Significant association of periodontal disease with anti-citrullinated peptide antibody in a Japanese healthy population –the Nagahama study.

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Abstract

Anti-citrullinated peptide antibody (ACPA) is a highly specific autoantibody to rheumatoid arthritis (RA). Recent studies have revealed that periodontal disease (PD) is closely associated with RA and production of ACPA in RA. Analyses of associations between PD and ACPA production in a healthy population may deepen our understandings. Here, we analyzed a total of 9,554 adult healthy subjects. ACPA and IgM-rheumatoid factor (RF) was quantified and PD status was evaluated using the number of missing teeth (MT), the Community Periodontal Index (CPI) and Loss of Attachment (LA) for these subjects. PD status was analyzed for its association with the positivity and categorical levels of ACPA and RF conditioned for covariates which were shown to be associated with PD, ACPA or RF. As a result, all of MT, CPI and LA showed suggestive or significant associations with positivity ($p=0.024$, 0.0042 and 0.037, respectively) and levels of ACPA ($p\leq0.00031$), but none of the PD parameters were associated with those of RF. These association patterns were also observed when we analyzed 6,206 non-smokers of the participants. The significant associations between PD parameters and positivity and levels of ACPA in healthy population support the fundamental involvement of PD with ACPA production.

Keywords: epidemioloical study, periodontal disease, ACPA, RF, association study, rheumatoid arthritis

1.**Introduction**

Rheumatoid arthritis (RA) is the most common cause of adult chronic autoimmune arthritis in the world affecting 0.5 to 1.0% of the population. Environmental and genetic factors have been shown to be important for the onset of RA[1, 2]. Anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF) are autoantibodies frequently found in patients with RA[3-5]. ACPA is a highly specific autoantibody to RA[5]. Smoking is an established environmental factor which is associated with RA development and ACPA positivity in RA[6, 7]. Although the pathological function of ACPA is not fully established, recent studies have revealed that some fractions of ACPA is pathogenic in experimental animal models[8].

The association between periodontal disease (PD) and RA development has been reported and PD is an emerging risk factor for RA[9]. PD affects more than 20% of the general population and its chronic inflammation leads to the damage of oral tissues including periodontal ligaments, resulting in periodontal pockets and ultimately, tooth loss[10]. Patients with RA were reported to have higher frequency of PD and severer PD than controls including patients with osteoarthirits[11, 12]. Since *Porphyromonas.gingivalis* (*P gingivalis*), a bacterial flora of PD, is the only microorganism ever found with citrullination enzyme peptidylarginine deiminase (PAD)[13], it has been hypothesized that PD is associated with the production of ACPA via citrullination of proteins by *P gingivalis* and the resultant RA[14, 15]. However, it is difficult to

assess whether PD is a cause or result of RA when analyzing data of patients with RA. In spite of a previous study reporting the presence of PD in patients with early RA naïve to DMARDs treatment[16], the possibility is undeniable that decreased activity of daily living due to early RA would lead to PD. Since RF and ACPA can be observed in patients with other diseases and even in healthy populations, studies should extend to healthy individuals to confirm the correlation between PD and production of RA-related autoantibodies and provide a clue to the causality of PD on the autoantibody production.

Recently, we reported detailed distribution and correlates of ACPA and RF using the data of the Nagahama Study, a Japanese community based prospective cohort[17]. Here, we analyzed the associations between PD status and RA-related autoantibodies especially ACPA using the data of the Nagahama study.

2.Materials and Methods

2.1Study Subjects

A total of 9,804 subjects were registered in the Nagahama Study. 201 subjects were excluded due to having or being suspected to have autoimmune diseases. 28 subjects were excluded due to insufficient data. As a result, 9,575 subjects in the Nagahama Study who did not have connective tissue diseases[17] were selected for the current study. The details of sample

selections were described in the previous study[17]. The current study was approved by the Ethics Committee, Kyoto University Graduate School and Faculty of Medicine and written informed consent was obtained from each participant.

2.2ACPA and RF quantification.

The details of the measurement of ACPA and RF were described previously[17]. Briefly, the 2nd generation anti-CCP antibody was quantified as ACPA by MESACUP CCP ELISA kit (Medical and Biological Laboratories Co., Ltd, Nagoya, Japan). IgM-RF was quantified as RF by latex turbidimetric immunoassay, IATRO-RF II (Mitsubishi Kagaku Medience, Co., Tokyo, Japan). The cut-off levels of the autoantibodies were set according to the manufacturer's instructions (ACPA<4.5U/ml, RF≤20IU/ml). The lower measuring limit of ACPA and RF were 0.6U/ml and 1IU/ml, respectively. RF levels of all subjects were within the measuring limit (≥1IU/ml). ACPA and RF were measured for all of the participants in the current study.

2.3PD parameters

Subjects in the Nagahama Study were evaluated by dentists for PD status. The number of missing teeth (MT), the Community Periodontal Index (CPI) and Loss of Attachment (LA) were evaluated as PD parameters for 9,554 out of 9,575 subjects (Supplementary Table 1). LA and

CPI were evaluated in the jaw sextants according to the WHO recommendations where the maximum score of LA and CPI was 4. Since we aimed to assess systemic PD and were not focusing on localized inflammation, we calculated the average score of LA or CPI in the sextants. When subjects had fractions of sextants we could not evaluate due to lack of teeth, a score of 4 was given to the regions.

2.4Statistical Analysis

Logistic regression analysis was performed to evaluate the effects of PD parameters on ACPA and RF positivity. Linear or Poisson regression analyses were applied to investigate the association between PD parameters and levels or categorical levels of ACPA and RF. Positivity, levels and categorical levels of ACPA and RF were used as dependent variables, and age, sex, smoking status, body mass index (BMI), alcohol drinking status, history of diabetes mellitus (DM), usage of insulin and anti-hyperglycaemic medications and the current working status were used as covariates based on the previous studies[17-19]. Ex- and current smokers were classified as smokers. Those who do not drink alcohol at all were regarded as non-drinkers. The current working status consists of three categories, namely, full-time worker, part-time worker and unemployed. Detailed distributions of the covariates are shown in Table 1. When levels of ACPA and RF were analyzed, the levels were natural logarithm-transformed and subjects whose

ACPA levels were under and over the measuring limit of the kit (0.6-100U/ml) were given 0.5 and 100U/ml of ACPA levels, respectively, for calculation.

When categorical levels of ACPA and RF were analyzed, levels under the measuring limit of ACPA and the lowest levels of RF were set as 0, levels of ACPA and RF which were lower than the cut-off levels and higher than category 0 were set as 1, levels higher than the cut-off levels and lower than three times the cut-off level were set as 2, levels higher than three times the cut-off level were set as 3 (Supplementary Table 1).

P-values less than 0.05 were regarded as suggestively significant. Although PD parameters were not independent from each other (Supplementary Table 2), conservational significant levels of p-values less than 0.017 based on the Bonferroni's correction were applied. Statistical analyses were performed by R statistical software or SPSS.

3.Results

3.1 Associations between PD and positivity of RF or ACPA

A total of 9,554 subjects were recruited from the 9,804 participants in the Nagahama study. The characteristics of the 9,554 study participants in the current study are summarized in Table 1 and detailed distributions of PD parameters are shown in Supplementary Table 3. In addition, we performed sub-analysis using 6,804 non-smokers among the 9,554 subjects. The detailed

process of sample exclusion and study design are shown in Supplementary Figure 1. Of the participants, 1.7 and 6.4% were positive for ACPA and RF, respectively. We analyzed whether positivity of ACPA and RF were associated with PD parameters in the participants. As a result, we found significant or suggestive positive associations between increasing positivity of ACPA and all of the PD parameters, namely, MT, CPI and LA conditioned with covariates ($p=0.024$, 0.0042 and 0.037, respectively, Table 2). On the contrary, none of the PD parameters revealed significant associations with RF positivity ($p\geq0.19$, Table 2). We also presented the association results between PD parameters and positivity of ACPA or RF without covariates (Table 2).

3.2 Associations between PD and levels of RF or ACPA

Next, whether levels of ACPA and RF are associated with PD parameters was analyzed. We observed significant associations between increasing ACPA levels and PD parameters ($p\leq0.010$, Supplementary Table 4). Since linearity of quantification of low titers around the lowest measuring limit was not fully guaranteed, we classified levels of ACPA and RF into four categories (for details, see Materials and Methods and Supplementary Table 1) and performed Poisson regression analysis to analyze increasing effects of PD parameters on ACPA and RF categorical levels. As a result, we found that PD parameters were significantly associated with increasing categorical levels of ACPA ($p\leq0.00031$, Table 2). On the contrary, we did not find

significant associations between PD parameters and crude or categorical levels of RF (Table 2 and Supplementary Table 4). Since the positivity of ACPA in the participants is low, linear regression model dividing ACPA levels into three groups of ACPA-negative, ACPA-low positive and ACPA-high positive resulted in very similar results to qualitative analysis of ACPA in the logistic regression models (data not shown).

Next, we classified PD parameters into four groups according to the severity of PD in order to confirm dose-dependent associations of PD parameters on positivity or categorical levels of ACPA and RF. We observed the above-mentioned associations in all combinations of PD parameters and positivity or categorical levels of ACPA and RF (Figure 1). CPI was shown to be associated with increasing ACPA positivity and levels when CPI was more than 2.

Finally, we performed a sub-analysis focusing on non-smoking subjects. 6,206 out of 9,554 subjects (65.0%) were non-smokers (Supplementary Figure 1). We found in the non-smokers the same association patterns between PD parameters and positivity or levels of ACPA and RF with comparable effect sizes to the results in 9,554 subjects (Supplementary Figure 2, Supplementary Table 5 and 6). These results confirmed that the above-mentioned associations were not confounded by smoking. While MT and LA showed suggestive negative associations

with categorical levels of RF (Supplementary Table 5), these associations were not clear for log-transformed RF (Supplementary Table 6) and CPI did not show a significant association.

4.Discussion

9,575 healthy subjects without connective tissue diseases were selected in this study. The 201 subjects excluded from the current study due to possibility of having connective tissue diseases were 2.1% of the Nagahama Study participants and 27.9, 34.8 and 70.1% of them were positive for ACPA, RF, and anti-nuclear antibody, respectively. The percentage of excluded samples seems reasonable considering prevalence of RA and safety margin to avoid contamination of patients with RA in the current study. We did not have information of sicca syndrome in the current study. However, considering high positivity of RF and low positivity of ACPA in subjects with sicca syndrome or Sjögren syndrome[20, 21], the association with ACPA and lack of association with RF cannot be explained by subjects with undiagnosed sicca or Sjögren syndrome.

This is the first study ever to analyze the association between PD parameters and positivity or levels of ACPA and RF in a large-scale healthy population. We found significant associations between PD parameters and positivity or levels of ACPA, but not between PD parameters and

positivity or levels of RF. These association patterns were also observed in non-smokers. The beta values of PD parameters on ACPA levels or positivity were not strong. However, since the positivity of ACPA in healthy subjects is very low, it is not realistic to assume a one-to-one association between ACPA and common factors such as PD. Thus, it is important to accumulate enough number of subjects to identify weak to moderate but significant associations between common factors and rare phenotypes.

Since detailed data for PD parameters in sextants were available, we adopted average CPI and LA as dependent variables which were expected to precisely reflect periodontal inflammation.

The covariates in the current study were selected based on previous studies and included major common covariates in the previous studies. While we did not have detailed socioeconomic data for the participants, we used the current working status as a covariate. Inclusion of people over 60 years old in the Nagahama study and the higher ratio of females in the participants might lead to increased ratio of unemployed population because many Japanese companies set retirement age as 60 and we categorized house wives as “unemployed”. A previous study showed that socioeconomic status in the Japanese population was not necessarily associated with disease risk observed in other countries due to the wide coverage of national health insurance system[22], so the influence of insufficient socioeconomic status in the current

study should be minimal.

Since shared epitope (SE), a susceptibility HLA-DRB1 allelic group, was not associated with ACPA and RF in our previous study[17], we did not use SE as a covariate. The associations of PD with ACPA or RF did not change in condition with SE when we analyzed 3,169 participants whose SE information were available (data not shown).

Considering the high specificity of ACPA and PD as a potential cause of RA, the positive association found in the current study may suggest that PD is associated with the production of ACPA and triggers the development of RA. It is interesting that we did not observe significant associations between PD parameters and RF positivity or levels. Not even a trend in increasing effects of PD parameters was observed on RF positivity and levels. We did find instead suggestive negative associations between LA or MT and categorical levels of RF in non-smokers, but the associations were inconclusive. Since RF positivity is much higher than that of ACPA, the lack of significant positive associations of RF cannot be explained by frequency. Involvement of PD with the process of ACPA production and not with that of RF could be a reasonable explanation. Although *P gingivalis* is a promising candidate to explain the involvement of PD with ACPA production, there is not yet enough evidence to conclude that *P gingivalis* in the PD-related bacterial flora is the cause of increasing positivity and level of

ACPA. A recent study suggested that fine-specific ACPA detected in healthy subjects may not be specific to citrullinated peptide[23]. There is a possibility that non-specific oral inflammation by PD is important for ACPA production through undetermined processes. While we do not have data of anti-*P gingivalis* antibody, a large-scale association study of healthy population with anti-*P gingivalis* antibody would conclude that the increasing effects of PD on ACPA is associated with nonspecific PD or PD caused by *P gingivalis*.

Recent studies have revealed that correlates of ACPA are different according to fine fractions of ACPA[24], and it will be interesting to quantify detailed fractions of ACPA in the study participants and compare the results among fractions of ACPA. It is feasible to replicate these results in other populations including Europeans.

5.Conclusions

ACPA positivity and levels are associated with PD in a healthy population. This association would support involvement of PD in the process of production of ACPA.

CONFLICT OF INTEREST: none

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FIGURE LEGEND

Figure 1. Significant associations of PD parameters with positivity and levels of ACPA but not with those of RF.

Y axes for categorical levels indicate adjusted levels by covariates.

P-values of logistic regression analysis or Poisson regression analysis in condition with covariates are indicated.

NS: not significant (p>0.05), MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor

TABLES

Table 1. Summary statistics of the study participants.

	Participants
Number	9,554
Female ratio	66.9%
Age*	53.29±13.43
MT**	0-28 (1)
CPI**	0-4 (0.833)
LA**	0-4 (0)
ACPA positivity	1.7%
ACPA category	0:7395, 1:1992, 2:100, 3:67
RF positivity	6.4%
RF category	0:6357, 1:2583, 2:486, 3:128
Body mass index*	22.30±3.28
Smoking	Smoker:3348, Non-smoker:6206
Alcohol drinking	Drinker:6134, Non-drinker:3419
Current working status***	Full-time:3013, Part-time:2052, Unemployed:4489
History of DM	5.2%
Usage of insulin	0.3%
Usage of anti-hyperglycaemic medications	2.8%

MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor

*Mean±standard deviation

**minimum-maximum (median)

***house wife is categorized as unemployed

Table 2. PD parameters, especially CPI are associated with positivity and levels of ACPA but not with those of RF.

	ACPA positivity		ACPA categorical levels			RF positivity		RF categorical levels		
PD parameters	OR (95%CI)	P	Beta	SE	P	OR (95%CI)	P	Beta	SE	P
Unadjusted										
MT	1.04 (1.02-1.06)	$8.0x10^{-5}$	0.019	0.0030	$6.2x10^{-10}$	1.00 (0.98-1.01)	0.81	-0.0037	0.0028	0.18
CPI	1.31 (1.15-1.48)	$2.0x10^{-5}$	0.111	0.017	$1.9x10^{-10}$	1.01 (0.94-1.09)	0.70	0.0068	0.014	0.64
LA	1.28 (1.12-1.46)	0.00033	0.126	0.020	$2.5x10^{-10}$	0.97 (0.88-1.07)	0.54	-0.017	0.018	0.36
Adjusted										
MT	1.03 (1.00-1.05)	0.024	0.013	0.0035	0.00031	0.99 (0.98-1.01)	0.27	-0.0043	0.0032	0.17
CPI	1.23 (1.07-1.42)	0.0042	0.081	0.020	$5.4x10^{-5}$	1.00 (0.92-1.09)	0.98	0.015	0.016	0.37
LA	1.18 (1.01-1.37)	0.037	0.088	0.022	$9.2x10^{-5}$	0.93 (0.84-1.03)	0.19	-0.016	0.020	0.43

ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor, PD:periodontal disease, MT:the number of missing teeth, CPI:the Community Periodontal Index, LA:Loss of Attachment, OR:odds ratio, CI:confidence interval, SE:standard error

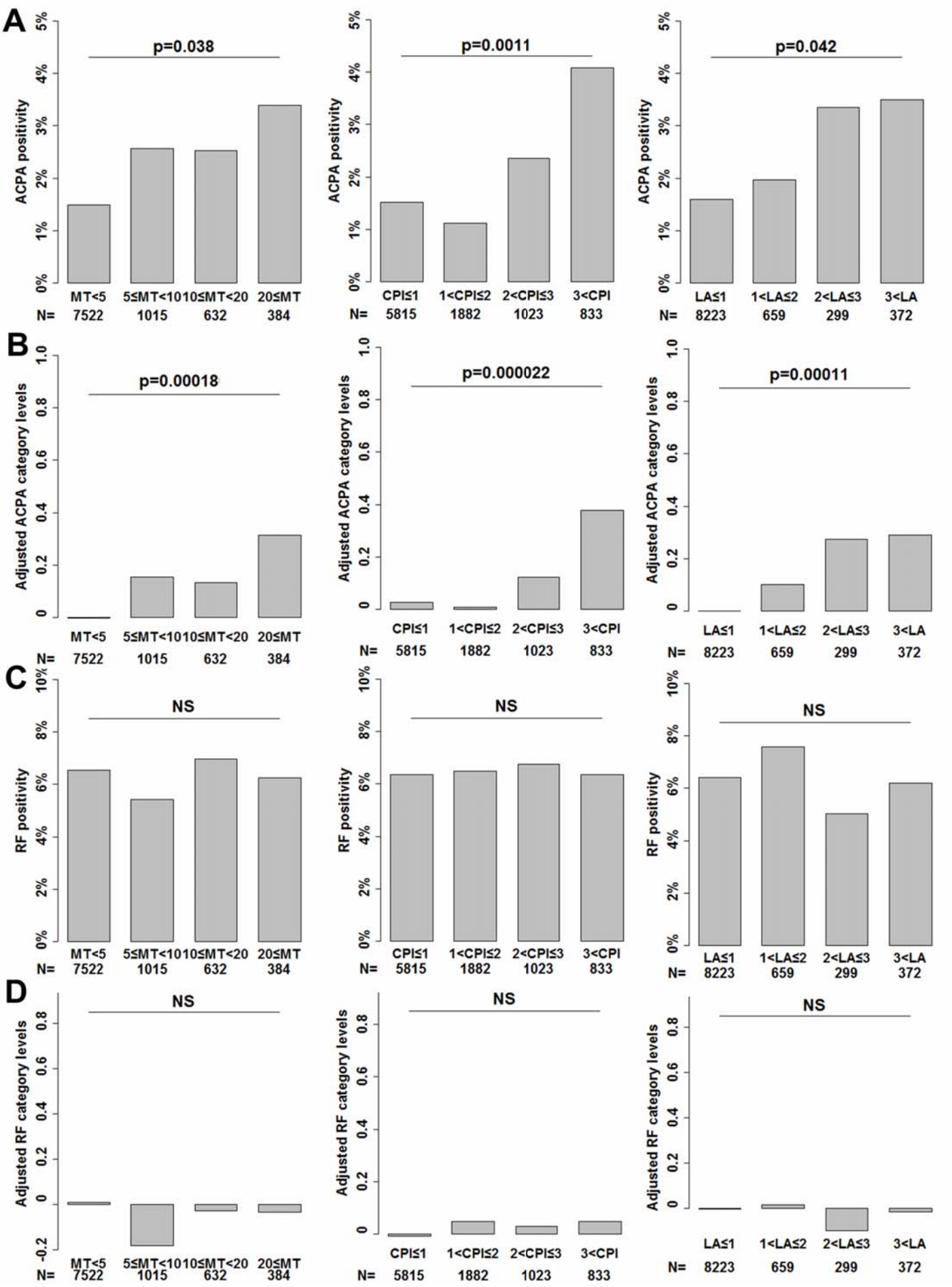
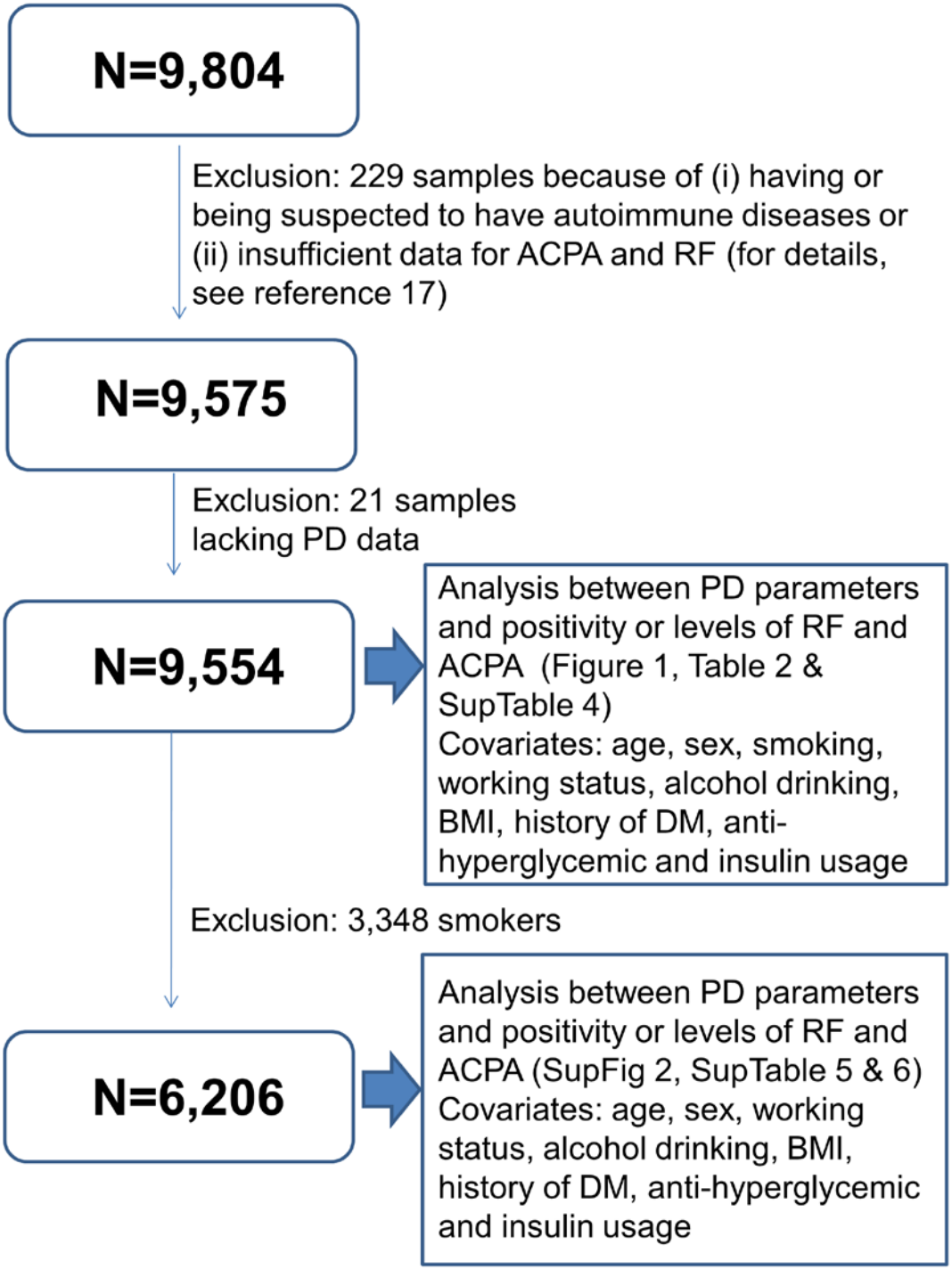

A
ACPA positivity
0%
1%
2%
3%
4%
5%
p=0.038
MT<5
5≤MT<10
10≤MT<20
20≤MT
N=
7522
1015
632
384
p=0.0011
CPI≤1
1<CPI≤2
2<CPI≤3
3<CPI
N=
5815
1882
1023
833
p=0.042
LA≤1
1<LA≤2
2<LA≤3
3<LA
N=
8223
659
299
372
B
Adjusted ACPA category levels
0
0.2
0.4
0.6
0.8
1.0
p=0.00018
p=0.000022
p=0.00011
C
RF positivity
0%
2%
4%
6%
8%
10%
NS
D
Adjusted RF category levels
-0.2
0
0.2
0.4
0.6
0.8

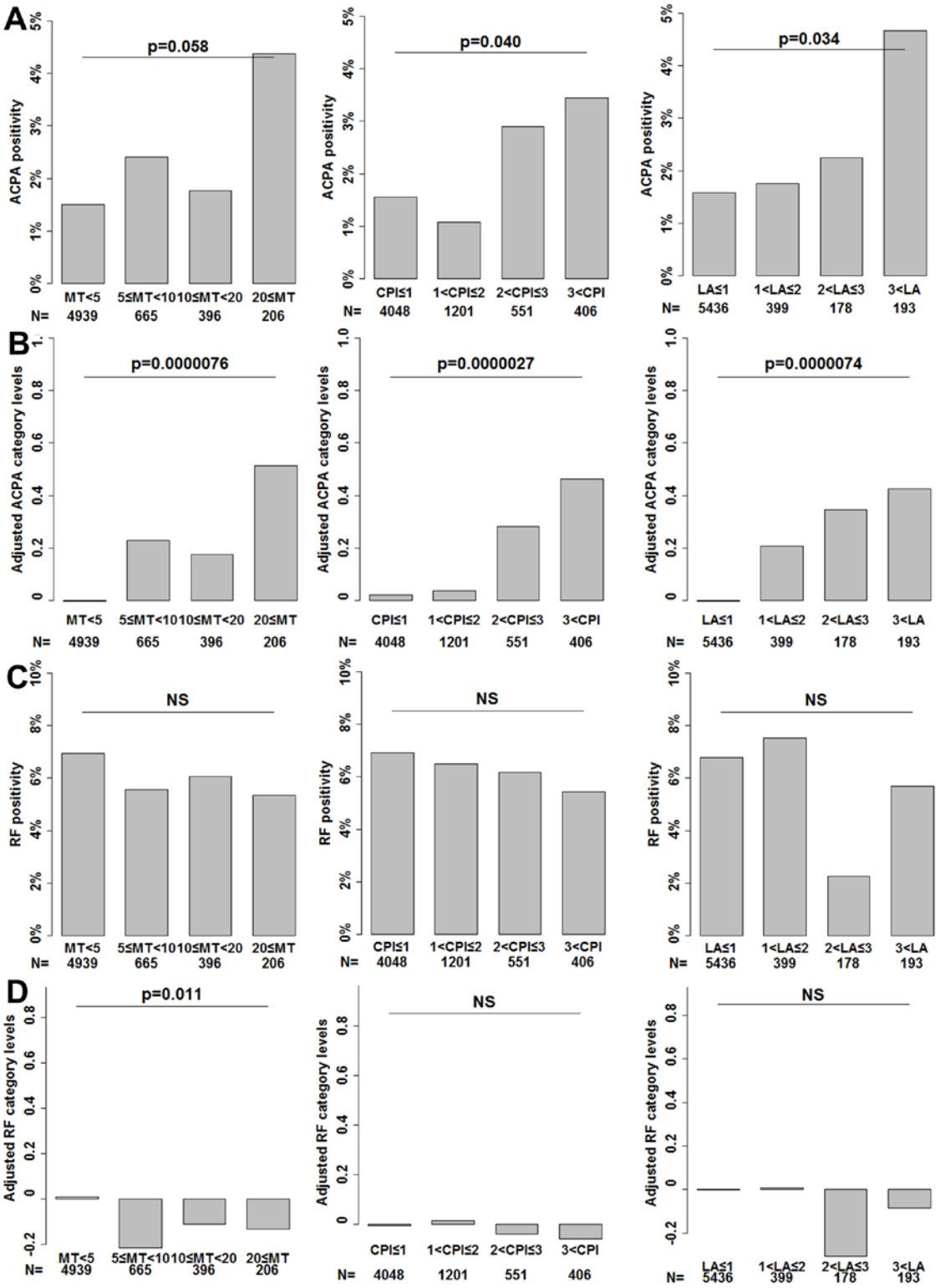
Figure 1

Supplementary Figure 1. Flow of the current study.



A schematic view of sample exclusion and data analysis is indicated.

Supplementary Figure 2. Associations between PD parameters and positivity or levels of ACPA and RF in non-smokers.



Y axes for categorical levels indicate adjusted levels by covariates.

P-values of logistic regression analysis or Poisson regression analysis in condition with covariates are indicated.

NS: not significant ($p>0.05$), MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor

Supplementary Table 1. Distributions of subjects in the current study according to categorical levels of ACPA and RF.

ACPA				
Positivity	Negative		Positive	
Levels	ACPA<0.6	0.6≤ACPA <4.5U/ml	4.5≤ACPA<13.5U/ml	13.5U/ml≤ACPA
Categorical levels	0	1	2	3
Number of subjects	7,395	1,992	100	67

RF				
Positivity	Negative		Positive	
Levels	RF=1IU/ml	1<RF≤20IU/ml	20<RF≤60IU/ml	60IU/ml <RF
Categorical levels	0	1	2	3
Number of subjects	6,357	2,583	486	128

Supplementary Table 2. Correlations between PD parameters.

	MT	CPI	LA
MT	-		
CPI	0.49	-	
LA	0.62	0.48	-

Supplementary Table 3. Distributions of the study participants according to PD parameters.

MT	0	1-5	6-10	11-15	16-20	21-25	26-
Subjects	4,301	3,527	809	330	236	150	201
CPI	0-≤0.5	0.5<-≤1	1<-≤1.5	1.5<-≤2	2<-≤2.5	2.5<-≤3	3<-
Subjects	4,023	1,793	1,078	804	598	425	833
LA	0-≤0.5	0.5<-≤1	1<-≤1.5	1.5<-≤2	2<-≤2.5	2.5<-≤3	3<-
Subjects	7,446	778	410	249	134	165	372

Supplementary Table 4. The associations between PD parameters and titers of ACPA and RF.

	ACPA titer			RF titer		
	Beta	SE	P	Beta	SE	P
Unadjusted						
MT	0.0055	0.0010	$8.3x10^{-8}$	-0.0015	0.0020	0.44
CPI	0.032	0.0054	$3.6x10^{-9}$	0.0087	0.011	0.41
LA	0.035	0.0067	$1.8x10^{-7}$	-0.0068	0.013	0.60
Adjusted						
MT	0.0030	0.0012	0.010	-0.0024	0.0012	0.28
CPI	0.020	0.0062	0.00096	0.012	0.012	0.31
LA	0.020	0.0075	0.0084	-0.0095	0.014	0.51

ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor, PD:periodontal disease, MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, OR:odds ratio, CI:confidence interval, SE:standard error

Supplementary Table 5. The associations between PD parameters and positivity or categorical levels of ACPA and RF in non-smoking population.

	ACPA positivity		ACPA categorical levels			RF positivity		RF categorical levels		
PD parameters	OR (95%CI)	P	Beta	SE	P	OR (95%CI)	P	Beta	SE	P
Unadjusted										
MT	1.04 (1.01-1.07)	0.0035	0.020	0.0040	$4.1x10^{-7}$	0.99 (0.97-1.01)	0.19	-0.0088	0.0038	0.022
CPI	1.26 (1.07-1.49)	0.0050	0.120	0.023	$1.5x10^{-7}$	0.95 (0.86-1.05)	0.28	-0.016	0.019	0.40
LA	1.29 (1.08-1.54)	0.0053	0.142	0.026	$5.3x10^{-8}$	0.87 (0.76-1.00)	0.050	-0.054	0.025	0.032
Adjusted										
MT	1.03 (1.00-1.06)	0.048	0.018	0.0045	$7.0x10^{-5}$	0.98 (0.96-1.00)	0.084	-0.0099	0.0043	0.022
CPI	1.20 (1.00-1.45)	0.049	0.110	0.026	$1.9x10^{-5}$	0.93 (0.83-1.04)	0.20	-0.012	0.021	0.57
LA	1.22 (1.00-1.50)	0.048	0.128	0.029	$1.1x10^{-5}$	0.84 (0.72-0.97)	0.022	-0.055	0.028	0.046

ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor, PD:periodontal disease, MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, OR:odds ratio, CI:confidence interval, SE:standard error

Supplementary Table 6. The associations between PD parameters and titers of ACPA and RF in non-smoking population.

	ACPA titer	RF titer

	Beta	SE	P	Beta	SE	P
Unadjusted						
MT	0.0057	0.0013	$1.3x10^{-5}$	-0.0045	0.0026	0.091
CPI	0.030	0.0070	$1.2x10^{-5}$	-0.0084	0.014	0.55
LA	0.038	0.0086	$1.4x10^{-5}$	-0.033	0.017	0.060
Adjusted						
MT	0.0046	0.0015	0.0016	-0.0055	0.0030	0.064
CPI	0.025	0.0077	0.0012	-0.0073	0.016	0.64
LA	0.031	0.0094	0.0010	-0.036	0.019	0.057

ACPA:anti-citrullinated peptide antibody, RF:rheumatoid factor, PD:periodontal disease, MT:the number of missing teeth, CPI:the Community Periodontal Index, LA: Loss of Attachment, OR:odds ratio, CI:confidence interval, SE:standard error