Effects of Tooth Loss and the Apolipoprotein E ε4 Allele on Mild Memory Impairment in the Fujiwara-kyo Study of Japan: A Nested Case-Control Study

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Accepted 23 August 2016

Abstract.
Background: Several studies have suggested that periodontal disease can exacerbate the pro-inflammatory status of the brain. Tooth loss is one of the alternative evaluation indices of periodontal disease. There are few data on the relationship between tooth loss and memory impairment, depending on the apolipoprotein E (APOE) ε4 genotype.
Objective: To determine if tooth loss is associated with mild memory impairment (MMI) and if this association is modified by the presence of the APOE ε4 allele.
Methods: A nested case-control study was conducted from 2007 to 2012 in Japan. Five hundred and thirty-seven Japanese subjects aged 65 years and over who were cognitively intact at baseline were analyzed. MMI at follow-up was evaluated.
Results: The median number of teeth at baseline was significantly lower in MMI participants (n = 179) than in controls (n = 358) (MMI: median 21.0, interquartile range 10.0–25.0 versus controls: 24.0, 14.0–27.0). After adjustment for demographics, vascular risk factors, and APOE ε4 allele, the multivariate adjusted odds ratio (OR) of ≤8 teeth was 1.97 (95% confidence interval [CI], 1.13–3.44) compared to 25–32 teeth. Participants with both the presence of at least 1 APOE ε4 allele and ≤8 teeth had a higher risk of MMI compared with those with neither (OR, 2.82; 95% CI, 1.15–6.91). Those with either risk factor alone did not have a higher risk of MMI.
Conclusions: A lower number of teeth is related to risk of MMI. This may be primarily true for those individuals with an APOE ε4 allele.

Keywords: APOE ε4 allele, community-based, memory decline, nested case-control study, tooth loss

INTRODUCTION

The prevalence of Alzheimer’s disease (AD) and dementia is on the increase all over the world. The conversion rate from mild cognitive impairment
(MCI) to dementia is reportedly 50% within 5 years [1, 2]. To reduce the number of patients developing dementia, it is crucial to identify modifiable risk factors of MCI for its prevention. The Nakayama study group [3] reported a simple diagnostic method for MCI using the Mini-Mental State Examination (MMSE) [4], in which the existence of mild memory impairment (MMI) is determined using a three-word delayed recall test (Recall; a subtest of the MMSE). This relatively short examination is suitable for community-based epidemiological surveys. The conversion rate from MMI to illness with dementia is higher than that from cognitively intact individuals, suggesting that MMI is comparable to MCI [3].

In the present study, we focused on periodontal disease as a candidate modifiable risk factor of MMI. Periodontal disease is a chronic inflammatory disease caused mainly by Gram-negative anaerobic bacteria. Locally, resorption of the alveolar bone supporting the teeth results in tooth loss. In that process, there is a possibility that periodontal disease-derived pro-inflammatory molecules, bacteria, and bacterial products increase the risk of developing an inflammatory state in the central nervous system [5]. Previously, some studies reported significant relationships between periodontal infection and lower Digit Symbol test scores in the Wechsler Adult Intelligence Scale [6], and between few teeth at midlife and an increased risk of AD and dementia [7, 8].

In our 5-year prospective cohort study, we reported that the risk of MMI was increased by 2.4-fold in edentulous individuals relative to those with multiple teeth [9]. Conversely, one study found no significant correlation between tooth loss and cognitive function due to the high impact of socioeconomic status [10].

The apolipoprotein E (APOE) ε4 allele (rs429358) promotes amyloid aggregation and deposition in the brain, and increases risk for AD [11]. No study has evaluated the correlations between periodontal disease, the APOE ε4 allele, and cognitive function, except for the reports from Stein et al. [12–14] and Kamer et al. [15]. However, none of these studies had large sample sizes nor evaluated East Asian individuals. In the present study, the number of remaining teeth and the depth of periodontal pockets were recorded to evaluate the long-term burden of periodontal disease and inflammation of the gingiva at the time of evaluation, respectively. We hypothesized that tooth loss might contribute to MMI. The purpose of this study was to investigate the combined effects of tooth loss and the APOE ε4 allele on MMI.

METHODS

Database

This study was approved by Ethics Review Board of Nara Medical University and Nara Medical University Human Genome and Gene Analysis Research Ethics Committee. We conducted a nested case-control study using longitudinal data collected in the Fujiwara-kyo study [16, 17], which started in 2007 and is an ongoing prospective cohort study of volunteer men and women who, at baseline, were independent elderly residents of Nara Prefecture, Japan, aged ≥65 years and able to walk unassisted. In 2007, 4206 people participated in the baseline health assessment. A follow-up assessment for cognitive function was conducted in 2012. Written informed consent was obtained from each subject prior to participation in the baseline and follow-up examinations.

Selection of cases and controls

Our study population included subjects who were cognitively intact at the baseline survey and had blood samples available for genotyping. Of the 4,206 participants, 3,696 were diagnosed as cognitively intact at baseline. Of these, 2,486 subjects participated in the follow-up assessment (159 died, 42 moved away, 15 were admitted to an institution, 260 were hospitalized or undergoing treatment, and 734 were non-responders). Among the 2,486 participants, 241 subjects who were determined to have MMI at follow-up were assessed first, and then suitable controls who were judged to be cognitively intact at follow-up were identified, using frequency matching on age (within 1 year) and sex. For each case, two controls were selected. MMI subjects with no useable blood samples (n = 20), who did not wish to undergo gene analysis (n = 31), or with no corresponding controls (n = 11) were excluded. In the present study, 179 cases and 358 controls were included.

Evaluation of cognitive function

Being cognitively intact was defined as: (1) no impairment of the activities of daily living (ADL); (2) normal general cognitive function, score ≥24, assessed by the MMSE (score range: 0–30) [18]; and (3) absence of objective memory impairment, score ≥2, assessed by the Recall test (score range: 0–3) in the MMSE, according to the Nakayama study. The MMSE was carried out by clinical psychologists or
and stored at –80°C. Buffy coats were prepared from whole blood collected at –80°C. Using TaqMan® SNP Assays (Life Technologies, Carlsbad, CA), single nucleotide polymorphism genotyping of the APOE e4 allele (rs429358) was conducted. Individuals with at least one e4 allele were determined as APOE e4 allele-positive carriers.

APOE genotypes

Buffy coats were prepared from whole blood collected at the follow-up survey in 2012. DNA was purified from buffy coats using a QIA symphony DSP DNA Midi Kit (QIAGEN, Hilden, Germany) and stored at –80°C. Using TaqMan® SNP Assays (Life Technologies, Carlsbad, CA), single nucleotide polymorphism genotyping of the APOE e4 allele (rs429358) was conducted. Individuals with at least one e4 allele were determined as APOE e4 allele-positive carriers.

Dental examinations

Dental examinations were carried out by two dentists calibrated as to the techniques using the single observer method, in a sitting position under artificial lighting [9]. The remaining teeth were defined as healthy, carious, or treated (including crowned, inlay, and abutment teeth for bridge work), inclusive of completely erupted third molars. The Community Periodontal Index (CPI) code of the World Health Organization (WHO) [23] was recorded to evaluate the depth of the periodontal pockets. The mouth was divided into 6 sextants: anterior and right and left posterior of the maxillomandibular; a sextant was examined only if there were ≥2 teeth present. The prescribed 10 representative teeth in the 6 sextants were examined at 4 sites on every tooth using a WHO probe. One of 5 code levels (code 0, healthy; code 1, gingival bleeding after probing; code 2, calculus present in the periodontal pocket; code 3, periodontal pocket 4–5 mm deep; and code 4, periodontal pocket at least 6 mm deep), or an ineligible sextant (sextant having 1 or zero remaining teeth) was assigned to each sextant. The highest code level identified was regarded as the maximum CPI code for the individual.

Other covariates at baseline

Vascular risk factors are predictors of cognitive impairment [24–26]. Each participant underwent an interview to record smoking habit, history of cerebrovascular disease, myocardial infarction, hypertension, diabetes mellitus, or dyslipidemia, and current medication. After sitting quietly for >5 min, blood pressure was determined twice at an interval of 30 s using an automatic blood-pressure manometer (ES-P2100; TERUMO Co., Tokyo, Japan). The average of 2 measurements was used in the analyses. Blood samples were collected from an antecubital vein after an overnight fast. Cerebrovascular disease and myocardial infarction were determined by medical history and current medication. Hypertension was defined according to the following Japanese Society of Hypertension criteria [27]: medical history, current use of antihypertensive medicine, and/or systolic/diastolic blood pressure ≥140/90 mmHg. Diabetes mellitus was defined by medical history, current antidiabetic medication, and/or by one of the following biochemical test results according to the guidelines of the Japan Diabetes Society [28]: fasting plasma glucose level ≥126 mg/dL or HbA1c level (NGSP) ≥6.5%. Dyslipidemia was
defined by medical history, current lipid-lowering medications, and/or by one of the following biochemical test results according to the Japan Atherosclerosis Society guidelines [29]: triglycerides $\geq 150$ mg/dL, low-density lipoprotein cholesterol $\geq 140$ mg/dL, or high-density lipoprotein cholesterol $<40$ mg/dL.

**Statistical analysis**

Statistical analysis was performed using SPSS (IBM, Armonk, NY) version 17.0. Two-tailed $p$-values were calculated in all analyses. The alpha level of significance was set at 0.05. Descriptive analyses are presented as proportions for categorical data and as medians (interquartile range, IQR) for continuous data. Baseline characteristics were compared between the participants with MMI and controls using the chi-square or Mann-Whitney test. Case-control analyses used a logistic regression. MMI at follow-up was used as a dependent variable. We divided the subjects into 4 tooth categories by 8 teeth: groups with 0–8, 9–16, 17–24, and 25–32 teeth. Tooth category at baseline, the number of remaining teeth at baseline, and CPI code (code 4 versus code 0, 1, 2, or 3) at baseline were used as independent variables. Dental variables were added separately to each model. Multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of each dental variable were calculated controlling for baseline age, sex, educational background ($\leq$ junior high school versus $\geq$ high school), baseline MMSE-total scores, baseline Recall (2 score versus 3 score), and baseline GDS ($\geq 6$ score versus $\leq 5$ score), smoking habit (current versus former and never), positive history of diseases, and $APOE$ e4 allele (any e4 versus no e4). The interaction between the number of teeth and $APOE$ e4 was assessed by adding an interaction term to the logistic regression model. Further, we examined the combined effects of $APOE$ e4 (presence or absence) and a lower number of teeth ($\leq 8$ versus $\geq 9$).

**RESULTS**

**Comparison between cases and controls**

Table 1 compares the baseline characteristics of the cases (MMI participants) and controls. The proportion of individuals with an education level lower than high school was significantly higher in the cases than

| Characteristic                        | Cases ($n = 179$) | Controls ($n = 358$) | $p$ value |
|--------------------------------------|------------------|----------------------|-----------|
| Age, median (IQR), years             | 72.0 (69.0–76.0) | 72.0 (69.0–76.0)     | 0.985     |
| Female                               | 58 (32.4%)       | 116 (32.4%)          | 1.000     |
| Education $\leq$ junior high school | 41 (22.9%)       | 54 (15.1%)           | 0.031     |
| MMSE total score, median (IQR)       | 28.0 (26.0–29.0) | 29.0 (27.0–30.0)     | $<0.001$  |
| Recall score 3                       | 98 (54.7%)       | 283 (79.1%)          | $<0.001$  |
| GDS score $\geq$ 6                   | 2 (1.1%)         | 8 (2.2%)             | 0.508     |
| Current smoker                       | 15 (8.4%)        | 40 (11.2%)           | 0.366     |
| Positive history of diseases         |                  |                      |           |
| Cerebrovascular disease              | 8 (4.5%)         | 19 (5.3%)            | 0.835     |
| Myocardial infarction                | 1 (0.6%)         | 10 (2.8%)            | 0.110     |
| Hypertension                         | 133 (74.3%)      | 246 (68.7%)          | 0.193     |
| Diabetes mellitus                    | 27 (15.1%)       | 42 (11.7%)           | 0.277     |
| Hyperlipidemia                       | 112 (62.6%)      | 206 (57.5%)          | 0.306     |
| Presence of at least 1 $APOE$ e4 allele | 43 (24.0%)    | 68 (19.0%)           | 0.177     |
| Tooth category                       |                  |                      |           |
| 25–32                                | 52 (29.1%)       | 163 (45.5%)          | 0.002     |
| 17–24                                | 64 (35.8%)       | 89 (24.9%)           |           |
| 9–16                                 | 23 (12.8%)       | 41 (11.5%)           |           |
| $\leq$8                              | 40 (22.3%)       | 65 (18.2%)           |           |
| Number of teeth, median (IQR)        | 21.0 (10.0–25.0) | 24.0 (14.0–27.0)     | 0.001     |
| CPI*                                 |                  |                      |           |
| n = 149                              |                  |                      |           |
| code 0, 1, 2, or 3                   | 111 (74.5%)      | 233 (72.4%)          | 0.657     |
| code 4                               | 38 (25.5%)       | 89 (27.6%)           |           |

IQR, interquartile range; Recall, three word delayed recall; GDS, geriatric depression scale; $APOE$, apolipoprotein E; CPI, Community Periodontal Index. Data are expressed as number (%) or median (IQR). Statistical analysis was performed by chi-square test or Mann-Whitney test. *We excluded people considered ineligible in all sextants in both maxillary and mandibular dentition from data analysis (cases, $n = 30$; controls, $n = 36$).
The combined effect of a lower number of teeth and the APOE e4 allele on MMI

We screened for the presence of an interaction between the number of teeth (a continuous variable) and APOE e4. The p-value for this interaction was 0.035 in the adjusted model (adjustment for age, sex, education, MMSE total score, Recall, GDS, smoking habit, and history of cerebrovascular disease, myocardial infarction, hypertension, diabetes mellitus, and hyperlipidemia). Given this significant interaction, we examined the combined effects of APOE e4 and a small number of teeth (Table 3). Participants with the presence of at least 1 APOE e4 allele and ≤8 teeth had a higher risk of MMI than those with neither (OR, 2.82, 95% CI, 1.15–6.91, p = 0.024). Those with either risk factor alone did not have a higher risk of MMI. There was no combined effect for the APOE e4 allele and CPI code 4.

DISCUSSION

In case-control analysis within a community-dwelling elderly cohort, we found that a lower number of teeth was related to risk of MMI, even after adjustment for covariates including the APOE e4 allele by logistic regression analysis. The combined effect of a lower number of teeth and the APOE e4 allele on risk of MMI was significant. To our knowledge, this is the first study of an East Asian population...
and degradation of AβoA-

fibrillary tangles. An imbalance in the production of cytokines can be directly toxic or stimulate further...aging chronic inflammation [30]. Pro-inflammatory cytokines related to the aggravation of both periodontal disease and dementia. Interleukin (IL)-1A (−889) and IL-1B (+3953) gene polymorphisms are associated with the severity of periodontal disease [37, 38] and the risk of AD [39]. Second, nutritional deficits occur due to the decrease in the regular intake of fish and fruit after tooth loss [40]. Finally, a decrease of sensory information due to the loss of periodontal ligaments and/or due to the decrease of mastication-induced stimulation can be listed. A decrease in the number of pyramidal cells was observed in the hippocampal CA1 and CA3 areas of molar-loss mice [41]. Tooth loss may contribute to AD pathogenesis via amyloid cascade-independent neuronal cell loss.

to investigate the effects of tooth loss and the APOE ε4 allele on memory decline. In the Nun study [13], it was found that there was an earlier decline in word recall scores in individuals with at least one APOE ε4 allele and fewer teeth, than in individuals with neither of these risk factors or with either risk factor alone. Our findings were in agreement with those of the Nun study. Therefore, it is essential to prevent tooth loss in adulthood and the elderly, especially in people with an APOE ε4 allele.

Multiple tooth loss at baseline implies the existence of a long-term inflammatory burden from periodontal disease. The CPI was used an evaluation index for gingival inflammation at the time of evaluation. There was no significant correlation between the CPI and MMI. It is suggested that the effects of gingival inflammation on memory decline were not observed clearly in a 5-year period.

As one of biological bases for the relationship between tooth loss and MMI, the burden of long-term inflammation due to periodontal disease can be listed first. The characteristics of AD brains are the accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles. An imbalance in the production and degradation of Aβ leads to its accumulation, which activates microglial cells and astrocytes, triggering chronic inflammation [30]. Pro-inflammatory cytokines can be directly toxic or stimulate further Aβ production [30]. Periodontal-derived pro-inflammatory molecules, pathogens, and their products are hypothesized to cross the blood-brain barrier and exacerbate AD [31, 32]. Indeed, a serological marker of periodontitis is associated with impaired delayed memory [33] and AD [15]. In addition, antibody levels to Fusobacterium nucleatum and Prevotella intermedia, which are oral flora organisms, are significantly increased in serum collected at baseline before AD onset in AD patients compared to controls [14]. Periodontal disease, which is defined by clinical attachment loss, is associated with brain Aβ load [34]. Leptomeningeal cells transfer peripheral inflammatory signals from macrophages activated by lipopolysaccharides from Porphyromonas gingivalis, which is a major etiological agent of periodontal disease, to microglial cells [35]. These results support the hypothesis that periodontal disease worsens inflammation of the brain. The APOE ε4 allele enhances the Aβ-induced inflammatory response [36], and compromises the integrity of the blood-brain barrier [32]. Therefore, the presence of both of the APOE ε4 allele and periodontal disease may increase the risk of MMI.

The association between tooth loss and MMI could also be explained by the following three reasons. First, the presence of a high-risk allele for proinflammatory cytokines related to the aggravation of both periodontal disease and dementia. Interleukin (IL)-1A (−889) and IL-1B (+3953) gene polymorphisms are associated with the severity of periodontal disease [37, 38] and the risk of AD [39]. Second, nutritional deficits occur due to the decrease in the regular intake of fish and fruit after tooth loss [40]. Finally, a decrease of sensory information due to the loss of periodontal ligaments and/or due to the decrease of mastication-induced stimulation can be listed. A decrease in the number of pyramidal cells was observed in the hippocampal CA1 and CA3 areas of molar-loss mice [41]. Tooth loss may contribute to AD pathogenesis via amyloid cascade-independent neuronal cell loss.

Table 3

| Cases | Controls | Adjusted OR (95% CI) | p value |
|-------|----------|----------------------|---------|
| APOE ε4 (−) and Number of teeth ≥ 9 | 110 | 236 | 1 |
| APOE ε4 (−) and Number of teeth ≤ 8 | 26 | 54 | 1.03 (0.59–1.81) | 0.919 |
| APOE ε4 (+) and Number of teeth ≥ 9 | 29 | 57 | 0.99 (0.58–1.68) | 0.969 |
| APOE ε4 (+) and Number of teeth ≤ 8 | 14 | 11 | 2.82 (1.15–6.91) | 0.024 |
| APOE ε4 (−) and CPI 0–3 | 87 | 187 | 1 |
| APOE ε4 (−) and CPI 4 | 30 | 74 | 0.73 (0.43–1.25) | 0.250 |
| APOE ε4 (+) and CPI 0–3 | 24 | 46 | 1.01 (0.56–1.83) | 0.979 |
| APOE ε4 (+) and CPI 4 | 8 | 15 | 0.96 (0.37–2.45) | 0.931 |

MMI, mild memory impairment; OR, odds ratio; CI, confidence interval; MMSE, Mini-Mental State Examination; Recall, three word delayed recall; GDS, Geriatric Depression Scale; APOE, apolipoprotein E; CPI, Community Periodontal Index. The OR was calculated by logistic analysis. Adjusted for age, sex, educational background, MMSE-total, Recall, and GDS, smoking habit, and history of cerebrovascular disease, myocardial infarction, hypertension, diabetes mellitus, and hyperlipidemia.
In this study, 62 of the 241 MMI subjects were excluded from the data analysis. The distribution of the number of teeth in these excluded subjects was 25–32 teeth in 14 cases (22.6%), 17–24 teeth in 20 cases (32.3%), 9–16 teeth in 7 cases (11.3%), and 0–8 teeth in 21 cases (33.9%), showing no significant difference with the distribution of the 179 people who were included in the data analysis ($p = 0.338$). In this study, 51 cases were excluded from the analysis because it was difficult to draw blood from some elderly subjects, resulting in an insufficient volume of blood for analysis. In addition, some patients declined consent for extra blood collection for gene analysis. To resolve these problems, buccal mucosa is considered to be a more suitable material than blood in this type of study. In the 11 cases who provided data for gene analysis but did not have the corresponding controls, 1 subject carried the $APOE\ e4$ allele (9.1%), indicating no significant difference with the presence of the $APOE\ e4$ allele in the 179 subjects who were included in the analysis ($p = 0.462$). In terms of the number of teeth and the presence of the $APOE\ e4$ allele, a similar trend was observed between the 62 excluded subjects and the 179 included subjects.

Three limitations of our study merit consideration. First, the MMSE and Recall scores can be affected by age and education. There is a possibility that some subjects showed a discrepancy between their actual condition and diagnosis due to the operational division of subjects into cognitively intact and MMI based on raw scores. Second, the evaluation of periodontal disease was conducted according to the number of remaining teeth and CPI in this study. If cost and time limitations had permitted, radiographic evaluation of alveolar bone resorption or clinical attachment loss of all teeth would have been more suitable to evaluate the burden of long-term inflammation. Third, we did not assess the extent to which dental caries or maxillofacial trauma accounted for tooth loss. Therefore, we may have overestimated the effects of the number of teeth on MMI. It is practically difficult to conduct a dental chart review to verify the reasons for the loss of every tooth in all participants.

**Conclusions**

We found that elderly individuals with a lower number of teeth had an increased risk of developing memory decline and that this risk remained after adjustment for demographics, vascular risk factors, and $APOE\ e4$ allele. This was primarily true for those individuals with an $APOE\ e4$ allele. Future studies will need to address whether preventing periodontal disease and tooth loss prevents memory decline in healthy elderly individuals.

**ACKNOWLEDGMENTS**

We would like to express deep appreciation to Visiting Lecturer Yoshiko Dohi, PhD, at Nara Medical University, Department of Biochemistry for her instruction on genetic analysis.

This work was supported by KAKENHI Grant Number 22790566, 24591726, 24249043, and 15K08814, by Nara Medical University Grant-in Aid for Collaborative Research Projects, and by a research grant from the Mitsui Sumitomo Insurance Welfare Foundation in 2007.

Japan Society for the Promotion of Science and The Ministry of Education, Culture, Sports, Science and Technology, and Nara Medical University had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, and in the preparation, review, and approval of the manuscript.

Authors’ disclosures available online (http://j-alz.com/manuscript-disclosures/16-0638r1).

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