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# Spontaneous periodontitis is associated with metabolic syndrome in rhesus monkeys

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ABSTRACT

*Objective:* The present study was designed to investigate (1) whether the non-human primate would be an appropriate animal model for the study of spontaneous periodontitis and its association with metabolic syndrome (MetS), and (2) whether microRNAs (miRNAs) play roles in the co-development of metabolic disorders and periodontitis.
*Design:* Rhesus monkeys (aged 12–29 years) with or without MetS were analyzed for the prevalence of periodontitis. The potential mechanisms underlying the association between MetS and periodontitis were explored using miRNA profiling of the gingival tissues from the MetS monkey groups with or without periodontitis as well as the age-matched controls.
*Results:* Among the 57 rhesus monkeys examined, 18 were diagnosed with periodontitis according to the inclusion criteria, with an overall prevalence of 31.6%. Moreover, the prevalence of periodontitis was 8.3% in the control group, 18.2% in the at-risk group, and 44.1% in the MetS group. The C-reactive protein level was doubled in the MetS periodontitis group, compared to the non-periodontitis sub-groups. Most importantly, only 3 miRNAs were confirmed to be differentially expressed between the MetS periodontitis and non-periodontitis subgroups while other miRNAs showed similar expression profiles.
*Conclusions:* The results indicate that the monkey with MetS is an ideal model for studies of spontaneous periodontitis and its association with MetS. miRNA profiling using this unique model showed that miRNAs play roles in the co-development of MetS and periodontitis.


## 1. Introduction

Periodontitis is a set of inflammatory diseases of the periodontium, *i.e.*, the tissues that surround and support the teeth. It involves progressive loss of the alveolar bone around the teeth, and can lead to their loosening and subsequent loss.[1] However, the pathogenesis of periodontitis still remains elusive despite all the research efforts that have been made. Evidences from human studies have identified an association of periodontitis with metabolic syndrome (MetS)[2] and its complications, such as diabetes[3] and cardiovascular diseases.[4,5] MetS is estimated to affect a quarter of the adult population worldwide in terms of impaired glucose regulation, abdominal obesity, dyslipidemia, and high blood pressure. These disease phenotypes are generally believed to originate from an overnutrition-induced pro-inflammatory state.[6] This pro-inflammatory state may also set up a scenario for the development of periodontitis. According to the

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third National Health and Nutrition Examination Survey (NHANESIII) by the US Centers for Disease Control and Prevention, the incidence of MetS in populations with no-to-mild, moderate, or severe periodontitis is 18%, 34%, and 37%, respectively. In addition, in the population aged over 45 years, the chance of periodontitis patients having MetS is 2.31 times higher than that of healthy subjects.[7] Morita et al. also found in their four-year longitudinal study of 1023 Japanese subjects that prevention and treatment of periodontitis lowers the risk of developing MetS.[8]

However, the mechanisms underlying the co-development of periodontitis and MetS have not been revealed, largely due to the lack of appropriate animal models. A number of rodent models have been used for the study of MetS and its complications, for example high-fat diet induced obese mice and db/db mice. Unfortunately, rodents are naturally resistant to periodontitis. Therefore, the animal models currently used for the study of periodontitis are established by imposed measures, such as orthodontic elastic ligatures or inoculation with human pathogens.[9] The pathological characteristics of the periodontitis imposed on animal models do not closely resemble those of the disease in human beings; thus, the findings obtained using these models may not be able to be generalized to human beings. In this study, a spontaneous MetS monkey model established in our previous study[10] was used to investigate chronic periodontitis and its association with MetS. Non-human primates such as monkeys have unique advantages over rodents in that non-human primates and humans have similar oral structures and microbial pathogens. Moreover, non-human primates have naturally-occurring dental plaques, biofilms, calculus, and periodontitis.[11,12] Most importantly, periodontitis and MetS develop spontaneously without any intervention in our monkey model and therefore, closely mimicking these diseases in human beings.

MicroRNAs (miRNAs) are a class of short non-coding RNAs functioning primarily as regulators of gene expression. They have been shown to modulate lipid and carbohydrate metabolism as well as inflammatory pathways.[13,14] In light of these findings, studies on human subjects have been performed to investigate the regulatory roles of miRNAs in periodontitis and a number have been found to be associated with periodontitis.[15–17] In this study, miRNA profiling of gingival tissues from the spontaneous MetS monkey model was performed to explore the mechanisms underlying the co-development of MetS and periodontitis.

## 2. Materials and methods

### 2.1. Housing of monkeys and ethics statement

The monkeys were individually housed on a 12-h light–dark cycle. The environmental temperature was between 18 °C and 24 °C, and the humidity between 40% and 70%. The monkeys had free access to water, and were fed *ad libitum* with national standard pellet monkey chow. The monkeys received routine oral hygiene care every 6 months. The study was approved by the Ethics Committee of Peking University and carried out in the animal facility of Peking University accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

### 2.2. Spontaneous metabolic syndrome monkey model

To investigate the association between periodontitis and MetS in non-human primates, rhesus monkeys with spontaneous MetS[10] were studied. These monkeys were identified by applying the following screening criteria for MetS: (1) waist >40 cm and waist/hip ratio >1, (2) blood pressure (BP) ≥130/80 mmHg, (3) high-density lipoprotein-cholesterol (HDL-c) <1.5 mmol/L, (4) triglycerides (TG) >0.8 mmol/L, and (5) fasting plasma glucose (FPG) >4.4 mmol/L. Monkeys fulfilling all three criteria were classified as having MetS, those displaying none or one MetS feature were classified as controls, and those displaying two MetS features were classified as at-risk. In total, 34 MetS monkeys, 12 age-matched controls, and 11 at-risk monkeys were included in the study. All the monkeys were male and ranged in age from 12 to 29 years.

### 2.3. Periodontitis diagnosis

The periodontal examination was conducted by the same investigator (M.A.R.) using a William's probe on two proximal sites (mesio- and disto-buccal) on each tooth, excluding the canines and third molars, and 48 sites in each monkey. Periodontitis was diagnosed according to the following inclusion criteria: at least 5 sites with probing depth (PD) >4 mm, attachment loss (AL) >2 mm, and bleeding on probing (BOP) over 10% of the probing site.

### 2.4. Blood sampling and biochemical tests

Blood samples were taken from a vein after 14–16 h of fasting and anaesthesia with ketamine (10 mg/kg BW). All measurements of plasma lipids and glucose were performed at the Department of Clinical Biochemistry of the 301 Hospital, Beijing, China, using Roche Modular Analytics E170 Combinations (Cobas 12017547 122). C-reactive protein (CRP) was analyzed by Roche Modular PE (Roche 03002012 122).

### 2.5. Tissue sampling and examination

To explore the potential mechanisms of periodontitis, miRNA expression was examined in inflamed and in healthy gingival tissues using microarrays. Gingival tissues were collected for biopsy from 4 MetS monkeys with periodontitis (MetS periodontitis group), 3 MetS monkeys without periodontitis (MetS non-periodontitis group) and 3 control monkeys with healthy gums (control group).

Briefly, monkeys were sedated with ketamine (14 mg/kg, i.m.) and diazepam (10 mg/kg, i.m.). Gingival tissue was incised with a scalpel blade, immediately snap-frozen in liquid nitrogen, and stored at −80 °C until use. A hemostatic sponge was immediately applied to the wound and pressed for at least 5 min to stop bleeding. Haematoxylin–eosin sections were prepared from gingival samples before a histological diagnosis of periodontitis was made.

### 2.6. miRNA profiling

miRNA profiling was performed using the Paraflo™ microRNA microarray assay (Rhesus monkey miRNA Array, Version No.