Received: 22 December 2020 | Revised: 20 April 2021 | Accepted: 20 April 2021

DOI: 10.1002/alz.12378

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

FEATURED ARTICLE

Effect of periodontal treatment on preclinical Alzheimer's disease—Results of a trial emulation approach

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[†]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Funding information
National Institutes of Health, Grant/Award Numbers: U01 AG024904, RF1 AG059421; European Union Joint Programme - Neurodegenerative Disease Research, Grant/Award Number: 01ED1615; Department of Defense award number, Grant/Award Number: W81XWH-12-2-0012; Federal Ministry of Education and Research, Grant/Award Number: 03IS2061A

Abstract

Introduction: We investigated the relationship between periodontal treatment and pre-clinical Alzheimer's disease (AD).

Methods: In this quasi-experimental design, 177 periodontally treated patients from the "Greifswald Approach to Individualized Medicine" cohort, which used the same protocols as the population-based Study of Health in Pomerania TREND (SHIP-TREND), and 409 untreated subjects from SHIP-TREND were analyzed. Subjects were younger than 60 years at the magnetic resonance imaging examination, with a median observation period of 7.3 years. Imaging markers for brain atrophy in late-onset AD and brain aging were used as the outcomes.

Results: Robust to sensitivity analyses, periodontal treatment had a favorable effect on AD-related brain atrophy (−0.41; 95% confidence interval: −0.70 to −0.12; $P = .0051$), which corresponds to a shift from the 50th to the 37th percentile of the outcome distribution. For brain aging, the treatment effect was uncertain.

Conclusion: Periodontitis is related to pre-clinical AD in our population.

KEYWORDS
aging, Alzheimer's disease, epidemiology, magnetic resonance imaging, periodontal diseases

1 | INTRODUCTION

The prevalence of Alzheimer's disease (AD) and other dementias worldwide is expected to reach about 135 million people in 2050.[1] Long-term strategies to reduce clinical manifestations by preventing the transition from asymptomatic to symptomatic pathology have been adapted to AD.[2,3] AD forms a continuum of disease severity,[2] including preclinical stages, mild cognitive impairment, and dementia, and can show deviations from "normal aging" already in earlier stages,[3] which corresponds to the concept of brain aging.[4,5]

Periodontitis has been suggested as a new risk factor for AD,[6] although relevant confounder information for this relationship has rarely been modeled in human studies.[7] Indeed, periodontitis and AD share risk factors, including age, obesity, diabetes mellitus, smoking, education, and alcohol consumption, as well as, possibly, depression, and nutrition.[7,8] While there is some evidence for causality, most longitudinal studies were not designed for the relationship of interest; the periodontal exposures lacked information, especially to model continuous dose-response relationships.[9] Periodontal treatment may have a tangible effect on preclinical AD because severe periodontitis is widespread—affecting 11% of the global population[10]—and treatment can in part be performed by dental auxiliaries.

Using data from the large population-based Study of Health in Pomerania (SHIP-TREND),[11] we generated hypotheses on the association between periodontitis and preclinical stages of AD such as brain aging and the recently developed AD score.[12] Following our working strategy,[13] we then transferred these hypotheses to the clinical sample of the periodontal disease cohort of the Greifswald Approach to Individualized Medicine (GANI_MED) from the same source population as SHIP-TREND.[11,13] We investigated the relationship between periodontal treatment and preclinical stages of AD by including treated subjects from GANI_MED and untreated subjects from SHIP-TREND in a quasi-experimental design.[14]

RESEARCH IN CONTEXT

1. **Systematic review**: Periodontitis has been suggested as a risk factor for Alzheimer's disease (AD), primarily based on animal studies. Most longitudinal studies in human populations were not designed for the aforementioned relationship, usually lacking information on relevant periodontal exposures and potentially important confounders. Recently developed statistical models enable researchers to emulate a clinical trial by estimating causal effects from observational data. Such models require data on treated subjects and untreated subjects who should have been treated but were, in fact, not treated for periodontitis. Epidemiologic literature on preventive strategies suggests dementia as a continuum of disease severity.
2. **Interpretation**: Our study provides several lines of evidence that periodontitis is independently related to preclinical AD.
3. **Future directions**: As randomized trials including intentionally untreated patients are hard to design for ethical reasons, further observational studies emulating a trial are needed.

2 | METHODS

The aim was to quantify the average treatment effect among the treated (ATET), which is, as an individual treatment effect, the within-subject difference defined by comparing the real outcome of the treated with the estimated potential outcome (counterfactual outcome) if he/she had not been treated.[14,15] The ATET is a causal effect,[14,15] whereas, contrary to common belief, "the improvement is not a causal effect."[15] Thus, a single measurement of the outcome is sufficient if the observation period between the exposure and the outcome is adequate. This prerequisite was met by choosing the periodontal treatment cohort from GANI_MED.[13] As the ATET is restricted to treated subjects, untreated subjects (who are needed for potential outcomes) can be chosen from a cross-sectional design if the outcome is worsening over time, thereby taking advantage of the bias toward zero. We selected untreated subjects from SHIP-TREND,[11] which ensures comparability across studies, including source population, inclusion criteria, and standardized methods (Table 1). At the start of the GANI_MED recruitment, the medical staff was trained and certified according to the standards of SHIP-TREND.[13] Importantly, we restricted the study samples to adults younger than 60 years to reduce reverse causality.[16]

2.1 | Quasi-experimental design

2.1.1 | Periodontal treatment and study population

Following German guidelines, patients were treated if they had at least three teeth with probing depth ≥3.5 mm after giving oral hygiene instructions and monitoring. The active treatment consisted of oral hygiene instructions; prophylaxis; subgingival scaling; and, if deemed necessary, access flap surgery. For maintenance sessions, which consisted of prophylaxis, repeated oral hygiene instructions, and rescaling in residual pockets ≥3.5 mm, patients visited the dental office between two and four times a year.

Data from GANI_MED patients having undergone active periodontal treatment were tracked back to the periodontal baseline examination between 1993 and 2012,[17] which was successful for 604 out of ≈2800 patients. Based on the intention-to-treat principle, we also included patients who dropped out during supportive treatment. The observation period was defined from the start of the active periodontal treatment to the magnetic resonance imaging (MRI) examination as part of GANI_MED between 2011 and 2014. To allow the periodontal treatment and its maintenance to have some effect on the outcomes, we included only patients having an observation period greater than

TABLE 1 Overview on the quasi-experimental design

	Quasi-experimental design			
	Patients treated for periodontitis	**Untreated subjects**	**Potential bias**	**Measures to reduce bias; comments**
Study	GANI_MED	SHIP-TREND		
Population coverage				
Definition of the population	Hospital-based	Population-based		
Catchment area or source population	Captures most of the patients of interest in West Pomerania as the periodontal unit is the only one in West Pomerania	Clearly defined by West Pomerania, a region in the northeast of Germany comprising about 200,000 inhabitants	Low to strong	Propensity score approach to make treated and untreated subjects comparable
Recruitment via	Treatment center, re-invited for GANI_MED examination	Population registries		
Study type	Treatment cohort, longitudinal for periodontal examinations, cross-sectional for MRI	Observational, cross-sectional	No observation period for untreated	Bias toward zero as the Alzheimer's disease score increases with age; sensitivity analyses to quantify this bias
Inclusion criteria				
Inclusion criteria for treatment acc. to German guidelines	At least three teeth with probing depth ≥3.5 mm			
Inclusion criteria used herein for untreated subjects who should have been treated to meet the positivity condition (the probability of receiving treatment is greater than zero, i.e., positive)		At least three teeth with probing depth ≥4 mm (half mouth)	Low to moderate for regression to the mean in untreated	Different cut-off points for the number of sites with probing depth ≥4 mm as an additional inclusion criterion for untreated
MRI examination	Yes	Yes	Low for conditioning on MRI examination (collider bias)	Periodontal treatment is unlikely to predict MRI examination; sensitivity analysis
Timeline and measurement				
Before treatment	First periodontal measurement, followed by oral hygiene instructions and monitoring if inclusion criteria met			Data not available
Pre-treatment variables to predict the treatment group by the propensity score approach (confounder set A)	1993 to 2012 before active periodontal treatment (using back-tracking in the treatment center to get data before July 7, 2011, the formal start of GANI_MED)	2008 to 2012	Low to moderate for the different time periods	Importantly, inclusion for and practice of periodontal treatment did not change over time; sensitivity analysis for cohort effect
Age, sex, education, smoking status, known diabetes mellitus	Standardized interviews across studies	Standardized interviews across studies	Low	For GANI_MED: based on interview data on change, smoking and diabetes were calculated back
Number of teeth	Maximum of 28 teeth	Maximum of 28 teeth	Very low	
Probing depth	Second measurement on six sites, full mouth; herein used only the same four sites as in SHIP-TREND; right/left differences within patients are negligible herein (Table 2)	Measured on four sites, half mouth	Low to moderate as some examiners were calibrated across studies	Sensitivity analyses based on some calibration data across studies; different cut-off points for the number of sites with probing depth ≥4 mm as an additional inclusion criterion for the untreated

(Continues)

TABLE 1 (Continued)

	Quasi-experimental design			
	Patients treated for periodontitis	**Untreated subjects**	**Potential bias**	**Measures to reduce bias; comments**
Income	Not available		Low	Patients of low income were treated inexpensively by students and monitored by periodontists; sensitivity analysis for unmeasured confounder
Marital status	Not available before GANI_MED examination		Low	Sensitivity analysis assuming marital status as time-invariant
Periodontal treatment	1993 to 2012, if inclusion criteria met for the second periodontal measurement	—		
Outcome	2011 to 2014	2008 to 2012		
Alzheimer's disease score, brain age gap	Standardized protocols across studies; MRI followed the core examination by days to months	Standardized protocols across studies; MRI followed the core examination by days to months	Very low	
Pre-treatment variables for the outcome model				
Probing depth, age, sex, education, smoking status, known diabetes mellitus, and body height (confounder set B)	Standardized interviews and examination protocols across studies	Standardized interviews and examination protocols across studies	Very low	
Further covariates of the outcome model that were pre-specified based on background knowledge; unknown confounders	Depression and alcohol consumption not assessed in this cohort of GANI_MED	Standardized interviews	Low to moderate	Sensitivity analyses for severe alcohol consumption and unknown confounders
N eligible for analysis	177	409	Low to moderate	Many sensitivity analyses

Abbreviations: GANI_MED, Greifswald Approach to Individualized Medicine; MRI, magnetic resonance imaging; SHIP-TREND, Study of Health in Pomerania, baseline examination of the second SHIP cohort.

2 years. Essentially, 177 subjects treated for periodontitis and younger than 60 years of age at MRI examination were eligible for analysis (Figure S1 in supporting information).

For the quasi-experimental design, the untreated subjects from SHIP-TREND had to meet the positivity criterion,[14] that is, the probability of receiving treatment is greater than zero (Table 1). Essentially, 409 untreated subjects younger than 60 years of age at MRI examination were eligible for analysis (Figures S1-2 in supporting information).

2.1.2 | Oral examination

In both studies, the number of teeth was counted, excluding third molars. Probing depth was measured full-mouth at six sites per tooth in GANI_MED and half-mouth at four sites per tooth in SHIP-TREND (either the two left or the two rights quadrants) using the periodontal probe PCP 15 (Hu-Friedy). For analysis, the same four sites were used in both studies (mesiobuccal, midbuccal, distobuccal, midlingual). As the staff of the periodontal unit (TK) was responsible for design, examination, and quality control of the periodontal examination in SHIP (starting in 1997) and SHIP-TREND,[11] some of the oral examiners were calibrated across the studies before the formal start of GANI_MED (Table 1).

2.1.3 | Magnetic resonance imaging and assessment of the outcomes

MRI scans were acquired with the same scanner in GANI_MED and SHIP-TREND (1.5T Siemens Magnetom Avanto).[13] T1-weighted MRI scans of the head were taken with the following set of parameters: axial plane, repetition time = 1900 ms, echo time = 3.4 ms, flip angle 15°, and resolution $1 \times 1 \times 1$ mm^3. Images showing structural abnormalities (e.g., tumors or cysts) and cases of cerebral stroke were excluded after visual inspection by expert radiologists (Figure S1).

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite, which is documented elsewhere.[12] Briefly, this processing includes removal of non-brain tissue segmentation of subcortical white matter and deep gray matter volumetric structures (including hippocampus and amygdala), tessellation of the gray matter–white matter boundary, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders. When the cortical models were completed, individual images were registered to a spherical atlas based on individual cortical folding patterns, and the cerebral cortex was parceled into 68 units with respect to gyral and sulcal structure. Cortical white matter, that is, white matter up to 5 mm below the gray matter boundary, was also parceled into 68 units by assigning each white matter voxel the label of the closest cortical voxel.

Based on FreeSurfer's whole-brain segmentation and cortical parcellation, we considered two preclinical outcomes, an imaging marker for late-onset AD, which has been recently developed based on data from the Alzheimer's Disease Neuroimaging Initiative (ADNI),[12] and brain age.[18] As brain age has to be adjusted for age at the time of MRI,[5,19] which is not a pre-treatment variable in the quasi-experimental design as required,[15] we calculated the brain age gap,[20] which by definition has a mean of zero throughout the age range (Text S1 and Figure S3 in supporting information).[21]

2.1.4 | Potential confounders and statistical analyses

For the relationship between periodontal treatment and the outcome, we present two possible confounder sets of pretreatment variables in a doubly robust model, such that only one set needs to be correct, thereby giving us "two chances to get it right."[14] The sets A and B are primarily (but not exclusively) related to the treatment and the outcome, respectively.[14,22] By predicting the treatment, confounder set A generates the propensity score to account for the non-random treatment assignment, which is effective in reducing bias, whereas the model for potential outcomes can increase efficiency.[23] Both confounder sets include age, sex, education, smoking, and known diabetes mellitus from the standardized interview, as well as probing depth. Confounder set A additionally includes the number of teeth to predict the treatment. Confounder set B additionally includes body height,[24] which was considered time-invariant, from the standardized medical examination.[11,13]

For the doubly robust model, we used the inverse-probability-weighted regression-adjustment (IPWRA) estimator in terms of Stata software (Stata release 16.1; Stata Corporation). Because Stata does not support multiple imputation for the IPWRA procedure, we adopted R code to estimate the ATET.[22] The whole procedure consisted of several steps. First, six multiple imputation sets were generated within treatment groups.[22,25,26] Second, the propensity score was calculated using confounder set A. Third, the subsample used in further analyses was restricted to overlapping regions of the propensity score across treatment groups, thereby improving confounder balance between groups considerably. Fourth, the propensity score was re-estimated for the subsample.[27] Fifth, based on the propensity score, inverse-probability weights were calculated and used in treatment-specific outcome models using confounder set B, that is, one model for the treated and one for the untreated, which serves for the estimation of the potential outcomes in the treated.[14,22] Finally, the difference in predictions between these two models was calculated for each treated; these differences were averaged over the treated subjects, which yielded the doubly robust ATET. The robust variance of the ATET was calculated across treated and untreated groups.

As naive linear models are often unreliable,[23] restricted cubic splines were used for continuous variables to model departures from linearity.[25] To preserve information, we used probing depth of the full mouth recording in the treatment group. Model assumptions and covariate balance were examined graphically and analytically using Stata and R software.[22,25,28] To evaluate covariate balance, we examined standardized differences and variance ratios.

Background knowledge and calibration data of SHIP[11] examiners and the four main examiners of GANI_MED between 1998 and 2012 suggested that periodontists measured slightly deeper periodontal pockets than SHIP examiners did. Therefore, we used a simple, transparent, and powerful approach to deal with potential examiner differences at the periodontal baseline examination in GANI_MED and SHIP-TREND by subtracting a constant (between 0.05 and 0.30 mm) from mean probing depth levels of the treatment group.[26]

Because unmeasured confounders are a serious problem in quasi-experimental designs, we presented the E-value, which is defined as "the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and the outcome […] to fully explain away a specific treatment-outcome association" and can be adapted for other scales.[29]

2.2 | Cross-sectional design for hypotheses generating

Herein, data from the SHIP-TREND baseline examination from 2008 to 2012 were analyzed.[11] Finally, 4420 participants were examined (Text S2 in supporting information);[11] data from 1323 participants were used for complete case analysis of the MRI sample of participants younger than 60 years of age (Figure S2). Details of design and analyses, for which we used R,[25,28,30] are described in Text S3 in supporting information. Although we provide 95% confidence intervals, we suspend a fixed α level.[31]

3 | RESULTS

3.1 | Treatment effects in the quasi-experimental design

Notably, periodontitis patients were treated long before reaching 50 years of age (median age 42.2 years at the start of the active

periodontal treatment; Table 2). Treatment groups differed by mean probing depth, age, sex, education, and smoking status (Table 2). Balance of these confounders was reached in two steps, thereby emulating a trial. First, the propensity scores, which predicted the treatment by confounder set A, were restricted to overlapping regions across treatment groups as non-overlapping regions indicate a lack of confounder balance (columns 4 and 5 in Table 2; Figure S4 in supporting information). Notably, there was sufficient overlapping although confounder set A predicted the treatment very well ($R\check{s}_{Nagelkerke}$ = 0.47 and c-index = 0.86 for 177 treated and 409 untreated).[32] Second, untreated subjects of the subsample were weighted differently (see column 6 in Table 2; maximum weight was 4.7) whereas treated subjects were weighted equally, which resulted in good confounder balance (Tables 2 and 3).

The ATET was −0.41 for the AD score, meaning that treated patients had on average a score of 0.41 less than if they had not been treated (Table 3). This effect of periodontal treatment corresponds to a shift from the 50th to the 37th percentile of the outcome distribution in the subsample. The ATET of −0.41 could be explained away by an unmeasured confounder that was associated with both the treatment and the outcome by an E-value of 2.0, above and beyond the measured confounders in sets A and B, but weaker confounding could not do so; the confidence limit of −0.12 corresponds to an E-value of 1.4.

The relationship between periodontal treatment and the AD score was robust to sensitivity analyses, including potential examiner differences (Table S1 in supporting information), cohort effects, putative confounders, and potential selection bias induced by conditioning on MRI examination (Text S4 in supporting information). To address the assumption of well-defined interventions,[14] we excluded patients who were additionally treated with antibiotics (Text S5 in supporting information). To account for the lack of an observation period in untreated subjects, we estimated the annual association between linear age and the AD score in SHIP-TREND (same predictors as used for Figure 1A), which was multiplied by the median observation period of the patients and then added to the score of the untreated subjects (0.025 × 7.3 = 0.18). This added score was transferred to an ATET = −0.59 (95% confidence interval: −0.88 to −0.30). This effect of periodontal treatment would correspond to a shift from the 50th to the 32nd percentile of the outcome distribution in the subsample. The E-values would be 2.4 and 1.8 for the estimate and the confidence limit, respectively.

Periodontal treatment was not associated with brain age gap (Table S2 in supporting information).

3.2 | Associations between periodontitis and subclinical outcomes in the general population

Here, we present those results of SHIP-TREND that led to the hypothesis for the quasi-experiment as well as an additional analysis to an open research question. Characteristics of study participants are shown in Table 4. Probing depth, but not calculus or dental plaque, was associated with brain age (Table S3 in supporting information). The APtreatment hypothesis was generated as probing depth formed a continuum of risk among the top half of the exposure range (Figure 1A–D). Severe or moderate cases of periodontitis, showing a prevalence of 16% and 31%, respectively, in our population restricted to subjects aged 20 to 59 years,[33] are involved in the continuum of disease severity represented by probing depth.[2]

The effect of probing depth was modified by apolipoprotein E (*APOE*) ε4 allele frequency; the three curves of the *APOE* ε4 carriers crossed close to the median of probing depth. Although this interaction was prespecified, it is wise to be skeptical of interaction effects even in large studies because interaction effects are prone to hidden assumptions in conventional statistics (Figure 1E and F).

4 | DISCUSSION

We found a moderate to strong effect of periodontal treatment and subsequent maintenance treatment on an imaging marker of AD in an analysis that emulates a trial.[14] Besides reversibility and temporality, additional evidence for a link between periodontitis and preclinical AD comes from the analyses of cross-sectional SHIP-TREND data. The clear dose–response relation restricted to the top half of the exposure range of probing depth meets a further criterion for causality. Specificity for subgingival probing depth contrary to supragingival calculus supports the hypothesis that periodontitis, but not merely poor oral hygiene as reflected in supragingival calculus, is involved in preclinical AD.

Insights in mechanisms linking periodontitis and AD brains gained from an experimental study suggest direct actions of *Porphyromonas gingivalis*,[34] which is the periodontal keystone pathogen.[35] The major burden of *P. gingivalis* resides in shallow pockets.[36] Probing depth quantifies the subgingival aspect of the tooth root, which harbors the subgingival biofilm, which drives inflammation in the periodontal tissues. Periodontal treatment aims primarily for the reduction in probing depth. Besides the quasi-experimental design, the specificity of mean probing depth and the dose–response relation restricted to the top 50% of this exposure in SHIP-TREND speaks also in favor of the bacterial translocation or inflammatory pathway.

The concept of preclinical AD is attractive and directly related to the subclinical disease framework.[2,3,5,19,37] The age restriction to subjects younger than 60 years supports the validity of the subclinical outcomes.[38] The AD score and brain age considered here are two of many possible measures to assess subclinical AD. The recently developed AD score, which has been developed based on data from the ADNI,[12] is an imaging marker for late-onset AD. This property may explain why the treatment affected this outcome but did not affect the brain age gap. There are, however, further explanations. The mean brain age gap is by definition zero throughout the age range of the underlying source population. More challenging to our quasi-experimental design, the variances of brain age gap did not increase with age.

Important in observational research, we examined the potential role of unmeasured confounding. As confounders that affect both treatment and outcome each by an effect size ≥2 "are not particularly

TABLE 2 Characteristics of treated subjects from GANI_MED and untreated subjects from SHIP-TREND

	Sample including untreated subjects with ≥3 periodontal pockets ≥4 mm		Subsample after improving covariate balance in the quasi-experimental design		
Variable	**Treated**	**Untreated**	**Treated, design step 1**	**Untreated, design step 1**	**Untreated, design step 2**
N	177	409	138	391	391
Weights	1	1	1	1	0.28 (0.13, 0.84)
Sum of weights	177	409	1	391	258
At treatment start					
Mean probing depth, mm					
Full mouth	3.38 (2.88, 3.85)		3.15 (2.75, 3.61)		
Half mouth, randomly		2.65 (2.46, 3.06)		2.66 (2.48, 3.07)	3.06 (2.67, 3.55)
Half mouth, right	3.38 (2.86, 3.92)		3.16 (2.73, 3.68)		
Half mouth, left	3.37 (2.85, 3.86)		3.16 (2.78, 3.54)		
Percentage of sites with probing depth ≥4 mm, %					
Full mouth	31.2 (18.5, 44.4)		25.5 (14.0, 38.8)		
Half mouth, randomly		16.1 (9.6, 29.2)		16.7 (9.6, 29.5)	28.7 (15.9, 45.8)
half mouth, right	31.2 (15.4, 45.0)		25.0 (13.5, 40.7)		
Half mouth, left	31.2 (17.9, 45.0)		26.3 (14.7, 38.2)		
Number of teeth	25 (23, 27)	25 (22, 27)	25 (23, 27)	25 (22, 27)	25 (22, 27)
Age (years)	42.2 (37.2, 46.5)	46.2 (40.1, 53.6)	42.8 (37.6, 48.0)	45.8 (39.6, 52.6)	43.3 (39.5, 46.9)
Sex, women, n (%)	102 (58)	176 (43.0)	72 (52.2)	171 (43.7)	(49.8)
Education					
< 10 years	6/176 (3)	35/408 (9)	6/137 (4)	30/390 (8)	(4)
10 years	104/176 (59)	271/408 (66)	89/137 (65)	260/390 (67)	(68)
> 10 years	66/176 (38)	102/408 (25)	42/137 (31)	100/390 (26)	(28)
Smoking					
Never	71/176 (40)	122/408 (30)	50 (36)	119/390 (31)	(34)
Ex	44/176 (25)	127/408 (31)	39 (28)	119/390 (31)	(30)
Current	61/176 (35)	159/408 (39)	49 (36)	152/390 (39)	(36)
Known diabetes mellitus[a]	9/176 (5)	18/407 (4)	5/137 (4)	18/389 (5)	(4)
Year of examination					
1993 to 1994	2 (1)	0 (0)	2 (1)	0 (0)	
1995 to 1999	29 (16)	0 (0)	23 (17)	0 (0)	
2000 to 2004	48 (27)	0 (0)	27 (20)	0 (0)	
2005 to 2007	38 (21)	0 (0)	30 (22)	0 (0)	
2008	17 (10)	31 (8)	15 (11)	29 (7)	
2009	18 (10)	155 (38)	17 (12)	146 (37)	
2010	13 (7)	124 (30)	13 (9)	120 (31)	
2011	11 (6)	73 (18)	11 (8)	71 (18)	
2012	1 (1)	26 (6)	0 (0)	25 (6)	
During periodontal treatment					
Use of adjunctive systemic antibiotics[a]	54/174 (31)		33/136 (24)		
Dropouts during supportive treatment	41 (23)		34 (25)		

(Continues)

TABLE 2 (Continued)

	Sample including untreated subjects with ≥3 periodontal pockets ≥4 mm		Subsample after improving covariate balance in the quasi-experimental design		
Variable	**Treated**	**Untreated**	**Treated, design step 1**	**Untreated, design step 1**	**Untreated, design step 2**
Treatment-related variables at final examination					
Observation period, years	7.3 (4.1, 12.2)		6.2 (3.7, 11.7)		
Mean probing depth, mm					
full mouth	2.47 (2.24, 2.76) [168]		2.44 (2.22, 2.75) [130]		
Percentage of sites with probing depth ≥4 mm, %					
Full mouth	10.1 (4.6, 17.0) [168]		9.7 (4.2, 16.4) [130]		
Number of teeth	24 (21, 27) [168]		24 (22, 27) [130]		
At final examination					
Total intracranial volume, liter	1.55 (1.46, 1.66)	1.60 (1.48, 1.71)	1.56 (1.46, 1.69)	1.60 (1.48, 1.71)	
Brain age gap (years)	0.5 (−3.8, 5.1)	0.5 (4.5, 5.0)	0.5 (−4.0, 5.4)	0.6 (−4.5, 5.1)	1.7 (−3.4, 6.5)
Alzheimer's disease score	−5.1 (−5.8, −4.3)	−4.8 (−5.6, −3.9)	−5.1 (−5.8, −4.3)	−4.8 (−5.6, −3.9)	−4.7 (−5.5, −3.8)
Age at MRI examination, years	50.2 (47.0, 54.0)	46.4 (40.1, 53.6)	49.9 (46.6, 54.4)	45.9 (39.7, 52.8)	43.3 (39.6, 47.0)
Body height, cm	171 (166, 178) [170]	174 (167, 179)	172 (166, 179) [133]	174 (167, 179)	174 (168, 180)
Marital status					
Single	11/176 (6)	48/408 (12)	10/137 (7)	48/390 (12)	
Married or living together	154/176 (87)	331/408 (81)	118/137 (86)	315/390 (81)	
Divorced or separated	10/176 (6)	25/408 (6)	8/137 (6)	23/390 (6)	
Widowed	1/176 (1)	4/408 (1)	1/137 (1)	4/390 (1)	
Smoking					
Never	71/176 (40)	122/408 (30)	50 (36)	119/390 (31)	
Ex	62/176 (35)	127/408 (31)	52 (38)	119/390 (31)	
Current	43/176 (24)	159/408 (39)	36 (26)	152/390 (39)	
Known diabetes mellitus	14/176 (8)	18/407 (4)	10/137 (7)	18/389 (5)	
HbA1c, %	5.4 (5.2, 5.7) [163]	5.2 (4.9, 5.5)	5.4 (5.2, 5.7) [129]	5.2 (4.9, 5.5)	
Waist circumference, cm	92 (83, 101) [173]	90 (81, 98) [408]	93 (82, 102) [135]	90 (81, 98) [390]	

Notes: Data are presented as median (interquartile range: 1st quartile, 3rd quartile), *n* (%), or, for sum of weights, (%); in case of missing values median (interquartile range) [*N*] or *n*/*N* (%).
Abbreviations: GANI_MED, Greifswald Approach to Individualized Medicine; SHIP-TREND, Study of Health in Pomerania, baseline examination of the second SHIP cohort.

common,"[29] the E-value of 2.0 for the estimate, but not the E-value of 1.4 for the confidence limit, does not support the assumption that unmeasured confounding is likely to affect the results. Accounting for the lack of an observation period in the untreated would further weaken this assumption. Some potential confounders were not modeled in the main analysis. Marital status (assumed as time-invariant) and severe alcohol consumption were examined in sensitivity analyses. The potential confounding by income, which is related to the treatment more directly than to the outcome, was diminished by pretreatment as well as the option of inexpensive treatment by students who were monitored by experienced periodontists (Table 1). Primarily outcome-related confounders such as obesity, waist circumference, or systemic inflammation were partially replaced by body height.[24] The confounding effect of depression could only be assessed using the E-value.

We add to the large variety of findings for the association between oral health and cognitive status by using a quasi-experimental design and continuous MRI-based outcomes, and by restricting the age range to reduce several types of bias.[7,39] Our results are in line with experimental findings, which also correspond to the concept of preclinical AD,[34] whereas evidence due to observational study design mainly comes from studies of elderly adults or clinical cases.[7] Because the onset of periodontitis is caused by the shift from a healthy to a dysbiotic biofilm, which drives the inflammatory destruction of periodontal tissues in early adulthood, the time interval between periodontal infection respective inflammation and AD can be shorter than assumed,[40] questioning that "*P. gingivalis* may spread slowly over many years" in the brain.[34] Tau pathology has already been shown in adults younger than 30 years, and the locus coeruleus,

TABLE 3 Effects of periodontal treatment on Alzheimer's disease score using a trial emulation approach; treated subjects from GANI_MED and untreated subjects from SHIP-TREND

Treated subjects from GANI_MED ($n = 177$) and untreated subjects from SHIP-TREND		Trial emulation approach after balancing covariates					
		Subsample based on the PS to balance covariates			Model for Alzheimer's disease score		
Periodontal pockets (half mouth) in an untreated subject (positivity criterion)	Untreated subjects acc. to the positivity criterion	Treated/ untreated subjects	Balance of confounder set A[a] between treated and untreated subjects	PS weighting for treated/ untreated subjects	PS weighting for confounder set A[a]	Doubly robust approach combining PS weighting for confounder set A[a] and regression adjustment for confounder set B[b]	
Number[c]	Number	Numbers	Balance[d]	Sums of weights	ATET (robust 95% CI)	ATET (robust 95% CI)	*P* value
≥3	409	138/391	good[e]	271/258	−0.38 (−0.68 – −0.07)	−0.41 (−0.70 – −0.12)	.0051
≥4	364	135/346	adequate[e,f]	245/236	−0.37 (−0.68 – −0.07)	−0.41 (−0.69 – −0.13)	.0041
≥5	316	140/300	good[e]	224/216	−0.33 (−0.64 – −0.01)	−0.37 (−0.64 – −0.10)	.0065
≥6	275	143/261	good[e]	205/199	−0.36 (−0.68 – −0.03)	−0.42 (−0.69 – −0.15)	.0025

Abbreviations: ATET, average treatment effect among the treated; CI, confidence interval; GANI_MED, Greifswald Approach to Individualized Medicine; PS, propensity score; SHIP-TREND, Study of Health in Pomerania, baseline examination of the second SHIP cohort.

[a]Confounder set A includes pre-treatment variables age, sex, mean probing depth, number of teeth, education, smoking, and diabetes mellitus.

[b]Confounder set B includes pre-treatment variables age, sex, mean probing depth, education, smoking, and diabetes mellitus, as well as body height.

[c]Numbers are related to sites with probing depth ≥4 mm in half-mouth assessment.

[d]Balance evaluation of confounder set A is based on (1) the maximum of the absolute value of the standardized mean differences between treated and untreated groups over each covariate (values < 0.10 indicate good covariate balance; values < 0.25 indicate adequate covariate balance); and (2) the variance ratio (good if between 0.8 and 1.2; adequate if between 0.5 and 2.0).

[e]The balance of body height was good.

[f]The balance was good except for probing depth (variance ratio = 0.75).

which is important for cognitive function, is especially vulnerable to infection.[41]

An open research question on the *APOE* genotype was analyzed only cross-sectionally. Two mechanisms for interaction between *APOE* genotype and *P. gingivalis* have been proposed.[34] Based on the observation that the three *APOE* genotype lines are largely parallel for probing depth values that represent periodontitis, but not parallel for probing depth values at the transition from periodontal health to periodontitis, it can be speculated that the effect of *P. gingivalis* on brain age is modified by the *APOE* genotype.[42,43]

Our quasi-experimental study has several limitations. Subjects were German residents of West Pomerania, thereby restricting the generalizability of the findings. Using a subsample, we sacrificed some external validity to focus more on internal validity as recommended.[27] Unmeasured known confounding, unknown confounding, selection bias, measurement error, and misclassification could have affected our results, which were investigated in several sensitivity analyses. We did not examine misclassification, confounding, and selection bias simultaneously.[44] Moreover, some assumptions, especially about loss to follow-up, are untestable.[14] We do not believe, however, that both treatment and outcome are strongly associated with the selection process as required for a large bias.[14]

Using a patient cohort from 1993 to 2012 is also subordinate in preference to a randomized trial, but even historical control patients can be made statistically comparable with later patients.[45] Indeed, the study design and the propensity score techniques used herein balanced covariates and enabled the statistical comparability of the two study groups. Of note, a randomized trial including intentionally untreated subjects is hard to design for ethical reasons. Periodontal treatment reduces pocket depths and the total subgingival biofilm,[46] but does not eradicate *P. gingivalis*. However, it reduces the presence and load of *P. gingivalis*, which is an invasive and evasive opportunistic pathogen.[42] Moreover, treatment may also affect the role of *P. gingivalis* and subgingival biofilm communities in orchestrating a host response.[42] Thus, active periodontal treatment and subsequent maintenance treatment represent a much broader approach than the use of a specific drug targeting gingipains from *P. gingivalis* in subjects with probable AD as done in an ongoing randomized, double-blind, placebo-controlled study.[47]

Our study has further strengths in design and analysis. We examined the role of known and unknown confounders using the E-value, which should supplement the *P*-value.[29] We avoided categorization and allowed for nonlinear dose-response relations,[2,25,48] thereby reducing residual confounding. We used several measures against reverse causality,[16] comprising exclusion of cases with brain injury, stroke, and Parkinson's disease; choosing a subclinical outcome;[2] and age restriction. Age restriction also ensured low survivor bias and facilitated a more concise investigation of the time interval between periodontitis and preclinical AD. Moreover, young subjects have a higher number of teeth and, therefore, a high reliability of periodontal measures on subject level. Finally, we estimated treatment effects in analyses that emulate a trial.[14,15]

Quantifying the effects of periodontitis on AD is challenging. Some major modifiable risk factors of AD point to periodontitis, which might be examined as their putative mediator using future studies with two follow-ups. Such a study design can also distinguish indirect (potentially bidirectional)[49] inflammatory effects from direct effects by

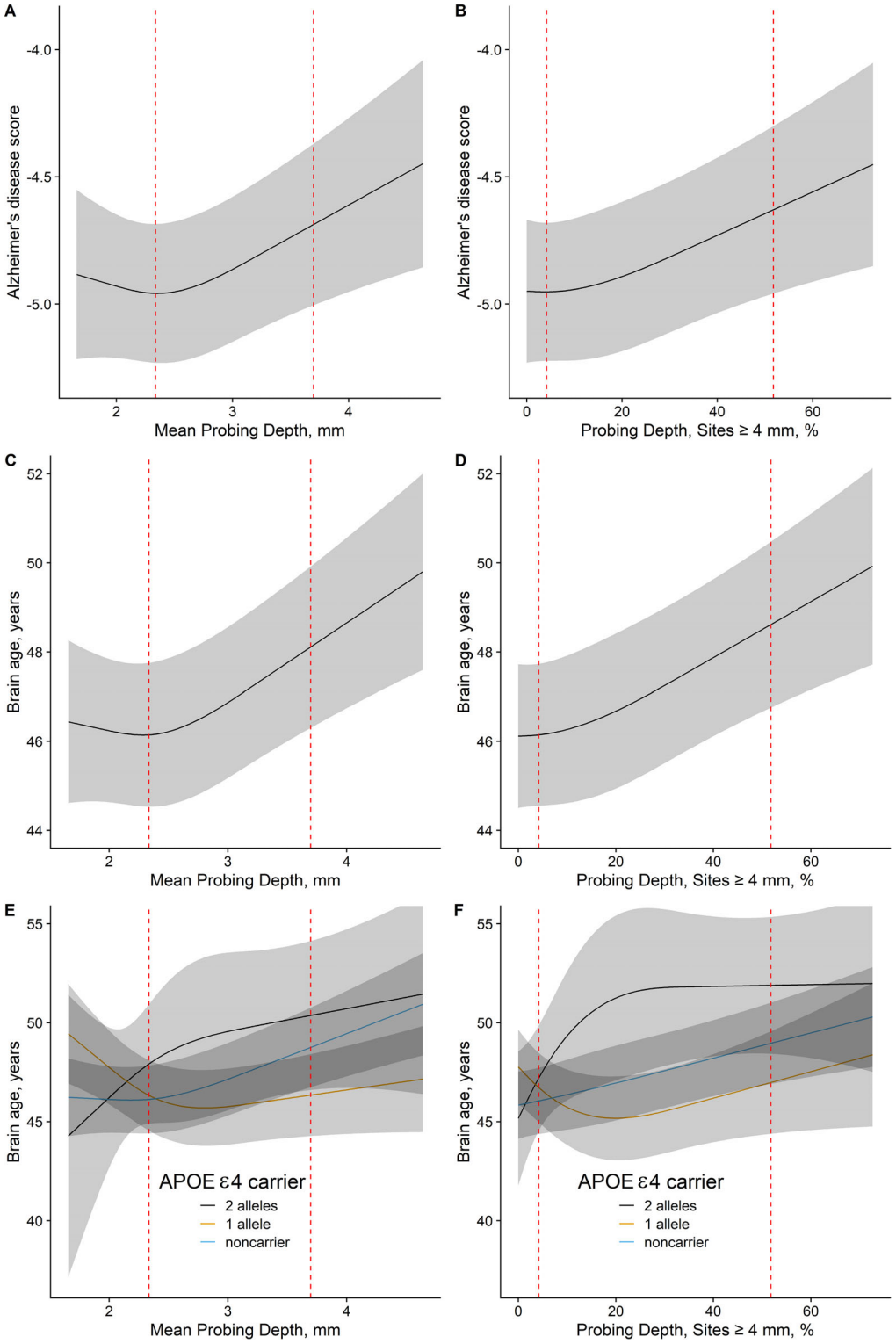

FIGURE 1 Associations between probing depth and the outcomes Alzheimer's disease score and brain age in SHIP-TREND participants younger than 60 years. Effects adjusted for age; sex; body height; intracranial volume; education; marital status; smoking; known diabetes mellitus; HbA1c; waist circumference; depression; alcohol consumption; the sex interactions with body height, intracranial volume, and waist circumference; as well as the interaction between diabetes mellitus and HbA1c in ordinary regressions (A: $n = 1335$, $P = .0065$; B: $n = 1335$, $P = .0042$; C: $n = 1323$, $P < .0001$; D: $n = 1323$, $P < .0001$). Additive interaction between *APOE* genotype and probing depth adjusted for the same confounders (E: $n = 1224$, $P = .0032$; F: $n = 1224$, $P = .0023$). Gray: 95% confidence intervals; dark gray: overlapping regions for confidence intervals. The median of the exposure was used as reference value (left vertical red line) to the top 5% (95th percentile; right red line). A, C, E) Mean probing depth (median = 2.34 mm; 95th percentile = 3.70). B, D, F) Proportion of sites with probing depth ≥4 mm (median = 4.2%; 95th percentile = 51.8%). *APOE*, apolipoprotein E; HbA1c, hemoglobin A1c; SHIP-TREND, Study of Health in Pomerania, baseline examination of the second SHIP cohort

TABLE 4 Characteristics of study participants with and without MRI from SHIP-TREND, 2008 to 2012

	MRI data not available		MRI data available					
			Periodontal data available		Periodontal data not available			
	MRI data not available ($n = 1348$)		Regular examination ($n = 1462$)		Mobile examination ($n = 19$)		Edentulous ($n = 16$)	
	n	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)
Teeth	1348	25 (21, 27)	1462	25 (22, 27)	19	25 (21, 28)	16	0 (0, 0)
Edentulism	1348	39 (2.9)	1462	0 (0)	19	0 (0)	16	16 (100)
No periodontal data in mobile examination	1348	178 (13.2)	1462	0 (0)	19	19 (100)	16	1 (6.2)
Mean probing depth (mm)	1098	2.3 (2.1, 2.8)	1438	2.3 (2.1, 2.7)	0	—	0	—
Sites with probing depth ≥4 mm (%)	1098	5.0 (0.0, 17.3)	1438	4.2 (0.0, 15.9)	0	—	0	—
Calculus (%)	1106	4.2 (0.0, 12.5)	1437	4.2 (0.0, 8.3)	0	—	0	—
Dental plaque (%)	1106	12.5 (4.2, 35.0)	1437	8.3 (0.0, 29.2)	0	—	0	—
Mean clinical attachment level (mm)	1069	1.6 (1.1, 2.5)	1403	1.7 (1.1, 2.6)	0	—	0	—
Sites with clinical attachment level ≥3 mm (%)	1069	15.0 (2.1, 47.5)	1403	19.6 (3.8, 50.0)	0	—	0	—
Removable dental prosthesis	1170	203 (17.4)	1462	179 (12.2)	19	0 (0.0)	15	15 (100)
Time since last dental visit	1169		1461		0		15	
≤6 months		749 (64.1)		980 (67.1)		—		9 (60.0)
7-12 months		242 (20.7)		345 (23.6)		—		2 (13.3)
>12 months		178 (15.2)		136 (9.3)		—		4 (26.7)
Last dental visit because of checkup	1147	588 (51.3)	1455	812 (55.8)	0	—	13	7 (53.8)
Total intracranial volume, liter	0	—	1462	1.59 (1.48, 1.71)	19	1.66 (1.56, 1.72)	16	1.53 (1.40, 1.58)
Brain age (years)	0	—	1370	46.5 (39.6, 53.3)	17	56.4 (34.6, 60.5)	15	57.5 (53.0, 63.8)
Alzheimer's disease score	0	—	1382	-4.9 (-5.7, -4.1)	17	-5.3 (-5.7, -3.9)	15	-3.8 (-4.9, -3.3)
Age (years)	1348	41.0 (32.0, 51.0)	1462	45.0 (37.0, 52.0)	19	45.0 (31.0, 56.0)	16	55.0 (53.8, 57.8)
Sex, women, n (%)	1348	721 (53.5)	1462	753 (51.5)	19	7 (36.8)	16	10 (62.5)
Body height, cm	1344	171.0 (165.0, 179.0)	1462	171.5 (165.0, 179.0)	19	172.0 (165.0, 180.0)	16	164.5 (158.9, 177.5)
Education	1345		1460		19		16	
< 10 years		202 (15.0)		87 (6.0)		1 (5.3)		0 (0.0)
10 years		837 (62.2)		921 (63.1)		13 (66.4)		13 (81.2)
> 10 years		306 (22.8)		452 (31.0)		5 (26.3)		3 (18.8)
Marital status	1345		1460		19		16	
Single		227 (16.9)		192 (13.2)		5 (26.3)		1 (6.2)
Married or living together		1006 (74.8)		1162 (79.6)		14 (73.7)		12 (75.0)
Divorced or separated		96 (7.1)		90 (6.2)		0 (0.0)		3 (18.8)
Widowed		16 (1.2)		16 (1.1)		0 (0.0)		0 (0.0)

(Continues)

TABLE 4 (Continued)

	MRI data not available		MRI data available					
			Periodontal data available		Periodontal data not available			
	MRI data not available ($n = 1348$)		Regular examination ($n = 1462$)		Mobile examination ($n = 19$)		Edentulous ($n = 16$)	
	n	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)
Smoking	1345		1460		19		16	
Never		374 (27.8)		528 (36.2)		6 (31.6)		4 (25.0)
Ex, < 1 cigarettes/day		177 (13.2)		237 (16.2)		0 (0.0)		0 (0.0)
Ex, 1–14 cigarettes/day		82 (6.1)		80 (5.5)		2 (10.5)		0 (0.0)
Ex, ≥15 cigarettes/day		158 (11.7)		175 (12.0)		4 (21.1)		2 (12.5)
Ex, unknown number		6 (0.4)		3 (0.2)		0 (0.0)		0 (0.0)
Current, < 1 cigarettes/day		46 (3.4)		70 (4.8)		0 (0.0)		0 (0.0)
Current, 1–14 cigarettes/day		236 (17.5)		184 (12.6)		3 (15.8)		6 (37.5)
Current, ≥15 cigarettes/day		266 (19.8)		183 (12.5)		4 (21.1)		4 (25.0)
Known diabetes mellitus	1345	93 (6.9)	1460	54 (3.7)	19	0 (0.0)	16	0 (0.0)
HbA1c, %	1344	5.1 (4.8, 5.4)	1460	5.1 (4.8, 5.4)	19	5.2 (4.8, 5.5)	16	5.5 (5.1, 5.7)
Waist circumference (cm)	1340	87.1 (76.9, 98.0)	1461	87.0 (77.6, 96.8)	19	91.5 (78.1, 102.0)	16	88.0 (79.8, 103.6)
Depression	1330		1449		19		16	
No major depressive disorder		1027 (77.2)		1,166 (80.5)		16 (84.2)		11 (68.8)
Single major depressive disorder		93 (7.0)		100 (6.9)		1 (5.3)		1 (6.2)
Recurrent major depressive disorder		210 (15.8)		183 (12.6)		2 (10.5)		4 (25.0)
Alcohol consumption last 30 days (g/day)	1333	3.9 (0.7, 12.5)	1450	4.5 (1.3, 11.0)	19	2.7 (0.0, 13.7)	16	1.9 (0.0, 4.0)
Physical activity	1344	869 (64.7)	1460	1013 (69.4)	19	10 (52.6)	16	11 (68.8)
Equivalent household income (€)	1290	1184 (775, 1761)	1411	1450 (1025, 1803)	19	1450 (778, 2050)	15	1096 (693, 1550)
Anti-inflammatory medication	1323	244 (16.9)	1445	216 (14.9)	19	1 (5.3)	16	4 (25.0)
Plasma fibrinogen concentration (g/L)	1329	2.9 (2.4, 3.4)	1447	2.8 (2.4, 3.3)	19	2.8 (2.4, 3.2)	15	3.5 (3.1, 3.9)
High-sensitive C-reactive protein (mg/L)	1294	1.3 (0.6, 3.2)	1424	1.1 (0.5, 2.3)	19	1.3 (0.4, 2.1)	16	2.7 (1.5, 5.0)
White blood cell count, Gpt/l	1342	6.1 (5.1, 7.2)	1459	5.5 (4.7, 6.7)	19	6.5 (4.7, 7.5)	16	7.7 (6.0, 10.1)
Anti-hypertensive medication	1345	292 (21.7)	1460	278 (19.0)	19	7 (36.8)	16	4 (25.0)
Systolic blood pressure (mmHg)	1341	123.0 (111.5, 135.5)	1458	122.5 (111.5, 133.5)	19	126.0 (115.0, 135.5)	16	123.8 (115.3, 139.5)
Diastolic blood pressure (mmHg)	1341	76.5 (70.0, 84.0)	1458	76.5 (70.5, 83.0)	19	79.0 (75.0, 86.0)	16	77.8 (72.8, 82.0)
LDL cholesterol (mmol/L)	1345	3.2 (2.6, 3.8)	1461	3.3 (2.7, 3.9)	19	3.7 (2.9, 4.5)	16	3.6 (3.2, 4.3)

(Continues)

TABLE 4 (Continued)

	MRI data not available		MRI data available					
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	n	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)	*n*	Median (1st, 3rd quartile) or number (percent)
HDL cholesterol (mmol/L)	1345	1.4 (1.1, 1.6)	1461	1.4 (1.2, 1.7)	19	1.5 (1.0, 1.8)	16	1.5 (1.1, 1.7)
APOE ε4 frequency	0		1350		18		16	
0		—		1023 (75.8)		11 (61.1)		13 (81.2)
1		—		293 (21.7)		7 (38.9)		2 (12.5)
2		—		34 (2.5)		0 (0.0)		1 (6.2)

Abbreviations: *APOE*, apolipoprotein E; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; SHIP-TREND, Study of Health in Pomerania, baseline examination of the second SHIP cohort.

P. gingivalis if the specificity of the germ is additionally designed. Design and analysis could be improved by considering instrumental variables (genetic factors; preference for or access to treatment, e.g., travel time).[14]

In conclusion, our studies provide several lines of evidence that periodontitis is related to preclinical AD, a notion that requires verification in independent samples.

ACKNOWLEDGMENTS

Data collection and sharing for this project was in part funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI; National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: AbbVie; Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen; Bristol-Myers Squibb Company; CereSpir, Inc.; Cogstate; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare; IXICO Ltd; Janssen Alzheimer Immunotherapy Research & Development, LLC; Johnson & Johnson Pharmaceutical Research & Development LLC; Lumosity; Lundbeck; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Takeda Pharmaceutical Company; and Transition Therapeutics. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Therapeutic Research Institute at the University of Southern California. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. GANI_MED was funded by the Federal Ministry of Education and Research (grant 03IS2061A) and by the Ministry of Education and Research of the State of Mecklenburg–West Pomerania. SHIP is part of the Community Medicine Research Network of the University Medicine Greifswald, which is supported by the German Federal State of Mecklenburg–West Pomerania. This study was further supported by the German Center for Neurodegenerative Diseases, the EU Joint Programme - Neurodegenerative Disease Research funding for BRIDGET (01ED1615), and by National Institutes of Health Grant RF1 AG059421.

CONFLICTS OF INTEREST

HJG has received travel grants and speaker honoraria from Fresenius Medical Care, Neuraxpharm, Servier, and Janssen Cilag as well as research funding from Fresenius Medical Care. HJG had personal contracts approved by the university administration for speaker honoraria and one IIT with Fresenius Medical Care.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Schwahn C, Frenzel S, Holtfreter B, et al. Effect of periodontal treatment on preclinical Alzheimer's disease—Results of a trial emulation approach. *Alzheimer's Dement*. 2021;1-15. https://doi.org/10.1002/alz.12378