MAEA rs6815464 polymorphism and periodontitis in postmenopausal Japanese females: a cross-sectional study

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Running title: *MAEA* polymorphism and periodontitis in postmenopausal women

Abbreviation: MAEA, Macrophage erythroblast attacher

Abstract

Objectives: Macrophage erythroblast attacher (MAEA) is a membrane protein that regulates the development of mature macrophages by mediating attachment with erythroblasts. A polymorphism rs6815464 (C/G) in *MAEA* gene was reported to be associated with type II diabetes. Along with diabetes, osteoporosis shows an increased prevalence in postmenopausal females, and both diseases have been reported to be associated with periodontitis. Therefore, we explored the relevance of the *MAEA* polymorphism to periodontitis, bone mineral density (BMD) and haemoglobin A1c (HbA1c).

Design: This was a cross-sectional study with the final sample comprised of 344 postmenopausal Japanese females. Probing pocket depth (PPD) and clinical attachment level (CAL) were measured. Genotype was determined by TaqMan assay. Blood biochemical parameters and BMD of the lumbar spine were evaluated.

Results: No differences were found in age, body mass index, HbA1c, BMD, number of teeth, bone metabolism parameters between the genotypes. Mean CAL and percentage of sites with PPD or CAL ≥5mm were higher in the G-allele carriers than in the non-carriers. Multiple logistic regression analyses revealed that G-allele carriage was associated with severe periodontitis (odds ratio = 3.68, 95% CI =1.35-10.05).

Conclusion: Our results suggested that the *MAEA* gene polymorphism was independently associated with severe periodontitis.

Keywords: Periodontitis, Women's health, Genetics, Geriatric dentistry, Diabetes, Osteoporosis

1. Introduction

Macrophage erythroblast attacher (MAEA) is an integral membrane protein with small extracellular and large cytoplasmic domains (Hanspal, Smockova, & Uong, 1998). MAEA protein is localized in the cell surface, nucleus, and the plasma membrane of macrophages (Soni, Bala, Kumar, & Hanspal, 2007). MAEA is an attacher of erythroblasts to macrophages and essential for the maturations of erythroid cells and macrophages (Hanspal et al., 1998, Soni et al., 2006). MAEA contributes to nuclear structure rearrangements and cell division both in macrophages and erythroblasts (Hanspal et al., 1998; Hanspal & Hanspal, 1994; Soni et al., 2006). A cell migration assay demonstrated that down-regulation of MAEA inhibited relocation of macrophages (Javan, Can, Yeboah, Lee, & Soni, 2016).

Macrophages are differentiated cells of the myeloid lineage that reside in all tissues (Gentek, Molawi, & Sieweke, 2014) and mediate phagocytosis, antigen presentation, and innate and adaptive immunity (Gordon., 2007). Polarized macrophages produce proinflammatory cytokines, such as tumour necrosis factor-α (TNF- α), interleukin-1β (IL-1β), and interleukin-8 (IL-8) inducing inflammation and damage in tissues (Barksby, Nile, Jaedicke, Taylor, & Preshaw, 2009). In contrast, macrophages also show anti-inflammatory functions and promote wound healing by secreting anti-inflammatory mediators, including interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1) (Fadok et al., 1998). In the pathogenesis of periodontal diseases, monocytes/macrophages are central players in immune responses against pathogens (Sima & Glogauer, 2013). Moreover, the number of macrophages is reported to be higher in periodontitis lesions than in gingivitis lesions (Thorbert-Mros, Larsson, & Berglundh, 2015). Therefore, we hypothesized that MAEA may have a role in the pathogenesis of periodontitis.

Periodontitis is a dysbiotic inflammatory disease that subverts the host immune response in susceptible individuals (Hajishengallis, 2015). Previous studies of genetic risk factors have indicated that periodontitis is related to multiple genes (Suzuki et al., 2004). In addition, a lot of studies have reported the association between periodontal and systemic diseases, such as obesity, diabetes, osteoporosis or cardiovascular disease (Mealey, Oates, & American, 2006; Iwasaki, Taylor, Nakamura, Yoshihara, & Miyazaki, 2013; Keller, Rohde, Raymond, & Heitmann, 2015; Nordendahl et al., 2018), whereas others reported no association (Phipps et al., 2007; de Castilhos et al., 2012; Henschel & Keenan, 2015). Patients with type II diabetes demonstrated three-fold increase in the risk of periodontitis compared with non-diabetes and are adjusted for factors such as age sex (Collin et al., 1998). A meta-analysis also showed a significant association between periodontitis and diabetes (Chavarry, Vettore, Sansone, & Sheiham, 2009). In addition, possible associations between

osteoporosis/osteopenia and periodontal disease parameters have been demonstrated in postmenopausal females (Iwasaki et al., 2013; Gomes-Filho et al., 2007). A longitudinal study in old adults indicated the association of bone mineral density (BMD) with progression of clinical attachment loss in three years (Yoshihara, Seida, Hanada, & Miyazaki, 2004). However, a cohort study of old females (mean age 75.5 years) provided insufficient evidence for an association of periodontal disease with longitudinal decrease of BMD (Famili, Cauley, Suzuki, & Weyant, 2005).

These inconsistent results on the association between periodontitis and systemic diseases may be partially due to the variable genetic background of study participants. Genetic polymorphisms in risk genes that are common for diabetes/osteoporosis and periodontitis can influence their relationship (Wang et al., 2013; Hanai et al., 2015, Yoshihara et al., 2015).

The human *MAEA* gene is located in chromosome 4p16.3. In 2012, a genome-wide association study (GWAS) with East Asians revealed that a single nucleotide polymorphism rs6815464 in an intron of the *MAEA* gene was associated with type II diabetes. Among eight new loci in various genes reported from the GWAS, *MAEA* rs6815464 showed the strongest signal (Cho et al., 2012). The association was also confirmed by a case-control study in Japanese subjects (Imamura et al., 2012). Taken together, *MAEA* gene polymorphisms may modulate how periodontal infections can impact systemic diseases.

Furthermore, menopause increases risks of osteoporosis and diabetes. In osteoporosis, postmenopausal osteoporosis caused by estrogen deficiency is the most common type (Davis et al., 2015). Because oestrogen regulates carbohydrate metabolism, estrogenic deficiency also contributes to increased susceptibility to diabetes in postmenopausal females (Davis et al., 2015).

Therefore, this study was conducted to evaluate the association between a single nucleotide polymorphism (rs6815464) in the *MAEA* gene and various periodontal indices in postmenopausal Japanese females, as well as the possible effects of the *MAEA* gene polymorphism on the associations between periodontitis and the markers of bone metabolism or diabetes mellitus.

2. Materials and methods

2.1. Study participants

Participants of this study included Japanese females who were residents of the Yokogoshi area, Niigata City, Japan. In 2005, all 1310 females between 55 and 76 years old received the invitation to participate in the study and 674 agreed to participate. The following females were excluded from the study participants has been reported in a previous study (Wang et al., 2013) : 13 females who had bilateral oophorectomy, 7 females receiving corticosteroid therapy, and 54 females treated with bisphosphonates, oestrogen, selective estrogenic receptor modulators, vitamin K, active vitamin D

analogues or calcitonin. After the exclusions, 600 females remained. In 2010, we conducted the follow-up examinations in which 407 females came. All participants were not institutionalized or ambulatory. Three edentulous females and a female with only one remaining tooth were excluded from this study. We isolated genomic DNA from blood samples of the 403 participants for genotyping assays. However, genomic DNA from 44 females was of insufficient quantity or quality. Therefore, *MAEA* genotyping assays were performed in 359 females and were successful in 358 females. Fourteen participants with missing data from the examinations were excluded. In this study, the final number of participants were 344 females, the mean age of 63.6 ± 5.4 years in 2010 (Figure 1).

All participants provided written informed consents. Procedures in the present study were in accordance with the Helsinki Declaration of 2002, and were approved by the Ethics Committee of Niigata University School of Medicine (No. 303). This was a human observational and genetic association study, and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines were followed.

2.2. Interview, physical examination, biochemical measurements and BMD measurement

All participants underwent interview to obtain information such as smoking status, menopausal age, systemic diseases, etc. Height and weight were measured for all participants. The body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height (m). Obesity was defined criteria as BMI $\geq$25kg/m^2 (Expert Panel on the Identification, 1998).

Because smoking has been reported as important risk factor of periodontitis (Ogawa, Yoshihara, Hirotomi, Ando, & Miyazaki, 2002), smoking status was interviewed (never/previous/current smoker). Since there was no current smoker in the final participants, previous smokers were defined as smoking (+).

As previously described (Nakamura et al., 2008), peripheral blood samples were collected six hours after a meal during the daytime and were stored immediately at 4°C. On the same day, serum from blood sample was collected and stored at -80°C. Serum C-reactive protein (hsCRP) level was determined by a high-sensitivity latex nephelometry assay (Behring Nephelometer II; Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The percentage of haemoglobin A1c (HbA1c) was measured by the latex aggregation assay by a clinical testing company (BML, Tokyo, Japan) and estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society) (%) + 0.4 (Seino et al., 2010). The definition criterion for hyperglycaemia was HbA1c $\geq$6.5% (International Expert, 2009).

The concentrations of serum bone metabolism markers were determined as previously

described (Nakamura et al., 2008). Serum osteocalcin concentration was measured by an immunoradiometry (Mitsubishi Kagaku Medical, Inc., Tokyo, Japan) (Reference value: 3.1 - 12.7 ng/ml, coefficients of variation: 6.6%). Type I collagen crosslinked N-telopeptides (NTx) concentration was measured with ELISA (Osteomark NTX Serum; Ostex International, Inc., Seattle, WA, USA) (Reference value: 10.7–24.0 nmol BCE/l, coefficients of variation: 2.8%). Serum intact parathyroid hormone (Pth) concentration was determined by immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA, USA) (coefficients of variation: 1.5%).

As previously described (Nakamura et al., 2008), BMD of the lumbar spine (L2 to L4) measurement by a dual-energy X-ray absorptiometry method was performed by a medical radiology technician. The coefficients of variation of the BMD values were 0.2%. BMD was expressed as a percentage of the young adult (20 to 44 years old) mean (YAM). Thus, low BMD in a female was defined as having BMD < 80% YAM.

2.3. Periodontal examination

All participants were evaluated clinically by two dentists to assess periodontal condition as described in a previous report (Iwasaki, Nakamura, Yoshihara, & Miyazaki, 2012). A calibration exercise for periodontal measurements has been carried out and the inter-observer agreement was assessed. The values of CAL with their difference ≤1 mm were considered to be in agreement. The coefficient was 0.76 (Kendall's coefficient of agreement: $\chi^2 = 95.2$, $p < 0.01$) and the kappa value was 0.199 ($p = 0.007$). Probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) were measured at the mesiobuccal and mid-buccal sites for each remaining tooth, including third molars. The mean PPD, mean CAL, and the BOP percentage were calculated for each female. Severe periodontitis was defined according to the modified criteria referred to by the Centres for Disease Control and Prevention—American Academy of Periodontology (CDC-AAP) case definition established in 2012 (Eke, Page, Wei, Thornton-Evans, & Genco, 2012). A female with only one remaining tooth was excluded from the study, because the definition of severe periodontitis in the above classification required at least two remaining teeth. Although the original definition performs six-site examinations per tooth and uses only interproximal measurements, we had only one interproximal measurement on mesiobuccal site per tooth. Specifically, definition of periodontitis according to CDC-AAP: 1) no periodontitis was defined as having no evidence of mild, moderate, or severe periodontitis; 2) mild periodontitis was defined as having at least two sites with CAL ≥3 mm, and ≥2 interproximal sites with PPD ≥4 mm (not on same tooth) or one site with PPD ≥5 mm; 3) moderate periodontitis was defined as having ≥2 interproximal sites with CAL ≥4 mm (not on same

tooth), or ≥2 interproximal sites with PPD ≥5 mm (not on same tooth); 4) severe periodontitis was defined as having at least two sites with CAL ≥6 mm (not on the same tooth) and having at least one site with PPD ≥5 mm (Eke et al., 2012).

2.4. Genotyping of the *MAEA* polymorphism

Genomic DNA was extracted from EDTA-treated peripheral blood. Genotyping for *MAEA* rs6815464 was performed with the TaqMan® SNP genotyping assays (Assay ID: C_29044379_10, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The genotyping was done blind to clinical diagnosis. The data from the genotyping assay were analysed with the Sequence Detection System v.2.4 software (Sequence Detection System v.2.4 software. Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and the quality value was set at >99%. The success rate of genotyping call was 99.7%.

2.5. Statistical analysis

The significance was set to a p-value of <0.05. Age, menopausal age, BMI, BMD, periodontal parameters and bone metabolic markers were compared between the *MAEA* genotypes with the Kruskal-Wallis test, and between the G-allele carrier group and non-carrier group with the Mann Whitney *U*-tests. For the analyses in contingency tables, exact tests or chi-square tests were performed. To assess the association between severe periodontitis as an outcome and risk factors including the G allele carriage of *MAEA* polymorphism, multiple logistic regression analysis was performed. General linear regression models were used to evaluate whether the *MAEA* polymorphism induced any changes in the relationship between mean CAL and BMD or HbA1c. The statistics software SPSS ver. 22.0 was used for all analyses (IBM Japan, Tokyo, Japan).

3. Results

The distribution of *MAEA* genotypes in females without severe periodontitis was in Hardy-Weinberg equilibrium (p >0.05). Since the number of GG homozygotes was limited, participants were divided into groups of the G-allele carriers and non-carriers and compared.

Table 1 shows the characteristics of participants by periodontitis status. The distribution of participants' periodontitis status was as follows: 1) no periodontitis, 131 individuals (38.1%); 2) mild periodontitis, 11 individuals (3.2%); 3) moderate periodontitis, 161 individuals (46.8%) and 4) severe periodontitis, 41 individuals (11.9%). There was no significant difference with age or menopausal age between the females with severe periodontitis and the other females.

As shown in Table 2, we compared characteristics of study participants with the *MAEA*

genotypes or in the G-allele carrier and non-carrier groups. The prevalence of obesity, diabetes and low BMD were 32%, 2% and 37%, respectively. There was no significant difference in age, menopausal age, number of smokers, hsCRP levels, BMI, HbA1c, BMD, osteocalcin, NTx, and Pth levels among *MAEA* genotypes nor between the G-allele carrier and non-carrier groups.

Table 3 shows the comparisons of periodontal parameters between *MAEA* genotypes or the G-allele carrier and non-carrier groups. There was no significant difference in the number of teeth, number of females with severe periodontitis and BOP amongst *MAEA* genotypes. Mean CAL, the percentage of CAL ≥5mm sites and the percentage of PPD ≥5mm sites were significantly higher in the G-allele carrier group than in the non-carrier group (Mann-Whitney *U* test, mean CAL: $p = 0.031$, the percentage of CAL ≥5mm sites: $p = 0.039$, the percentage of PPD ≥5mm sites: $p = 0.022$).

Multiple logistic regression models for severe periodontitis as the outcome were shown in Table 4. Age, menopausal age, smoking, HbA1c ≥6.5%, and BMD <80%YAM were entered as independent variables and had no significant association with severe periodontitis. The G-allele carriage was significantly associated with severe periodontitis (adjusted odds ratio = 3.68; 95% confidence interval = 1.35-10.05).

Table 5 shows general linear models to evaluate the effects of *MAEA* gene polymorphism on the association between mean CAL as an independent valuable and BMD or HbA1c as a dependent valuable. There was no significant association between mean CAL and BMD, or between mean CAL and HbA1c, after adjustment for the confounding factors in both G-allele carrier and non-carrier groups.

4. Discussion

This is the first study that investigated the possible role of MAEA in human diseases other than diabetes. Our results showed that severe periodontitis was significantly associated with the G-allele carriage after adjustment for covariates. Our results suggested G allele to be associated with periodontitis which was the opposite allele from the one previously reported as the risk for diabetes (Imamura et al., 2012). The association between the *MAEA* polymorphism and severe periodontitis in this study did not depend on the existence of diabetes or osteoporosis as a linkage. In previous studies, the authors did not discuss possible mechanisms of association between diabetes and the *MAEA* polymorphism specifically (Cho et al., 2012; Imamura et al., 2012). MAEA is important for the development of mature macrophages (Soni et al., 2007). During the response against periodontal pathogens, macrophages produce an array of cytokines, chemokines, and growth factors, which contribute to the resolution of inflammation, the activation of adaptive immunity and the mediation of alveolar bone resorption and apposition (Sima & Glogauer, 2013). The function of MAEA is well

studied in erythroblast enucleation, maturation and correlate with erythroblastic island. however, the MAEA-mediated pathway that regulates macrophage function remains to be elucidated, which might help to understand the mechanism of tissue destruction in inflammatory diseases such as periodontal diseases.

Mean CAL, the percentage of CAL ≥5mm sites and the percentage of PPD ≥5mm sites in females with the G allele were significantly higher than those in females without the G allele. It seems to be consistent with the results demonstrated for the logistic regression analysis, though after Bonferroni correction (α=0.05/10=0.005), differences shown in the univariate analyses would not be considered significant. In this study, the percentages of CAL ≥6mm and PPD ≥6mm sites showed no significant difference between the genotypes, probably because of the narrow range close to the lower limit of the possible value.

Although we tried to evaluate the possible effects of the *MAEA* polymorphism to modify the relationships between mean CAL and HbA1c or BMD, no association was found between them in both G allele carries and non-carriers. The results might be caused by relatively high BMD and and low HbA1c in the females participated in this study. Because bone structure is reduced and altered due to oestrogen deficiency, osteoporosis as a result of menopause in females is evident (Davis et al., 2015). However, the number of females with osteoporosis was relatively small in this study compared to other studies (Oka et al., 2018). Additionally, the number of females with high HbA1c was also small (Oka et al., 2018). The limited numbers of patients with diabetes and osteoporosis may account for the negative results in our study.

An advantage of this study was the relatively homogeneous genetic background of the participants, because only Japanese females were included. Sample homogeneity is relevant because race and sex are known to affect the development of periodontal diseases (Eke et al., 2015). We included participants from 55 to 75 years old, because older age is another contributor factor to periodontal diseases (Eke et al., 2016). Furthermore, we controlled for known confounding factors in the statistical analyses.

The present study has some limitations. The number of females with severe periodontitis was small (n = 41). Therefore, subgroup of G allele carriers (severe periodontitis, n = 29) and G allele non-carriers (severe periodontitis, n = 12) was even limited. Additionally, we could not perform periodontal examinations at six sites per tooth (Eke, Thornton-Evans, Wei, Borgnakke, & Dye, 2010). Only one site from each interproximal site per tooth was examined with the assuming these measurements represent full–mouth measurements (Borrell & Talih, 2012). Therefore, the prevalence of severe periodontal disease could have been underestimated in this study. On the other hand, because there is no other information about the socio-economic status such as income and education

of the participants and oral health such as plaque scores, history of dental treatment etc, resulting in that other important potential confounding factors cannot be included in the analysis and a complete assessment of periodontal disease and other diseases cannot be performed.

According to our cross-sectional study in postmenopausal Japanese women, *MAEA* gene polymorphism *rs6815464* was suggested to be associated with severe periodontitis. The association was not depending on the co-existence of diabetes or osteoporosis. The *MAEA* polymorphism did not show significant effects on the relations between periodontal parameters and BMD or HbA1c. This is the first study to evaluate a role of *MAEA* in human diseases other than diabetes. Further studies are required with larger and diverse populations, complete information of periodontitis, various polymorphisms of the *MAEA* gene to confirm and extend these observations.

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Conflict of interest

The authors report no conflicts of interest.

Author contributions

Yulan Che contributed to the conception of the study and wrote the manuscript. Noriko Sugita directed genotyping of *MAEA* and performed statistical analyses and advised preparation of the manuscript. Akihiro Yoshihara, Masanori Iwasaki and Hideo Miyazaki conducted and performed collections of medical and periodontal data and peripheral blood and serum samples, and additionally, advised statistical analyses. Kazutoshi Nakamura planned and managed recruitment of participants and supervised medical aspects of the study. Hiromasa Yoshie gave scientific advices on the study design. All authors discussed the results and contributed to the final manuscript.

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Figure captions

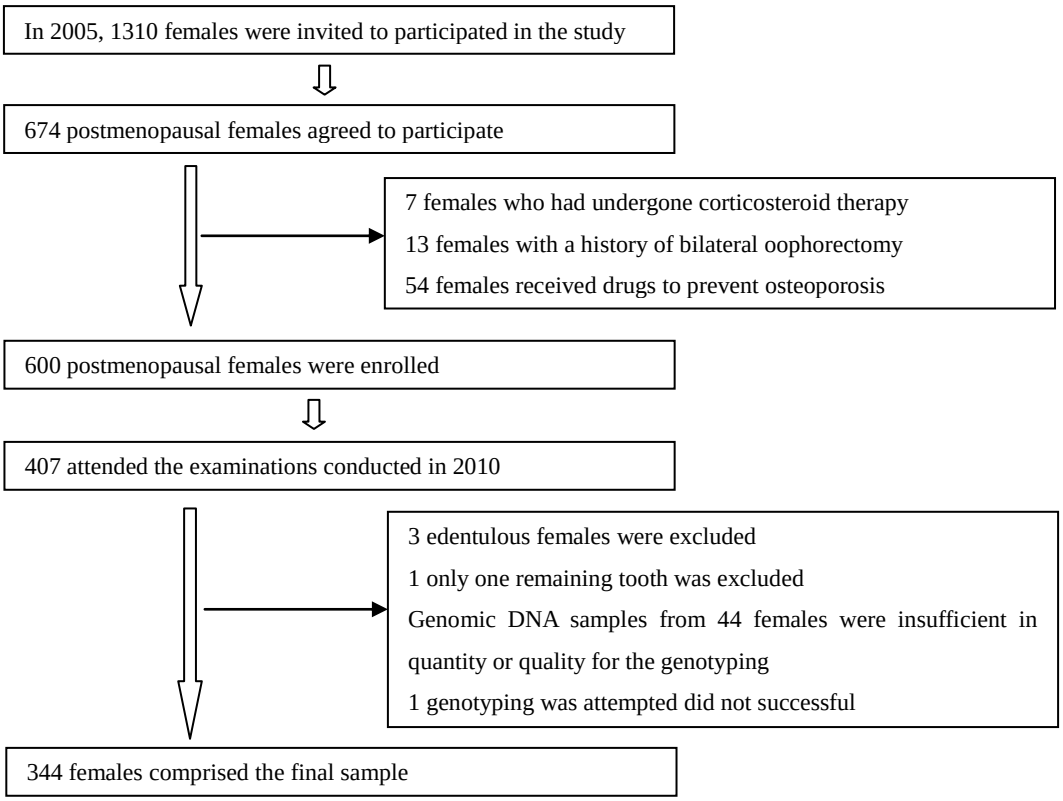

Figure 1. Flow diagram of the study.

Table 1. Selected characteristics by periodontal disease severity

Variables	No periodontitis (n = 131)	Mild+Moderate periodontitis (n =172)	No+Mild+Moderate periodontitis (n = 303)	Severe periodontitis (n = 41)
Age	62.7±5.2	64.4±5.3	63.6±5.3	63.6±5.5
Menopausal age	49.6±4.1	20.2±4.0	49.9±4.0	50.3±3.8
Mean CAL (mm)	2.20±0.30	2.81±0.43	2.55±0.48	3.85±1.01
Percentage of CAL ≥4mm sites	3.2±5.5	20.8±12.6	13.2±13.4	47.9±23.9
Percentage of CAL ≥5mm sites	0.6±1.7	6.8±7.7	4.1±6.6	27.2±18.1
Percentage of CAL ≥6mm sites	0.03±0.31	2.2±3.9	1.3±3.1	19.0±13.8
Mean PPD (mm)	1.99±0.23	2.4±0.4	2.25±0.41	3.29±0.81
Percentage of PPD ≥4mm sites	1.5±2.8	12.5±10.6	7.7±9.8	34.8±20.0
Percentage of PPD ≥5mm sites	0.1±0.9	3.9±5.3	2.2±4.4	20.4±16.4
Percentage of PPD ≥6mm sites	0.0±0.3	1.2±2.5	0.7±1.9	13.4±11.6
Bleeding on probing (%)	7.0±5.9	11.0±9.5	9.3±8.4	20.4±14.5

CAL, Clinical attachment loss; PPD, Probing pocket depth.

No periodontitis was defined as having no evidence of mild, moderate, or severe periodontitis; mild periodontitis was defined as having at least two sites with CAL ≥3 mm, and ≥2 interproximal sites with PPD ≥4 mm (not on same tooth) or one site with PPD ≥5 mm; moderate periodontitis was defined as having ≥2 interproximal sites with CAL ≥4 mm (not on same tooth), or ≥2 interproximal sites with PPD ≥5 mm (not on same tooth); severe periodontitis was defined as having at least two sites with CAL ≥6 mm (not on the same tooth) and having at least one site with PPD ≥5 mm.

Values represent the mean ± SD.

Table 2. Characteristics of MAEA genotypes

Variables	CC (n=145)	CG (n=159)	GG (n=40)	*P* Value	G allele carriers (n=199)	G allele non-carriers (n=145)	*P* Value
Age (years)	63.7±5.5	63.3±5.0	65.1±5.8	0.230	63.6±5.2	63.7±5.5	0.940
Menopausal age (years)	50.3±3.7	49.9±4.3	48.9±3.9	0.057	49.7±4.2	50.3±3.7	0.207
Number of smokers (%)	7 (5)	11 (7)	2 (5)	0.817	13 (7)	7 (5)	0.640
hsCRP (mg/l)	0.87±2.26	0.98±3.05	0.44±0.55	0.141	0.87±2.74	0.87±2.26	0.750
BMI (kg/m^2)	22.7±3.8	22.9±3.37	22.7±3.54	0.517	22.8±3.40	22.7±3.84	0.316
Number of BMI ≥25kg/m^2 (%)	43 (30)	53 (33)	14 (35)	0.715	68 (34)	43 (30)	0.483
HbA1c (%)	5.11±0.44	5.16±0.59	5.02±0.37	0.481	5.13±0.56	5.11±0.44	0.819
Number of HbA1c ≥6.5% (%)	3 (2)	5 (3)	0 (0)	0.681	5 (3)	3 (2)	1.000
BMD (g/cm^2)	0.86±0.13	0.86±0.14	0.82±0.17	0.312	0.85±0.14	0.86±0.13	0.648
Number of low BMD	55 (38)	54(34)	19 (48)	0.278	73 (37)	55 (38)	0.822
Osteocalcin (ng/ml)	7.75±2.52	7.65±2.72	7.80±1.84	0.578	7.68±2.56	7.75±2.52	0.759
NTx (nmol BCE/l)	18.6±5.5	18.9±5.4	19.9±6.9	0.695	19.1±5.7	18.6±5.5	0.441
Pth (pmol/l)	3.8±1.4	4.1±1.7	3.8±1.6	0.178	4.0±1.7	3.8±1.4	0.397

BMD, bone mineral density; BMI, body mass index; HbA1c, haemoglobin A1c; hsCRP, high sensitivity C-reactive protein; NTx, serum type I collagen crosslinked N-telopeptides; Pth, serum intact parathyroid hormone.

BMD was measured at the lumbar spine (L2 to L4).

Low BMD was defined as BMD <80%YAM (%).

Values represent the number of women or mean ± SD.

The Kruskal-Wallis tests were performed between MAEA genotypes. The Mann-Whitney *U* tests were performed comparing the G allele carriers and non-carriers. Chi-square tests for 2X3, exact tests for 2X2 contingency tables were performed.

* *P* Value <α, $\alpha = 0.05$.

Table 3. Periodontal parameters in MAEA genotypes

Variables	CC (n=145)	CG (n=159)	GG (n=40)	*P* Value	G allele carriers (n=199)	G allele non-carriers (n=145)	*P* Value
Number of teeth	23.0±5.5	22.6±5.7	22.1±5.4	0.472	22.5±5.6	23.0±5.5	0.313
Severe periodontitis (+) (%)	12 (8)	25 (16)	4(10)	0.125	29 (15)	12 (8)	0.092
Mean CAL (mm)	2.63±0.69	2.76±0.75	2.76±0.61	0.084	2.76±0.72	2.63±0.69	0.031*
Percentage of CAL ≥4mm sites	15.5±17.8	18.6±19.7	19.1±18.0	0.276	18.7±19.3	15.5±17.8	0.137
Percentage of CAL ≥5mm sites	5.9±11.9	7.6±11.3	7.5±10.9	0.109	7.6±11.2	5.9±11.9	0.039*
Percentage of CAL ≥6mm sites	3.1±8.9	3.7±7.7	3.0±5.8	0.285	3.5±7.3	3.1±8.9	0.115
Mean PPD (mm)	2.35±0.63	2.38±0.56	2.45±0.50	0.098	2.39±0.55	2.35±0.63	0.070
Percentage of PPD ≥4mm sites	10.4±15.6	11.2±13.5	12.3±14.2	0.222	11.4±13.6	10.4±15.6	0.099
Percentage of PPD ≥5mm sites	4.0±10.6	4.7±8.1	4.5±7.1	0.072	4.7±7.9	4.0±10.6	0.022*
Percentage of PPD ≥6mm sites	2.2±7.1	2.3±5.4	1.6±3.5	0.415	2.2±5.0	2.2±7.1	0.214
Bleeding on probing (%)	10.3±10.1	10.8±10.1	10.7±9.3	0.854	10.8±9.9	10.3±10.1	0.582

CAL, Clinical attachment loss; PPD, Probing pocket depth;

Severe periodontitis was defined as having at least two sites with CAL ≥6 mm (not on the same tooth) and having at least one site with PPD ≥5 mm.

Values represent the number of women or mean ± SD.

The Kruskal-Wallis tests were performed between MAEA genotypes.

The Mann-Whitney *U* tests were performed comparing the G allele carriers and non-carriers. Chi-square tests for 2X3, exact tests for 2X2 contingency tables were performed.

* *P* Value <α, $\alpha = 0.05$.

Table 4. Multiple logistic regression analyses for severe periodontitis as the outcome

	Variables	Adjusted Odds Ratio	95% CI
Model 1	Age	0.99	0.92-1.07
	Menopausal age	1.00	0.90-1.10
	Smoking (+)	1.50	0.40-5.67
	HbA1c ≥ 6.5 %	1.56	0.17-13.77
	Low BMD	0.88	0.37-2.13
	BMI ≥ 25	0.69	0.27-1.76
Model 2	G allele carriage	3.73	1.36-10.19
	Age	0.99	0.91-1.07
	Menopausal age	1.01	0.91-1.11
	Smoking (+)	1.50	0.39-5.83
	HbA1c ≥ 6.5 %	1.56	0.17-14.56
	Low BMD	0.86	0.35-2.12
	BMI ≥ 25	0.66	0.25-1.71

BMD, bone mineral density; HbA1c, haemoglobin A1c, BMI, body mass index.

BMD was measured at the lumbar spine (L2 to L4).

Low BMD was defined as BMD <80%YAM (%).

Severe periodontitis was defined as having at least two sites with CAL ≥6 mm (not on the same tooth) and having at least one site with PPD ≥5 mm. * *P* Value <α, $\alpha = 0.05$.

Table 5. General linear regression models between BMD or HbA1c and mean CAL in different MAEA genotypes

Outcome		G allele-carriers (n=199)			G allele non-carriers (n=145)		
		B	95%95% CI	P Value	B	95% CI	P Value
BMD	Age	-0.302	-0.01-0.00	0.000*	-0.284	-0.01-0.00	0.005*
	Smoking (versus never smoker)	0.209	0.03-0.18	0.007*	-0.019	-0.10-0.09	0.843
	HbA1c	0.014	-0.03-0.04	0.857	-0.033	-0.06-0.04	0.723
	Mean CAL	0.091	-0.01-0.05	0.233	-0.019	-0.04-0.04	0.848
HbA1c	Age	-0.029	-0.02-0.02	0.718	-0.059	-0.02-0.11	0.562
	Smoking (versus never smoker)	0.030	-0.28-0.41	0.718	0.037	-0.28-0.42	0.703
	BMI	0.183	0.00-0.06	0.025*	0.197	0.00-0.05	0.046*
	Mean CAL	-0.083	-0.19-0.06	0.306	0.093	-0.08-0.21	0.351

BMD, bone mineral density; BMI, body mass index; CAL, Clinical attachment loss; HbA1c, haemoglobin A1c.

BMD was measured at the lumbar spine (L2 to L4).

* *P* Value<α, $\alpha = 0.05$.