Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA

The Journal of DNA Mapping, Sequencing, and Analysis

ISSN: 1940-1736 (Print) 1940-1744 (Online) Journal homepage: http://www.tandfonline.com/loi/imdn20

Association of mitochondrial DNA displacement loop polymorphisms and aggressive periodontitis in a Chinese population: a pilot study

Xiaoxuan Wang, Yuan Guo & Qingxian Luan

To cite this article: Xiaoxuan Wang, Yuan Guo & Qingxian Luan (2015) Association of mitochondrial DNA displacement loop polymorphisms and aggressive periodontitis in a Chinese population: a pilot study, Mitochondrial DNA, 26:3, 389-395

To link to this article: http://dx.doi.org/10.3109/19401736.2013.840589

Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, 2015; 26(3): 389–395
 DOI: 10.3109/19401736.2013.840589

informa
healthcare

RESEARCH ARTICLE

Association of mitochondrial DNA displacement loop polymorphisms and aggressive periodontitis in a Chinese population: a pilot study

Xiaoxuan Wang, Yuan Guo, and Qingxian Luan

Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China

Abstract

To examine whether certain mitochondrial DNA (mtDNA) haplogroups and/or alterations affect susceptibility to aggressive periodontitis (AgP), we analyzed the mtDNA D-Loop region in a Han Chinese population. The mtDNA haplogroups were analyzed in 58 patients with AgP and 50 periodontally healthy controls. The frequency of haplogroups A in AgP group was significantly higher than that in the control group ($p = 0.007$). Furthermore, the frequency of haplogroup D was higher in the control group than that in AgP group ($p = 0.007$). The frequencies of D-Loop polymorphisms m.16126T>C, m.16290C>T and m.152T>C were significantly higher in patients with AgP compared with controls ($p = 0.029$, 0.014 and 0.022, respectively). Additionally, the frequencies of three other D-Loop polymorphisms, m.16223C>T m.489T>C and m.515CA deletion (del) were significantly lower in patients with AgP compared with controls ($p = 0.042$, 0.003 and 0.026, respectively). Our study showed for the first time, an association between AgP and mtDNA haplogroups. Haplogroups A was implicated as a risk factor for AgP, while haplogroups D exhibited a protective effect in this disease. These observations may provide a new perspective on the study of the pathogenesis of periodontitis.

Keywords

AgP, D-loop, haplogroup, hypervariable region, mtDNA

History

Received 4 June 2013
Revised 29 July 2013
Accepted 30 August 2013
Published online 9 October 2013

Introduction

Aggressive periodontitis (AgP) is a rare and severe form of periodontal disease that is characterized by early onset, rapid disease progression and familial aggregation (Armitage, 1999). It is generally considered that an imbalance between dental microbial plaque formation and host immune responses contribute to the periodontal tissue destruction in AgP, including attachment loss and alveolar bone loss. Different host responses to microbial plaque lead to differences in the quality and quantity of local inflammatory and immune reactions, which causes periodontal tissue destruction (Page et al., 1997). Genetic factors are partly responsible for the host response to microbial plaque and, to date, numerous nuclear genes related to inflammatory and immune reactions have been involved to study the susceptibility of AgP (Kinane & Hart, 2003; Loos et al., 2005).

The mitochondrial genome, which is unique in that it exists outside the nucleus in humans, is a circular double-stranded DNA molecule of 16,569 bp found adjacent to the mitochondrial inner membrane. It encodes 37 genes composed of 2 rRNA genes, 22 tRNA genes and 13 genes essential for oxidative phosphorylation. The displacement loop (D-loop) is the only noncoding region (nucleotide position [np] 16,024–576 = 1122 bp) of the mitochondrial genome which is known to accumulate alterations at a higher frequency than the coding region (Michikawa et al., 1999). The D-Loop region is a hot spot for mitochondrial DNA (mtDNA) alterations containing three highly polymorphic hypervariable segments: HV1 (np 16,024–16,383), HV2 (np 57–333) and HV3 (np 438–574; Imaizumi et al., 2002; Lutz et al., 2000; Penta et al., 2001; Yakes & van Houten, 1997).

mtDNA haplogroups are characteristic of specific ethnic groups. However, there are differences in the mtDNA sequence between individuals of the same ethnic group. Most mitochondrial mutations occur in pre-existing haplogroups and have essential implications for the investigation of disease associations (Graven et al., 1995; Lertrit et al., 1994; Obermaier-Kusser et al., 1994). It is increasingly found that certain mtDNA clusters are related to distinct disorders (Sun et al., 2012; Torroni et al., 1997). However, the relationship between mtDNA haplogroups, alterations and susceptibility to AgP has not yet been reported. In this study, we analyzed the mitochondrial D-Loop region in a Han Chinese population to examine whether certain mtDNA haplogroups and/or alterations affect susceptibility to AgP.

Materials and methods

Subjects

The study was approved by Institutional Review Board and Ethics Committee of Peking University Health Science Center and each patient signed informed written consent. A total of 108 unrelated subjects were recruited at the Department of Periodontology, Peking University School and Hospital of Stomatology (Beijing, China). Fifty-eight patients with AgP (mean age, 27.29 ± 4.453 years; 36 females and 22 males) were enrolled in the study according to the recommendations of the 1999 International Classification Workshop (Armitage, 1999). Fifty control subjects (mean age $\pm$ SD, 26.96 ± 5.387 years; 30 females and 20 males) were periodontally healthy or showed minimal signs of periodontal disease. Exclusion criteria were as follows: pregnancy, the

Correspondence: Qingxian Luan, Peking University School and Hospital of Stomatology, Department of Periodontology, 22 Zhongguancun Nandajie, Haidian District, Beijing 100081, P.R. China. Tel: + 8610 82195497. Fax: + 8610 62173402. E-mail: kqluanqx@126.com

presence of systemic diseases, obvious malocclusion or a history of orthodontic treatment.

DNA extraction

Peripheral blood samples were collected from fasting participants into a tube containing EDTA by venipuncture. Genomic DNA was extracted from peripheral blood samples using a DNA isolation kit (DP1801, Bioteke, Beijing, China) according to manufacturer's instructions and then stored at −20 °C for later use.

Genotyping of the entire mtDNA D-loop region

Two pairs of corresponding primers (Table 1) were synthesized and purified (Shanghai Sangon Corporation, China) according to previously described methods (Andrews et al., 1999). The mtDNA D-Loop region was amplified in two separate PCRs that were performed in a 25 µl reaction volume containing 5 µl 5 × PrimeSTAR Buffer (Mg2 + Plus), 35 ng genomic DNA, 200 µm/each dNTP (TaKaRa, Japan), 0.2 µm/each primer, 0.625 units PrimeSTAR® HS DNA Polymerase (TaKaRa) and 15.75 µl sterile distilled water. The single set of thermal cycling conditions was as follows: initial denaturation at 98 °C for 5 min, 30 cycles of 98 °C for 30 s, 61 °C for 10 s and 72 °C for 1 min, followed by a single elongation incubation at 72 °C for 10 min. Reactions were performed using a DNA Engine PTC-200 (Bio-Rad, Hercules, CA). After amplification, PCR products (765 bp and 954 bp, respectively) were separated by 1% agarose gel electrophoresis of of Goldview (SBS, Beijing, China) stained samples and visualized using a UV transilluminator.

PCR products were purified by ethanol precipitation and sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Carlsbad, CA) on an ABI 3730 automated sequencer according to manufacturer's instructions.

Data analysis

Sequences were compared with the revised Cambridge Reference Sequences (rCRS; GenBank accession number NC_012920) using Nucleotide BLAST in NCBI (Anderson et al., 1981; Andrews et al., 1999; http://blast.ncbi.nlm.nih.gov/) and MITOMAP (http://www.mitomap.org). Sequence variations found in both AgP patients and controls were checked against the MITOMAP. Alterations that appeared in the database were reported as polymorphisms and those not recorded in the database were categorized as novel mutations. The haplogroup classification was based on the phylogeny proposed by van Oven & Kayser (2009). The ages of AgP patients and control subjects were compared by the unpaired Student's *t*-test. Chi-square tests were used to assess the differences in the sex ratio, frequencies of haplogroups and alterations among the two groups. Binary logistic regression models were used to analyze the associations. Bonferroni corrections were applied for multiple testing. Data were presented as mean ± SD and p values below 0.05 were considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science program (SPSS13.0 for Windows (Chicago, IL)).

Results

Patient background and clinical periodontal characteristics

The genotyping of the mtDNA D-Loop region was completed in 58 patients with AgP and 50 controls. Comparisons of the background characteristics, including age and sex ratio, showed that there was no difference between the AgP and control groups (Table 2).

The typical clinical periodontal characteristics are shown in Figure 1(A). The full mouth X-ray investigation showed severe, generalized alveolar bone loss in all erupted teeth of a 23-year-old female with AgP. The alveolar bone loss was indicated as a radiolucent projection extending from the crest into the interdental septum. In contrast, normal appearance of interdental septa was revealed in a 25-year-old healthy female (Figure 1B).

A total of 13 haplogroups were found in both AgP patients and controls including Z, F, N9a, G, R, A, B, C, Y, Uk, T, D and M (Table 3). The phylogenetic tree of mitochondrial haplogroup classification, including rCRS showed the significant differences in the frequencies of haplogroups A and D ($p < 0.01$) between the AgP and control groups, while none were detected between the other types of haplogroups (Figure 2).

Haplogroups A and D are associated with AgP

A significant difference in the frequency of haplogroup A was detected between the AgP patients (8/58) and controls (0/50; $p < 0.01$). A significant difference in the frequency of haplogroup D was also detected between the AgP patients (8/58) and controls (18/50; $p < 0.01$, odds ratio (OR) = 0.284; Figure 3).

D-Loop alterations in AgP

MtDNA alterations in D-Loop region were analyzed in blood samples from all 58 AgP patients and 50 controls. Patients with AgP showed comparatively higher alteration frequency compared with the numbers of alterations identified in each individual than controls (Table 2). In this study, we identified nine novel mutations in the D-Loop region of patients with AgP, among which three were nucleotide deletions, five were nucleotide insertions, and one was base substitutions (Supplemental Table). In addition, we identified 171 reported polymorphisms in the D-Loop region of AgP patients and/or controls (Supplemental Table). Overall, among the identified 171 reported polymorphisms, 155 were base substitutions, seven were nucleotide deletions, and nine were nucleotide insertions. All the alterations in the D-Loop region that had statistically significant differences in frequency between AgP and control subjects are polymorphisms and listed in Table 4. The frequencies of D-Loop polymorphisms m.16126T > C, m.16290C > T and m.152 T > C were significantly higher in patients with AgP compared with controls ($p = 0.029$, 0.014 and 0.022, respectively). Additionally,

Table 1. PCR primers.

Primer name	Primer sequence 5′–3′	3′ position[a]	Length
1F	TCATTGGACAAGTAGCATCC	15811	765
1R	GAGTGGTTAATAGGGTGATAG	5	
2F	CACCATTCTCCGTGAAATCA	16420	954
2R	AGGCTAAGCGTTTTGAGCTG	775	

[a]Position of 3′-end of primer.

Table 2. Characteristics of total study subjects.

	Control group, $n = 50$	AgP group, $n = 55$	p Value[a]
Mean age at diagnosis	26.96 ± 5.387	27.29 ± 4.453	0.726
Sex ratio (female to male)	30:20	36:22	0.826
Number of alterations	11.5 ± 2.279	12.62 ± 2.758	0.025

[a]Values observed after ''*t* test.''
AgP, aggressive periodontitis.

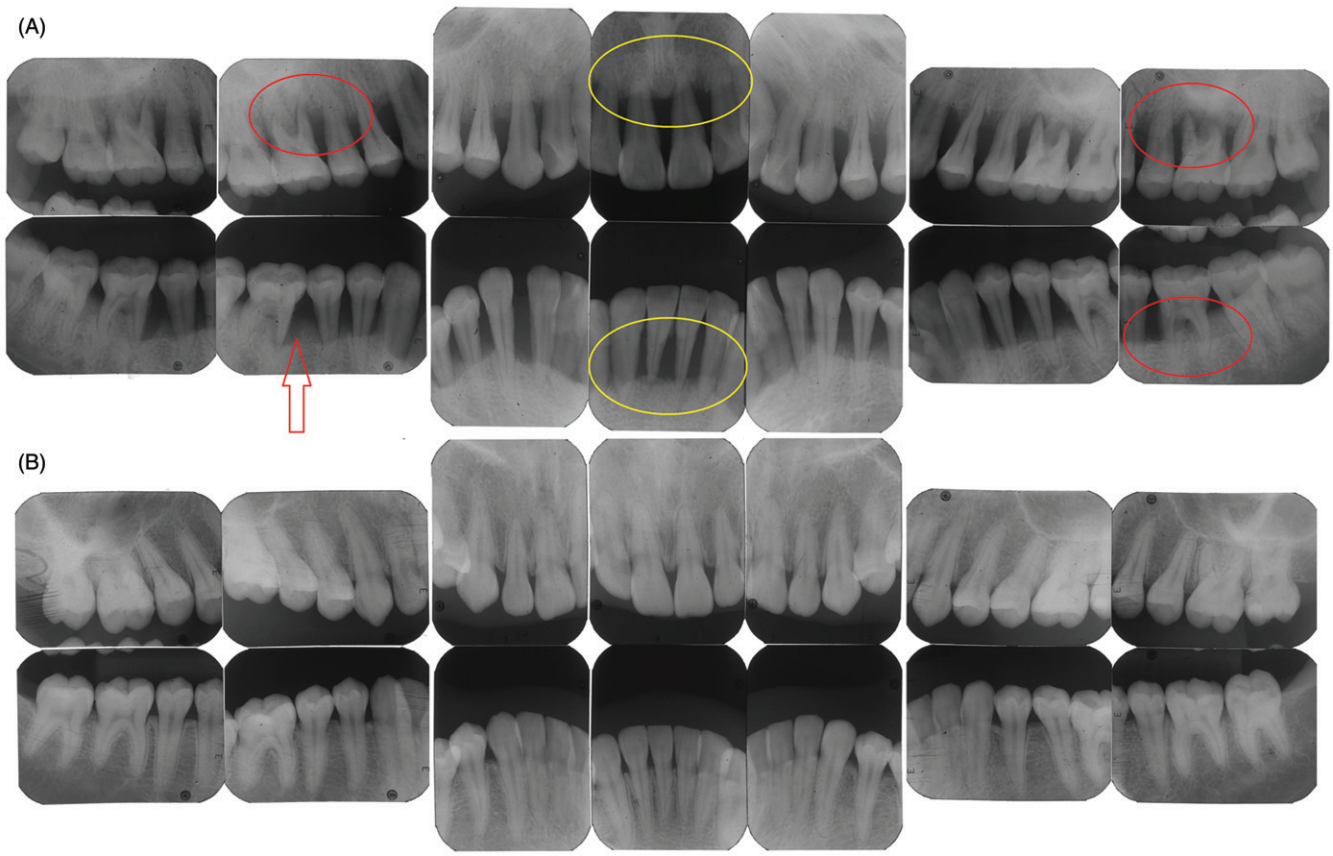

Figure 1. Full mouth X-ray films of a severe generalized AgP patient and a healthy control individual. (A) Severe generalized aggressive periodontitis in a 23-year-old female patient. Radiolucent projection from the crest into the interdental septum indicates alveolar bone loss. Arc-shaped loss of alveolar bone in the first molar extends from the distal surface of the second premolar to the mesial surface of the second molar (red oval box). The arrow shows the wedge-shaped alveolar bone loss. The bone resorption of incisors extended to the root tip (yellow oval box) and alveolar bone loss of the other tooth can also be observed. (B) Normal radiopaque lamina dura around the roots and interdental septum in a 25-year-old female control individual.

Table 3. Haplogroups frequencies, Chi-square test and binary logistic regression test in patients with AgP and controls.

Haplogroup	AgP group	Control group	*p* Value[a]	OR	95%CI	*p* Value[b]
Z	3/58	2/50	1.000	–	–	–
F	10/58	5/50	0.278	–	–	–
N9a	3/58	4/50	0.839	–	–	–
G	2/58	3/50	0.865	–	–	–
R	6/58	3/50	0.642	–	–	–
A	8/58	0/50	**0.007**	–	–	**–**
B	5/58	4/50	1.000	–	–	–
C	4/58	2/50	0.815	–	–	–
Y	1/58	0/50	1.000	–	–	–
Uk	1/58	0/50	1.000	–	–	–
T	1/58	0/50	1.000	–	–	–
D	8/58	18/50	**0.007**	0.284	0.111–0.731	**0.009**
M	6/58	9/50	0.251	–	–	**–**

[a]Chi-square test was applicated.
[b]If the distribution frequencies of haplogroups were significantly different between AgP group and control group, binary logistic regression models were further used to analyze the association between the mtDNA haplogroups and AgP.
The boldface values represent that there is no need to do the binary logistic regression test or the data cannot be analyzed by the test.

the frequencies of three other D-Loop polymorphisms, m.16223C > T m.489 T > C and m.515CA deletion (del) were significantly lower in patients with AgP compared with controls ($p = 0.042$, 0.003 and 0.026, respectively). M.16290C > T was linked to mitochondrial haplogroup A while m.16126T > C, m.152 T > C, 16223C > T and m.489 T > C were linked with multiple mtDNA haplogroups. However, m.515CA del was not linked to haplogroups (van Oven & Kayser, 2009).

Polymorphism in the C-stretch of the mtDNA HV1, HV2 and HV3 regions of controls

Nineteen types of C-stretch sequences detected in np 16,184–16,193, np 303–315 and np 568–573 are shown in Table 5. The type with the highest frequency was CCCCCTCCCC-CCCCCCCCCTCCCCCC- CCCCCC (14/50), followed by CCCCCTCCCC- CCCCCCCTCCCCCC- CCCCCC (8/50; Figure 4A–D). No cases were found with the same structure as the rCRS.

Polymorphism in the C-Stretch of the mtDNA HV1, HV2 and HV3 regions of patients with aggressive periodontitis

Eighteen types of C-stretch sequences detected in np 16,184–16,193, np 303–315 and np 568–573 are shown in Table 6. As with the control group, the type with the highest frequency was CCCCCTCCCC- CCCCCCCCTCCCCCC- CCCCCC (17/58), followed by CCCCCTCCCC- CCCCCCCTCCCCCC- CCCCCC (10/58; Figure 3A–D). No cases were found with the same structure as the rCRS.

Discussion

A variety of nuclear genetic factors are known to be associated with various diseases. For example, the frequency of haplotype

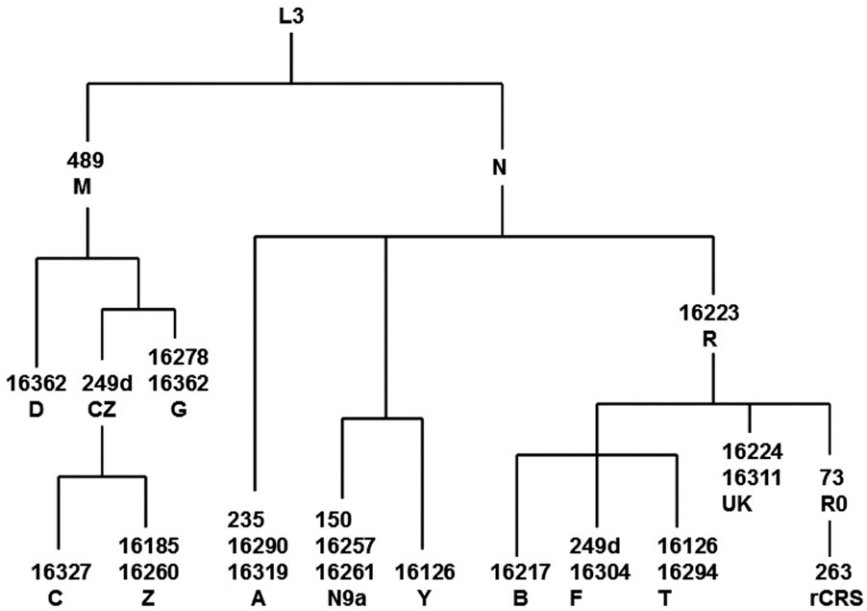

Figure 2. The phylogenetic tree of mitochondrial haplogroup classification, including revised Cambridge Reference Sequence (rCRS). The diagnostic mitochondrial D-Loop region DNA variation markers used for haplogroup classification, according to the mitochondrial DNA rCRS, are reported along the tree branches. The tree is rooted in macrohaplogroup L3. Suffix «d» indicates deletion.

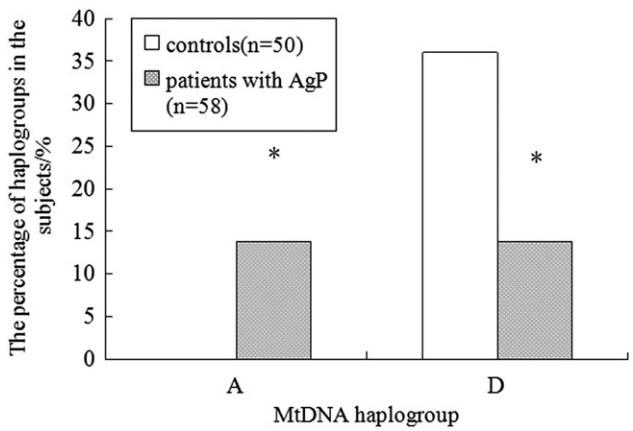

Figure 3. Differential distribution of frequencies of mtDNA haplogroups in the AgP and control groups. The x-axis shows two mtDNA haplogroups and the y-axis shows percentage frequencies of subjects. *Represents $p < 0.01$.

Table 4. Polymorphisms in mtDNA D-Loop region with statistically significant differences between AgP patients and controls.

Mitochondrial polymorphisms	AgP group	Control group	*p* Value[a]	OR	95%CI	*p* Value[b]
16126	6/58	0/50	0.029	–	–	–
16223	32/58	37/50	0.042	0.432	0.191–0.979	0.044
16290	7/58	0/50	0.014	–	–	–
152	22/58	9/50	0.022	2.784	1.137–6.816	0.025
489	23/58	34/50	**0.003**	0.309	0.140–0.684	**0.004**
515	4/58	12/50	0.026	0.235	0.070–0.783	0.018

[a]Chi-square test was applicated.

[b]If the distribution frequencies of polymorphisms were significantly different between AgP group and control group, binary logistic regression models were further used to analyze the association between the polymorphisms and AgP.

The boldface values represent that the data cannot be analyzed by the binary logistic regression test.

''-12915T-301 G-546 C'' (formyl peptide receptor 1 gene) was significantly higher in AgP patients than in healthy control subjects (Gunji et al., 2007). However, the role of nuclear DNA mutations in diseases is highly complex; mtDNA may provide a better defined approach to the exploration of these effects. The human complete mitochondrial genome was first sequenced in 1981 (Anderson et al., 1981) and subsequently, details of numerous polymorphisms, mutations, haplogroups and haplotypes have been reported (Sun et al., 2012).

The mitochondrial D-Loop is a hot spot for mtDNA alterations and thus sequence alterations of the D-Loop may contribute to altered replication and/or transcription of mitochondrial genes (Clayton, 2000). This directed our attention to this mtDNA region. MtDNA haplogroups can be classified according to the D-Loop polymorphisms. Certain mtDNA haplogroups are known contribute to the genetic susceptibility to various disorders (Hofmann et al., 1997). For diabetes mellitus, haplogroup M is a protective factor due to the mitochondrial A10398G polymorphism (Liao et al., 2008), while haplogroup N is a risk factor for breast and esophageal cancer due to the mtDNA G10398A polymorphism (Darvishi et al., 2007). It has been suggested that haplogroups A and M7 are risk factors for chronic obstructive pulmonary disease (COPD), while haplogroups D, F, and M9 are related to individual COPD resistance in Han Chinese populations (Zheng et al., 2012a). Furthermore, haplogroups D and F have been implicated in decreased risk of lung cancer, whereas haplogroups G and M7 are risk factors (Zheng et al., 2012b). Haplogroups D, D4a, and D5 might increase the susceptibility for esophageal cancer in the two high-risk areas in China (Li et al., 2011). Some studies have reported the sequences of the HV1, HV2 and HV3 regions of mtDNA in recurrent oral ulceration (ROU) patients and indicated that haplogroups D5 and R function as protective factors for ROU (Sun et al., 2012). This phenomenon has not yet been described for AgP patients. Accumulation of D-Loop alterations has also been reported in several complex human diseases (Chen et al., 2012; Michikawa et al., 1999; Mueller et al., 2011; Wang et al., 2011), but they have not yet been systematically characterized in AgP. In this study, we report for the first time a comprehensive study of D-Loop alterations in AgP.

We identified haplogroups associated with AgP by sequencing the D-Loop region containing the HV1, HV2 and HV3 regions. In our study, the distribution frequencies of haplogroups A in AgP patients was significantly higher than that in the controls, thus implicating haplogroup A as a risk factor that increase the incidence of AgP ($p = 0.007$). In contrast, the frequency of haplogroup D in AgP patients was lower than that in the controls, indicating that haplogroup D is a protective factor for AgP

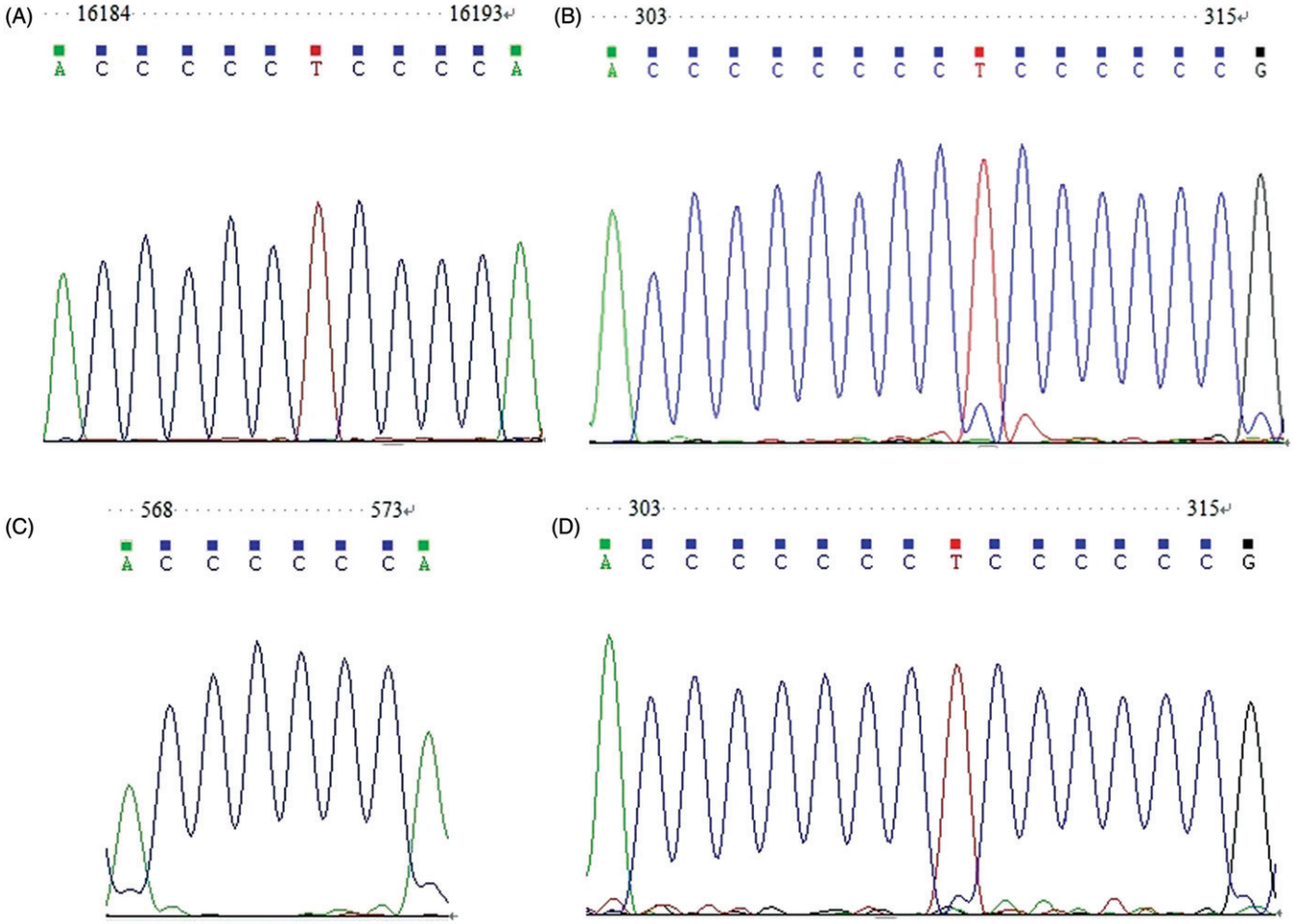

Figure 4. The sequences of mtDNA np 16,184–16,193, np 303–315 and np 568–573. (A) np 16,184–16,193, CCCCCTCCCC; (B) np 303–315, CCCCCCCCCTCCCCCC; (C) np 568–573, CCCCCC; (D) np 303–315, CCCCCCCCTCCCCCC.

Table 5. C-stretch sequences and frequencies of mtDNA HV1, HV2 and HV3 regions in the controls.

	C-Stretch sequences				
	np 16,184–16,193	np 305–315	np 568–573	*n*	%
1	CTCCCTCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/50
2	TCCCCTCCCC	CCCCCCCTCCCCCC	CCCCCC	3	3/50
3	CCCCCTCCCC	CCCCCCCCCTCCCCCC	CCCCCC	3	3/50
4	CCCCCTCCCC	CCCCCCCTCCCCCC	CCCCCC	8	8/50
5	CCCCCTCCCC	CCCCCCCCTCCCCCC	CCCCCC	14	14/50
6	CTCCCTCCCC	CCCCCCCCTCCCCCC	CCCCCC	2	2/50
7	CCCCCCCCCC	CCCCCCCCTCCCCCC	CCCCCCCCCCC	1	1/50
8	CCCCCCCCCCC	CCCCCCCCCTCCCCCC	CCCCCC	2	2/50
9	CCCCCCCCCCCC	CCCCCCCCCTCCCCCC	CCCCCC	3	3/50
10	CCCCCCCCCCC	CCCCCCCCTCCCCCC	CCCCCC	2	2/50
11	CCCCCCCCCC	CCCCCCCCCTCCCCCC	CCCCCC	1	1/50
12	CCCCCTCCTC	CCCCCCCCTCCCCCC	CCCCCC	2	2/50
13	CCCCTTCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/50
14	CCCCCCCCCdel	CCCCCCCCCTCCCCCC	CCCCCC	1	1/50
15	CCCCCTCCTC	CCCCCCCTCCCCCC	CCCCCC	1	1/50
16	CCCCCCCCCCC	CCCCCCCTCCCCCC	CCCCCC	2	2/50
17	CCCCCTCCCT	CCCCCCCCTCCCCCC	CCCCCCCCCCC	1	1/50
18	TCCCCCCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/50
19	CCCCCCCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/50

np, frequency of each C-stretch observed. del, nucleotide deletion, $n = 50$.

[$p = 0.009$, OR = 0.284(0.111–0.731)]. To our knowledge, it is the first study to trace mtDNA H variants in patients with AgP.

The haplogroup A is characterized by several D-Loop polymorphisms such as m.235 A > G, m.16290C > T, and m.16319G > A, which we used for genotyping. And the haplogroup D is characterized by D-Loop polymorphism m.16362T > C. We found that the frequency of D-Loop polymorphism m.16126T > C, m.16290C > T and m.152 T > C was higher in patients with AgP than in controls ($p = 0.029$, 0.014 and 0.022, respectively). Furthermore, m.16223C > T m.489 T > C

Table 6. C-stretch sequences and frequencies of mtDNA HV1, HV2 and HV3 regions in patients with AgP.

	C-Stretch sequences				
	np 16,184–16,193	np 305–315	np 568–573	*n*	%
1	CTCCCTCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/58
2	CCCCCTCCCC	CCCCCCCCCTCCCCCC	CCCCCC	5	5/58
3	CCCCCTCCCC	CCCCCCCTCCCCCC	CCCCCC	10	10/58
4	CCCCCTCCCC	CCCCCCCCCTCCCCCC	CCCCCC	17	17/58
5	CTCCCTCCCC	CCCCCCCCCTCCCCCC	CCCCCC	1	1/58
6	CCCCCTCCCC	CCCCCCCTCCCCCC	CCCCCCCCCCC	1	1/58
7	CCCCCCCCCCCC	CCCCCCCCTCCCCCC	CCCCCC	5	5/58
8	CCCCCCCCCC	CCCCCCCTCCCCCC	CCCCCC	1	1/58
9	CCCCCCCCCdel	CCCCCCCCTCCCCCC	CCCCCC	2	2/58
10	CCCCCTCCCC	CCCCCCCTCCCCCC	CCCCCCCCCCCCC	1	1/58
11	CCCCCCCCCCCCC	CCCCCCCCCTCCCCCC	CCCCCC	1	1/58
12	CCCCCCCCCCCCC	CCCCCCTCCCCCC	CCCCCC	2	2/58
13	CCCCCCCCCC	CCCCCCCCTCCCCCC	CCCCCC	1	1/58
14	CCCCCCCCCCC	CCCCCCCCTCCCCCC	CCCCCC	1	1/58
15	CCCCCCCCCCC	CCCCCCCTCCCCCC	CCCCCC	2	2/58
16	CCCCTCCCCC	CCCCCCCCCTCCCCCC	CCCCCC	2	2/58
17	CCCCGTCCCC	CCCCCCCCTCCCCCC	CCCCCC	2	2/58
18	TCCCCTCCCC	CCCCCCCCTCCCCCC	CCCCCC	3	3/58

np: frequency of each C-stretch observed. del, nucleotide deletion, $n = 58$.

and m.515CA del were lower in AgP patients than in controls ($p = 0.042$, 0.003 and 0.026, respectively). Interestingly, m.16290C > T is present only in the A haplogroup, but m.515CA deletion is not linked with haplogroups. Other four polymorphisms are present in several haplogroups among the global population. In detail, m.152 T > C is present in haplogroup A, etc; m.489 T > C in haplogroup D, etc; m.16223C > T in haplogroup A, D and others. However, m.16126T > C is not present in haplogroup A and D. (Coskun et al., 2003). Therefore, the results of the mitochondrial D-Loop polymorphism analysis are consistent with the results of mitochondrial haplogroup frequencies. Mitochondrial polymorphisms appear to be common in this Han Chinese population, with an average of 11–13 for each individual in reference to rCRS. The actual number of polymorphisms may be less if the reference sequence was of Han Chinese origin.

Substitutions in the D-Loop may constitute a haplotype with mutations in the coding region of mtDNA. Polymorphisms in the D-Loop may be selectively neutral; however, they are responsible for increased risk of disease or the production of a severe clinical outcome when combined specifically with established pathogenic mtDNA mutations. Compared with the relatively constant characteristics of the mtDNA coding region, the high degree of variability in HV1, HV2 and HV3 presents useful criteria for pathogenetic studies (Arnestad et al., 1999).

During this investigation of mtDNA haplogroups and alterations, we observed the interesting phenomenon of the C-Stretch generation. Polymorphism in these sequences result from infidelity in the polymerase-catalyzed mtDNA replication process and regulation of nuclear code factors (Tuo et al., 2007). It has been reported that the polymorphism of the C-stretch is maternally inherited based on the observation of a similar distribution along the maternal lineage (Malik et al., 2002a). Along with an increase in the number of cytosines, polymorphism of the C-Stretch becomes more unstable and the mtDNA replication fidelity declines significantly (Malik et al., 2002b). However, no significant differences in the polymorphisms of the C-Stretch in mtDNA were detected between the AgP and control group.

Conclusions

Pathogenesis of AgP is complicated and its heredity is not well characterized. For the first time, our results demonstrate an association between AgP and mtDNA haplogroups. Haplogroups A is implicated as risk factors for AgP, while haplogroups D appears to exert a protective effect against AgP. These observations may provide a new perspective for the investigation of the pathogenesis of periodontitis. A larger sample size is needed to further confirm the relation between AgP susceptibility and haplogroup A and D.

Acknowledgements

We are grateful to Central Laboratory, Peking University School and Hospital of Stomatology, for providing relative platform and instruments.

Declaration of interest

This study was funded by the National Nature Science Foundation of China (30872883; 81271148). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, et al. (1981). Sequence and organization of the human mitochondrial genome. Nature 290:457–65.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147.

Armitage GC. (1999). Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1–6.

Arnestad M, Opdal SH, Torgersen H, Vege A, Rognum TO. (1999). Substitutions in mitochondrial (MT) DNA D-Loop insides are due to inheritance – not to somatic mutations. Pediatric Res 45:29.

Chen JB, Yang YH, Lee WC, Liou CW, Lin TK, Chung YH, Chuang LY, et al. (2012). Sequence-based polymorphisms in the mitochondrial D-loop and potential SNP predictors for chronic dialysis. PLoS One 7: e41125.

Clayton DA. (2000). Transcription and replication of mitochondrial DNA. Hum Reprod 2:11–17.

Coskun PE, Ruiz-Pesini E, Wallace DC. (2003). Control region mtDNA variants: Longevity, climatic adaptation, and a forensic conundrum. Proc Natl Acad Sci USA 100:2174–6.

Darvishi K, Sharma S, Bhat AK, Rai E, Bamezai RN. (2007). Mitochondrial DNA G10398A polymorphism imparts maternal Haplogroup N a risk for breast and esophageal cancer. Cancer Lett 249:249–55.

Graven L, Passarino G, Semino O, Boursot P, Santachlara-Benerecetti AS, Langaney A, Excoffier L. (1995). Evolutionary correlation

between control region sequence and restriction polymorphisms in the mitochondrial genome of a large Senegalese Mandenka population. Mol Biol Evol 12:334–45.

Gunji T, Onouchi Y, Nagasawa T, Katagiri S, Watanabe H, Kobayashi H, Arakawa S, et al. (2007). Functional polymorphisms of the FPR1 gene and aggressive periodontitis in Japanese. Biochem Biophys Res Commun 364:7–13.

Hofmann S, Jaksch M, Bezold R, Mertens S, Aholt S, Paprotta A, Gerbitz KD. (1997). Population genetics and disease susceptibility: Characterization of central European haplogroups by mtDNA gene mutations, correlation with D loop variants and association with disease. Hum Mol Genet 6:1835–46.

Imaizumi K, Parsons TJ, Yoshino M, Holland MM. (2002). A new database of mitochondrial DNA hypervariable regions I and II sequences from 162 Japanese individuals. Int J Legal Med 116:68–73.

Kinane DF, Hart TC. (2003). Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med 14:430–49.

Lertrit P, Kapsa RM, Jean-Francois MJ, Thyagarajan D, Noer AS, Marzuki S, Byrne E. (1994). Mitochondrial DNA polymorphism in disease: A possible contributor to respiratory dysfunction. Hum Mol Genet 3:1973–81.

Li XY, Guo YB, Su M, Cheng L, Lu ZH, Tian DP. (2011). Association of mitochondrial haplogroup D and risk of esophageal cancer in Taihang Mountain and Chaoshan areas in China. Mitochondrion 11:27–32.

Liao WQ, Pang Y, Yu CA, Wen JY, Zhang YG, Li XH. (2008). Novel mutations of mitochondrial DNA associated with type 2 diabetes in Chinese Han population. Tohoku J Exp Med 215:377–84.

Loos BG, John RP, Laine ML. (2005). Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol 32:159–79.

Lutz S, Wittig H, Weisser HJ, Heizmann J, Junge A, Dimo-Simonin N, Parson W, et al. (2000). Is it possible to differentiate mtDNA by means of HVIII in samples that cannot be distinguished by sequencing the HVI and HVII regions? Forensic Sci Int 113:97–101.

Malik S, Sudoyo H, Pramoonjago P, Sukarna T, Darwis D, Marzuki S. (2002a). Evidence for the de novo regeneration of the pattern of the length heteroplasmy associated with the T16189C variant in the control (D-loop) region of mitochondrial DNA. J Hum Genet 47:122–30.

Malik S, Sudoyo H, Pramoonjago P, Suryadi H, Sukarna T, Njunting M, Sahiratmadja E, Marzuki S. (2002b). Nuclear mitochondrial interplay in the modulation of the homopolymeric tract length heteroplasmy in the control (D-loop) region of the mitochondrial DNA. Hum Genet 110:402–11.

Michikawa Y, Mazzucchelli F, Bresolin N, Scarlato G, Attardi G. (1999). Aging dependent large accumulation of point mutations in the human mtDNA control region for replication. Science 286:774–9.

Mueller EE, Eder W, Ebner S, Schwaiger E, Santic D, Kreindl T, Stanger O, et al. (2011). The mitochondrial T16189C polymorphism is associated with coronary artery disease in Middle European populations. PLoS One 6:e16455.

Obermaier-Kusser B, Lorenz B, Schubring S, Paprotta A, Zerres K, Meitinger T, Meire F, et al. (1994). Features of mtDNA mutation patterns in European pedigrees and sporadic cases with Leber hereditary optic neuropathy. Am J Hum Genet 55:1063–6.

Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. (1997). Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. Periodontol 2000;14:216–48.

Penta JS, Johnson FM, Wachsman JT, Copeland WC. (2001). Mitochondrial DNA in human malignancy. Mutat Res 488:119–33.

Sun M, Fu SM, Wang LF, Dong GY, Wu D, Wang GX, Wu Y. (2012). Hypervariable region polymorphism of mtDNA of recurrent oral ulceration in Chinese. PLoS One 7:e45359.

Torroni A, Petrozzi M, D'Urbano L, Sellitto D, Zeviani M, Carrara F, Carducci C, et al. (1997). Haplotype and phylogenetic analysis suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 60:1107–21.

Tuo Y, Liu QB, Li SB. (2007). Sequence-length polymorphism research of C-stretch in mitochondrial DNA hyper-variant Region. Fa Yi Xue Za Zhi 23:123–6.

van Oven M, Kayser M. (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30: 386–94.

Wang C, Zhang F, Fan H, Peng L, Zhang R, Liu S, Guo Z. (2011). Sequence polymorphisms of mitochondrial D-loop and hepatocellular carcinom a outcome. Biochem Biophys Res Commun 406: 493–6.

Yakes FM, van Houten B. (1997). Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA 94: 514–19.

Zheng S, Wang C, Qian G, Wu G, Guo R, Li Q, Chen Y, et al. (2012a). Role of mtDNA haplogroups in COPD susceptibility in a southwestern Han Chinese population. Free Radic Biol Med 53: 473–81.

Zheng S, Qian P, Li F, Qian G, Wang C, Wu G, Li Q, et al. (2012b). Association of mitochondrial DNA variations with lung cancer risk in a Han Chinese population from southwestern China. PLoS One 7: e31322.

Supplementary material available online

Supplementary Table