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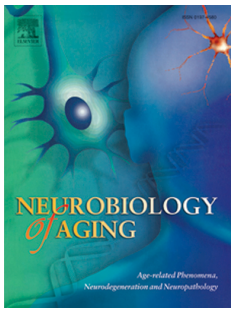

Tooth loss is associated with accelerated cognitive decline and volumetric brain differences: a population-based study

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Tooth loss is associated with accelerated cognitive decline and volumetric brain differences: a population-based study

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ABSTRACT

Tooth loss has been related to cognitive impairment, however its relation to structural brain differences in humans is unknown. Dementia-free participants (*n*= 2715) age ≥60 years were followed for up to 9 years. A sub-sample (*n*=394) underwent MRI at baseline. Information on tooth loss was collected at baseline and cognitive function was assessed using the Mini-Mental State Examination (MMSE) at baseline and at follow-ups. Data were analyzed using linear mixed-effects models and linear regression models. At baseline, 404 (14.9%) participants had partial tooth loss, and 206 (7.6%) had complete tooth loss. Tooth loss was significantly associated with a steeper cognitive decline (β: -0.18, 95% CI: -0.24 to -0.11), and remained significant after adjusting for or stratifying by potential confounders. In cross-sectional analyses, persons with complete or partial tooth loss had significantly lower total brain volume (β: -28.89, 95% CI: -49.33 to -8.45) and gray-matter volume (β: -22.60, 95% CI: -38.26 to -6.94). Thus, tooth loss may be a risk factor for accelerated cognitive aging.

Keywords: Cohort Study; Neuroimaging; Tooth Loss; Cognitive Aging; Gray-Matter.

1. INTRODUCTION

The global population is living longer, which has led to a rise in the prevalence of dementia. Worldwide, the number of people suffering from dementia is around 46.8 million, and this number is predicted to double every 5 years (Livingston et al., 2017). Cognitive decline in old age is a common phenomenon, however it remains unclear why certain people decline more and faster than others. Treating dementia once the diagnosis has been established is challenging and limited to symptomatic alleviation. It is therefore imperative to target modifiable factors, which may exacerbate cognitive decline and subsequent dementia.

Recently, poor dental health has been proposed as such a factor. Globally, the prevalence of edentulism (complete tooth loss) is 2.4% (Kassebaum et al., 2014) and around 26% in adults over 75 (Dye et al., 2015). Tooth loss in older adults represents the final outcome of dental conditions, such as caries and periodontitis (Petersen et al., 2005) and has been related to worse performance on cognitive tests, as well as the Mini-Mental State Examination (MMSE) in longitudinal studies (Cerutti-Kopplin et al., 2016), but with some inconsistent findings (Naorungroj et al. 2015; Tsakos et al. 2015). Additionally, an increased risk of cognitive impairment and dementia in individuals with severe tooth loss has been reported in some studies (Gatz et al., 2006; Kim et al, 2007; Luo et al., 2015; Okamoto et al., 2010; 2015; 2017; Park et al., 2013; Peres et al., 2014; Saito et al, 2013; and Shimazaki et al., 2001; Yamamoto et al, 2012). Other studies have not found such associations (Shimazaki et al., 2001; Avlund et al., 2004; Chen et al., 2010; Elsig et al., 2015; Hansson et al., 2014; Matthews et al., 2011; Starr et al., 2008).

The discrepant findings among these studies may be due to differences in study design (population-based or clinical settings), length of follow-up time, different assessments of tooth loss and cognitive function and insufficient adjustment for potential confounders.

Tooth loss is often a marker of chronic oral inflammation and has therefore been suggested as a pathway for systemic inflammation and atherosclerosis conditions, such as cardiovascular diseases (CVDs) and cerebrovascular disease (de Oliveira et al., 2010; Desvarieux et al., 2003; Joshipura et al., 2003; Paquette et al., 2007). However, the role of systemic inflammation and vascular diseases in the relationship between tooth loss and cognitive decline remains unclear.

Although recent investigations suggest that there may be a link between poor dental health and accelerated cognitive decline, studies on the impact of tooth loss on brain aging are lacking. Volumetric brain changes following tooth loss have been observed in mice, compared to those with no tooth loss (Avivi-Arber et al., 2017). To date, structural brain differences in relation to tooth loss have not been investigated in humans. In this study, we aimed to: 1) investigate the longitudinal association between tooth loss and cognitive decline, using 9-year follow-up data from a population-based cohort study; 2) examine the role of inflammation, vascular diseases and other potential confounders in the tooth loss-cognitive decline association; and 3) explore the cross-sectional relationship between tooth loss and structural brain differences, using Magnetic Resonance Imaging (MRI).

2. METHODS

2.1 Study population. The study participants were derived from the Swedish National study on Aging and Care-Kungsholmen (SNAC-K), a longitudinal project focusing on the aging process and the Swedish care system (Lagergren et al., 2004). SNAC-K participants were a random sample of individuals aged 60+ years living at home or in institutions in the Kungsholmen district, a central area in Stockholm, Sweden. Because of more rapid changes in health among older age groups, the sampling is was stratified by age cohort. Assessments took place at 6-year intervals for younger cohorts (60, 66, and 72 years), and at 3-year

intervals for older cohorts (78, 81, 84, 87, 90, 93, 96, and 99+ years). Of the 5111 persons initially invited to participate, 4590 were alive and eligible and 3363 (73.3%) agreed to be part of the baseline survey (March 2001 through June 2004). Of the 3363 participants at baseline, we excluded 240 participants with definite dementia, 70 with questionable dementia, 41 with neurological/developmental disorders, 282 with missing data on dental status and 15 with missing Mini-Mental State Examination (MMSE) score, leaving 2715 participants for the current study. During the 9-year follow-up, 782 were examined at the first follow-up (2004–2007). At the second follow-up (2007-2010), 1803 were examined, and 600 were examined at the third follow-up (2010-2013). Throughout the follow-up period, 861 died and 307 dropped out (Figure A.1). Written informed consent was obtained from all participants or from informants when the person was cognitively impaired. The Regional Ethical Review Board and the Central Ethical Review Board in Stockholm, Sweden approved the protocols for data collection at baseline and follow-ups.

2.2 Baseline data collection. Data on demographic factors (age, sex, and education) and lifestyle factors (smoking and alcohol consumption), were collected through personal interviews by nurses following a structured protocol (http://www.snac-k.se/). Educational level was categorized into elementary school, high school, or university. Smoking status (non-smokers for those who had never smoked, former smokers or current smokers) and alcohol consumption (low/never, moderate or heavy) were trichotomized (Wang et al., 2014). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. As a measure of functional ability, walking speed was tested by asking participants to walk 6 meters in their usual pace, or 2.4 m if the participant reported walking quite slowly, and recorded in meters per second. In this test, a walking aid was allowed (Welmer et al., 2014). Chronic diseases, including diabetes and CVDs (including heart failure, arrhythmia, bradycardias and conduction diseases, cardiac valve disease and ischemic heart disease), were

ascertained from examinations by physicians, self-reported medical history, medication use, or linkage with the Swedish National Patient Register. The tenth revision of the International Classification of Disease, (ICD-10) was used in the registry. Multimorbidity was defined as the presence of 2+ chronic diseases (Calderón-Larrañaga et al., 2016). Peripheral blood samples were taken from all participants. Apolipoprotein (*APOE*) allelic status was dichotomized into any epsilon 4 (ε4) carriers or ε4 non–carriers (Laukka et al., 2013). Haemoglobin (Hb) and Albumin (Alb) were measured as markers of nutritional status. Hb was measured using the Sodium Lauryl Sulphate method (Sysmex XE-5000, Sysmex Corp., Kobe, Japan). World Health Organization criteria for the diagnosis of anaemia (Hb concentration <130 g/l in men and <120 g/l in women) was used to dichotomize serum Hb levels (Patel, 2008). Albumin (Alb) was measured by Bromcresol Purple dye method (DXC800, Beckman Coulter, Brea, CA, USA) and hypoalbuminemia was defined as Alb concentrations <37 g/l (Fried et al, 1998). C-reactive protein (CRP) was measured as a marker of inflammation at baseline and at follow-ups, using a turbidimetric method (DXC800, Beckman Coulter) and was dichotomized as normal (0–5mg/l, lab reference value), or high (>5 mg/l).

2.2.1 Assessment of tooth loss. During the nurse interview at baseline, the participants were asked the following question: "Do you have your own natural teeth only or removable denture?", and the answer was classified as: 1) own teeth only; 2) own teeth with removable denture; 3) own teeth with removable denture in one jaw and full denture in one jaw; 4) complete tooth loss; 5) complete tooth loss and full denture in one or both jaws; or 6) implants. Dental status was categorized as no tooth loss (1), partial tooth loss (2; 3; 6), or complete tooth loss (4; 5) and these categories were used in the analyses (Welmer et al., 2017). Chewing ability was assessed with the following question: "Can you chew hard food such as hard bread or apples?" The answer was classified according to one of the options as:

1) yes, without difficulty; 2) yes, but I must be careful; and 3) no, not at all. These answers were categorized as: 1= No chewing difficulty; 2= Having mild chewing difficulty; and 3= Having severe chewing difficulty.

2.2.2 SNAC-K MRI sub-study. During the baseline survey (September 2001 to October 2003), non-institutionalized, non-disabled, and non-demented participants in SNAC-K were invited to undertake a structural brain MRI. In total, 555 individuals were scanned on a 1.5T scanner (Philips Intera, the Netherlands). Out of these, 394 participants free of dementia and neurological disorders, with available information on tooth loss and MMSE assessment were included in the analysis (Figure A.1).

The MRI protocol included an axial 3D T1-weighted fast field echo and an axial turbo fluid attenuated inversion recovery (FLAIR) sequence (Wang et al., 2014). Total gray-matter volume (GMV) and white-matter volume (WMV) were determined after automatic segmentation of the T1 images in native space using the SPM12 software (http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab (The Mathworks) (Ashburner and Friston, 2005). All gray matter, white matter and cerebral spinal fluid (CSF) segments were individually scrutinized for verification of the segmentation accuracy. Total brain volume (TBV) was defined as the sum of gray matter and white matter. Hippocampal volume (HV) was obtained through freesurfer automatic segmentation. White-matter hyperintensities (WMHs) were manually delineated on the FLAIR images (for details about the procedure, see Köhncke et al., 2016). Total intracranial volume (TIV) was calculated by summing up of GMV, WMV and CSF. All MRI measurements were adjusted for TIV and age (Raz et al., 2005). The total volumes of gray matter, white matter, hippocampus and WMHs were measured as the investigation broadly examined the associations of tooth loss and MRI markers of neurodegeneration and cerebral small vessel disease, so as to generate hypotheses for future prospective studies.

2.3 Assessment of cognitive functioning. Cognitive functioning was assessed at baseline and at each follow-up with the MMSE, a 30-point test that is used to measure global cognitive functioning (Folstein et al., 1975). This test includes questions about different functions such as orientation to time and place, attention, calculation, memory, language, and visuospatial ability. Dementia was diagnosed at baseline and at follow-ups following the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM–IV) criteria, using a validated three–step procedure. Two physicians independently made a preliminary diagnosis and, in case of disagreement, a third opinion was sought from a senior physician to reach a concordant diagnosis (Fratiglioni et al., 1992). Based on the DSM-IV criteria for dementia, participants who fulfilled all the criteria were classified as "definite" dementia, whereas when impairment in memory or other cognitive abilities was questionable, they were classified as "questionable" dementia.

2.4 Statistical analysis

Participant characteristics across dental status groups were compared using the chi-square ($\chi 2$) tests for categorical variables and one-way ANOVAs for continuous variables. The change in MMSE score over the study period was obtained from the repeated measures of individual MMSE scores at baseline and follow-ups, and was considered as the main outcome. Mixed-effects linear regression was used to examine the association of each tooth loss category with MMSE changes over time. The fixed effects of the model included the exposure of interest, linear annual follow-up time, and their interaction term. Random effects included intercept and slope for time. Model 1 was adjusted for demographic factors (age, sex and education) and model 2 was further adjusted for smoking, alcohol consumption, multimorbidity, walking speed, diabetes, *APOE* ε4 allele, MMSE score at baseline, Hb and Alb. Model 3 was further adjusted for CRP at baseline and follow ups (time-varying), and model 4 was additionally adjusted for CVDs and cerebrovascular disease at baseline. To further test whether CVDs,

cerebrovascular disease, or inflammation might account for the association between tooth loss and cognitive decline, stratified analyses were performed.

For the cross-sectional MRI data analysis, linear regressions were performed to measure the relationship between tooth loss and regional brain volumes and WMHs (TIV and age-adjusted). The linear regression models were adjusted for sex, education, CVDs, CRP, multimorbidity and *APOE* ε4 allele status. From a power calculation for repeated measures, based on the mean MMSE differences in the tooth loss groups at baseline and the correlation between the repeated MMSE assessments with $\alpha = 0.05$, power= 0.80, the sample size of SNAC-K (*n*=2715), was deemed sufficient to verify our hypotheses. All statistical analyses were performed using Stata SE 14.0 for Windows (StataCorp LP).

3. RESULTS

3.1 Characteristics of the study population. Out of the 2715 participants, 1706 (62.8%) were women. There were 1587 participants (58.5%) in the younger cohorts (aged 60-72 years), and 1128 (41.5%) in the older cohorts (aged ≥78 years) at baseline. The prevalence of any tooth loss was 22.5%, including 14.9% for partial loss and 7.6% for complete tooth loss (Table 1). Compared to participants with no tooth loss, those with partial or complete tooth loss were more likely to be older and smokers, have more vascular diseases, hypoalbuminemia, anemia, CRP level above 5 mg/l, greater multimorbidity, slower walking speed and lower education and baseline MMSE score (Table 1).

(Insert Table 1 here)

3.2 Longitudinal association between tooth loss and cognitive decline. At baseline, complete tooth loss was significantly associated with lower MMSE score compared to no tooth loss (Table 2, Model 1), however the association was no longer significant with further

adjustment (Table 2). Over the 9 year follow-up, both partial (β: -0.13, 95% CI: -0.20 to -0.05) and complete (β: -0.30, 95% CI: -0.42 to -0.18) tooth loss were significantly associated with steeper MMSE decline, compared to the no tooth loss group after multi-adjustment (Table 2, Model 4). In the multi-adjusted model, any tooth loss (partial or complete) was associated with an increased decline of 0.18 (95% CI: -0.24 to -0.11) MMSE score per year, compared to the no tooth loss group (Table 2, Model 4).

(Insert Table 2 here)

3.3 The role of vascular diseases and inflammation in the tooth loss-cognitive decline association. Adjustment for CRP >5 m/l as a time-varying variable, attenuated the association between tooth loss and cognitive decline, however it remained significant (Table 2, Model 3). In Model 4 we further adjusted for cardiovascular and cerebrovascular diseases, which did not modify the association. Furthermore, stratified analyses by CVDs, cerebrovascular disease and CRP >5 m/l (time-varying) were performed. The direction of the tooth loss-cognitive decline association was not affected by CVDs or CRP stratification, and became nonsignificant among people with cerebrovascular disease, likely due to lack of power (Table 3).

(Insert Table 3 here)

3.4 Cross-sectional association between tooth loss and structural brain differences. Compared to the full study population, the MRI sub-sample was significantly younger, had higher education, less CVDs, cerebrovascular disease, diabetes, hypoalbuminemia, anemia, multimorbidity and higher baseline MMSE, and walking speed (Table A.1). In multi-adjusted linear regression analyses, participants with complete tooth loss had significantly lower multi-adjusted TBV (β: -52.70, 95% CI: -8.79 to -16.61) compared to the no tooth loss group. In addition, significantly lower GMV was observed among the partial (β: -17.10, 95% CI: -33.54

to -0.67) and complete tooth loss group (β: -36.77, 95% CI: -62.78 to -10.77) compared to the no tooth loss group. A lower HV was observed in those with tooth loss compared no tooth loss, however this association did not reach significance, likely due to lack of power. There was no significant association between tooth loss and WMV or WMHs (Table 4 and Figure 1).

(Insert Table 4 and Figure 1 here)

3.5 Additional analysis. In additional analyses, to explore the possibility of reverse causation, we repeated the longitudinal analysis with the exclusion of participants who developed dementia during the 9-year follow-up period. After removing incident dementia cases (*n*= 283), the association of tooth loss with MMSE decline remained in the same direction but was no longer significant (Table A.2). The association between tooth loss and GMV remained significant (Table A.3). Further, we repeated all analysis by additional adjustment for, and stratification by chewing ability, dichotomized as: chewing difficulty (*n*= 366) and no chewing difficulty (*n*= 2,345), which showed similar results to those from the main analysis (data not shown).

4. DISCUSSION

In this large population-based cohort study, including an MRI sub-sample, we found that: 1) tooth loss is associated with accelerated cognitive decline over 9 years, especially in those with complete tooth loss; 2) the association between tooth loss and cognitive decline is not fully accounted for by CVDs, or higher inflammation; and 3) tooth loss is cross-sectionally associated with smaller TBV and GMV. Future studies with prospective MRI data are needed to further explore the role of neurodegeneration in the relation between tooth loss and cognitive decline.

A few population-based longitudinal studies have investigated the impact of tooth loss on cognitive decline or risk of cognitive impairment and dementia, however there is no clear evidence of a causal relationship. In a systematic review by Cerutti-Kopplin et al. (2016) 6 out of the included 10 studies, reported that number of teeth independently predicted cognitive impairment or dementia. Three other studies found no such association, and one found a lower risk of dementia attributable to edentulism among participants with low education. These discrepancies might be due to several reasons, such as differences in the age of the study population, assessment of tooth loss, and inclusion of people with preclinical dementia. In our study, we found an association between tooth loss and cognitive decline, with the novel finding of the cross-sectional association between tooth loss and lower TBV and GMV. However, the results from these exploratory MRI analyses should be interpreted with caution as the data were cross-sectional.

Previous studies have shown that tooth loss is related to elevated inflammatory biomarkers (Paraskevas et al., 2008; Passoja et al., 2010), which have been associated with cognitive impairment and dementia (Kamer et al., 2009; 2012; Sparks et al., 2012) as well as higher brain amyloid load in cognitively normal older adults (Kamer et al., 2015). In the main analysis, adjusting for inflammatory status (CRP >5 m/l) at baseline and follow-ups attenuated the association between tooth loss and cognitive decline, however stratifying by CRP did not modify the association in participants with complete tooth loss. Moreover, vascular diseases have been proposed as mechanisms in the relationship between poor dental health and cognitive decline. Several studies indicate that poor dental health is associated with the risk of CVDs and cerebrovascular disease (de Oliveira et al., 2010; Desvarieux et al., 2003; Joshipura et al., 2003; Paquette et al., 2007). In the present study, the relationship of tooth loss to cognitive decline remained statistically significant when adjusting for or stratifying by CVDs. Furthermore, tooth loss was significantly associated with cognitive

decline only in those without cerebrovascular disease. However, this might be due to power issues, given the low number of participants who had cerebrovascular disease in our study sample. These findings suggest that the effect of tooth loss on cognitive decline in our study may not be accounted for by vascular diseases or elevated CRP levels.

The possible neurobiological mechanisms behind the association between tooth loss and accelerated cognitive decline are not well known, however several hypotheses exist. Animal research suggests that edentulism may lead to neurodegeneration and impaired neurogenesis, particularly in the hippocampus (Almeida et al., 2012; Hirai et al., 2010; Onozuka et al., 2000; Kawahata et al., 2014) as well as widespread volumetric changes (Avivi-Arber et al., 2017). Up to date, no studies have examined structural brain differences in relation to tooth loss in humans. In our cross-sectional MRI sub-study, we found that dementia-free participants with tooth loss had lower TBV and GMV in comparison to those without tooth loss. Participants with complete tooth loss had lower HV compared to the no tooth loss group, and while it did not reach statistical significance, it supports findings from the animal studies (Almeida et al., 2012; Hirai et al., 2010; Onozuka et al., 2000; Kawahata et al., 2014). Furthermore, the association between tooth loss and TBV and GMV were not modified by inflammatory status or CVDs. Given that there were only two participants with cerebrovascular disease, we did not adjust for this factor. These findings suggest that tooth loss may be a risk factor for neurodegenerative processes in dementia-free older adults, however these exploratory cross-sectional findings need to be validated in future longitudinal MRI studies, in order to further investigate this relationship.

A possible pathway in the relationship between tooth loss, cognitive decline and brain atrophy may be reduced sensory stimulation through mastication. Animal models of edentulism show a reduction in chewing-induced sensory stimulation, as well as reduced volume, in cortical brain regions involved in somatosensory, motor, cognitive and emotional functions (Almeida

et al., 2012; Hirai et al., 2010; Onozuka et al., 2000 and Kawahata et al., 2014; Avivi-Arber et al., 2017). This can lead to neurodegeneration, reduced neurogenesis, and, ultimately, cognitive decline in aged mice and rats. In addition, functional MRI studies in humans have shown increased brain activity in the prefrontal cortex, anterior cingulate cortex, and left frontal gyrus during chewing (Hirano et al., 2013; Higaki et al., 2016). Furthermore, Lin et al. (2016) showed that GMV and resting-state functional connectivity in the dorsolateral PFC is positively related to masticatory ability. The smaller GMV observed among our participants with tooth loss in the current results is consistent with these findings. Taken together, this evidence suggests that loss of teeth may be a risk factor for accelerated cognitive decline, by loss of sensory stimulation in cognitively relevant brain areas.

However, adjusting for or stratifying by chewing difficulty did not modify the associations between tooth loss and cognitive decline and lower TBV and GMV in our study. This suggests that chewing difficulty caused by tooth loss may be different from that caused by other age-related conditions. Indeed, the function of the jaw muscles in relation to sensorimotor control of masticatory movements may be affected by aging-related declines such as reduced biting forces, impaired chewing, and swallowing (Avivi-Arber and Sessle, 2018). Therefore, impairment in chewing ability through such pathways, may have an impact on cognitive decline separate to that of tooth loss. Related to this, Lexomboon et al. (2012) found that chewing difficulty, but not multiple tooth loss was related to higher odds of cognitive impairment, however this study was cross-sectional. Future longitudinal studies including more detailed assessment for both tooth loss and chewing ability should address the relationship between these two measures of oral health and their impact on cognitive aging.

Another possibility is that tooth loss is related to brain volume differences and cognitive decline due to poor nutrition. We attempted to account for this by adjusting for markers of

poor nutrition (anaemia, hypoalbuminemia and BMI), which did not modify the association. However, future studies should include a more thorough assessment on dietary intake.

The strengths of this study were the community-based design with a relatively long-term follow-up and MRI data available in a sub-sample, adjustment for multiple potential confounders, and stratification for vascular and inflammatory factors to explore potential modifiers. However, several limitations need to be pointed out. First, tooth loss was self-reported using a questionnaire during the nurse interview, which could be a less accurate assessment than a clinical dental examination. Nevertheless, several studies investigating self-reported dental status have shown moderate- to high-accurate validity, compared to clinical dental examinations (Douglas et al., 1991; Pitiphat et al., 2002; Matsui et al., 2017). Moreover, we did not have information on which teeth had been lost, which may have implications in masticatory ability. Second, we used MMSE for the assessment of cognitive function. MMSE is a test for global cognitive function, and originally developed as a screening instrument for cognitive impairment. Therefore, it may lack the sensitivity to detect subtle changes in cognitive performance, as well as decline in specific cognitive domains. This might have underestimated the tooth loss-cognitive decline association. Third, we used CRP as a measure of inflammation which might not have captured a history of chronic inflammation, such as that associated with periodontitis, the main cause for tooth loss. Other inflammatory biomarkers (including tissue necrosis factor (TNF)-alpha, interleukin (IL)-6, IL-10, IL-2 among others) were not explored and could be valuable to examine this relationship. In addition, CRP was tested at baseline and at follow-up examinations, and thus might not reflect the inflammatory status at the occurrence of tooth loss. Fourth, it is important to acknowledge the possibility of reverse causality in regards to our findings. The MRI brain differences between those with tooth loss compared to no tooth loss at baseline, could be marker evidence of pre-clinical dementia, with tooth loss relating to inattention to

oral health rather than adverse oral health causing cognitive decline. Excluding incident dementia cases weakened the association between tooth loss and MMSE decline, while the cross-sectional association with lower GMV remained significant. Therefore, the findings of this study remain to be confirmed in future prospective MRI studies. Finally, the generalizability of the findings may be limited. The study population consisted of highly educated and relatively healthy older adults. This is true in particular for the MRI sub-sample, which was healthier and more independent than the full study population. Nevertheless, tooth loss was significantly more common in persons with low education, therefore educational attainment, as a proxy of socioeconomic status (SES), was controlled for and stratified by, but did not modify the association of tooth loss with cognitive decline or lower TBV and GMV. However, future studies should further investigate the possible contribution of SES in the association between tooth loss and cognitive aging.

In conclusion, this population-based longitudinal study of community dwelling dementia-free people over 60 years of age, provides evidence of a detrimental effect of tooth loss on cognitive aging, and that this association appears to be independent of current vascular diseases and inflammatory status. Furthermore, we found that tooth loss is associated with neurodegenerative markers using MRI. Our findings highlight the need of timely monitoring of cognitive functioning among older adults with tooth loss, for early detection and prevention of accelerated cognitive decline.

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614 **Table 1**. Characteristics of the baseline study population (*n*= 2715).

Characteristic	Tooth loss			*p* value
	None	Partial	Complete	
	n=2105 (77.5%)	*n*=404 (14.9%)	*n*=206 (7.6%)	
Age	70.9 (±9.7)	79.5 (±9.5)[a]	84.3 (±8.2)[a]	<0.000
Women	1296 (61.6)	272 (67.3)	138 (67.00)	0.040
Education				
Elementary	206 (9.8)	116 (28.8)[a]	93 (45.4)[a]	<0.000
High school	1033 (49.1)	210 (52.1)	89 (43.4)[a]	
University	866 (41.1)	77 (19.1)	23 (11.2)	
Vascular disease				
Cardiovascular diseases	389 (18.5)	132 (32.8)[a]	92 (44.7)[a]	<0.000
Cerebrovascular diseases	122 (5.8)	32 (7.9.9)	19 (9.2)	0.061
Diabetes	159 (7.6)	49 (12.1)	18 (13.6)	<0.000
C- reactive protein >5 mg/l	447 (21.2)	124 (30.7)[a]	79 (38.4)[a]	<0.000
Smoking status				
Never	982 (46.9)	178 (44.2)[a]	83 (40.5)[a]	0.020
Former	824 (39.4)	154 (38.2)[a]	78 (38.1)[a]	
Current	286 (13.7)	71 (17.6)	44 (21.5)	
MMSE score	28.9 (±1.5)	28.1 (±2.03)[a]	27.2 (±2.5)[a]	<0.000
Alcohol consumption				
Never/occasional	572 (27.3)	199 (49.4)[a]	135 (65.9)[a]	<0.000
Light/moderate	1129 (53.9)	151 (37.5)[a]	60 (29.3)[a]	
Heavy	395 (18.9)	53 (13.2)[a]	10 (4.9)[a]	
Walking speed m/s	1.3 (±0.4)	0.9 (±0.4)[a]	0.7 (±0.4)[a]	<0.000
BMI	25.7 (±4.1)	25.6 (±4.4)	25.2 (±4.4)	0.210
Multimorbidity	3.5 (±2.3)	4.7 (±2.5)[a]	5.4 (±2.6)[a]	<0.000
Hypoalbuminemia	138 (6.7)	52 (13.6)[a]	36 (18.9)[a]	<0.000
Anemia	124 (6.1)	32 (8.4)[a]	23 (12.1)[a]	0.003
APOE ε4 allele carriers	583 (29.7)	88 (24.5)[a]	44 (25.9)	0.090

615 Data are numbers (percent) or means (standard deviation). MMSE= Mini Mental State
616 Examination, BMI= body mass index
617 [a] Significant pairwise comparison comparing partial and complete tooth loss with no tooth
618 loss group as reference (p<0.05).

Table 2. Estimated annual mean change in Mini-Mental State Examination score by tooth loss over 9-years (*n*= 2715).

	β-coefficients (95% CI)			
Tooth Loss	**Model 1[a]**	**Model 2[b]**	**Model 3[c]**	**Model 4[d]**
None	Reference	Reference	Reference	Reference
Any	-0.23 (-0.41 to -0.04)	0.06 (-0.06 to 0.18)	0.14 (-0.03 to 0.31)	0.14 (-0.03 to 0.31)
Partial	0.04 (-0.25 to 0.17)	0.10 (-0.03 to 0.23)	0.16 (-0.03 to 0.35)	0.16 (-0.03 to 0.35)
Complete	-0.65 (-0.94 to -0.37)	-0.03 (-0.32 to 0.16)	-0.08 (-0.21 to 0.38)	-0.08 (-0.21 to 0.38)
Tooth loss x time				
None x time	Reference	Reference	Reference	Reference
Any x time	-0.29 (-0.37 to -0.20)	-0.30 (-0.39 to -0.21)	-0.18 (-0.24 to -0.11)	-0.18 (-0.24 to -0.11)
Partial x time	-0.19 (-0.28 to -0.09)	-0.21 (-0.31 to -0.11)	-0.13 (-0.20 to -0.05)	-0.13 (-0.20 to -0.05)
Complete x time	-0.55 (-0.70 to -0.40)	-0.54 (-0.70 to -0.39)	-0.30 (-0.42 to -0.18)	-0.30 (-0.42 to -0.18)

Abbreviations: CI= confidence interval. β-coefficients and 95% CIs are derived from linear mixed-effects models

[a] Model 1: age, sex and education.

[b] Model 2: further adjustment for, smoking, alcohol consumption, multimorbidity, walking speed, Apolipoprotein ε4 allele, anemia, hypoalbuminemia and diabetes.

[c] Model 3: further adjustment for C-reactive protein levels at baseline and at follow ups.

[d] Model 4: further adjustment for cardiovascular diseases and cerebrovascular disease.

628 **Table 3.** Estimated annual mean change in MMSE score by tooth loss over 9-years, stratified
629 by CVDs, cerebrovascular disease and time-varying CRP levels (*n*= 2715).

	β-coefficients (95% CI)	**β-coefficients (95% CI)**
Tooth loss	**No CVDs (*n*= 2067)**	**CVDs (*n*= 648)**
Any	0.06 (-0.09 to 0.21)	0.12 (-0.11 to 0.35)
Partial	0.12 (-0.04 to 0.28)	0.11 (-0.16 to 0.37)
Complete	-0.13 (-0.38 to 0.13)	0.16 (-0.18 to 0.49)
Tooth loss x time		
Any	-0.24 (-0.33 to -0.15)	-0.28 (-0.54 to -0.01)
Partial	-0.21 (-0.32 to -0.11)	-0.02 (-0.32 to 0.28)
Complete	-0.32 (-0.49 to -0.15)	-0.76 (-1.14 to -0.37)
Tooth loss	**No Cerebrovascular disease (*n*= 2543)**	**Cerebrovascular disease (*n*= 173)**
Any	0.08 (-0.12 to 0.28)	-0.25 (-1.14 to 0.65)
Partial	0.11 (-0.03 to 0.25)	0.07 (-0.57 to 0.71)
Complete	-0.04 (-0.29 to 0.22)	0.10 (-0.87 to 1.07)
Tooth loss x time		
Any	-0.24 (-0.31 to -0.17)	-0.40 (-0.86 to 0.05)
Partial	-0.22 (-0.31 to -0.12)	0.58 (-0.30 to 1.46)
Complete	-0.56(-0.71 to -0.41)	-0.59 (-1.84 to 0.67)
Tooth loss	**CRP <5 mg/l (*n*= 2065)**	**CRP >5 mg/l (*n*= 513)**
Any	0.10 (-0.12 to 0.32)	-0.11 (-0.54 to 0.32)
Partial	0.18 (-0.01 to 0.38)	-0.20 (-0.75 to 0.34)
Complete	0.05 (-0.28 to 0.39)	-0.16 (-0.41 to 0.74)
Tooth loss x time		
Any	-0.34 (-0.43 to -0.24)	-0.15 (-0.31 to 0.00)
Partial	-0.14 (-0.22 to -0.06)	-0.08 (-0.35 to 0.18)
Complete	-0.27 (-0.22 to -0.06)	-0.30 (-0.59 to -0.02)

630 Abbreviations: CI= confidence interval, CVDs= cardiovascular diseases, CRP= C-reactive
631 protein. MMSE= Mini-mental state examination.
632 β-coefficients and 95% CIs are derived from linear mixed-effects models adjusted for age,
633 sex, education, smoking, alcohol consumption, multimorbidity, walking speed,
634 Apolipoprotein ε4 allele, anemia, hypoalbuminemia and diabetes.
635

Table 4. Cross-sectional association of tooth loss with adjusted regional brain volumes and white-matter hyperintensities *(n* = 394).

	β-coefficients (95% CI)				
Tooth loss	**TBV**	**GMV**	**HV**	**WMV**	**WMHs**
None (*n*=336)	Reference	Reference	Reference	Reference	Reference
Any (*n*= 58)	-28.89 (-49.33 to -8.45)	-22.13 (-36.84 to -7.42)	-0.16 (-0.39 to 0.08)	-5.15 (-25.03 to 14.72)	0.16 (-0.24 to 0.55)
Partial (*n*=43)	-20.70 (-43.53 to 2.09)	-17.10 (-33.54 to -0.67)	-0.08 (-0.35 to 0.18)	-3.65 (-26.82 to 19.50)	0.10 (-0.34 to 0.54)
Complete (*n*=15)	-52.70 (-8.79 to -16.61)	-36.77 (-62.78 to -10.77)	-0.39 (-0.81 to 0.04)	-8.56 (-42.07 to 24.95)	0.34 (-0.35 to 1.03)

Abbreviations: CI= confidence interval, TBV= total brain volume, HV= hippocampal volume, GMV= gray-matter volume, WMV= white-matter volume, and WMHs= white-matter hyperintensities.

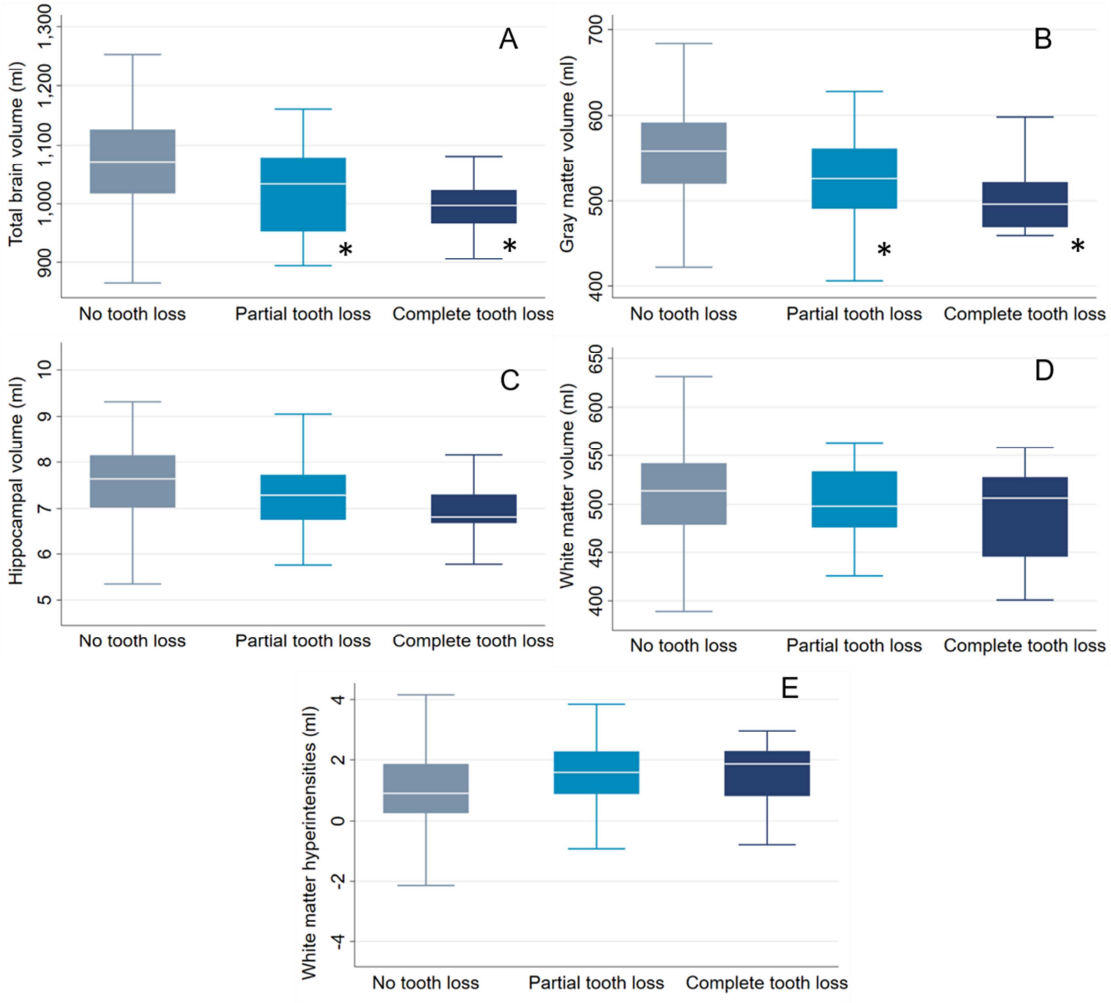
All volumes were adjusted for total intracranial volume and age.

β-coefficients and 95% CIs are derived from linear regression adjusted for sex, education, cardiovascular diseases, C-reactive protein, multimorbidity, apolipoprotein ε4 allele and baseline Mini-Mental State Examination score.

643 **Figure 1.** Regional brain volumes (ml) and white-matter hyperintensities by tooth loss status among dementia-free participants at baseline.

644

645 **Figure 1. Legend:** (A) Total brain volume (TBV), (B) Gray-matter volume (GMV), (C) White-matter volume (WMV), (D) Hippocampal
646 volume (HV), (E) White-matter hyperintensities volume (WMHs). Boxplots represent the means and standard deviations of TBV (A), GMV (B),
647 WMV (C), HV (D) and WMHs (E) in n=394 SNAC-K participants. All volumes were adjusted for total intracranial volume and age.
648 * Significant group differences derived from linear regression models with tooth loss as categorical predictor variable (reference group: no tooth
649 loss) and regional brain volumes and white-matter hyperintensities as continuous outcome variables, respectively. Models were adjusted for sex,
650 education, cardiovascular diseases, C-reactive protein, multimorbidity, apolipoprotein ε4 allele and baseline Mini-Mental State Examination
651 score.
652 *p*-value <0.05.


A
Total brain volume (ml)
900
1,000
1,100
1,200
1,300
No tooth loss
Partial tooth loss
Complete tooth loss
*
*
B
Gray matter volume (ml)
400
500
600
700
No tooth loss
Partial tooth loss
Complete tooth loss
*
*
C
Hippocampal volume (ml)
5
6
7
8
9
10
No tooth loss
Partial tooth loss
Complete tooth loss
D
White matter volume (ml)
400
450
500
550
600
650
No tooth loss
Partial tooth loss
Complete tooth loss
E
White matter hyperintensities (ml)
-4
-2
0
2
4
No tooth loss
Partial tooth loss
Complete tooth loss

Highlights

- Tooth loss is associated with faster cognitive decline in older adults.
- This association is not accounted for by vascular disorders or inflammation.
- Tooth loss is associated with lower total brain volume and gray-matter volume.