Association of the *OGG1* Ser326Cys Polymorphism With Tooth Loss

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Although tooth loss is a serious health problem for elderly people, little is known about the genetic basis for susceptibility to it. In the present study we aimed to find a single nucleotide polymorphism (SNP) associated with tooth loss. DNA samples from 119 outpatients (mean age = 78.8 years) were genotyped on seven polymorphisms (tumor necrosis factor- α –1031T/C, interleukin-1 β –511C/T, interleukin-6 –634C/G, macrophage migration inhibitory factor –173G/C, interleukin-1 receptor antagonist variable number of tandem repeat in intron 2, matrix metalloproteinase-1 –16071G/2G, and oxoguanine glycosylase 1 (*OGG1*) Ser326Cys (1245C/G)), and the

results were statistically evaluated. Of the seven polymorphisms tested, only *OGG1* Ser326Cys was revealed to associate with tooth loss at a statistically significant level ($P=0.0086$). In addition, a multivariate logistic regression analysis in which age, gender, body mass index (BMI), and ischemic heart disease were included as independent variables indicated that Ser326Cys could be an independent factor affecting tooth loss (OR, 3.191; 95%CI, 1.174–8.672). The data suggest that the *OGG1* Ser326Cys polymorphism may be associated with tooth loss. *J. Clin. Lab. Anal.* 20:47–51, 2006. © 2006 Wiley-Liss, Inc.

Key words: oxoguanine glycosylase 1; polymorphism; tooth loss

INTRODUCTION

Tooth loss is a serious health issue for the elderly. Continuing increases in life expectancy portend an increasing incidence, especially in industrial nations, despite improvements in dental health care (1,2). The major causes of tooth loss are dental caries, dental injuries, and periodontal diseases throughout an individual's lifespan (3). In addition, age, female gender, deteriorated general health, systemic bone loss, and age-related hearing loss have been reported to be associated with tooth loss in elderly people (2). Although some of these factors are preventable by further improvements in personal hygiene, there seems to be a remarkable individual difference in the severity of pathological conditions, such as periodontitis (4) and resorption of the residual ridge (1). The ability to more accurately identify the disease process that causes tooth loss in individuals might enable us to design a program to more efficiently prevent tooth loss.

It is thought that single nucleotide polymorphisms (SNPs) may be the genetic basis underlying individual differences in susceptibility to many common diseases. A number of studies on periodontitis, one of the major causes of tooth loss, have recently been conducted to find susceptibility SNPs by analyzing a variety of genes, including cytokines (5–7), Fc gamma receptor IIIa (8,9), vitamin D receptor (10), and matrix metalloproteinase (MMP)-1 (11). However, only a few reports revealed an association between tooth loss and SNPs, and those studies were focused on the genes related to bone loss, vitamin D receptor (12,13), or matrix Gla protein (14). In this study we investigated the relationship between

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Received 16 August 2005; Accepted 1 December 2005

DOI 10.1002/jcla.20101

Published online in Wiley InterScience (www.interscience.wiley.com).

tooth loss and seven SNPs in cytokine, MMP-1, and DNA repair enzyme genes.

MATERIALS AND METHODS

A total of 119 outpatients who were seen at the Department of Oral and Maxillofacial Surgery, Yamaguchi University Hospital, from April 2003 to August 2004 were enrolled in this study. Of 164 eligible patients, 45 declined to participate, resulting in a participation rate of 72.6% (119/164). Since the patients were all native Japanese and were drawn from Yamaguchi and the surrounding prefectures, similar environmental and social factors were assumed. The patients' characteristics are shown in Table 1. The mean age of the patients was 78.8 years (range = 60–100 years), and the male:female ratio was 49:70. Information on the body mass index (BMI), community periodontal index (CPI) (15), and number of residual teeth was obtained from each patient during the examination. Most patients (99/119) had chronic diseases (mean number per patient = 1.97). The chronic diseases found in more than 10% of the patients were as follows: 32 cases of ischemic heart disease (26.9%), 25 cases of essential hypertension (21%), and 14 cases of diabetes mellitus (11.8%). A history of malignancy was found in seven patients

(5.9%). All patients provided written informed consent. The experimental protocol was approved by the Institutional Ethics Committee of the Yamaguchi University School of Medicine.

DNA was extracted from peripheral blood by a conventional NaI method (16) and stored at -70°C until SNP analysis was performed. A tetra-primer ARMS-PCR assay (16) was used to genotype six polymorphisms: tumor necrosis factor (TNF)- α -1031T/C, interleukin (IL)-1 β -511C/T, IL-6 -634C/G, macrophage migration inhibitory factor (MIF) -173G/C, *MMP-1* -16071G/2G, and *OGG1* Ser326Cys (1245C/G). The primers for TNF- α -1031T/C, IL-1 β -511C/T, IL-6 -634C/G, MIF -173G/C (16), and *MMP-1* (17) have been previously described. For detection of the *OGG1* Ser326Cys, a set of four primers consisting of forward outer (FO): 5'-GCCCCAGGTGGCCCTAAAGG-3', reverse outer (RO): 5'-GAGGGGAAGGTGCTTGGGGA-3', forward inner (FI): 5'-GTGCCGACCTGCGCATTTC-3', and reverse inner (RI): 5'-TGGCTCC TGAGCATGGCAGC-3' was used. PCR was carried out in a total volume of 10 μL consisting of 80 ng/ μL DNA template, 200 μM dNTP, 2 mM MgCl_2 , 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 1U Taq polymerase (TaKaRa Ex Taq; TAKARA, Tokyo, Japan) with 3 pmol primer FO, 10 pmol RO, 30 pmol FI, and 6 pmol RI. A Program Temp Control System PC-808 (ASTEC, Fukuoka, Japan) was used in this study. The PCR cycling conditions were as follows: 2 min at 95°C , followed by 10 cycles of 30 sec at 94°C , 30 sec at 63°C and 30 sec at 72°C ; then 23 cycles of 30 sec at 94°C , 20 sec at 57°C and 30 sec at 72°C ; and with a final step at 72°C for 10 min to allow for the complete extension of all fragments. The PCR products were separated by electrophoresis on a 2.0% agarose gel, and subsequently stained with ethidium bromide.

The *IL-1RA* variable number of tandem repeats (VNTR) in intron 2 was genotyped by PCR and polyacrylamide gel electrophoresis as described previously (18).

The distribution of SNP genotypes tested in this study did not show any departure from Hardy-Weinberg equilibrium when the samples used in this study ($n = 119$) were examined before analysis ($P > 0.05$). Association of each polymorphism with clinical parameters was evaluated by Fisher's exact test. A multivariate logistic regression analysis was carried out to determine whether the *OGG1* polymorphism was an independent factor affecting edentulousness, and to obtain odds ratios (ORs) and 95% confidence intervals (CIs). A P -value < 0.05 was considered as statistically significant. The StatView statistical software system (version 5; SAS Institute Inc., Cary, NC) was used for analyses.

TABLE 1. Patient characteristics

No. of cases	119
Age (years, mean $\pm$ SD)	78.8 $\pm$ 8.5
Range (years)	
60–69	15
70–79	54
80–89	29
90–100	21
Gender (M/F)	49/70
BMI	
≤ 21	71
> 21	37
CPI	
0	4 (3.4%)
1	18 (15.1%)
2	11 (9.2%)
3	25 (2.1%)
4	18 (15.1%)
n.d.	43 (36.1%)
No. of residual teeth	
0	38 (31.9%)
1–10	33 (27.7%)
11–20	27 (22.7%)
> 20	21 (17.6%)
Medical profiles	
Ischemic heart disease	32 (26.9%)
Essential hypertension	25 (21.0%)
Diabetes mellitus	14 (11.8%)

BMI, body mass index; CPI, community periodontal index; n.d., not determined due to the loss of corresponding tooth.

RESULTS

We genotyped seven polymorphisms (*TNF-α* -1031T/C, *IL-1β* -511C/T, *IL-1RA* VNTR in intron 2, *IL-6* -634C/G, *MIF* -173G/C, *MMP-1* -16071G/2G, and *OGG1* Ser326Cys (1245C/G); Table 2) and evaluated the relation of each polymorphism with six clinical parameters (age, gender, BMI, CPI, tooth loss, and the presence or absence of ischemic heart disease). As shown in Table 3, the frequency of the Cys/Cys genotype of the *OGG1* Ser326Cys polymorphism in edentulous patients was significantly higher than in dentate (one tooth or more) ones ($P = 0.0086$). There was no significant association between these clinical parameters and the other six polymorphisms (data not shown).

The *OGG1* Ser326Cys remained significant (OR, 3.191; 95%CI, 1.174–8.672) after a multivariate logistic regression analysis for age, gender, BMI and ischemic heart disease (Table 4) was performed, indicating that it could be an independent factor affecting tooth loss.

DISCUSSION

The present data are the first to demonstrate the possibility that the *OGG1* Ser326Cys polymorphism is associated with tooth loss. The genotype distribution in our patients was in Hardy-Weinberg equilibrium, and when compared to that in a previously reported younger Japanese population ($n = 241$; mean age = 56.8 years;

TABLE 2. Genotype distribution of 7 polymorphisms tested

Polymorphism	Genotype	Distribution ^a
<i>TNF-α</i> -1031	T/T	88 (73.9)
	T/C	26 (21.8)
	C/C	5 (4.2)
<i>IL-1β</i> -511	C/C	43 (36.1)
	C/T	47 (39.5)
	T/T	29 (24.4)
<i>IL-1RA</i> -VNTR	allele 2/allele 4	9 (7.6)
	allele 3/allele 4	1 (0.8)
	allele 4/allele 4	108(90.8)
	allele 4/allele 5	1 (0.8)
<i>IL-6</i> -634	C/C	68 (57.1)
	C/G	42 (35.3)
	G/G	09 (7.6)
<i>MIF</i> -173	G/G	81 (68.1)
	G/C	33 (27.7)
	C/C	5 (4.2)
<i>MMP-1</i> -1607	2G/2G	62 (52.1)
	2G/1G	47 (39.5)
	1G/1G	10 (8.4)
<i>OGG1</i> Ser326Cys	Ser/Ser	33 (27.7)
	Ser/Cys	57 (47.9)
	Cys/Cys	29 (24.4)

^aValues are n (%).

TABLE 3. Genotypes of patients with tooth loss divided by clinical parameters

	<i>OGG1</i> Ser/Ser+Ser/ Cys (n = 90)	<i>OGG1</i> Cys/Cys (n = 29)	<i>P</i> *
Age			
< 79 years	48	16	0.5134
≥ 79 years	42	13	
Gender			
Male	39	10	0.9911
Female	51	19	
BMI			
≤ 21	53	18	0.8955
> 21	29	8	
CPI			
0–2	27	6	0.8272
3–4	36	7	
No. of residual teeth			
0	23	15	0.0086
≥ 1	67	14	
Ischemic heart disease			
Presence	24	8	>0.9999
Absence	66	21	

* χ^2 test.

TABLE 4. Multivariate logistic regression analysis of clinical parameters including *OGG1* polymorphism in edentulous patients

	OR (95% CI)	<i>P</i>
Age (< 79 years vs. ≥ 79 years)	5.264 (1.970–14.071)	0.0009
Gender (Male vs. female)	0.795 (0.295–2.142)	0.6636
BMI (≤ 21 vs. > 21)	1.029 (0.404–2.623)	0.9610
Ischemic heart disease (presence vs. absence)	0.869 (0.326–2.315)	0.7794
<i>OGG1</i> Ser326Cys (Cys/Cys vs. Ser/Ser+Ser/Cys)	3.191 (1.174–8.672)	0.0229

TABLE 5. Genotype distribution of Ser326Cys in our patients and an independent Japanese population

Genotype	Distribution ^a	
	Our patients (n = 119)	Ito et al.(19) ^b (n = 241)
Ser/Ser	33 (27.7)	68 (28.3)
Ser/Cys	57 (47.9)	118 (49.2)
Cys/Cys	29 (24.4)	54 (22.5)

^aValues are n (%).

^bA hospital control without a history of cancer. The mean age was 56.8 years (range 39–69) and male/female was 118/123.

range = 39–69 years) (19), there was no statistical difference between the two groups (Table 5). This suggests that the population used in this study may be

representative of the entire Japanese population as regards the *OGG1* polymorphism.

In addition to the *OGG1* polymorphism, age, gender, BMI, and ischemic heart disease were included as independent variables for multivariate logistic regression analysis, since all of these factors have been shown to be associated with tooth loss (4,20–23). However, in this study only age and the *OGG1* Ser326Cys were found to be independent factors affecting tooth loss. Although age was a strong factor for tooth loss (Table 4), no association was found between age and Ser326Cys, as shown in Table 3. CPI was not included in the multivariate logistic regression analysis because 43 patients (36.1%) could not be evaluated for this due to the lack of teeth for an examination.

The human *OGG1* gene encodes a DNA glycosylase that is involved in base excision repair of 7,8-dihydro-8-oxoguanine (8-oxoG) from oxidatively damaged DNA (24), and is located on chromosome 3p25. Kohno et al. (25) reported a C/G polymorphism at nucleotide position 1245 in exon 7 of the *OGG1* gene, resulting in a nonsynonymous amino acid substitution from Ser to Cys at codon 326. Furthermore, the 8-oxoG repair activity of *OGG1* was found to be greater for Ser326 than Cys326 by a complementation assay of an *E. coli* mutant defective in 8-oxoG repair (25). This finding led to molecular epidemiological studies on the relation of the *OGG1* polymorphism with various diseases, and a significant association was demonstrated in a variety of cancers (26–28).

Additional studies have indicated the functional effect of Ser326Cys on the enzyme activity of *OGG1*. Yamane et al. (29) recently demonstrated that mutations by 8-oxoG were more efficiently suppressed in *OGG1*-Ser326-transduced cells than in *OGG1*-Cys326-transduced cells. Aka et al. (30) revealed that the *OGG1* variants Ser/Cys and Cys/Cys genotypes showed slower in vitro DNA repair than the Ser/Ser genotype, and that the repair capacity was influenced by the *OGG1* polymorphism in a control population, according to a multivariate analysis.

The molecular mechanism that connects tooth loss with *OGG1* remains to be determined. It is well known that the number of lost teeth is correlated with age. For example, in Sweden elderly people lose 1.4 teeth every 5 years and 1.7–2.6 teeth every 10 years (2). Our present data also show that age is a strong factor affecting tooth loss. On the other hand, accumulating evidence suggests that oxidative DNA damage may be implicated in the aging process (31). In homozygous *OGG1*–/– null mice, the steady-state level of oxidative DNA modifications, including 8-oxoG, increases continuously over the whole lifespan (32), suggesting an age-dependent increase of oxidative damage in the absence of *OGG1*. Oxidative

damage to mitochondrial DNA has been suggested to be a key factor in the age-associated decline in tissue functions (the mitochondrial theory of aging), since the major source of reactive oxygen species in cells is mitochondria, the genomes of which are more susceptible to oxidative damage than the nuclear genome (33). The human *OGG1* gene codes for two alternative spliced isoforms, *OGG1*- α and *OGG1*- β , and only *OGG1*- β is targeted to mitochondria (34). Rachek et al. (35) demonstrated that transfection of the *OGG1* gene with mitochondrial localization sequence into HeLa cells results in enhanced cell survival following oxidative stress. Cockayne syndrome (CS) is a segmental premature aging syndrome in humans that can be divided into two complementation groups: CSA and CSB (36). It was recently reported that expression of recombinant CSB specifically increases incision and repair of 8-oxoG in the mitochondria of CSB-deficient human cells (36), suggesting a relationship between decreased *OGG1* activity due to CSB deficiency and premature aging. From these findings, it may be possible to speculate that decreased *OGG1* activity due to the DNA polymorphism may be involved in individual differences in normal aging processes, and may eventually affect an individual's edentulous level.

Although further studies with a larger sample size are necessary, the present findings may help elucidate the mechanism behind individual differences in tooth loss.

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