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Increase in Sequence Variation of the D310 Region and Decrease of the Copy Number of Mitochondrial DNA in Gingival Tissues From Patients With Chronic Periodontitis

Yi-Shing Ma[1], *Yi-Hung Lin*[2], *Jung-Tse Yang*[1], *Jui-Chang Huang*[2,*], *Yau-Huei Wei*[1,*]

[1]Center for Mitochondrial Medicine and Free Radical Research, Changhua Christian Hospital, Changhua, Taiwan
[2]Department of Dentistry, Changhua Christian Hospital, Changhua, Taiwan

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[*]**Corresponding author**

Yau-Huei Wei, Ph.D.

Center for Mitochondrial Medicine and Free Radical Research, Changhua Christian Hospital

No. 176, Zhonghua Rd., Changhua City, Changhua County 500, Taiwan

E-mail: yhweibabi@gmail.com

Jui-Chang Huang, D.D.S.

Department of Dentistry, Changhua Christian Hospital

No. 135, Nanxiao St., Changhua City 500, Taiwan

E-mail: 90375@cch.org.tw

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ABSTRACT

Chronic periodontitis (CP) is a chronic disease caused by complex interactions between oral microbes and host immunity. Enhanced oxidative stress and mitochondrial dysfunction also play roles in the pathogenesis of the disease. We used Sanger sequencing to determine whether CP is associated with specific sequence variations, including length heteroplasmy (LHP), in the displacement loop (D-loop) region of mitochondrial DNA (mtDNA) in the gingival tissues. The copy number of mtDNA and pathogenic bacteria Porphyromonas gingivalis (Pg) were also determined by qPCR. We enrolled 54 CP patients and 55 healthy controls (HC) for comparison. Pg infection was found in 89% of the gingival tissue in the CP group as compared with only 16.7% in the HC group. The occurrence frequencies of T16298C and T199C were positively and negatively correlated with CP, respectively. The occurrence frequencies of total LHP and the specific C_8TC_6 variant in the D310 region were significantly higher in the CP patients, implying that mtDNA with the D-loop mutations might be positively selected in the gingival tissues of patients. We also found that the copy number of mtDNA was decreased significantly in the gingival tissues of CP patients. Its content was negatively associated with the copy number of Pg genomic DNA, although without significance ($\rho = -0.195$, $p = 0.149$). The findings of this study supported the notion that infection with Pg is a risk factor in the development of CP and possibly links to a decrease in the quality and quantity of mtDNA in the pathogenesis of this dental disease.

Introduction

Chronic periodontitis (CP) is considered a response to oral dysbiosis and chronic inflammation of the periodontal tissue in conjunction with influences of environmental factors and human aging [1,2]. The progression of the disease is usually relatively slow. Later in the disease progression, patients often develop deep pockets between teeth and gingival tissues, indicating the loss of attachment of periodontal tissue. If left untreated, patients may also suffer bone destruction around the teeth, eventually leading to tooth loss.

Histologically, gingival tissues are composed of the overlying epithelium and the underlying connective tissue. Gingival fibroblasts (GFs) and periodontal ligament fibroblasts are the predominant cell types in the superficial gingival and deep periodontal connective tissues, respectively, which play crucial roles in tissue repair and the inflammatory response [3]. The adhesion and colonization of pathogenic bacteria and their subsequent penetration across the local epithelium barrier initiate the inflammation cascade that recruits circulating neutrophils and other immunocytes into the affected tissues [4]. Infection with *Porphyromonas gingivalis* (Pg) triggers the host innate immune system response by activating inflammation consisting of the NLRP3 inflammasome [5]. It is evident that the overreaction of neutrophils and the accumulation of reactive oxygen species (ROS), cytokines, and matrix metalloproteinases are important factors contributing to the pathogenesis of CP and the destruction of periodontal tissue [6]. Evidence has shown that CP is highly associated with red-complex bacteria in subgingival plaque [7], and infection with Pg in gingival tissue cells also induces intracellular production of ROS [8,9]. This also suggests that excess ROS increases local gingival oxidative stress, which in turn builds up a ROS-elicited oxidative stress inflammation cascade [4]. Smoking, diabetes mellitus, and aging have been mentioned as risk factors for CP through modulation of the host innate immunity [10-12]. Mitochondrial dysfunction and the release of ROS and mitochondrial DNA (mtDNA) into the cytosol are additional key upstream events implicated in NLRP3 activation [13]. Increases in damaged and dysfunctional mitochondria following impairment of mitophagy enhance NLRP3 inflammasome activation. Mitochondrial oxidative metabolism and the responses of cells to xenobiotics, cytokines, and bacterial invasion may result in the accumulation of ROS, which can lead to oxidative stress and oxidative damage due to excess production of ROS that exceeds the cellular antioxidant capacity [14].

Human mtDNA is a double-stranded circular molecule with a length of 16,569 bp [15]. A functional electron transport chain is made by coordinated expressions of many genes in the nucleus and mitochondrial genome. However, the probability of replication errors and oxidative damage of mtDNA is increased during the aging of the human body, resulting in mitochondrial defects and functional decline of affected cells, and eventually culminating in symptoms of aging or disease. For these reasons, mtDNA is constantly attacked by ROS and other free radicals generated as by-products of normal oxidative metabolism, and thus has a high probability of mutation [16-18].

The displacement loop (D-loop) region of mtDNA contains three hypervariable regions and regulates the replication and transcription of mtDNA [15]. It is a hot spot of genetic variation, including single nucleotide variation, insertion, deletion, and poly-C length heteroplasmy (LHP). Sequence analysis of these regions of mtDNA is used not only in forensic analyses but also in the clinical diagnosis and follow-up of cancer and other diseases. It has been shown that the D-loop region is highly vulnerable to electrophilic attack by ROS and other free radicals in comparison with other regions of mtDNA [19]. The region of the nucleotide position (np) 303 to 309 poly-C tract in particular is more likely to cause profound mtDNA oxidation damage and lead to a significant increase in the abundance of D310 C-tract variants. In light of the important role of ROS-induced oxidative stress in the pathogenesis of periodontal diseases, the mtDNA mutation associated with CP has been studied previously [20,21]. A recent study demonstrated a decrease in the mtDNA copy number, abnormal mitochondrial structure and function in the gingival tissues and gingival tissue-derived fibroblasts in patients

with CP [22]. In this study, we intended to extend the understanding of the changes in the quality and quantity of mtDNA in the gingival tissues of patients with CP by sequencing of the D-loop region of mtDNA and analysis of the mtDNA copy number of the tissues.

Methods

Study subjects

In this study, 55 CP patients and 54 ethnically-matched periodontally healthy volunteers were recruited at the Periodontology Department of Changhua Christian Hospital between October 2019 and May 2022. All participants were non-smokers without diabetes or cardiovascular disease according to a survey by questionnaire and medical history review. Informed consent documents were obtained from all subjects recruited under protocols approved by the Institutional Review Board of Changhua Christian Hospital (permit numbers: 190106 and 191109). Periodontal diagnostic criteria for CP were assigned accordingly [23]. Gingival tissues of the CP patients showed inflammation and more than 30% loss of alveolar bone. Loss of periodontal ligament occurred in the CP patients, recorded as having a pocket depth (PD) ≥ 5 mm at more than 2 sites. The PD of the control subjects receiving crown lengthening procedures was recorded at 6 sites. The main indications of the crown lengthening procedure included a short clinical abutment, subgingival caries, and tooth fractures. Surgically-collected gingival tissues were stored in a −80°C freezer before further processing.

DNA isolation

Tissues were ground in a frozen state and the broken tissues were lysed in 0.5 mL of lysis buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1% SDS, 100 μg/mL RNase A, and 100 μg/mL proteinase K at 55°C for 6 hr. DNA was isolated using the phenol/chloroform extraction method. The DNA sample was kept at −20°C until use.

Sanger sequencing and sequence analysis

The D-loop region of mtDNA was amplified using KAPA HiFi HotStart ReadyMix (Roche Sequencing Solution Inc., Pleasanton, CA, USA) according to the manufacturer's protocol. Sanger sequencing was performed by Mission Biotech (Taipei, Taiwan). The nucleotide sequences of primers used for amplification and sequencing are listed in Table 1. A heteroplasmy at a np was defined as the peak height of a secondary peak being more than 10% of the primary peak. The patterns

Table 1. Oligonucleotide primers and probes used in this study

Name of the primer	5' to 3' sequence
For D-loop amplification and sequencing	
H16036	GAAGCAGATTTGGGTACCAC
L640	GGGGTGATGTGAGCCCGTCT
H111	ACCCTATGTCGCAGTATCTG
L308	GGGGGGTTTGGTGGAAATTTTTTG
For total mtDNA	
D-loop-F	ACATCACGATGGATCACAGGTCTAT
D-loop-R	CCCCCAGACGAAAATACCAA
Probe	Hex-CCACTCACGGGAGCTCTCCATGC-BHQ1
For genomic DNA	
B2M-F	TGCTGTCTCCATGTTTGATGTATCT
B2M-R	TCTCTGCTCCCCACCTCTAAGT
Probe	Hex-TTGCTCCACAGGTAGCTCTAGGAGG-BHQ1

B2M: beta-2-microglobulin; D-loop: displacement loop; mtDNA: mitochondrial DNA.

of CnTCn sequence variants in the D16189 and D310 regions, including predominant variants, were determined as described previously [24-26]. The nucleotide sequences were aligned against the revised Cambridge Reference Sequence (rCRS, NC_012920.1). The haplogroup was defined by the Mitomaster tool according to each D-loop sequence of mtDNA [27]. Multiple sequence alignment was conducted by MEGA 11 using the ClustalW algorithm. Analysis of the population genetic diversity was performed using the DnaSP v6.12 [28].

Quantification of mtDNA copy number

The copy number of mtDNA was determined by taking the ratio between the amount of the polymerase chain reaction (PCR) product of the D-loop of mtDNA and that of a nuclear beta-2-microglobulin gene (D-loop/B2M) on a CFX96 Connect Real-Time PCR system (Bio-Rad Laboratories Inc., Taiwan Branch, Taipei, Taiwan). Reaction mixtures included 100 ng of DNA, 600 nM of each primer, 100 nM of the probe (GeneDireX, Inc., Miaoli County, Taiwan), and 1× TaqMan Universal PCR Master Mix II, no UNG (Applied Biosystems Inc., Foster City, CA, USA) in a final volume of 20 μL. The efficiency of the reaction was determined using serial dilutions of the DNA samples or plasmids cloned with the target DNA fragment. A spike-in DNA sample from a patient was run on all plates as an internal control for normalization to all samples. Cycle threshold (Ct) values from triplicate reactions of all samples were within the linear range. We determined the relative mtDNA copy number by the following equation: Relative mtDNA copy number = $2^{\Delta Ct}$, where ΔCt = Ct B2M – Ct D-loop.

Quantification of Pg

PCR was performed in 20 μL of a reaction mixture containing 1× BioRad iQ SYBR Green Supermix, 50 ng total DNA, and 400 nM of each primer, as described by Kato et al. [29]. A standard curve was generated using a series of ten-fold diluted standards (MBD0004, Sigma-Aldrich Chemical Co., St. Louis, MO, USA) with the copy number ranging from 4.13×10^4 (0.1 ng) to 4.13 (0.01 pg). Linearity with an R^2 value over 0.997 was routinely observed.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 7.0 software (GraphPad Software Inc., Boston, MA, USA). Comparison of groups in the demographic table was performed using the non-parametric Mann-Whitney U test. Fisher's exact test and odds ratios (ORs) with 95% confidence intervals (CIs) were applied to compare the differences in categorical variables between the HC and CP groups. Multiple logistic regression was performed to identify variants associated with CP after adjustment for age and gender. Differences in the measured parameter values between the HC and CP groups with $p < 0.05$ were considered statistically significant.

Results

Study population

There were 51 males (46.8%) and 58 females recruited for this study (Table 2). The proportion of males (61.8%) was significantly higher in the CP group than in the HC group (37.0%; $p < 0.05$), which indicated that men have a higher chance of developing CP as compared with women. The average age of the subjects was also higher in the CP group than in the HC group ($p < 0.05$). Patients showed a significantly higher PD, and the prevalence of the pathogenic bacteria Pg in the gingival tissues of the CP patients was higher than that in the HC subjects ($p < 0.001$).

Sequence variations in the mtDNA D-loop

Single-nucleotide variations (SNVs)

We detected 151 SNVs within our study population with a nucleotide diversity (π) of 0.01039. A total of 114 and 99 SNVs were identified in the HC and CP groups, respectively. We found rather high frequencies of A73G (100%), A263G (100%), T489C (55%), T16519C (56.9%), and C16223T (55%) (Table S1). We selected five SNVs

(T199C, C16184T, C16266T, T16298C, T16311C) for further study, as the other variations showed very low frequencies or even distributions in the two groups. After Fisher's exact test analysis, only T199C and T16298C showed significant differences in the occurrence frequency between the CP and HC groups ($p < 0.05$ and $p < 0.01$, respectively; Table 3). We then conducted the next multiple logistic regression analysis for these two variants. After we adjusted for age and gender, significant differences remained in variants T199C (OR = 0.2709, $p < 0.05$, 95% CI: 0.0692–0.8745) and T16289C (OR = 6.888, $p < 0.05$, 95% CI: 1.729–46.200) between the CP and HC groups.

Insertion and deletion and poly-C LHP

We identified 10 insertions and 9 deletions in the study subjects (Table S2). In the subjects of the HC group, 8 insertions and 5 deletions were detected, while 6 insertions and 8 deletions were found in the controls. In Figure 1, we present the representative electrophoretograms of Sanger sequencing to show a polymorphic poly-C stretch in the D16189, D310, and D568 regions of mtDNA. Insertion of at least one C residue at np 309 (C_8TC_6, C_9TC_6, and $C_{10}TC_6$) was found in 51.9% and 65.5% of the subjects in the HC and CP groups, respectively (Table S2 and Table 4). It is worth noting that the occurrence frequency of D310 sequence variant C_8TC_6 was significantly higher in the CP group (47.3% vs. 27.8%, $p < 0.05$). In addition, the occurrence frequency of poly-C LHP in the D310 region was significantly higher in the subjects of the CP group as compared with the HC group (72.7% vs. 53.7%, $p < 0.05$). The significance of the association remained solid after logistic regression analysis of the values adjusted for age and gender (adjusted OR = 2.728, p = 0.021, 95% CI: 1.179–6.583). There was no difference in the occurrence frequency of LHP in the D16189 poly-C region of mtDNA between the two groups (27.8% vs. 29.1%, p = 0.834). In the D568 region, LHP was detected only in 4 controls, including $C_{9\text{-}13}$ LHP in 3 subjects and $C_{8\text{-}10}$ LHP in 1 subject. As to the (CA) n repeats at np 514–523, the occurrence frequency of $(CA)_3$ to $(CA)_7$ repeats in the subjects of the HC

Table 2. Characteristics of the study subjects

Characteristic	HC	CP
Age (years)[a]	48.1 ± 15.5	54.7 ± 12.0*
Gender ratio (male:female)	20:34	31:24*
PD (mm)[a]	2.6 ± 0.3	5.6 ± 1.4***
Pg (positive:negative)[b]	9:45	49:6***

[a]Data are presented as mean ± standard deviation.

[b]"Pg-positive" was defined when more than five copies of the *Porphyromonas gingivalis* genome were detected in 50 ng total cellular DNA of gingival tissues.

***$p < 0.001$, *$p < 0.05$ by the Mann-Whitney U test.

CP: chronic periodontitis; HC: healthy controls; PD: pocket depth; Pg: *Porphyromonas gingivalis*.

Table 3. Selected mitochondrial D-loop variants for univariate logistic regression analysis

rCRS position	rCRS NT	Variant	CP, N (%)	HC, N (%)	OR	p value	95% CI
16298	T	C	13 (23.2)	2 (3.7)	8.048	0.004**	1.850–36.880
16184	C	T	7 (12.5)	1 (1.9)	7.729	0.06	1.285–88.490
16311	T	C	8 (14.5)	3 (5.6)	2.894	0.202	0.800–10.470
199	T	C	4 (7.3)	12 (22.2)	0.2745	0.032*	0.092–0.846
16266	C	T	1 (1.8)	6 (11.1)	0.1481	0.06	0.013–0.981

*$p < 0.05$; **$p < 0.01$ by Fisher's exact test.

CI: confidence interval; CP: chronic periodontitis; HC: healthy controls; NT: nucleotide; OR: odds ratio; rCRS: revised Cambridge Reference Sequence.

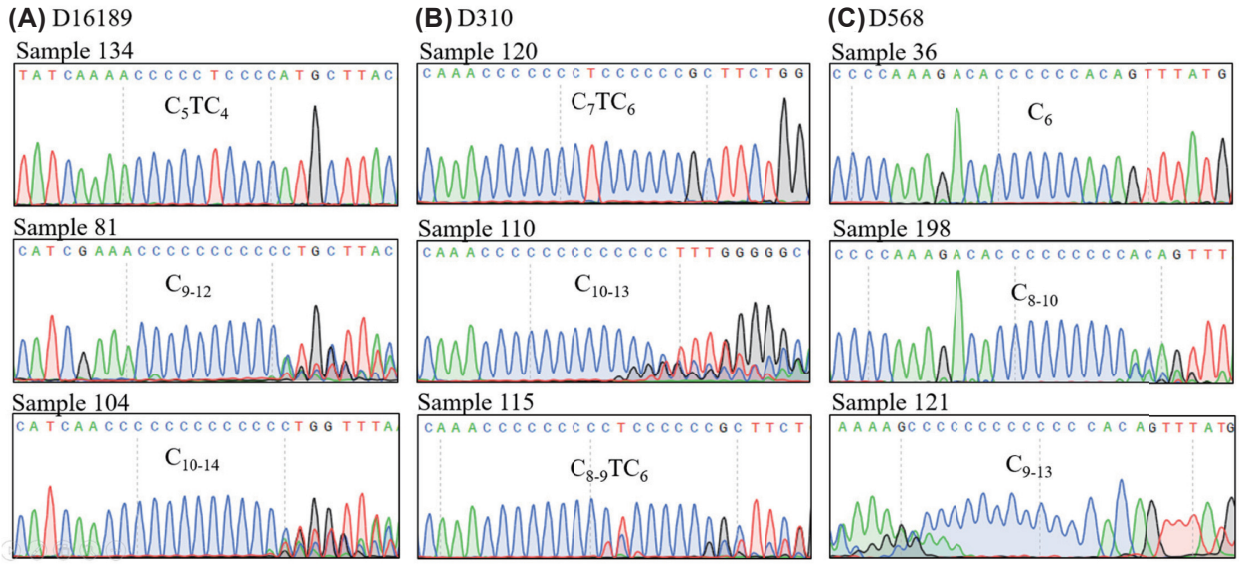

Figure 1. Determination of variants from simple sequence repeat regions of the D-loop of mtDNA. Panels (A) to (C) show the sequencing electrophoretograms of the homoplasmic or heteroplasmic poly-C stretch in D16189 (np 16184–16193), D310 (np 303–315), and D568 (np 568–573), respectively.

Table 4. The occurrence frequencies of LHP in the D16189 and D310 regions of the D-loop of mtDNA in the study subjects

Variant	HC			CP		
	N	Hetero	%[a]	N	Hetero	%[a]
D310 sequence						
C_7TC_6	26	1	3.8	17	2	11.8
C_8TC_6	15	15	100.0	26*	26	100.0
C_9TC_6	11	11	100.0	10	10	100.0
$C_{10}TC_6$	2	2	100.0			
T310d				2	2	100.0
Total	54	29	53.7	55	40	72.7*
D16189 sequence						
C_4TC_4				6	0	0.0
C_5TC_4	37	0	0.0	33	0	0.0
T16189C	17	15	88.2	15	15	100.0
T16189d				1	1	100.0
Total	54	15	27.8	55	16	29.1

[a]The percentage "%" denotes "percentage of heteroplasmy."
*$p < 0.05$ by Fisher's exact test.
CP: chronic periodontitis; HC: healthy controls; Hetero: heteroplasmy.

group was 1.9%, 53.7%, 42.6%, 0.0%, and 1.9%, respectively, and 1.8%, 30.9%, 61.8%, 5.5%, and 0.0%, respectively, in the subjects of the CP group. Only the occurrence frequency of the $(CA)_4$ repeat in subjects between the HC and CP groups showed a significant difference ($p = 0.033$).

Haplogroups of mtDNA

We identified 97 mtDNA haplogroups in all subjects with a haplotype diversity of 0.996, of which 51 haplogroups were found in the HC group and 49 were found in the CP group (Table S3). We identified 11 mtDNA major haplogroups including A, B, C,

D, F, G, I, M, N, R, and Y. Of these, M, B, D, and F covered 85.3% of the major haplogroups of mtDNA in all subjects, but there was no difference in the distribution between the two groups (Figure 2).

Decreased mtDNA copy number and negative correlation with the Pg copy number in the gingival tissues of patients in the CP group

The relative mtDNA copy number was significantly lower in the subjects of the CP group (91.78 ± 38.66) as compared with the HC group (130.95 ± 50.32) ($p < 0.001$) (Figure 3A). The DNA samples in the CP group contained higher Pg genomic DNA (1,773 ± 3,677 copy/50 ng DNA) than did those of the HC group (108 ± 515 copy/50 ng DNA) (Figure 3B). In the subjects of the CP group, the Pg copy number was positively correlated with the age of the subject but negatively correlated with the mtDNA copy number (Figure 3B and 3D).

Discussion

Previous studies have revealed mitochondrial genome instability including large-scale deletions and SNVs in periodontal tissues [20,21,30,31]. Our study first linked CP with an increase of D310 LHP, which is a possible biomarker of oxidative stress and has been associated with various oxidative stress-related diseases such as Alzheimer's disease [25,32] and Parkinson's disease [33]. This region of mtDNA has also been identified as a mutational hotspot in several tumors [24,34,35]. We found that there were differences in the distributions of T16298C and T199C between the subjects in the CP and control groups (Table 3). The compositions of the major haplogroups in our study subjects were similar to previous studies [36]. Further analysis revealed that T16289C is one of the definite mutations located in the D-loop for haplogroup M8. Although we did not find an association of a specific major haplogroup with CP, the occurrence frequency of haplogroup M8a in the CP group was found to be four times higher than that in the HC group ($p = 0.0499$, Chi-square test). The presence of T16184C further assigned the haplogroup M8 to M8a2′3. The OR of haplogroup M8a2′3 was 7.729, the same as that of T16184 (Table 3). Separately, T199C defined M7b1a and M7C1 in our study subjects. Therefore, the occurrence frequencies of these two sub-haplogroups in the HC group were higher than those in the CP group according to the occurrence frequency of T199C shown in Table 3. There remains limitation in our study with respect to the resolution of haplogroups determined merely by sequencing of the D-loop region. Future study using next-generation sequencing to completely sequence mtDNA or with a larger sample size is warranted to confirm this finding. It is worth noting

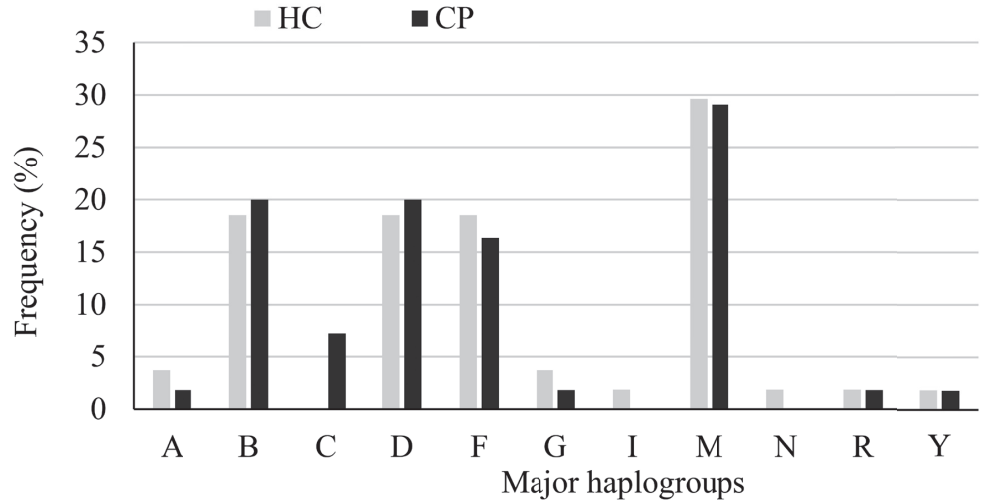

Figure 2. The occurrence frequencies of the mtDNA major haplogroups in the healthy controls (HC) and chronic periodontitis (CP) patients. The nucleotide sequence of the D-loop region of mtDNA was analyzed using the Mitomaster program to determine the haplogroups of mtDNA.

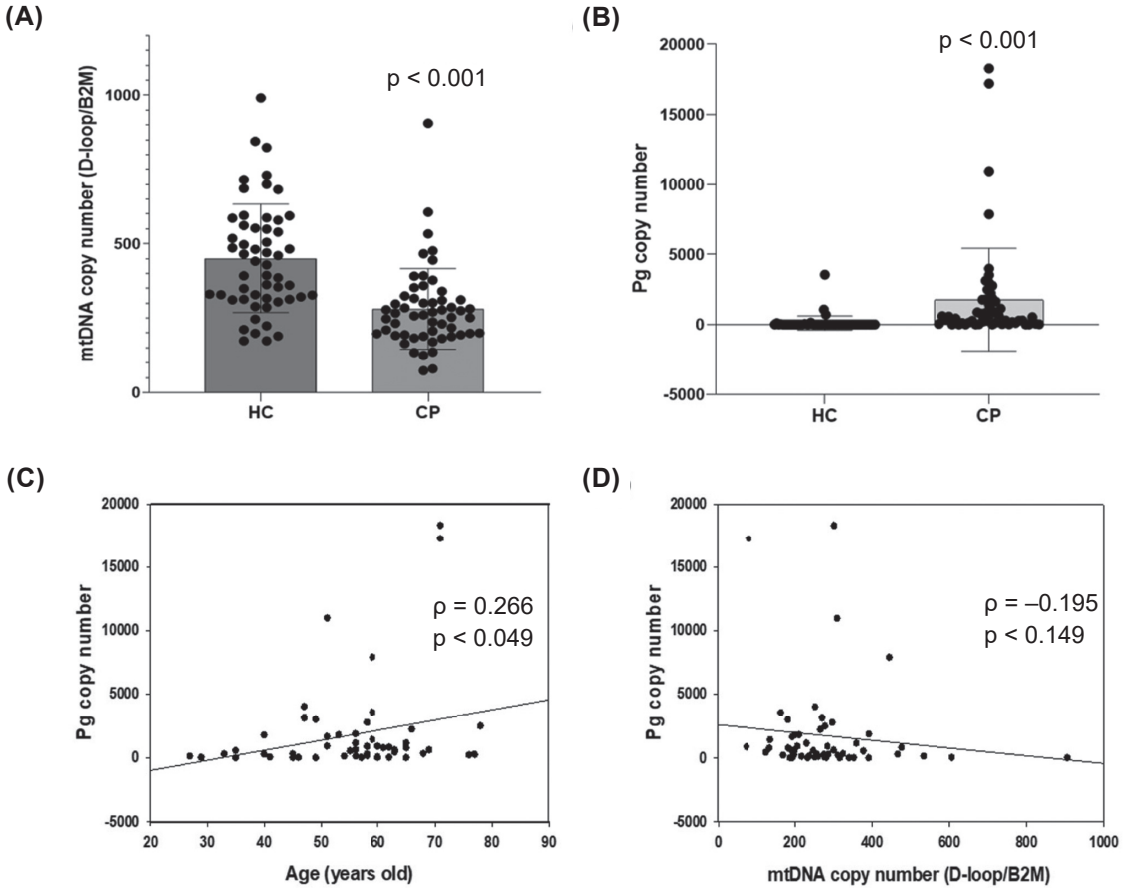

Figure 3. The copy number of mtDNA was decreased and negatively correlated with the *Porphyromonas gingivalis* (Pg) copy number in the subjects of the chronic periodontitis (CP) group. (A) The copy number of mtDNA was significantly decreased in the subjects of the CP group. (B) The copy number of Pg genomic DNA was significantly increased in the gingival tissues of the subjects of the CP group. (C) The copy number of Pg genomic DNA was positively correlated with the age of the subjects in the CP group. (D) The copy number of mtDNA and Pg genomic DNA showed a negative correlation in the gingival tissues of the subjects in the CP group.
B2M: beta-2-microglobulin; D-loop: displacement loop; HC: healthy control; ρ: Spearman's rank correlation coefficient.

that T16126TC, C16290T, T152C, and haplogroup A were identified as risk factors for early-onset aggressive periodontitis in a Chinese population [37]. The effects of those SNVs in the regulation of mitochondrial function warrant future study.

In the analysis of mtDNA copy number, the ND1 gene is the most frequently used in the literature [38]. However, SNVs located in the region of primer and probe may decrease the efficiency of PCR and lead to erroneous determination of the mtDNA copy number. Previously, we conducted a multiplex real-time PCR assay that simultaneously amplified the ND1 gene and D-loop region of mtDNA on a set of 157 different DNA samples isolated from gingival tissues. In the top 11 samples with the lowest delta Ct (Ct of D-loop assay minus Ct of ND1 assay) ranging from 0.99 to –5.02, at least one base mismatch to the sequence of primer or probe for the ND1 gene was identified by Sanger sequencing (unpublished data). Separately, four samples with the highest delta Ct ranging from 22.32 to 2.93 showed one to three sequence variations in the D-loop against the primer or probe used for the D-loop analysis. These findings revealed that mtDNA SNVs cannot be ignored in population studies using qPCR to determine the mtDNA copy number. Such a problem can be resolved by a well-designed primer pair and probe to exclude SNVs with high occurrence frequencies in the study subjects. In the current study, sequence analysis confirmed that no SNVs were present in the regions that the primers or probes annealed to. The D-loop

shows a unique triple-strand characteristic structure and controls the replication and transcription of the mitochondrial genome [9]. Alteration in the mtDNA copy number in the D-loop region may be related to an altered cell cycle or mtDNA replication, rather than a change of the whole mtDNA molecule. These changes were modest when compared to single-cell mtDNA heterogeneity [39]. The mtDNA copy number determined by the D-loop region was reported to be about 1.0- to 1.6-fold of that determined by the ND1 gene or other genes in mtDNA using droplet digital PCR [39,40]. Although the mtDNA copy number of the D-loop region cannot truly reflect the content of mtDNA, the level of ND1 and D-loop was linearly correlated in stimulated T cells from 17 centenarians ($R^2 = 0.9265$) [39].

Findings from *in vitro* studies in which cultured GFs were exposed to Pg have suggested that periodontal pathogens participate in the induction of ROS-mediated autophagy [22,41]. Induction of autophagy can facilitate the survival of Pg [42]. However, hyperactivated autophagy may lead to the destruction of mitochondria and decrease of mtDNA. The findings of this study confirmed that infection of Pg in the gingival tissues is highly prevalent in patients with CP (Table 1). The copy number of Pg in the gingival tissues in the patients was increased in an age-dependent manner, which suggested that aging is an important factor in the development of CP. CP represents a co-morbidity that contributes to aging. People with ineffective oral hygiene exhibited 2- to 5-fold increase in the risk of developing periodontitis [43]. Therefore, maintaining good hygiene is important for the decrease of the occurrence of CP in elderly subjects.

The finding of a negative correlation between the copy number of mtDNA and Pg also links bacterial infection with mtDNA depletion, which is possibly a result of mtDNA mutations and oxidative damage induced by oxidative stress [15]. Although more than 700 bacterial species are present in the oral cavity, the red complex has been considered a prototype polybacterial pathogenic consortium, consisting of Pg, *Treponema denticola*, and *Tannerella forsythia* [7]. We only measured the copy number of Pg, which is the most studied oral pathogenic bacterium regarding CP in the literature. There was no significant difference between HC and CP groups in the negative correlation between the copy number of mtDNA and Pg. Pg alone may not be sufficient to reflect the real biofilm in patients with CP. A more comprehensive analysis of the bacteria infecting the gingival tissues may help to link the deleterious effect of bacterial infection to the copy number of mtDNA in the host cells. Moreover, we noticed that thrombus formation was variable in content among different tissue biopsies. Gingival tissues comprise epithelial cells, fibroblasts, blood, and immune cells. We intend to believe that a decrease in the mtDNA copy number in the CP group was contributed mainly by GFs [3]. Although the mtDNA copy number of blood cells changes with time of day, aging, and disease [44,45], they are minor cell populations in gingival tissues.

In conclusion, the findings of this study support the notion that infection with Pg is a risk factor in the development of CP and possibly links to a decrease in the quality and quantity of mtDNA in the pathogenesis of this dental disease. A more sophisticated study is required to manipulate these factors to better understand the mechanism of pathogenesis of CP.

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Supplement

Table S1. The frequencies of occurrence of SNVs in the D-loop region of mtDNA of the study subjects

rCRS position	rCRS NT	Variant	HC frequency (%), N = 54	CP frequency (%), N = 55
72	T	C	1.9	0.0
73	A	G	100.0	100.0
93	A	G	3.7	1.8
103	G	A	1.9	3.6
125	T	C	1.9	0.0
127	T	C	1.9	0.0
128	C	T	1.9	0.0
131	T	C	0.0	1.8
146	T	A	5.6	0.0
146	T	C	11.1	10.9
150	C	T	20.4	16.4
151	C	T	5.6	3.6
152	T	C	18.5	20.0
153	A	G	1.9	1.8
183	A	G	1.9	0.0
185	G	A	1.9	0.0
189	A	G	3.7	0.0
193	A	G	3.7	1.8
194	C	T	1.9	0.0
195	T	C	3.7	9.1
197	A	G	0.0	1.8
198	C	T	1.9	0.0
199	T	C	22.2	7.3
200	A	G	1.9	3.6
204	T	C	5.6	9.1
207	G	A	11.1	9.1
210	A	G	1.9	0.0
215	A	G	1.9	0.0
217	T	C	1.9	3.6
234	A	G	0.0	1.8
235	A	G	3.7	1.8
250	T	C	1.9	0.0
251	G	A	1.9	1.8
260	G	A	1.9	0.0
263	A	G	100.0	100.0
275	G	A	0.0	1.8
310	T	C	51.9	65.5
318	T	C	3.7	0.0
326	A	G	1.9	0.0
374	A	G	0.0	1.8

Table S1. The frequencies of occurrence of SNVs in the D-loop region of mtDNA of the study subjects (continued)

rCRS position	rCRS NT	Variant	HC frequency (%), N = 54	CP frequency (%), N = 55
385	A	G	0.0	1.8
456	C	T	0.0	1.8
466	T	C	1.9	0.0
489	T	C	51.9	58.2
499	G	A	3.7	9.1
499	G	C	0.0	1.8
507	T	C	0.0	1.8
533	A	G	1.9	0.0
551	A	G	0.0	1.8
593	T	C	0.0	1.8
16086	T	C	1.9	0.0
16092	T	C	5.6	3.6
16093	T	C	7.4	10.9
16094	T	C	1.9	0.0
16104	C	T	0.0	1.8
16108	C	T	1.9	0.0
16111	C	T	0.0	3.6
16126	T	C	3.7	1.8
16129	G	A	27.8	23.6
16136	T	C	3.7	7.3
16140	T	C	1.9	5.5
16145	G	A	1.9	0.0
16147	C	C	0.0	1.8
16150	C	T	1.9	0.0
16153	G	A	1.9	1.8
16157	T	C	1.9	1.8
16162	A	G	7.4	3.6
16164	A	G	5.6	1.8
16167	C	T	1.9	0.0
16168	C	T	0.0	1.8
16172	T	C	18.5	14.5
16174	C	T	1.9	0.0
16179	C	T	3.7	3.6
16180	A	G	1.9	1.8
16182	A	C	18.5	18.2
16183	A	C	24.1	23.6
16184	C	A	0.0	1.8
16184	C	T	1.9	12.7
16185	C	T	0.0	1.8
16186	C	T	1.9	0.0
16188	C	T	1.9	0.0
16189	T	C	31.5	27.3

Table S1. The frequencies of occurrence of SNVs in the D-loop region of mtDNA of the study subjects (continued)

rCRS position	rCRS NT	Variant	HC frequency (%), N = 54	CP frequency (%), N = 55
16192	C	T	5.6	1.8
16194	A	C	0.0	1.8
16195	T	C	0.0	1.8
16207	A	G	1.9	0.0
16209	T	C	3.7	0.0
16213	G	A	0.0	1.8
16214	C	T	0.0	1.8
16217	T	C	14.8	14.5
16218	C	T	0.0	1.8
16221	C	T	0.0	1.8
16223	C	T	50.0	60.0
16231	T	C	1.9	3.6
16232	C	A	1.9	1.8
16234	C	T	1.9	9.1
16235	A	G	0.0	3.6
16239	C	T	0.0	1.8
16243	T	C	3.7	5.5
16245	C	T	1.9	0.0
16248	C	T	0.0	1.8
16249	T	C	3.7	5.5
16256	C	T	0.0	3.6
16257	C	A	1.9	0.0
16260	C	T	0.0	5.5
16261	C	T	13.0	0.0
16263	T	C	0.0	9.1
16266	C	A	1.9	0.0
16266	C	T	11.1	1.8
16271	T	C	3.7	1.8
16274	G	A	1.9	0.0
16278	C	T	7.4	5.5
16287	C	T	1.9	0.0
16290	C	T	7.4	3.6
16291	C	T	0.0	1.8
16292	C	T	1.9	0.0
16293	A	G	1.9	0.0
16293	A	T	1.9	0.0
16294	C	T	1.9	0.0
16295	C	T	7.4	3.6
16297	T	C	7.4	5.5
16298	T	C	3.7	23.6
16299	A	G	3.7	1.8
16300	A	G	1.9	0.0

Table S1. The frequencies of occurrence of SNVs in the D-loop region of mtDNA of the study subjects (continued)

rCRS position	rCRS NT	Variant	HC frequency (%), N = 54	CP frequency (%), N = 55
16304	T	C	20.4	18.2
16309	A	G	1.9	0.0
16311	T	C	5.6	14.5
16318	A	G	0.0	1.8
16318	A	T	0.0	1.8
16319	G	A	11.1	18.2
16325	T	C	1.9	0.0
16327	C	T	1.9	7.3
16335	A	G	1.9	0.0
16335	A	G	0.0	5.5
16343	A	G	1.9	0.0
16354	C	T	1.9	0.0
16355	C	T	1.9	5.5
16356	T	C	3.7	1.8
16357	T	C	3.7	1.8
16360	C	T	1.9	0.0
16362	T	C	31.5	30.9
16391	G	A	1.9	0.0
16399	A	G	1.9	0.0
16463	A	G	1.9	1.8
16465	C	T	1.9	1.8
16470	G	A	1.9	0.0
16471	G	A	1.9	0.0
16473	G	A	1.9	0.0
16497	A	G	3.7	0.0
16519	T	C	51.9	61.8
16526	G	A	0.0	1.8
16558	G	A	0.0	1.8

CP: chronic periodontitis; HC: healthy control; NT: nucleotide; rCRS, revised Cambridge Reference Sequence.

Table S2. The frequencies of occurrence of insertions and deletions in the D-loop region of mtDNA in the study subjects

rCRS position	rCRS NT	INDELs	HC frequency (%), N = 54	CP frequency (%), N = 55
241	A	—	0.0	1.8
249	A	—	18.5	25.5
309	C	CCCCT	3.7	0.0
309	C	CCCT	20.4	18.2
309	C	CCT	27.8	47.3
310	T	—	0.0	3.6
315	C	CC	48.1	30.9
502	C	CCCC	0.0	1.8
513	GC	—	5.6	1.8
521	ACAC	—	1.9	1.8
523	AC	—	46.3	29.1
524	C	CAC	0.0	5.5
524	C	CACAC	1.9	0.0
573	C	CCCC	1.9	0.0
573	C	CCCCC	3.7	0.0
16166	A	—	0.0	1.8
16182	A	AC	5.6	1.8
16189	T	—	0.0	1.8
16216	A	—	1.9	0.0

CP: chronic periodontitis; HC: healthy control; INDELs: insertions and deletions; NT: nucleotide; rCRS: revised Cambridge Reference Sequence.

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
34	HC	B5a	A73G, A210G, A263G, C315CC, AC523-, C16188T, T16189C, C16266A, C16327T, T16519C
105	HC	A (A+152+16362)	A73G, T152C, A235G, A263G, C309CCT, T310C, AC523-, T16086C, C16150T, C16223T, C16290T, G16319A, T16362C
134	HC	A14 (A14)	A73G, C151T, T152C, A200G, A235G, A263G, C315CC, AC523-, C16176T, C16223T, C16290T, G16319A, T16362C, A16463G
36	HC	D1	A73G, A263G, C315CC, T489C, C16223T, T16311C, T16325C, T16362C
29	HC	D2c	A73G, A263G, C309CCT, T310C, T489C, T16092C, C16223T, T16362C, T16519C
146	HC	D2c (D2c)	A73G, T152C, A263G, C309CCCT, T310C, T489C, T16093C, G16129A, C16223T, T16362C, T16519C
2	HC	D4a (D4a3)	A73G, T152C, A263G, C309CCCT, T310C, T489C, G16129A, C16223T, T16249C, T16362C
9	HC	D4b (D4b2b7)	A73G, C194T, A263G, C309CCT, T310C, T489C, AC523-, T16209C, C16223T, C16266T, T16362C, T16519C
144	HC	D4h (D4h1)	A73G, A263G, C315CC, T489C, T16126C, T16172C, C16174T, C16223T, A16343G, T16362C
112	HC	D4o (D4o2a1)	A73G, T195C, A263G, C315CC, T489C, A533G, T16093C, C16223T, C16232T, C16290T, T16362C
23	HC	F1a (F1a)	A73G, G185A, A249d, A263G, C315CC, AC523-, T16093C, G16129A, T16172C, T16304C, T16519C
25	HC	F1a (F1a1)	A73G, C151T, A249d, A263G, C315CC, AC523-, G16129A, A16162G, T16172C, T16243C, C16292T, T16304C, T16519C
50	HC	F1a (F1a1)	A73G, A249d, G251A, A263G, C315CC, AC523-, G16129A, A16162G, T16172C, C16294T, T16304C, A16335G, T16519C
71	HC	F1a (F1a1)	A73G, C151T, A153G, A249d, A263G, C315CC, AC523-, G16129A, A16162G, T16172C, C16266T, T16304C, T16519C
137	HC	F1a (F1a1a)	A73G, C150T, A249d, A263G, C315CC, AC523-, C16108T, G16129A, A16162G, T16172C, T16304C, T16519C
149	HC	F1a (F1a2)	A73G, A249d, A263G, C315CC, AC523-, T16172C, T16304C, T16519C
135	HC	F2c (F2c2)	A73G, A249d, A263G, C309CCT, T310C, T16271C, T16304C
91	HC	F4a (F4a1a)	A73G, T146C, T152C, G207A, A249d, A263G, C309CCT, T310C, A16207G, T16304C, C16360T, T16362C, A16399G, A16497G
7	HC	G1a (G1a)	A73G, C150T, A263G, C315CC, T489C, C16223T, T16249C, T16362C, T16519C
106	HC	G2a (G2a1d2)	A73G, G260A, A263G, C309CCCT, T310C, T489C, C16223T, C16278T, T16362C
198	HC	I2 (I2'3)	A73G, T152C, A189G, T199C, T204C, G207A, T250C, A263G, C315CC, C573CCCC, G16129A, C16223T, G16391A, T16519C

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA (continued)

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
76	HC	M10a (M10a1a1)	A73G, T146C, A263G, C315CC, T489C, AC523-, C573CCCCCC, T16093C, G16129A, T16209C, C16223T, T16311C, T16357C, A16497G, T16519C
125	HC	M12a (M12a1)	A73G, T125C, T127C, C128T, A263G, C309CCT, T310C, T318C, T489C, GC513-, C16223T, C16234T, C16287T, C16290T, T16362C
103	HC	M7b (M7b1a1)	A73G, C150T, T199C, A263G, C309CCCT, T310C, T489C, G16129A, C16223T, T16271C, T16297C
22	HC	M7b (M7b1a1+(16192))	A73G, C150T, T199C, A263G, C315CC, T489C, G16129A, C16192T, C16223T, T16297C
49	HC	M7b (M7b1a1+(16192))	A73G, C150T, A189G, T199C, A263G, C315CC, T489C, G16129A, C16192T, C16223T, T16297C
72	HC	M7b (M7b1a1+(16192))	A73G, C150T, A183G, T199C, A263G, C315CC, T466C, T489C, G16129A, C16192T, C16223T, T16297C
156	HC	M7c (M7c1)	T72C, A73G, T146C, T199C, A263G, C309CCCT, T310C, T489C, AC523-, C16223T, A16293T, C16295T
14	HC	M7c (M7c1a3)	A73G, T146C, T199C, A263G, C315CC, T489C, AC523-, C16295T, G16319A, T16519C
141	HC	M7c (M7c1a3)	A73G, T146C, T199C, A263G, C315CC, T489C, AC523-, C16295T, G16319A, T16519C
101	HC	M7c (M7c1c2)	A73G, T146A, T199C, A263G, C315CC, T489C, AC523-, T16519C
95	HC	M7c (M7c1c2)	A73G, T146A, T199C, A263G, C309CCT, T310C, T489C, AC523-, T16519C
193	HC	M7c (M7c1c2)	A73G, T146A, T152C, T199C, T204C, G207A, A263G, C309CCT, T310C, T489C, AC523-, T16519C
92	HC	M8a (M8a)	A73G, T199C, A263G, C309CCT, T310C, T489C, C16223T, T16298C, G16319A
126	HC	M9 (M9)	A73G, A263G, C309CCT, T310C, T489C, C16223T, T16362C
145	HC	N9a (N9a4)	A73G, C150T, A263G, C309CCCT, T310C, C524CACAC, G16145A, T16172C, C16223T, C16245T, C16257A, C16261T
100	HC	R9c (R9c1)	A73G, A263G, C309CCT, T310C, T16157C, T16304C
27	HC	Y1 (Y1)	A73G, T146C, T152C, G207A, A263G, C309CCCT, T310C, T16126C, T16231C, C16266T, T16519C
48	HC	B4b (B4b1a+207)	A73G, G207A, A263G, C315CC, G499A, T16136C, A16182AC, A16183C, T16189C, T16217C, A16309G, C16354T, T16519C
140	HC	B4h (B4h)	A73G, A93G, A263G, C315CC, GC513-, G16129A, A16182C, A16183C, T16189C, T16217C, C16261T, T16356C
83	HC	B5b (B5b)	A73G, G103A, A263G, C309CCT, T310C, AC523-, T16140C, A16182AC, A16183C, T16189C, T16243C, T16311C, C16355T, T16519C
11	HC	F1e (F1e3)	A73G, A249d, A263G, C309CCT, T310C, AC523-, G16153A, A16182AC, A16183C, T16189C, C16278T, A16300G, T16304C, T16357C, T16519C

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA (continued)

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
82	HC	D5a (D5a2a1b1)	A73G, C150T, A263G, C315CC, T489C, AC523-, T16092C, A16164G, C16167T, T16172C, A16182C, A16183C, T16189C, C16223T, C16266T, G16274A, C16278T, A16293G, T16362C
73	HC	B4a (B4a4)	A73G, A193G, A263G, C309CCCT, T310C, AC523-, T16094C, A16182C, A16183C, T16189C, T16217C, C16261T, A16299G, T16519C
104	HC	B4a (B4a4)	A73G, T152C, A193G, A263G, C309CCCT, T310C, AC523d, A16182C, A16183C, T16189C, T16217C, C16261T, A16299G, T16519C
116	HC	B4h (B4h)	A73G, A93G, A263G, C315CC, GC513-, G16129A, A16182C, A16183C, T16189C, T16217C, C16261T, T16356C
121	HC	B4 (B4+16261)	A73G, A263G, C309CCCT, T310C, C573CCCCC, A16182C, A16183C, T16189C, A16216d(=A16215d†), T16217C, C16261T, T16519C
4	HC	B4 (B4+16261)	A73G, A263G, C309CCCCT, T310C, AC523-, C573CCCCC, A16182C, A16183C, T16189C, T16217C, C16261T, C16295T, T16519C
19	HC	B4b (B4b1a+207)	A73G, C150T, T204C, G207A, A263G, C309CCCCT, T310C, G499A, T16136C, C16179T, A16182C, A16183C, T16189C, T16217C, T16519C
1	HC	D5a (D5a2a1+@16172)	A73G, C150T, A263G, C309CCCT, T310C, T489C, AC523-, A16164G, A16182C, A16183C, T16189C, C16223T, C16266T, T16362C
80	HC	M8a (M8a2a1)	A73G, T152C, A263G, C315CC, T489C, C16184T, T16189C, C16223T, C16278T, T16298C, G16319A, G16470A, G16471A, G16473A
81	HC	F1a (F1a2a)	A73G, T217C, A249d, A263G, C309CCT, T310C, ACAC521-(=ACAC515d†), T16172C, A16180G, T16189C, T16304C, C16465T, T16519C
192	HC	M11a (M11a)	A73G, T195C, C198T, A215G, A263G, C315CC, T318C, A326G, T489C, T16189C, C16223T
191	HC	D5a (D5a2a1+@16172)	A73G, C150T, A263G, C309CCT, T310C, T489C, AC523-, T16092C, A16164G, A16182C, A16183C, C16186T, T16189C, C16223T, C16266T, T16362C
5	CP	M8a (M8a2'3)	A73G, A263G, C315CC, T489C, C16184T, C16223T, T16298C, G16319A
44	CP	M8a (M8a2'3)	A73G, A263G, C315CC, T489C, C16184T, C16223T, T16298C, G16319A
45	CP	M8a (M8a2'3)	A73G, A263G, C315CC, T489C, C16184T, C16223T, T16298C, G16319A
173	CP	M8a (M8a2'3)	A73G, A263G, C315CC, T489C, C16184T, C16223T, T16298C, G16319A
186	CP	M8a (M8a2'3)	A73G, A263G, C315CC, T489C, C16184T, C16223T, T16298C, G16319A
180	CP	M8a (M8a2'3)	A73G, A263G, C309CCT, T310C, T489C, C16184T, C16223T, T16298C, G16319A, T16519C

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA (continued)

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
179	CP	A (A+152+16362)	A73G, T152C, A235G, A263G, C315CC, AC523-, T16172C, C16223T, C16290T, G16319A, T16362C, T16519C
60	CP	C	A73G, A249d, A263G, C309CCT, T310C, T489C, C16223T, T16298C, C16327T, T16519C
87	CP	C4a (C4a2b2)	A73G, T195C, A249d, A263G, C309CCT, T310C, T489C, C16223T, T16298C, A16318G, C16327T, T16357C, T16519C
163	CP	C4b (C4b1)	A73G, T146C, A249d, A263G, C309CCT, T310C, T489C, C16223T, T16298C, C16327T, T16519C
66	CP	D2c (D2c)	A73G, T152C, A263G, C309CCCT, T310C, T489C, G16129A, C16223T, T16362C, T16519C
79	CP	D4a (D4a)	A73G, T152C, A263G, C315CC, T489C, T16093C, G16129A, C16223T, T16362C, T16519C
93	CP	D4a (D4a3b2)	A73G, T152C, A263G, C309CCT, T310C, T489C, C524CAC, G16129A, C16223T, T16249C, C16278T, T16311C, T16362C
157	CP	D4a (D4a3b2)	A73G, T152C, A263G, C309CCT, T310C, T489C, C524CAC, G16129A, C16223T, T16249C, C16278T, T16311C, T16362C
158	CP	D4a (D4a6)	A73G, T146C, T217C, A263G, C309CCT, T310C, T489C, C16223T, C16234T, T16362C
117	CP	D4a (D4a7)	A73G, T152C, A263G, C309CCT, T310C, T489C, T507C, G16129A, C16223T, T16263C, T16362C, T16519C
123	CP	D4h (D4h4a)	A73G, T152C, A263G, C309CCT, T310C, T489C, C16223T, T16311C, C16355T, T16362C
114	CP	D4q (D4q)	A73G, A200G, A263G, C309CCT, T310C, T489C, C16223T, C16234T, C16256T, T16311C, T16362C, T16519C
169	CP	F1a (F1a)	A73G, A200G, A249d, A263G, C315CC, AC523-, G16129A, T16172C, C16295T, T16304C
172	CP	F1a (F1a)	A73G, A249d, A263G, C309CCT, T310C, AC523-, G16129A, T16172C, T16304C, T16519C
51	CP	F1a (F1a1)	A73G, A249d, G251A, A263G, C309CCT, T310C, AC523-, G16129A, A16162G, T16172C, T16304C, A16335G, T16519C, G16558A
61	CP	F1a (F1a1)	A73G, C151T, T195C, A249d, A263G, C309CCT, T310C, AC523-, G16129A, A16162G, T16172C, T16243C, T16304C, T16519C
178	CP	F2 (F2+195)	A73G, T195C, A249d, A263G, C309CCT, T310C, AC523-, T16304C, T16519C
109	CP	F2b (F2b1)	A73G, A249d, A263G, C309CCCT, T310C, AC523-, T16092A, C16291T, T16304C
74	CP	F2i	A73G, T152C, T195C, A249d, A263G, G275A, C309CCT, T310C, C16221T, T16304C, T16519C
63	CP	F3a (F3a1)	A73G, G207A, A249d, A263G, C309CCCT, T310C, C16260T, T16298C, T16311C, C16355T, T16362C
78	CP	G1c	A73G, A263G, C309CCT, T310C, T489C, T593C, C16223T, T16362C, T16519C

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA (continued)

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
170	CP	M33c (M33c)	A73G, A263G, T310C, T310TTC, T489C, T16093C, C16104T, C16111T, C16223T, A16235G, T16362C, T16519C
6	CP	M72 (M72)	A73G, A263G, C315CC, T489C, T16093C, A16166d(=A16162d†), C16214T, C16223T, T16249C, C16278T, T16519C
120	CP	M7b (M7b1a1)	A73G, C150T, T199C, A263G, C315CC, T489C, G16129A, C16223T, T16297C
181	CP	M7b (M7b1a1+(16192))	A73G, C150T, T199C, A263G, C309CCCT, T310C, T489C, G16129A, C16192T, C16223T, T16297C, G16526A
153	CP	M7b (M7b1a1b)	A73G, C150T, T199C, T204C, A263G, C309CCT, T310C, T489C, C16223T, T16297C, T16519C
131	CP	M7c (M7c1)	A73G, T146C, T199C, A263G, C309CCT, T310C, T489C, G499C, C502CCCC, AC523-, C16223T, C16295T, T16519C
162	CP	M8a (M8a)	A73G, T152C, A263G, C309CCT, T310C, T489C, G499A, C16223T, T16298C, G16319A
138	CP	M8a (M8a2'3)	A73G, T195C, A263G, C315CC, A374G, T489C, C16168T, C16184T, C16223T, T16298C, G16319A, T16519C
166	CP	M9a (M9a4a2)	A73G, T146C, C151T, A153G, A263G, C309CCT, T310C, T489C, C16223T, C16234T, C16260T, T16271C, T16362C
26	CP	M9a (M9a5)	A73G, C150T, A263G, C315CC, A385G, T489C, T16093C, C16223T, C16234T, T16362C, T16519C
183	CP	R9c (R9c1a)	A73G, T152C, A234G, A249d, A263G, C315CC, T16157C, C16256T, T16304C, A16335G
115	CP	Y1 (Y1)	A73G, T146C, T152C, G207A, A263G, C309CCCT, T310C, T16126C, T16231C, C16266T, T16519C
53	CP	B4b (B4b1a+207)	A73G, G207A, A241d(=A240d†), A263G, C309CCCT, T310C, G499A, AC523-, T16136C, A16182C, A16183C, T16189C, T16217C, T16519C
122	CP	B5b (B5b)	A73G, G103A, T204C, A263G, C315CC, AC523-, T16140C, G16153A, A16183C, T16189C, T16243C, A16318T, T16519C
133	CP	B4b (B4b1b'c)	A73G, A263G, C315CC, G499A, T16093C, T16136C, A16182AC, A16183C, T16189C, T16217C, C16218T, C16239T, C16248T, T16519C
69	CP	B4c (B4c1b2a)	A73G, T146C, C150T, A263G, C315CC, AC523-, T16092C, T16140C, A16182C, A16183C, T16189C, T16311C, A16335G, T16519C
196	CP	B4h (B4h)	A73G, A93G, A263G, C309CCT, T310C, GC513-, G16129A, A16182C, A16183C, T16189C, T16217C, C16261T, T16356C
77	CP	B4a (B4a4)	A73G, A193G, A263G, C309CCCT, T310C, AC523-, A16182C, A16183C, T16189C, G16213A, T16217C, C16261T, A16299G, T16519C
110	CP	B4 (B4+16261)	A73G, A263G, T310d, AC523-, A551G, A16182C, A16183C, T16189C, T16217C, C16261T, T16519C
129	CP	B4b (B4b1a+207)	A73G, C150T, T204C, G207A, A263G, C309CCCT, T310C, G499A, T16136C, C16179T, A16182C, A16183C, T16189C, T16217C, T16519C

Table S3. The haplogroups of mtDNA predicted by the Mitomaster program based on the variants in the D-loop region of mtDNA (continued)

Sample code	Group	Haplogroup	Variants in the D-loop of mtDNA
124	CP	B4b (B4b1a+207)	A73G, C150T, T204C, G207A, A263G, C309CCCT, T310C, G499A, T16136C, C16179T, A16182C, A16183C, T16189C, T16217C, T16519C
164	CP	D5a (D5a2)	A73G, C150T, A263G, C309CCT, T310C, T489C, AC523-, A16164G, T16172C, A16182C, A16183C, T16189C, C16223T, C16259T, T16362C
168	CP	D5b (D5b4)	A73G, C150T, A263G, T310d, C456T, T489C, C524CAC, A16182C, A16183C, T16189C, C16223T, T16311C, T16362C
15	CP	B5b (B5b2)	A73G, G103A, T131C, T204C, A263G, C309CCCT, T310C, AC523-, T16093C, C16111T, G16129A, T16140C, T16172A, A16182C, A16183C, T16189C, T16231C, C16234T, T16243C, C16261T, T16304C, A16463G, T16519C
118	CP	B4c (B4c2)	A73G, A263G, C309CCT, T310C, C16147T, A16183C, C16184A, T16189C, T16217C, A16235G, C16290T, T16519C
195	CP	F1a (F1a2a)	A73G, T217C, A249d, A263G, C309CCT, T310C, ACAC521-(=ACAC515d†), T16172C, A16180G, T16189d, A16194C, T16195C, T16304C, C16465T, T16519C
111	CP	C4a (C4a2b2)	A73G, A197G, A249d, A263G, C309CCT, T310C, T489C, T16189C, C16223T, T16298C, T16311C, C16327T, C16355T, T16519C
167	CP	D4b (D4b1)	A73G, A263G, C315CC, T489C, AC523-, C16185T, T16189d, C16223T, C16232A, G16319A, T16362C

CP: chronic periodontitis; D-loop: displacement loop; HC: healthy control; mtDNA: mitochondrial DNA.