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Correlation study on mtDNA polymorphisms as potential risk factors in aggressive periodontitis by NGS

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Abstract

Objectives: Using next-generation sequencing (NGS) to determine whether aggressive periodontitis is associated with specific mitochondrial polymorphisms.

Materials and Methods: A total of 165 unrelated Han Chinese were enrolled in the study. We analyzed the mitochondrial DNA (mtDNA) in 97 patients with aggressive periodontitis and 68 healthy controls by NGS. The mitochondrial DNA was L-PCR-amplified and subsequently sequenced by an Illumina Genome Analyzer (NGS). Chi-square tests were used to assess the differences between the two groups. In cases of significant difference, multivariate logistic regression models were further used to analyze the association between mtDNA polymorphisms and aggressive periodontitis.

Results: Significant association was observed between aggressive periodontitis and eight mitochondrial polymorphisms: "8860G-10400C" (OR = 2.828, p = .002), "8701A" (OR = 2.308, p = .005), "12705C-10398A" (OR = 2.683, p = .002), "9540C" (OR = 3.838, p = .001) and "10873T-15043G" (OR = 4.375, p = .001).

Conclusions: The pathogenesis of aggressive periodontitis is complicated, and its heredity is not well characterized. Our study was the first to use next-generation sequencing and found that 8860G-10400C, 8701A, 12705C-10398A, 9540C, and 10873T-15043G are associated with aggressive periodontitis in the Han Chinese population.

KEYWORDS

aggressive periodontitis, mitochondrial DNA, mitochondrial polymorphisms, next-generation sequencing

1 | INTRODUCTION

Periodontitis is an inflammatory disease caused by microbial plaque. Inflammation can lead to the destruction of periodontal tissue, including loss of alveolar bone and supporting connective tissue. This disease is classified into chronic periodontitis and aggressive periodontitis. Aggressive periodontitis (AgP) is a relatively rare type of periodontitis, which has the characteristics of special reactions between plaque microorganisms and the host, rapid disease progression, and familial aggregation (Armitage, 1999). The imbalance of the host immune responses to microbial plaque is the main cause of periodontal tissue destruction in patients with AgP. Progression and severity of the disease depend on interacting risk factors such as genetic, microbiological, immunological, and environmental factors (Nunn, 2010). The degree of periodontal tissue destruction of the host varies in terms of the quality and quantity of local inflammation and the immune response caused by the host response to microbial plaque (Page, Offenbacher, Schroeder, Seymour, & Kornman, 2010). Genetic changes can influence the host

response to dental plaque. Many genes related to inflammation have been studied as possible susceptibility factors for AgP. For example, the role of the nuclear factor-kappa B (NF-κB) gene has been studied in AgP. Innate immune responses are induced by periodontopathogens, which can lead to activation of the nuclear transcription factor NF-κB through toll-like receptor 2 (TLR2). The NF-κB polymorphism of the del/del genotype is related to AgP, and del/del carriers have a higher risk of subgingival colonization with Aggregatibacter actinomycetemcomitans (Schulz et al., 2010). To date, research on genetic factors of AgP has mainly focused on nuclear DNA, but there is still no strong evidence to explain the influence of genetic factors on AgP.

Human mitochondrial DNA (mtDNA) is the only extranuclear genetic material, consisting of circular double-stranded molecules of 16.6 KB. mtDNA encodes 37 genes, including 13 oxidative phosphorylation system (OXPHOS) essential polypeptides (the respiratory chain complex I, III, IV, and V subunits), 22 tRNAs, and two ribosomal RNAs (12S and 16S). The D-loop region does not encode any product necessary for cellular function, containing a promoter region and a replication origin. It plays a regulatory role in DNA replication and transcription (Taanman, 1999). mtDNA is susceptible to environmental factors due to the lack of histone protection and a complete repair system and its variations at several times the rate of nuclear DNA, including missense mutations, insertions, gene deletions, and frameshift mutations. mtDNA is more susceptible to damage-induced variations in its structure and function. Multiple mtDNA polymorphisms, large fragment deletions, and pathogenic point mutations have been found to be associated with a variety of human diseases (Singh & Kulawiec, 2009), such as Alzheimer's disease, hereditary optic nerve lesions, Parkinson's disease, mitochondrial myopathy, and cardiomyopathy. mtDNA variations can affect the normal oxidative respiratory function and release a large amount of reactive oxygen species (ROS), increasing the risk of various inflammatory disorders, such as periodontitis (Canakci, Cicek, & Canakci, 2005). The negative effects of increased levels of oxidative stress are oxidative damage, usually caused by decreased exposure to relatively high concentrations of ROS and/or anti-oxidant defense systems. More and more evidence indicates that ROS participate in the pathological processes of many inflammatory diseases. Some studies have found that ROS are also involved in the pathological process of periodontitis. Furthermore, mitochondrial dysfunction has been found in gingival fibroblasts in patients with chronic periodontitis (Govindaraj et al., 2011). Dysfunction is manifested by a decrease in the mitochondrial membrane potential and increase in ROS, which may provide a basis for the association between mtDNA variations and diseases, but whether these mitochondrial dysfunctions are related to mitochondrial gene polymorphisms has not been reported. There are few studies on mtDNA, mainly focusing on the deletion of large fragments of mtDNA associated with chronic periodontitis. In 2011, Govindaraj first reported the relationship between chronic periodontitis and mtDNA; 14-point mutation sites were found in gingival tissues of chronic periodontitis patients (Govindaraj et al., 2011). Our group has used traditional Sanger sequencing to detect mtDNA coding regions in peripheral blood and found two polymorphic sites associated with AgP (Wang, Luan, Chen, Zhao, & Guo, 2014).

The aim of this study was to investigate the correlation between AgP and point mutations or mtDNA polymorphisms through next-generation sequencing (NGS). NGS can meet a large number of repeated sequencing standards, with high genome coverage, so the mitochondrial genome-wide sequence can be detected easily by NGS because of its short length. Therefore, we used NGS technology to investigate the whole mitochondrial genome in peripheral blood samples collected from both aggressive patients and normal age-matched controls in this study.

2 | MATERIALS AND METHODS

2.1 | Subject information

The Institutional Review Board and Ethics Committee of Peking University Health Science Center approved the study. Informed consent forms were described in detail for each subject. A total of 165 subjects were enrolled in this study. All participants of this study were patients visiting the Department of Periodontology, Peking University School and Hospital of Stomatology, China. Ninety-seven AgP patients (42 males and 55 females; mean age, 27.13 ± 4.52 years) were enrolled according to diagnostic criteria based on the 1999 International Classification Workshop (Armitage, 1999). Sixty-eight control subjects (31 males and 37 females; mean age, 26.73 ± 4.29 years) were periodontally healthy. Subject details were collected, including historical, clinical, radiographic, and laboratory characteristics.

2.2 | Preparation of mtDNA

Peripheral blood samples of each subject were collected rapidly by venipuncture. Total DNA was extracted from blood samples by using the Genomic DNA Isolation Kit (Bioteke) and stored until analysis was performed.

Table 1 contains the sequence details of two pairs of PCR primers that were designed for complete enrichment and amplification

TABLE 1 L-PCR primers

Primers	Sequence 5′–3′	3′ position	Length
F2817/R11570	GCGACCTCGGAGCAGAAC	2817	8754
	GTAGGCAGATGGAGCTTGTTAT	11570	
F10796/R3370	CCACTGACTATGACTTTCCAA	10796	7923
	AGAATTTTTCGTTCGGTAAG	3370	

of mtDNA from the extracted total genomic DNA. The reaction system and conditions were as follows: 50 μl reaction system with 2 μM 2 × Prime STAR Buffer (Mg2 + Plus), 50 ng of genomic DNA, 200 μM/each dNTP (Roche), 0.2 μM/each primer, 3 units of Roche Expanded Long Range DNA Polymerase (Roche), and 15.75 μl of distilled water. The reaction conditions were as follows: 2 min; 10 cycles of denaturation at 94°C for 15 s, annealing at 62.3°C for 30 s, extension at 68°C for 8 min; 72°C extension for 8 min; and 4°C ∞ (DNA Engine PTC-200). After amplification, the PCR products were detected by 1.5% agarose gel electrophoresis, and then color development was performed with Goldview, followed by purification of the fragments.

2.3 | Next-generation sequencing and sequence analysis

After purification and gel extraction of purified mtDNA, the sample concentrations were measured in line with library construction requirements with a volume and concentration of 50 μl and 50 ng/μl, respectively. The samples were used for NGS on a HiSeq2500 platform (Illumina) with a coverage of 35,000 reads per pair on average. An indexed paired-end DNA library was prepared using the TruSeq DNA HT Sample Preparation Kit (Illumina). The amplicon was fragmented to 300 bp and repaired with AMPure XP beads, 3'-adenylation and adaptor ligation with the TruSeq DNA HT sample preparation kit (Illumina). DNA samples were enriched by PCR following the protocol. Then, the library was quantified by using the KAPA Library Quantification Kit (Kapa Biosystems). All indexed DNA libraries were sequenced with 100-bp pairwise reading chemistry and pooled at the same molar ratio in a single pathway of flowing cells on a HiSeq 2,500 (Illumina). We used BLASTn in NCBI to compare these sequences with the revised Cambridge (NC_012920) sequence (Anderson et al., 1981; Andrews et al., 1999). In addition, we used mitomap (http://www.mitomap.org) and MitoTool (http://www.mitotool.org/) to find possible mutations and mtSNP sites. The selected variations were amplified again and reverse-sequenced to confirm their accuracy.

2.4 | Statistical analysis

The sex, age, and blood test results and smoking status between the AgP group and the control group were compared by using an unpaired *t* test. $p < .05$ was considered to indicate statistical significance. After comparing the sequencing results with the modified Cambridge sequence, a total of 773 variants were found, of which 14 polymorphic sites were selected for focused investigation. The chi-square test was used to evaluate the differences in allele frequencies of SNP, with the Holm method to correct multiple comparisons. Then, we found that the frequencies of 9 SNPs in the AgP group and the control group were different. After adjustment for age, sex, and smoking status, multivariate logistic regression (regression method) was used to analyze the correlation between mtDNA variants and aggressive periodontitis.

3 | RESULTS

A total of 165 subjects participated in the study. We compared the characteristics of the participants, including age, sex ratio, smoking status, and other blood test results. Quantitative data were expressed as the mean ± standard deviation (*SD*). There were no statistically significant differences in characteristics between the AgP groups and the control groups, except for platelet counts and mean platelet volume (Table 2).

3.1 | Association of variants with AgP

Among the 773 variants obtained by sequencing on the NGS platform (Table S1), we selected 14 SNPs (T6392C, T7501C, A8860G, A8701G, T9540C, G10310A, A10398G, C10400T, T10873C, C12705T, C13967T, T14783C, G15043A, and G15301A) for further study, as all other variations occurred at relatively low frequencies (<10%). These 14 SNPs were all located in the coding region. After chi-square test analysis, there were 9 positions that

TABLE 2 Characteristics of the study subjects

Characteristics	AgP group ($n = 97$)	Control group ($n = 68$)	*p* value
Age, mean (year)	27.13 ± 4.52	26.73 ± 4.39	.686
Sex ratio (female:male)	55:42	37:31	.253
Smoking status (non-smoking:smoking)	82:15	58:10	.418
WBC, mean	6.52 ± 1.66	6.11 ± 1.61	.062
Glucose, mean	4.92 ± 0.74	4.81 ± 0.68	.359
PLT, mean	232.42 ± 31.67	246.85 ± 33.52	.005*
MPV, mean	9.98 ± 0.69	10.32 ± 0.79	.004*

Abbreviations: Glucose, fasting blood glucose level; MPV, mean platelet volume; PLT, platelet count; WBC, white blood cell count.

p values observed after "*t* test" for age, sex ratio, smoking status, WBC, glucose, PLT, and MPV.

*$p < .05$.

had significantly different frequencies of allele distribution between the AgP and control groups: A8860G, G8701A, A10398G, T9540C, T10400C, C10873T, T12705C, C14783T, and A15043G were higher in the AgP group (p = .002, 0.003, 0.004, 0.001, 0.002, 0.002, 0.004, 0.041, and 0.002; Table 3). Independent logistic regression analysis was performed to evaluate the correlation between these 9 alleles and AgP. Since the distribution frequencies of "A8860G-T10400C, T12705C-G10398A, and C10873T-A15043G" were the same in the two separate groups, these six SNPs were enrolled as three single separate entities in the logistic regression analysis. Therefore, "A8860G-T10400C, G8701A, T9540C, T12705C-G10398A, C10873T-A15043G, and C14783T" were taken as six factors in the next logistic regression analysis. There was a significant correlation between AgP and five of these factors adjusted for confounding factors: "8860G-10400C" (OR = 2.828, p = .002), "8701A"(OR = 2.308, p = .005), "12705C-10398A" (OR = 2.683, p = .002), "9540C" (OR = 3.838, p = .001), and "10873T-15043G" (OR = 4.375, p = .001), as shown in Table 4. Among them, A8860G, G8701A. and G10398A are missense mutations that will cause changes in the encoded amino acids, resulting in 112 amino acids changing from alanine to threonine, and 59 and 114 amino acids from threonine to C. amino acid.

TABLE 3 Univariate data analysis of 14 SNPs

Gene	Site	AgP	Control	p value[a]
COX1	6392T	88	64	.425
	6392C	9	4	
ATP6	8860A	70	62	.002*
	8860G	27	6	
ATP6	8701G	63	55	.003*
	8701A	34	13	
COX3	9540C	54	55	.001*
	9540T	43	13	
ND3	10310G	85	64	.166
	10310A	12	4	
ND3	10398G	71	62	.004*
	10398A	26	6	
ND3	10400T	70	62	.002*
	10400C	27	6	
ND4	10873C	72	63	.002*
	10873T	25	5	
ND5	12705T	71	62	.004*
	12705C	26	6	
ND6	13967T	88	65	.331
	13967C	9	3	
Cytb	14783C	59	52	.041
	14783T	38	16	
Cytb	15043A	72	63	.002*
	15043G	25	5	
Cytb	15301A	88	65	.331
	15301G	9	3	
tRNA	7501T	77	57	.145
	7501C	20	10	

[a]p value—a chi-squared test was used.

*p < .05.

4 | DISCUSSION

Aggressive periodontitis is defined as a group of rare, severe, rapidly progressing kinds of periodontitis, which differ from chronic periodontitis in terms of clinical manifestations and laboratory examination. Aggressive periodontitis shows an imbalance between plaque and the host response, leading to the destruction of periodontal tissue in AgP patients, including alveolar bone resorption and attachment loss. Different degrees of host response caused by plaque result in varying degrees and extent of local inflammation and immune-inflammatory response, which in turn cause the destruction of periodontal tissue. In the new classification of periodontal and peri-implant diseases and conditions, the forms of the disease previously recognized as “chronic” or “aggressive” are now grouped under a single category, “periodontitis.” The new classification characterizes periodontitis based on a multidimensional staging and grading system (Caton et al., 2018). A workshop concluded that although aggressive periodontitis and chronic periodontitis differ in clinical phenotype, there is still little evidence in biological studies that they are separate entities, and it is more likely that they are variations in the same disease process. Workshop attendees expressed the idea that a diagnosis is made in order to support prognostication and treatment strategy, so it is not appropriate to include both of these factors as separate entities in the classification system. In our study, we screened patients before a new classification of periodontal disease was available, so the periodontal diagnosis of subjects with AgP was established based on the 1999 International Classification Workshop. Bone loss estimation was radiographically performed in each patient to assess the extent and severity of alveolar bone loss. Under the new classification, our AgP patients should be diagnosed with stage III or stage IV periodontitis based on clinical and radiographic evidence, with a grade C representing high-risk progression. Our control subjects had no radiographic evidence of bone loss or any sign of past and present periodontitis, and they should be diagnosed with clinical gingival health based on an intact periodontium or gingivitis associated with dental biofilm alone (Caton et al., 2018; Dietrich et al., 2019).

In our study, the mean platelet count and mean platelet volume in the AgP group were lower than those in the control group, and the platelet count and mean platelet volume were significantly different between the two groups. Wang found that the average platelet volume of patients with severe chronic periodontitis is lower than that of healthy controls. After periodontal treatment, the mean platelet volume increased again (Xian'E et al., 2015), which is similar to our findings. Aggressive periodontitis patients

TABLE 4 Multivariate logistic regression analysis

		AgP (*n* = 97)		Control (*n* = 68)			
SNP	**AA variant**	***N***	**%**	***N***	**%**	**OR (95% CI)**	***p* value**
A8860G	T112A	27	27.83	6	8.82	2.828 (1.417–6.064)	.002**
T10400C	Synonymous						
G8701A	A59T	34	35.05	13	19.12	2.308 (1.341–3.972)	.005*
T9540C	Synonymous	43	44.33	13	19.12	3.838 (1.932–7.176)	.001**
C10873T	Synonymous	25	25.77	5	7.35	4.375 (2.287–8.375)	.001**
A15043G	Synonymous						
G10398A	A114T	26	26.80	6	8.82	2.683 (1.421–6.078)	.002**
T12705C	Synonymous						
C14783T	Synonymous	38	39.18	16	23.53	2.193 (1.216–5.483)	.022

Note: Multivariate logistic regression analysis was adjusted for age, sex, and smoking status.
*$p < .01$.
**$p < .005$.

are highly susceptible to periodontal disease. Individual susceptibility to periodontal disease is affected by gene polymorphisms. Some nuclear genes have been found to be associated with aggressive periodontitis in certain populations. In recent years, some studies have found that a gene polymorphism of CD28 is associated with aggressive periodontitis (Cifcibasi et al., 2015; e Silva et al., 2013). In addition, the osteoclast inhibitor gene polymorphism G1181C, a lactoferrin gene polymorphism, a monocyte chemoattractant protein-1 gene polymorphism, and a vitamin D receptor fokI polymorphism also enhanced susceptibility to periodontitis (Zhu et al., 2012). Schaefer used genome-wide association studies to identify GLT6D1 as a risk gene factor for aggressive periodontitis (Schaefer et al., 2010). There have also been some other genome-wide association studies that have identified risk gene factors, such as DEFA1A3, SIGLEC5, and ANRIL (Masumoto et al., 2019; Vaithilingam et al., 2014). However, the genetic factors of aggressive periodontitis are not fully understood.

In fact, there is a genome outside the nucleus: the mitochondrial genome, which is the only genome outside the human nucleus. Mitochondrial genes are more susceptible to damage than nuclear genomes. In previous studies, mitochondrial DNA was shown to be associated with a variety of clinical syndromes (Duno et al., 2013; Emmanuele et al., 2013; Gupta et al., 2013; Mancuso et al., 2013; Martikainen, Kytovuori, & Majamaa, 2013), indicating the importance of mitochondrial DNA polymorphisms. In some studies, mitochondrial DNA polymorphisms have been shown to be associated with mitochondrial dysfunction in diabetic patients (Sun et al., 2017; Ye et al., 2013). The Govindaraj research group analyzed the mitochondrial DNA heterogeneity of blood cells and gingival tissues in 30 chronic periodontitis patients for the first time by traditional Sanger sequencing and showed that 14 point mutations were only found in the periodontal tissue, suggesting that oxidative stress had an effect on the periodontal tissue. This provides evidence for mitochondrial dysfunction in chronic periodontitis (Govindaraj et al., 2011). Our group also used traditional Sanger sequencing to detect mitochondrial DNA coding regions in 64 patients with aggressive periodontitis, and two mitochondrial polymorphisms were found to be significantly different between the AgP group and the control group: 8701A - 9540T- 10400C- 10873T- 14783T- 15043G and 10398A may be risk factors for aggressive periodontitis (Wang et al., 2014). Candidate gene association studies were widely performed in previous periodontal research, focusing mainly on candidate genes related to immunity, inflammation, or bone metabolism that were thought to be associated with aggressive periodontitis. In contrast, a genome-wide association methodology is capable of searching for candidate genetic risk factors on the genome-wide scale, allowing the identification of genetic variants that are independent of inflammatory and immune genes and could not be detected in early candidate-targeted studies (Masumoto et al., 2019; Munz et al., 2017; Vaithilingam et al., 2014). In the present study, we began to use NGS to detect the whole mitochondrial genome of patients with aggressive periodontitis on the basis of enlarged sample size. There was a significant correlation between AgP and five of these factors adjusted for confounding factors: "8860G-10400C" (OR = 2.828, $p = .002$), "8701A" (OR = 2.308, $p = .005$), "12705C-10398A" (OR = 2.683, $p = .002$), "9540C" (OR = 3.838, $p = .001$), and "10873T-15043G" (OR = 4.375, $p = .001$). Compared with our previous study, A8860G and T12705C are newly discovered polymorphisms, which may be related to the expansion of sample size and changes in sequencing methods. A8860G, G8701A, and G10398A are non-synonymous polymorphisms. A8860G (Thr112Ala) and G8701A (Ala59Thr), located in the ATP synthase 6 (ATP6) gene, belong to the oxidative phosphorylation complex V, and G10398A (Ala114Thr) is located in the nicotinamide adenine dinucleotide dehydrogenase subunit 3 (ND3) gene, belonging to the oxidative phosphorylation complex I. Base mutations at these three sites will cause changes in the corresponding amino acids.

Point mutations or polymorphisms in mitochondrial DNA cause dysfunction in mitochondria. Variations in the genes encoding certain complexes in mitochondria can cause amino acid changes in the

complex, affecting protein activity and leading to decreased mitochondrial ATP synthesis, resulting in insufficient cell function and a series of diseases. On the other hand, variations in a certain gene in mitochondria can also alter the function of mitochondria-encoded proteins, resulting in increased ROS production. One of the most widely studied mitochondrial SNPs is G10398A. Related studies reported that 10398A was associated with some systemic diseases, increasing the risk of Parkinson's disease, breast cancer, and other types (Chia-Wei et al., 2012; Gui et al., 2012; Juo et al., 2010; Li et al., 2016; Mims et al., 2006; van der Walt et al., 2003). A recent study showed that cognitive decline was not related to any usual risk factor but to the G allele of A10398G in mtDNA (Monte et al., 2017), and another study suggested that the mitochondrial DNA 10398 A/G polymorphism plays a possible role in the genetic etiology of attention deficit hyperactivity disorder (ADHD) in Korean children (Hwang et al., 2017). Studies have found that 10398A can cause functional changes in the oxidative phosphorylation of respiratory complex I, leading to increased ROS production (Mims et al., 2006; Ross et al., 2001; van der Walt et al., 2003). All conditions that change ROS production can affect oxidative stress directly and critically (Figueira et al., 2013; Kowaltowski, Souza-Pinto, Castilho, & Vercesi, 2009). Oxidative damage has been observed in patients with chronic periodontitis, but it is not associated with any mtSNP (Çanakçi et al., 2009; Govindaraj et al., 2011). A study found that a mtDNA complex group composed of specific combinations of 8701/10398 alleles may potentially lead to pathophysiological changes and disease generation by affecting the mitochondrial matrix pH and intracellular calcium dynamics (An-A et al., 2006). Some authors suggested that the G10398A polymorphism may affect various pathological processes by affecting mitochondrial matrix pH and intracellular calcium dynamics, and the results indicated that the ND3 gene has complex interactions with nuclear DNA or environmental factors (Chu, Luo, Zhan, Ren, & Pang, 2015). A Chinese pedigree study found that mitochondrial dysfunction caused by the A8701G polymorphism may promote the pathophysiology of cardiovascular disease alone or synergistically (Zhu, Gu, & Xu, 2016). These observations from cell models suggest that specific mtDNA variations credibly underlie the etiology of the pathogenesis of medical diseases. Combined with our study's results, this may explain the susceptibility of AgP patients with 8701G or 10398A. The clinical phenotypes associated with the G8701A polymorphism have been reported to include hereditary optic neuropathy, mitochondrial encephalomyopathy, Parkinson's syndrome, deafness, and metabolic syndrome; of these, deafness was identified in a pedigree study (Chalkia et al., 2018; Li, Zhang, Li, & Wang, 2014; Martikainen et al., 2013; Mkaouar-Rebai, Tlili, Masmoudi, Charfeddine, & Fakhfakh, 2008). The A8860G polymorphism has been detected in many diseases, but evidence for its association with these diseases is not strong (Bahreini, Houshmand, Modarressi, & Akrami, 2018; Chia-Wei et al., 2012; Li et al., 2016, 2014; Masserrat et al., 2018). Some authors found that A8860G may reduce the stability of ATPase6 (Houshmand et al., 2011), but another study suggested that it is a benign point mutation with no effect on protein function (Masserrat et al., 2018). This may be because A8860G is located in a less conserved region. Therefore, in some of the above studies, A8860G is found in a high proportion in both the control group and the disease group.

To correlate possible mtDNA polymorphisms with AgP, this study conducted mitochondrial genome-wide analysis on 68 control samples and 97 AgP samples. We first used the next-generation sequencing method to investigate the relationship between mitochondrial polymorphisms and aggressive periodontitis. The results showed that there were significant differences in the distribution of alleles at 9 loci between the AgP and the control groups. After relative risk analysis, 8 variations were found to have a negative correlation probability with aggressive periodontitis. Oxidative phosphorylation is a biochemical process in which the energy released into the body is supplied to ADP and inorganic phosphorus to synthesize ATP. This coupling reaction is mainly carried out in mitochondria. The eight mitochondrial polymorphisms associated with aggressive periodontitis in our study are important for the oxidative phosphorylation complex.

Our study is a preliminary exploratory study to investigate whether any mitochondrial gene polymorphism is associated with AgP in the Han Chinese population. It represents the first effort to reveal the genome-wide mtDNA in AgP, using the NGS method for detection. In conclusion, we found that "8860G-10400C," "8701A," "12705C-10398A," "9540C," and "10873T-15043G" are associated with AgP susceptibility in the Han Chinese population. We hypothesize that 8860G-10400C, 8701A, 12705C-10398A, 9540C, and 10873T-15043G might be candidate genes for the study of aggressive periodontitis in the Chinese population.

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AUTHOR CONTRIBUTIONS

Q. Shi and Q. Luan designed the research. Q. Shi and X. Wang participated in the acquisition of data. Q. Shi analyzed data and interpreted the data with Q. Luan, Y. Cai, and X. Wang. Q. Shi drafted the paper, and the other three authors revised the paper critically. All authors have approved the submitted version.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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