e., CAL3) and the relevant covariates (Stein et al., 2007) were the independent variables. Demographic data (age, gender, and education), systemic factors (comorbidities), and oral (brushing, flossing, and dentist visits) and social (smoking) measures were obtained by a standardized examiner-conducted interview at the time of the oral examination. For high systemic blood pressure (SBP) and diastolic blood pressure (DBP), body mass index, cholesterol, and high-density lipoproteins, standard cutoffs were used (Barba et al., 2008; National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002). ApoE genotype (carriers vs. noncarriers of ApoE ϵ 4 allele [ApoE4]) was obtained as previously reported (Mosconi et al., 2012; Osorio et al., 2014), and smoking was classified as never or former/current smokers. Cognitive performance was assessed by Logic2 of Wechsler Memory Scale—Revised Test (De Santi et al., 2008).

Our initial approach tested the previous covariates, and only significant covariates in at least 1 ROI were retained in the final models. These covariates included age, ApoE, and smoking. These analyses were repeated with additional measures of periodontal disease (CAL4, PD3, BOP, and Perio1 as independent variables).

The partial correlations, unstandardized β coefficients (β), and the 95% confidence interval (95% CI) of the β coefficients are obtained. All models were checked for linearity, independence, homoscedasticity, and normality. The log transformation was used to normalize the distribution of the MaskAD SUVR. Regression plots are presented in which the axes represent the standardized residuals for the dependent (MaskAD SUVR) and independent variables (CAL3 and CAL4) adjusted for covariates. Statistical significance was set at $p \leq 0.05$. Statistical analyses were performed using IBM SPSS, version 21 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Characteristics of the population

Table 1 summarizes the subject characteristics. Briefly, the mean years of age was 61.3 (standard deviation [SD] = 8.1; range, 44–79; and 5 subjects <55), mean years of education 17.6 (SD = 2.2; range, 13–22; and 4 subjects <16 years of education), 68% were female, and 42% were carriers of an ApoE4. The subjects were relatively healthy with 55% reporting no medical conditions and 73% no smoking history. Three subjects had high SBP and 2 DBP, 5 were obese (body mass index > 30), and none of the subjects had diabetes. Most subjects reported good oral hygiene practices and regular visits to the dentist (Table 1). The age, gender, education, and ApoE adjusted means for Logic2 scores were not different within the oral health categories (brushing: $p = 0.581$; flossing: $p = 0.447$; and dental visits: $p = 0.727$).

3.2. Measures of periodontal disease associate with amyloid load in AD-vulnerable areas

The mean of CAL3 and CAL4 was 125.4 and 79.4, respectively (SD = 30.2 and 29.7) meaning that 125.4 and 79.4 dental sites had a

Table 1

Characteristics of the subject population

| Characteristics | | |
|--|--------------|-------------|
| Demographic data | | |
| Number of subjects (n) | 38 | |
| Female, n (%) | 26 (68.4) | |
| Age, mean (SD) | 61.3 (8.1) | |
| Years of education, mean (SD) | 17.6 (2.2) | |
| ApoE carriers—total, n (%) | 16 (42.1) | |
| Systemic health findings, means (SD) | | n (%), high |
| BMI (n = 38) | 24.8 (4.2) | 5 (13.2) |
| SBP (n = 37) | 116.2 (16.6) | 3 (8.1) |
| DBP (n = 36) | 68.4 (9.1) | 2 (5.6) |
| Total cholesterol (n = 37) | 198.7 (33) | 20 (54.1) |
| HDL (n = 37) | 65.4 (22.6) | 34 (91.1) |
| Smoking (n = 38) | | 10 (26.3) |
| Oral hygiene behavior characteristics (n = 38) | | |
| Brushing (%) | | |
| ≤ Once per day | 31.6 | |
| > Once per day | 68.4 | |
| Flossing (%) | | |
| < Once per day | 63.2 | |
| ≥ Once per day | 36.8 | |
| Visits to dentist (%) | | |
| ≤ 6 mo | 71.1 | |
| > 6 mo | 28.9 | |
| Periodontal examination findings, mean (SD) | | |
| Tooth number | 25.86 (5.1) | |
| CAL ≥ 3 mm | 125.4 (30.2) | |
| CAL ≥ 4 mm | 79.4 (29.7) | |
| CAL ≥ 5 mm | 35.3 (23.6) | |
| CAL ≥ 6 mm | 10.2 (11.5) | |
| PD ≥ 3 mm | 96.3 (22.3) | |
| PD ≥ 4 mm | 26.3 (14.9) | |
| PD ≥ 5 mm | 6.9 (6.5) | |
| PD ≥ 6 mm | 1.7 (3.0) | |
| BOP | 36.6 (18.8) | |
| Perio1, n (%) | 13.0 (36.1) | |

Key: ApoE, apolipoprotein E; BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment loss; DBP, diastolic blood pressure; HDL, high-density lipoprotein; Perio1, CAL ≥ 3 mm at ≥ 66% sites/PD ≥ 5 mm; PD, probing depth; SBP, systolic blood pressure; SD, standard deviation.

clinical attachment loss of 3 and ≥ 4 mm (maximum possible number of sites = 192). The mean for PD3 was 96.3 (SD = 22.3) and for BOP 36.6 (SD = 18.8). The range of CAL3, CAL4, PD3, and BOP was 60–159, 27–135, 44–139, and 6–76, respectively. Using the definition of Perio1 (CAL3 at ≥ 66% sites/PD5), 13 subjects had extensive moderate-to-severe periodontitis. Among the covariates tested (SBP, DBP, obesity, cholesterol, high-density lipoprotein, comorbidities, Logic2, plaque index, tooth loss, brushing, flossing, visits to the dentist, and time difference between periodontal examination and PIB), none were found significant in any of the models. The regression analyses with each of these covariates are presented in Supplementary Table 1.

The primary measure of exposure, CAL3, was significantly correlated with PIB retention in MaskAD after controlling for age, ApoE, and smoking (adjusted partial correlation $r = 0.50$, $p = 0.002$, $\beta = 0.011$, 95% CI = 0.004–0.017). Figure 1A shows the regression plot for CAL3 and MaskAD. Thus, addition of CAL3 to the model predicting PIB retention led to a statistically significant increase in R^2 (ΔR^2) of 0.22 ($p = 0.002$), indicating that 22% of the variance of the PIB retention in the AD-vulnerable regions could be attributed to CAL3. The significance of this observation is also supported by the consistency of the results in each of the regions comprising the MaskAD (Supplementary Table 2). After controlling for age, ApoE, and smoking, CAL4 and Perio1 also show consistent and significant results, whereas PD3 correlations were not significant (CAL4: $r = 0.410$, $p = 0.015$, $\beta = 0.008$, 95% CI = 0.002–0.015; Perio1: $r = 0.412$, $p = 0.017$, $\beta = 0.482$, 95% CI = 0.084–0.881; and PD3: $r = 0.299$, $p = 0.085$, $\beta = 0.009$, 95% CI = –0.001 to 0.019). Figure 1B shows the

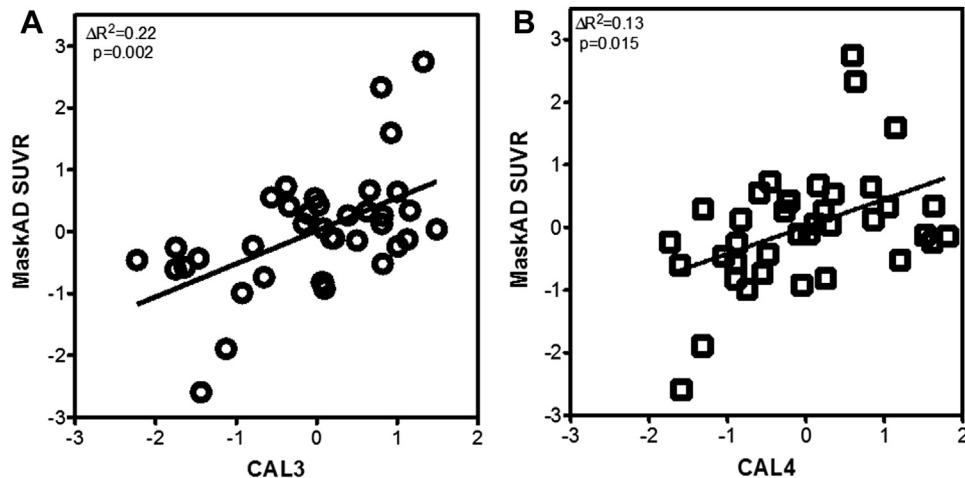


Fig. 1. Partial regression plots show the relationships between clinical attachment loss (CAL) ≥ 3 mm (CAL3) (A) and CAL ≥ 4 mm (CAL4) (B) and ^{11}C -Pittsburgh compound B (PIB) retention in PIB_{AD} mask (MaskAD). The x and y axes are the residuals for CAL3 and CAL4, respectively, and PIB retention is adjusted for age, apolipoprotein E carrier status, and smoking. The changes in R^2 (ΔR^2) and p values for these changes are shown. MaskAD is defined by combining the standardized uptake value ratios (SUVRs) of inferior parietal lobule, lateral temporal lobe, middle frontal gyrus, posterior cingulate cortex/precuneus, and prefrontal cortex. The CAL3-SUVR and CAL4-SUVR associations are significant.

regression plot for CAL4 and MaskAD. By comparison, BOP did not associate with PIB retention in MaskAD ($r = -0.096$, $p = 0.584$, $\beta = -0.003$, 95% CI = -0.003 to 0.008). Regression models showing the adjusted partial correlation coefficients and the unstandardized β coefficients with the 95% CI for age, ApoE, and smoking adjusted partial correlation coefficients are presented in [Supplementary Tables 2 and 3](#). No significant interaction was found between ApoE genotype and any of the periodontal measures. [Figure 2](#) illustrates the PIB-PET scans of 4 normal individuals: 2 of them are ApoE4 carriers with high versus low CAL and 2 of them are non-carriers of ApoE4 with high versus low CAL.

4. Discussion

To our knowledge, this is the first study to show that clinical measures of periodontal disease in cognitively normal healthy elders are positively associated with the magnitude of brain amyloid accumulation assessed by [^{11}C]PIB-PET. This conclusion was reached after showing that neither medical confounds, smoking,

oral health behaviors, tooth loss, and memory performance nor ApoE genotype accounted for this association. These results are consistent with the hypothesis that chronic periodontal inflammation/infection contributes to brain amyloid load and that these associations are not secondary to impaired cognition.

4.1. Periodontal inflammation/infection associates with amyloid load in AD-vulnerable amyloid areas

Periodontal disease is a chronic, peripheral, polymicrobial infection ([Socransky and Haffajee, 1997](#)) characterized by local and systemic inflammations ([Paraskevas et al., 2008](#)). Approximately 85% of the bacteria colonizing the subgingival biofilm are Gram negative ([Socransky and Haffajee, 2002](#)) that are rich in lipopolysaccharide. Among them, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, and *Treponema denticola* are important periodontal pathogens ([Haffajee and Socransky, 2006](#); [Socransky and Haffajee, 1997](#)). Periodontal disease is more prevalent in adulthood and elderly, but it can start in

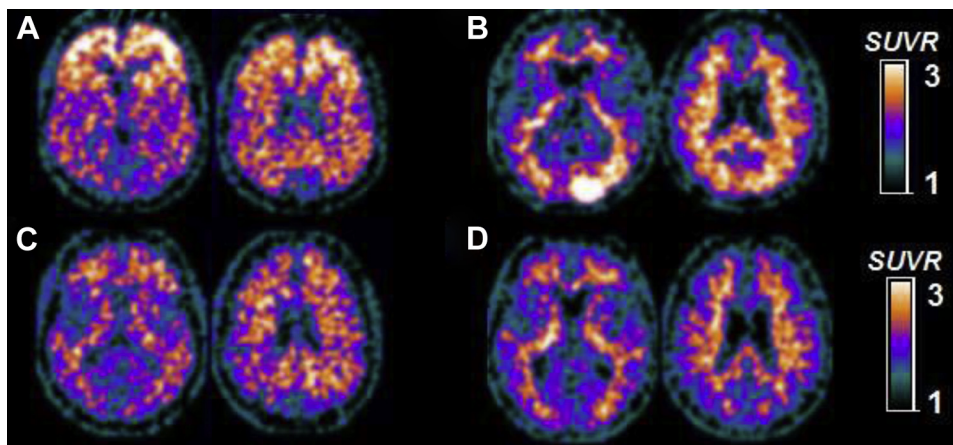


Fig. 2. PIB-positron emission tomography (PET) scans of 4 representative normal individuals: 2 of them of age 72 and 75 are apolipoprotein E (ApoE) carriers with high CAL ≥ 3 mm (CAL3) (A) and low CAL3 (C) and 2 of them age 79 and 76 are ApoE noncarriers with high CAL3 (B) and low CAL3 (D). The subjects in the upper row have significant amyloid accumulation and high CAL3 measures, whereas the subjects in the lower row have low brain amyloid accumulation and low CAL3. PIB-PET scans for each subject are displayed as axial sections encompassing basal ganglia (first slide) and inferior parietal level (second slide), respectively. PIB measures are standardized uptake value ratios to cerebellar gray matter. The ApoE $\epsilon 4$ allele (ApoE4)-positive subject presents with a positive PIB pattern, whereas the ApoE4-negative subject presents with an “emerging PIB pattern” in the right temporal-occipital cortex. (A) PIB-positive $\epsilon 4$ carrier, (B) PIB-positive $\epsilon 4$ noncarrier, (C) PIB-negative $\epsilon 4$ carrier, and (D) PIB-negative $\epsilon 4$ noncarrier. (For interpretation of the references to color in this Figure, the reader is referred to the Web version of this article.)

childhood. Approximately 64% of adults aged 65 years or older have chronic moderate and severe periodontitis (Eke et al., 2012). It is known that periodontal-derived pro-inflammatory molecules, bacteria, and bacterial products can reach the brain via systemic circulation and/or neural pathways (Holmes and Cotterell, 2009; Kamer et al., 2008b; Rivest, 2003) and increase brain cytokine levels. These types of inflammatory changes are separately known to contribute to brain amyloid accumulation and cognitive dysfunction (Kamer et al., 2008b; Perry et al., 2003). Our previous clinical data and other studies have linked periodontal disease and AD with moderate odds ratios (Gatz et al., 2006; Grabe et al., 2009; Kamer et al., 2012; Kaye et al., 2010; Noble et al., 2009; Stein et al., 2007; Stewart and Hirani, 2007). For example, in a survey of >6000 subjects with a broad age range, CAL3 associated with cognitive dysfunction (Stewart and Hirani, 2007). In a sample of 197 subjects, antibodies to known periodontal pathogens predicted the development of AD years before its clinical diagnosis (Sparks Stein et al., 2012). However, previous clinical studies included subjects with a broad range of cognitive performance by including cognitively impaired subjects. Our study used robust standardized criteria to define medically and cognitively “normal” subjects, thus minimizing the effect of cognitive and other confounds on periodontal and brain health.

The strongest correlation between measures of periodontal disease and amyloid retention was achieved using 3 mm as the threshold for the clinical attachment loss. Although not as strong, CAL4 correlation to amyloid was also significant, and PD3 approached statistical significance in some areas. BOP, a marker of current inflammation, was not significant. When interpreting our results, it is important to bear in mind that our subject population is receiving good oral care. These findings are consistent with our hypothesis that long-term inflammatory burden is more important than current inflammation. These results also suggest that even mild cases of disease can have long-term effects as found previously (Demmer et al., 2008).

Considerable in vitro animal model and clinical data show that peripheral inflammations and infections are sufficient to increase brain amyloid load possibly by augmenting A β synthesis (Weintraub et al., 2013), disrupting the brain blood barrier, and/or A β trafficking (Erickson et al., 2012). Further clinical evidence for the importance of inflammation in brain amyloid accumulation is suggested by the data coming from the Alzheimer's Disease Neuroimaging Initiative. This study reported that inflammatory molecules such as chemokine ligand 13, interleukin (IL)-17, fibrinogen, alpha-1-antitrypsin, complement C3, IL-3, and IL-13 were associated with PIB retention (Kiddle et al., 2012).

Infection-induced brain amyloid load has also been reviewed critically in the literature (Miklosy, 2008, 2011a, 2011b). It has been reported that spirochetal and chronic bacterial infections can cause cognitive decline and brain amyloid deposition (Miklosy, 2008, 2011a; Miklosy et al., 2004, 2006). A classic example of infection is the atrophic form of general paresis caused by *Treponema pallidum*, a spirochete that presents with progressive dementia and brain amyloid deposits. Miklosy (1993); Miklosy et al. (1994) proposed that oral spirochetes may be the possible candidates to invade the brain and cause cognitive impairment in AD. Riviere et al. (2002) detected 6 different periodontal pathogen treponemes in the brains of >90% of the 16 AD cases analyzed. Moreover, *P. gingivalis*-derived lipopolysaccharide was also detected in the brains of AD patients (Poole et al., 2013). It is accepted that periodontal bacteria can be found at distant sites (Cavrini et al., 2005; Haraszthy et al., 2000; Okuda et al., 2001; Reichert et al., 2013). These data indicate that peripheral inflammation/infections can lead to infection/inflammation in the brain and promote amyloid accumulation. Whether the amyloid constitutes an immune

protective molecule, an injurious agent, or both remains to be established (Castellani et al., 2009; Soscia et al., 2010; White et al., 2014).

Our results can also have other explanations. Reduced masticatory abilities because of periodontal disease may result in dietary deficiencies and increased stress response, and these may lead to increased A β beta (Ekuni et al., 2013). It is also possible that the relationship between CAL and amyloid load is related to a relationship between CAL and cognitive dysfunction. However, our subjects are cognitively normal, and the inclusion of Logic2 was not significant in the models. It is also possible that people with periodontal disease also have poorer systemic health. However, our use of exclusion criteria mitigated this effect. Still another explanation may be related to host response (hyperinflammatory) that could affect both periodontal disease and brain pathology (Kamer et al., 2008a, 2008b).

Among the multiple covariates investigated, smoking associated with PIB retention in MaskAD but only at trend level ($p = 0.067$). Smoking is a risk factor for periodontal disease, and we expected that smoking would negatively confound the association between CAL and PIB retention. Contradictory to our prediction, smoking did not downregulate the CAL effect on PIB retention. Controversy exists regarding the associations between smoking and AD. Some studies found that smoking may provide a protective effect (Brenner et al., 1993), whereas most studies showed a deleterious effect (Anstey et al., 2007; Cataldo et al., 2010; Reitz et al., 2011). Moreover, in an animal model, it was shown that smoking was able to upregulate brain amyloid possibly through brain inflammation (Moreno-Gonzalez et al., 2013). It is tempting to speculate that perhaps a common inflammatory mechanism for CAL and smoking leading to amyloid increase may explain these results. However, this sample size is small. Additional longitudinal studies are needed to untangle the relationship among smoking, periodontal disease, and AD pathology.

4.2. Strengths and weaknesses

As in most cross-sectional studies, reverse causation should also be considered, as the direction of the associations observed in these studies cannot be determined. The observed association between CAL and brain amyloid accumulation and cognitive dysfunction may reflect the effects of amyloid load/cognition on periodontal health. Equally possible is that people with poor cognition have poorer oral health, and several studies have shown that oral health measures such as tooth loss, caries level, and plaque control are impaired in subjects with cognitive impairment or dementia (Ellefsen et al., 2008, 2009; Ship, 1992). However, our subjects are defined cognitively normal by robust tests. The presence of periodontal disease in individuals with lower cognitive function or dementia is contradictory (Ship, 1992; Yu and Kuo, 2008). However, the potential effect of cognition on periodontal condition cannot be ignored (Kaye et al., 2010).

Our sample size was modest; therefore, the results of this study should be considered in this context. We observed statistically significant associations between measures of periodontal disease and brain amyloid load even after controlling for covariates because of our population characteristics. Our sample was quite homogeneous with respect to having excluded confounding measures. The cohort had high education, good systemic health, high cognitive function, and lost few teeth. On the other hand, this homogeneity allowed us to detect statistically significant differences despite the limited number of subjects. All medical and dental examinations were standardized, and 1 trained periodontist performed all periodontal evaluations blind to both PIB retention data, thus minimizing observer bias. A potential weakness is that some

measurement misclassification of exposure variables was possible as oral health behaviors were assessed by subject report and recollection. An additional bias may be related to the participants themselves. Although our sample was derived from the community, the participants were self-selected, thus introducing a potential bias. Notably, 95% of our subjects were white, 42% were ApoE carriers, and most of them currently have good oral care. Finally, although homogeneity of the study population constituted a strength of our project, it also limited the generalizability of our findings.

In conclusion, we showed that after accounting for the relevant confounds, measures of periodontal disease were associated with amyloid accumulation in brain in areas that are prone to amyloid accumulation in patients with AD. Our results suggest that periodontal inflammation/infection may increase the risk for brain amyloid deposition. Future longitudinal and therapeutic studies involving changes in periodontal disease could potentially reveal what is the cause and what is the effect.

Disclosure statement

No conflict of interest is reported for A.R. Kamer, P. Corby, R.G. 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 New York University (NYC), 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 Inc. Dr L. Glodzik 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.

Acknowledgements

This study was supported by NIH/National Institutes on Aging grants AG035137, AG032554, AG12101, AG022374, and AG13616; NIH DE023139-02; Alzheimer's Association New Investigator Research Grant (NIRG-12-173937); NIH/NCATS 8 UL1 TR000038; NYU College of Dentistry; and Bluestone Center for Clinical Research. Contributors: ARK, MJdeL, RGC, LG, and DS designed the study; ARK and EP analyzed the data with assistance from MJdeL; ARK, MJdeL, and LP interpreted the data; ARK wrote the manuscript with assistance from MJdeL, LG, RGC, and LM; PM and SW performed medical examinations and collected the cognitive data; ARK performed the oral examinations assisted by PC, RL, SS, and CS; and 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.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2014.10.038>.

References

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T., 2000. Inflammation and Alzheimer's disease. *Neurobiol. Aging* 21, 383–421.

Alzheimer's Association, 2014. 2014 Alzheimer's disease facts and figures. *Alzheimers Dement.* 10, e47–e92.

Anstey, K.J., von Sanden, C., Salim, A., O'Kearney, R., 2007. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. *Am. J. Epidemiol.* 166, 367–378.

Barba, R., Zapatero, A., Losa, J.E., Valdes, V., Todoli, J.A., Di Micco, P., Monreal, M., Riete, I., 2008. Body mass index and mortality in patients with acute venous thromboembolism: findings from the RIETE registry. *J. Thromb. Haemost.* 6, 595–600.

Beck, J.D., Elter, J.R., Heiss, G., Couper, D., Mauriello, S.M., Offenbacher, S., 2001. Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. *Arterioscler. Thromb. Vasc. Biol.* 21, 1816–1822.

Beekly, D.L., Ramos, E.M., Lee, W.W., Deitrich, W.D., Jacka, M.E., Wu, J., Hubbard, J.L., Koepsell, T.D., Morris, J.C., Kukull, W.A., NIA Alzheimer's Disease Centers, 2007. The National Alzheimer's Coordinating Center (NACC) database: the Uniform Data Set. *Alzheimer Dis. Assoc. Disord.* 21, 249–258.

Berg, L., 1984. Clinical dementia rating. *Br. J. Psychiatry* 145, 339.

Braak, H., Braak, E., 1997. Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol. Aging* 18, 351–357.

Brenner, D.E., Kukull, W.A., van Belle, G., Bowen, J.D., McCormick, W.C., Teri, L., Larson, E.B., 1993. Relationship between cigarette smoking and Alzheimer's disease in a population-based case-control study. *Neurology* 43, 293–300.

Castellani, R.J., Lee, H.G., Siedlak, S.L., Nunomura, A., Hayashi, T., Nakamura, M., Zhu, X., Perry, G., Smith, M.A., 2009. Reexamining Alzheimer's disease: evidence for a protective role for amyloid-beta protein precursor and amyloid-beta. *J. Alzheimers Dis.* 18, 447–452.

Cataldo, J.K., Prochaska, J.J., Glantz, S.A., 2010. Cigarette smoking is a risk factor for Alzheimer's disease: an analysis controlling for tobacco industry affiliation. *J. Alzheimers Dis.* 19, 465–480.

Cavirini, F., Sambri, V., Moter, A., Servidio, D., Marangoni, A., Montebugnoli, L., Foschi, F., Prati, C., Di Bartolomeo, R., Cevenini, R., 2005. Molecular detection of *Treponema denticola* and *Porphyromonas gingivalis* in carotid and aortic atheromatous plaques by FISH: report of two cases. *J. Med. Microbiol.* 54 (Pt 1), 93–96.

Cockrell, J.R., Folstein, M.F., 1988. Mini-mental state examination (MMSE). *Psychopharmacol. Bull.* 24, 689–692.

De Santi, S., Pirraglia, E., Barr, W., Babb, J., Williams, S., Rogers, K., Glodzik, L., Brys, M., Mosconi, L., Reisberg, B., Ferris, S., de Leon, M.J., 2008. Robust and conventional neuropsychological norms: diagnosis and prediction of age-related cognitive decline. *Neuropsychology* 22, 469–484.

Demmer, R.T., Kocher, T., Schwahn, C., Volzke, H., Jacobs Jr., D.R., Desvarieux, M., 2008. Refining exposure definitions for studies of periodontal disease and systemic disease associations. *Community Dent. Oral Epidemiol.* 36, 493–502.

Eke, P.I., Dye, B.A., Wei, L., Thornton-Evans, G.O., Genco, R.J., CDC Periodontal Disease Surveillance Workgroup: James Beck, Gordon Douglass, Roy Page, 2012. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J. Dent. Res.* 91, 914–920.

Ekuni, D., Endo, Y., Tomofuji, T., Azuma, T., Irie, K., Kasuyama, K., Morita, M., 2013. Effects of apoE deficiency and occlusal disharmony on amyloid-beta production and spatial memory in rats. *PLoS One* 8, e74966.

Ellefsen, B., Holm-Pedersen, P., Morse, D.E., Schroll, M., Andersen, B.B., Waldemar, G., 2008. Caries prevalence in older persons with and without dementia. *J. Am. Geriatr. Soc.* 56, 59–67.

Ellefsen, B., Holm-Pedersen, P., Morse, D.E., Schroll, M., Andersen, B.B., Waldemar, G., 2009. Assessing caries increments in elderly patients with and without dementia: a one-year follow-up study. *J. Am. Dent. Assoc.* 140, 1392–1400.

Elter, J.R., Champagne, C.M., Offenbacher, S., Beck, J.D., 2004. Relationship of periodontal disease and tooth loss to prevalence of coronary heart disease. *J. Periodontol.* 75, 782–790.

Erickson, M.A., Hartvigson, P.E., Morofuji, Y., Owen, J.B., Butterfield, D.A., Banks, W.A., 2012. Lipopolysaccharide impairs amyloid beta efflux from brain: altered vascular sequestration, cerebrospinal fluid reabsorption, peripheral clearance and transporter function at the blood-brain barrier. *J. Neuroinflammation* 9, 150–165.

Gatz, M., Mortimer, J.A., Fratiglioni, L., Johansson, B., Berg, S., Reynolds, C.A., Pedersen, N.L., 2006. Potentially modifiable risk factors for dementia in identical twins. *Alzheimers Dement.* 2, 110–117.

Grabe, H.J., Schwahn, C., Volzke, H., Spitzer, C., Freyberger, H.J., John, U., Mundt, T., Biffar, R., Kocher, T., 2009. Tooth loss and cognitive impairment. *J. Clin. Periodontol.* 36, 550–557.

Griffin, W.S., Sheng, J.G., Royston, M.C., Gentleman, S.M., McKenzie, J.E., Graham, D.I., Roberts, G.W., Mrak, R.E., 1998. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol.* 8, 65–72.

Haffajee, A.D., Socransky, S.S., 2006. Introduction to microbial aspects of periodontal biofilm communities, development and treatment. *Periodontol.* 2000 42, 7–12.

Haraszthy, V.I., Zambon, J.J., Trevisan, M., Zeid, M., Genco, R.J., 2000. Identification of periodontal pathogens in atheromatous plaques. *J. Periodontol.* 71, 1554–1560.

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., Jones, R.W., Bullock, R., Love, S., Neal, J.W., Zotova, E., Nicoll, J.A., 2008. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 372, 216–223.

Holmes, C., Butchart, J., 2011. Systemic inflammation and Alzheimer's disease. *Biochem. Soc. Trans.* 39, 898–901.

Holmes, C., Cotterell, D., 2009. Role of infection in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 23, 993–1002.

- Jack Jr., C.R., Lowe, V.J., Weigand, S.D., Wiste, H.J., Senjem, M.L., Knopman, D.S., Shiung, M.M., Gunter, J.L., Boeve, B.F., Kemp, B.J., Weiner, M., Petersen, R.C., 2009. Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. *Brain* 132 (Pt 5), 1355–1365.
- Jonsson, D., Spinell, T., Vrettos, A., Stocklin-Wasmer, C., Celenti, R., Demmer, R.T., Kebschull, M., Papapanou, P.N., 2014. Circulating endothelial progenitor cells in periodontitis. *J. Periodontol.* 85, 1–14.
- Kamer, A.R., Craig, R.G., Dasanayake, A.P., Brys, M., Glodzik-Sobanska, L., de Leon, M.J., 2008a. Inflammation and Alzheimer's disease: possible role of periodontal diseases. *Alzheimers Dement.* 4, 242–250.
- Kamer, A.R., Dasanayake, A.P., Craig, R.G., Glodzik-Sobanska, L., Bry, M., de Leon, M.J., 2008b. Alzheimer's disease and peripheral infections: the possible contribution from periodontal infections, model and hypothesis. *J. Alzheimers Dis.* 13, 437–449.
- Kamer, A.R., Morse, D.E., Holm-Pedersen, P., Mortensen, E.L., Avlund, K., 2012. Periodontal inflammation in relation to cognitive function in an older adult Danish population. *J. Alzheimers Dis.* 28, 613–624.
- Kaye, E.K., Valencia, A., Baba, N., Spiro 3rd, A., Dietrich, T., Garcia, R.I., 2010. Tooth loss and periodontal disease predict poor cognitive function in older men. *J. Am. Geriatr. Soc.* 58, 713–718.
- Kiddle, S.J., Thambisetty, M., Simmons, A., Riddoch-Contreras, J., Hye, A., Westman, E., Pike, I., Ward, M., Johnston, C., Lupton, M.K., Lunnon, K., Soininen, H., Kłoszewska, I., Tsolaki, M., Vellas, B., Mecocci, P., Lovestone, S., Newhouse, S., Dobson, R., Alzheimers Disease Neuroimaging Initiative, 2012. Plasma based markers of [11C] PiB-PET brain amyloid burden. *PLoS One* 7, e44260.
- Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergstrom, M., Savitcheva, I., Huang, G.F., Estrada, S., Aussen, B., Debnath, M.L., Barletta, J., Price, J.C., Sandell, J., Lopresti, B.J., Wall, A., Koivisto, P., Antoni, G., Mathis, C.A., Langstrom, B., 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.* 55, 306–319.
- Kok, E., Haikonen, S., Luoto, T., Huhtala, H., Goebeler, S., Haapasalo, H., Karhunen, P.J., 2009. Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. *Ann. Neurol.* 65, 650–657.
- Landau, S.M., Marks, S.M., Mormino, E.C., Rabinovici, G.D., Oh, H., O'Neil, J.P., Wilson, R.S., Jagust, W.J., 2012. Association of lifetime cognitive engagement and low beta-amyloid deposition. *Arch. Neurol.* 69, 623–629.
- Li, Y., Rinne, J.O., Mosconi, L., Pirraglia, E., Rusinek, H., DeSanti, S., Kemppainen, N., Nagren, K., Kim, B.C., Tsui, W., de Leon, M.J., 2008. Regional analysis of FDG and PIB-PET images in normal aging, mild cognitive impairment, and Alzheimer's disease. *Eur. J. Nucl. Med. Mol. Imaging* 35, 2169–2181.
- McGeer, P.L., Rogers, J., McGeer, E.G., 2006. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. *J. Alzheimers Dis.* 9 (3 Suppl), 271–276.
- Miklossy, J., 1993. Alzheimer's disease—a spirochetosis? *Neuroreport* 4, 1069.
- Miklossy, J., 2008. Chronic inflammation and amyloidogenesis in Alzheimer's disease—role of Spirochetes. *J. Alzheimers Dis.* 13, 381–391.
- Miklossy, J., 2011a. Alzheimer's disease—a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. *J. Neuroinflammation* 8, 90–106.
- Miklossy, J., 2011b. Emerging roles of pathogens in Alzheimer disease. *Expert Rev. Mol. Med.* 13, e30–e64.
- Miklossy, J., Kasas, S., Janzer, R.C., Ardizzone, F., Van der Loos, H., 1994. Further ultrastructural evidence that spirochaetes may play a role in the aetiology of Alzheimer's disease. *Neuroreport* 5, 1201–1204.
- Miklossy, J., Khalili, K., Gern, L., Ericson, R.L., Darekar, P., Bolle, L., Hurlimann, J., Paster, B.J., 2004. Borrelia burgdorferi persists in the brain in chronic Lyme neuroborreliosis and may be associated with Alzheimer disease. *J. Alzheimers Dis.* 6, 639–649; discussion 73–81.
- Miklossy, J., Kis, A., Radenovic, A., Miller, L., Forro, L., Martins, R., Reiss, K., Darbinian, N., Darekar, P., Mihaly, L., Khalili, K., 2006. Beta-amyloid deposition and Alzheimer's type changes induced by Borrelia spirochetes. *Neurobiol. Aging* 27, 228–236.
- Moreno-Gonzalez, I., Estrada, L.D., Sanchez-Mejias, E., Soto, C., 2013. Smoking exacerbates amyloid pathology in a mouse model of Alzheimer's disease. *Nat. Commun.* 4, 1495.
- Morgan, D., 2011. Immunotherapy for Alzheimer's disease. *J. Intern. Med.* 269, 54–63.
- Mosconi, L., Andrews, R.D., Matthews, D.C., 2013a. Comparing brain amyloid deposition, glucose metabolism, and atrophy in mild cognitive impairment with and without a family history of dementia. *J. Alzheimers Dis.* 35, 509–524.
- Mosconi, L., Rinne, J.O., Tsui, W.H., Berti, V., Li, Y., Wang, H., Murray, J., Scheinin, N., Nagren, K., Williams, S., Glodzik, L., De Santi, S., Vallabhajosula, S., de Leon, M.J., 2010. Increased fibrillar amyloid- β burden in normal individuals with a family history of late-onset Alzheimer's. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5949–5954.
- Mosconi, L., Rinne, J.O., Tsui, W.H., Murray, J., Li, Y., Glodzik, L., McHugh, P., Williams, S., Cummings, M., Pirraglia, E., Goldsmith, S.J., Vallabhajosula, S., Scheinin, N., Viljanen, T., Nagren, K., de Leon, M.J., 2013b. Amyloid and metabolic positron emission tomography imaging of cognitively normal adults with Alzheimer's parents. *Neurobiol. Aging* 34, 22–34.
- Mosconi, L., Tsui, W.H., De Santi, S., Li, J., Rusinek, H., Convit, A., Li, Y., Boppana, M., de Leon, M.J., 2005. Reduced hippocampal metabolism in MCI and AD: automated FDG-PET image analysis. *Neurology* 64, 1860–1867.
- Mosconi, L., Tsui, W., Murray, J., McHugh, P., Li, Y., Williams, S., Pirraglia, E., Glodzik, L., De Santi, S., Vallabhajosula, S., de Leon, M.J., 2012. Maternal age affects brain metabolism in adult children of mothers affected by Alzheimer's disease. *Neurobiol. Aging* 33, 624.e1–624.e9.
- Muller-Gartner, H.W., Links, J.M., Prince, J.L., Bryan, R.N., McVeigh, E., Leal, J.P., Davatzikos, C., Frost, J.J., 1992. Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. *J. Cereb. Blood Flow Metab.* 12, 571–583.
- National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 106, 3143–3421.
- Noble, J.M., Borrell, L.N., Papapanou, P.N., Elkind, M.S., Scarmeas, N., Wright, C.B., 2009. Periodontitis is associated with cognitive impairment among older adults: analysis of NHANES-III. *J. Neurol. Neurosurg. Psychiatry* 80, 1206–1211.
- Okuda, K., Ishihara, K., Nakagawa, T., Hirayama, A., Inayama, Y., Okuda, K., 2001. Detection of *Treponema denticola* in atherosclerotic lesions. *J. Clin. Microbiol.* 39, 1114–1117.
- Osorio, R.S., Pirraglia, E., Gumb, T., Mantua, J., Ayappa, I., Williams, S., Mosconi, L., Glodzik, L., de Leon, M.J., 2014. Imaging and cerebrospinal fluid biomarkers in the search for Alzheimer's disease mechanisms. *Neurodegener. Dis.* 13, 163–165.
- Ozudogru, S.N., Lippa, C.F., 2012. Disease modifying drugs targeting beta-amyloid. *Am. J. Alzheimers Dis. Other Dement.* 27, 296–300.
- Page, R.C., Eke, P.I., 2007. Case definitions for use in population-based surveillance of periodontitis. *J. Periodontol.* 78 (7 Suppl), 1387–1399.
- Paraskevas, S., Huizinga, J.D., Loos, B.G., 2008. A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J. Clin. Periodontol.* 35, 277–290.
- Perry, V.H., Newman, T.A., Cunningham, C., 2003. The impact of systemic infection on the progression of neurodegenerative disease. *Nat. Rev. Neurosci.* 4, 103–112.
- Poole, S., Singhrao, S.K., Kesavalu, L., Curtis, M.A., Crean, S., 2013. Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *J. Alzheimers Dis.* 36, 665–677.
- Reichert, S., Haffner, M., Keyser, G., Schafer, C., Stein, J.M., Schaller, H.G., Wienke, A., Strauss, H., Heide, S., Schulz, S., 2013. Detection of oral bacterial DNA in synovial fluid. *J. Clin. Periodontol.* 40, 591–598.
- Reisberg, B., Ferris, S.H., de Leon, M.J., Crook, T., 1982. The Global Deterioration Scale for assessment of primary degenerative dementia. *Am. J. Psychiatry* 139, 1136–1139.
- Reitz, C., Brayne, C., Mayeux, R., 2011. Epidemiology of Alzheimer disease. *Nat. Rev. Neurol.* 7, 137–152.
- Rivest, S., 2003. Molecular insights on the cerebral innate immune system. *Brain Behav. Immun.* 17, 13–19.
- Riviere, G.R., Riviere, K.H., Smith, K.S., 2002. Molecular and immunological evidence of oral Treponema in the human brain and their association with Alzheimer's disease. *Oral Microbiol. Immunol.* 17, 113–118.
- Ship, J.A., 1992. Oral health of patients with Alzheimer's disease. *J. Am. Dent. Assoc.* 123, 53–58.
- Silness, J., Loe, H., 1964. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol. Scand.* 22, 121–135.
- Socransky, S.S., Haffajee, A.D., 1997. The nature of periodontal diseases. *Ann. Periodontol.* 2, 3–10.
- Socransky, S.S., Haffajee, A.D., 2002. Dental biofilms: difficult therapeutic targets. *Periodontol* 2000 28, 12–55.
- Soscia, S.J., Kirby, J.E., Washicosky, K.J., Tucker, S.M., Ingelsson, M., Hyman, B., Burton, M.A., Goldstein, L.E., Duong, S., Tanzi, R.E., Moir, R.D., 2010. The Alzheimer's disease-associated amyloid β -protein is an antimicrobial peptide. *PLoS One* 5, e9505–e9515.
- Sparks Stein, P., Steffen, M.J., Smith, C., Jicha, G., Ebersole, J.L., Abner, E., Dawson 3rd, D., 2012. Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. *Alzheimers Dement.* 8, 196–203.
- Stein, P.S., Desrosiers, M., Donegan, S.J., Yepes, J.F., Kryscio, R.J., 2007. Tooth loss, dementia and neuropathology in the Nun Study. *J. Am. Dent. Assoc.* 138, 1314–1322.
- Stewart, R., Hirani, V., 2007. Dental health and cognitive impairment in an English national survey population. *J. Am. Geriatr. Soc.* 55, 1410–1414.
- Tanzi, R.E., 2012. The genetics of Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 2, 1–10.
- Tonetti, M.S., Claffey, N., European Workshop in Periodontology group, C., 2005. Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *J. Clin. Periodontol.* 32 (Suppl 6), 210–213.
- Weintraub, M.K., Bisson, C.M., Nouri, J.N., Vinson, B.T., Eimerbrink, M.J., Kranjac, D., Boehm, G.W., Chumley, M.J., 2013. Imatinib methanesulfonate reduces hippocampal amyloid- β and restores cognitive function following repeated endotoxin exposure. *Brain Behav. Immun.* 33, 24–28.
- White, M.R., Kandel, R., Tripathi, S., Condon, D., Qi, L., Taubenberger, J., Hartshorn, K.L., 2014. Alzheimer's associated beta-amyloid protein inhibits influenza A virus and modulates viral interactions with phagocytes. *PLoS One* 9, e101364.
- Yu, Y.H., Kuo, H.K., 2008. Association between cognitive function and periodontal disease in older adults. *J. Am. Geriatr. Soc.* 56, 1693–1697.