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Interactions among moderate/severe periodontitis, ADIPOQ-rs1501299, and LEPR-rs1137100 polymorphisms on the risk of type 2 diabetes in a Chinese population

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is a complex disease influenced by genes and the environment. Periodontitis a demonstrated risk factor of T2DM. Previous studies related to gene-environment interactions on the risk of T2DM mainly focused on gene-obesity interactions. However, the impact of gene-periodontitis interaction on the risk of T2DM has not yet been investigated. This study aimed to investigate gene-environment interactions among moderate/severe periodontitis, polymorphisms of adiponectin (ADIPOQ)-rs1501299, and leptin receptor (LEPR)-rs1137100 on T2DM risk in Chinese subjects.

Design: A case-control study was conducted in 239 Chinese participants from Beijing Hypertension Association Institute (BHAL). After full-mouth periodontal examinations, the participants underwent bilateral buccal swabs for DNA testing. ADIPOQ-rs1501299 and LEPR-rs1137100 were used for genotyping. Generalised multifactor dimensionality reduction (GMDR) and logistic regression were used to examine the interactions among single nucleotide polymorphisms (SNPs) and moderate/severe periodontitis on the risk of T2DM.

Results: The risk of T2DM was higher in moderate/severe periodontitis [adjusted odds ratio (AOR) = 3.67, 95% confidence interval (95%CI): 1.26–10.71] in ADIPOQ-rs1501299 GG genotype (AOR = 3.42, 95%CI: 1.81–6.46) and LEPR-rs1137100 GG genotype (AOR = 3.16, 95%CI: 1.56–6.39). The GMDR model indicated that there was a significant three-factor model ($p = 0.001$) involving rs1501299, rs1137100, and moderate/severe periodontitis, demonstrating a potential gene-environment interaction among periodontitis, polymorphisms of rs1501299, and rs1137100 influencing the risk of T2DM. Moderate/severe periodontitis patients with rs1501299-GG and rs1137100-GG have the highest T2DM risk after adjusting for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status, and hypertension (AOR = 20.39, 95%CI: 2.64–157.26).

Conclusions: Interactions among moderate/severe periodontitis, rs1501299-GG, and rs1137100-GG were associated with an increased risk of T2DM. This study may provide a new insight into the effect of gene-environment interactions on T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM), characterised by disorders in insulin production and hyperglycaemia, is an increasing global health burden with a high prevalence of 8.3% among those ages 20˜70 (Guariguata et al., 2014). It is estimated that every year, over 3.8 million people die from T2DM and its complications (Yoon, Kwok, & Magkidis, 2013). The International Diabetes Federation (IDF) reported that the number of people aged 20˜79 years affected with diabetes is expected to reach 642 million by 2040 (Ogurtsova et al., 2017). Therefore, it is of great importance to explore the risk factors for T2DM.

T2DM is a multifactorial complex disease that is influenced by genotype, including gene-gene interactions and environmental factors, such as age, smoking, socio-economic status, and obesity, among other factors. T2DM is also influenced by the interactions between genotype and the environment (G × E) (Hu & Jia, 2018). In the last 10 years, numerous investigators used generalised multifactor dimensionality reduction (GMDR) (Lou et al., 2007) to explore the potential gene-gene or gene-environment interactions to discover the possible biological pathways of complex diseases (Liang et al., 2017; Lv et al., 2017; Qi

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et al., 2016). To date, obesity is considered an environmental risk factor to interact with susceptible genes on the risk factors for T2DM (Lv et al., 2017). However, whether periodontitis interacts with susceptible genes on T2DM has not yet been investigated.

Periodontitis is a complex chronic inflammatory disease of infectious origin affecting the tooth-supporting tissue. Previous studies suggested that periodontitis is a factor that increases the risk of diabetes (Winning, Patterson, Neville, Kee, & Linden, 2017). Outcomes from longitudinal studies showed that severe periodontitis was associated with poor glycaemic control and diabetes-related complications during follow-up periods (Seppala, Seppala, & Ainamo, 1993; Taylor et al., 1996a). Moreover, periodontal therapy could lead to a significant reduction in HbA1c levels (Darre, Vergnes, Gourdy, & Sixou, 2008) and insulin resistance (Sun et al., 2011).

ADIPOQ is located on chromosome 3q27.3. A prospective study reported that ADIPOQ polymorphism was associated with insulin resistance, type 2 diabetes, and adiponectin levels in plasma (Fumeron et al., 2004). ADIPOQ-rs1501299 is located in intron 2 (G vs T). It was reported as a susceptible gene of T2DM for Chinese population (Guariguata et al., 2014; Li, Zhang et al., 2015). Adiponectin is encoded by the ADIPOQ gene and can alter glucose metabolism and insulin sensitivity (Ziemke & Mantzoros, 2010). Low levels of adiponectin in serum predict decreased insulin sensitivity, increased fasting glucose, insulin resistance (IR), and the risk of T2DM (Ghoshal & Bhattacharyya, 2015; Tschritter et al., 2003).

Leptin is an important hormone protein in regulating food intake and energy expenditure for energy balance, fertility, and metabolism, and is mediated by the cell surface leptin receptor (Klok, Jakobsdottir, & Drent, 2007). The cell surface leptin receptor is encoded by the LEPR gene on chromosome 1p31.3. Several functional variants of the LEPR gene with possible biological effects on metabolic regulation have been extensively investigated for their genetic predispositions on diabetes and its complications (Liao et al., 2012; Mohammadzadeh et al., 2013). LEPR-rs1137100 is located in exon 4 and leads to an amino acid change in K109R, which was assessed for its role in T2DM susceptibility in 3 previous studies conducted in Chinese populations (Jiang et al., 2014; Liao et al., 2012; Qu et al., 2007). None of the studies demonstrated a significant association. A meta-analysis of the studies also did not show a statistically signification relationship between LEPR-rs1137100 SNP and T2DM risk in Chinese subjects (Yang & Niu, 2018).

Circulating levels of adiponectin are reduced in periodontitis (Furugen et al., 2008; Zimmermann, Bastos, Dias Goncalves, Chambrone, & Duarte, 2013), and periodontal therapeutic intervention significantly led to increased serum adiponectin levels via decreased inflammation (Sun et al., 2011). Upregulated leptin in periodontitis suppressed the secretion of insulin from islets and contributed to insulin resistance (Kamohara, Burcelin, Halaas, Friedman, & Charron, 1997; Kieffer, Heller, Leech, Holz, & Habener, 1997). Thus, our hypothesis is that there may be biological associations among adiponectin, leptin, and periodontitis related to the risk of T2DM.

Therefore, based on the GMDR model, the present study aimed to explore gene-environment interactions among periodontitis, polymorphisms of ADIPOQ-rs1501299, and LEPR-rs1137100 on the risk of T2DM in a Chinese population.

2. Materials and methods

2.1. Subjects

According to the inclusive and exclusive criteria, 239 subjects from a cohort from the Beijing Hypertension Association Institute (BHAL) were enrolled in this study. The selection of case and control groups are shown in Fig. 1. The participants were then divided into a T2DM group (individuals affected with T2DM, n = 129) and a non-diabetes mellitus (NDM) group (n = 110) according to the 1999 World Health Organisation (WHO) criteria for the diagnosis of T2DM (Alberti & Zimmet, 1998).

Inclusion criteria

- Age > 40 years
- No systemic infectious diseases except periodontitis

Exclusion criteria

- Systemic diseases except T2DM and hypertension
- Pregnant or lactating females
- Systemic use of antibiotics in the preceding 3 months
- Inability to cooperate with the examination
- Initial periodontal therapy in the preceding 6 months
- Metabolic syndrome (MS), the diagnosis of which was determined based on the IDF definition (Force, 2006)

The definition of chronic periodontitis (CP) was according to the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Organization, 1999). According to the criteria of the Centres for Disease Control and Prevention (CDC) and the American Academy of Periodontology (Page & Eke, 2007), the subjects were classified as no/mild periodontitis (N/M-CP) or moderate/severe periodontitis (M/S-CP).

All of the subjects provided written informed consent to participate in the study. The protocol was approved by the Ethics Committee (IRB00001052-05106), Medical Science Centre of Peking University.

2.2. Physical examination and periodontal examinations

Data regarding demographic information, such as age and gender, lifestyle risk factors, including smoking and alcohol consumption, the systemic health condition, and medication history were obtained using a questionnaire administered by trained staff. Body weight, height, waist circumference (WC), and hip circumference (HC) were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in metres, and the waist-to-hip ratio (WHR) was calculated as the WC divided by the HC. Periodontal examinations were conducted by a trained and experienced periodontist. The plaque index (PLI), probing depth (PD), bleeding index (BI), clinical attachment loss (CAL), and bleeding on probing (BOP) were recorded at the mesial-buccal and distal-lingual sites for each examined tooth using a Williams probe (apart from for the third molars). Blood samples were collected the morning after at least 8 h of fasting. The serum samples were used for biochemical detection in the same unit.

2.3. Sampling DNA extraction and genotyping

The collection methods using oral mucosa swab were as follows: sterilised cotton swabs were used to rotate and wipe the buccal mucosa bilaterally in the same direction for approximately 10–15 seconds. The swabs were dried naturally at room temperature in a 30-minute UV-disinfected Super-Clean Bench, and then stored at -20°C after sealing. A TIANamp Swab DNA Kit (Tiangen Biotech Co. Ltd, Beijing, China) was used to extract DNA from the swab samples. The DNA was then stored at 4°C for later analysis (Dashash, Drucker, Hutchinson, Bazrafshani, & Blinkhorn, 2007). The concentration and purity of the DNA was estimated at 260 nm using a spectrophotometer. Two selected single nucleotide polymorphisms (SNPs) were genotyped using the Sequenom MassARRAY system (Shanghai Benegene Biotechnology Co. Ltd) based on matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) technology. The sequences of the two SNPs are shown in Table 1. To confirm the genotyping results, the samples were genotyped twice. The consistency of the replicated measurements of ADIPOQ rs1501299 and LEPR rs1137100 were 99.7% and 99.4%, respectively.

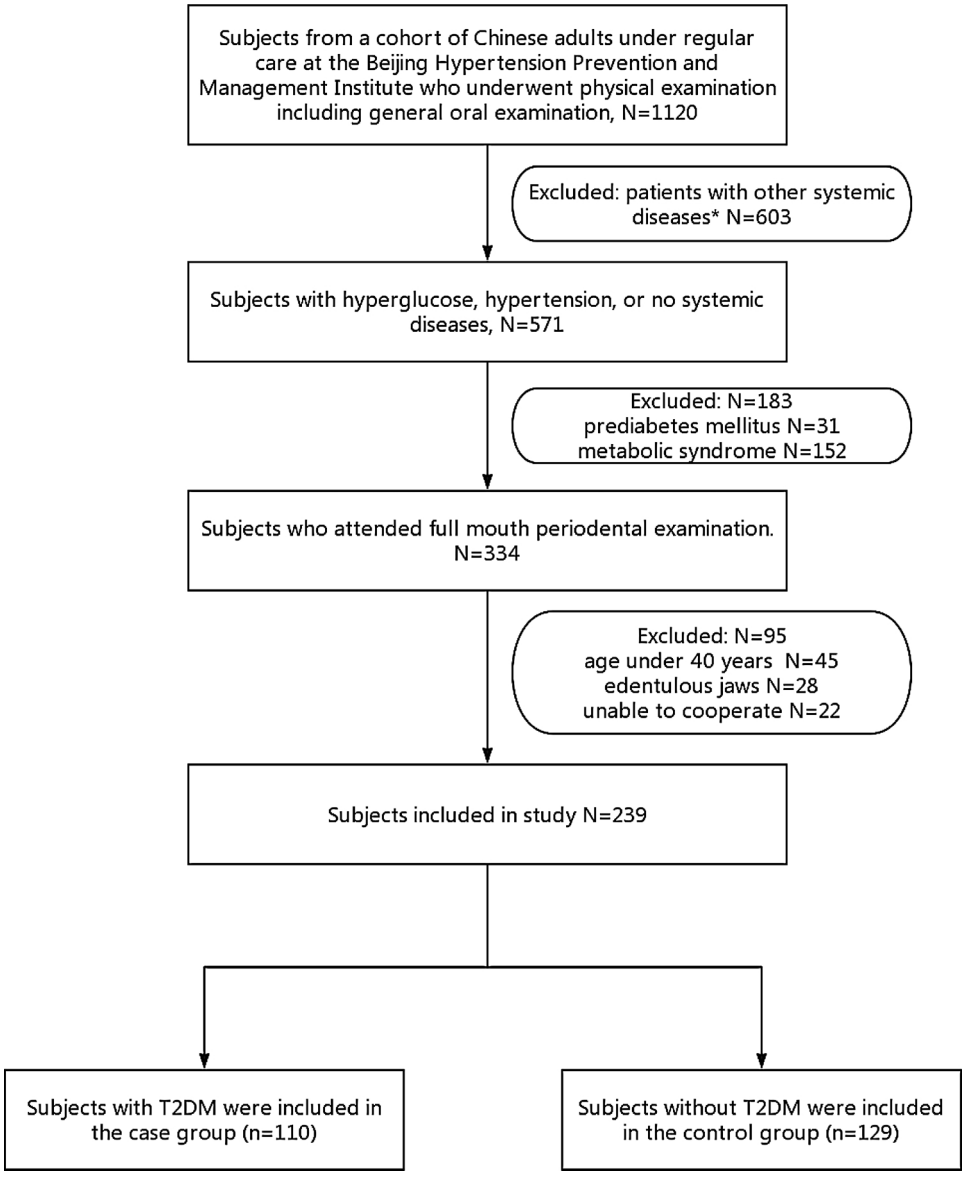

Fig. 1. Flow chart of study population enrolment.
* Other systemic diseases include: coronary heart disease, myocardial infarction, cerebral thrombosis, cerebral hemorrhage, nephropathy and peripheral vascular thrombosis

2.4. Statistical analysis

Continuous and categorical variables were presented as mean ± standard deviation (SD) and N (%), respectively. The characteristics of the subjects, fasting blood glucose (FBG), and clinical periodontal parameters were compared between the T2DM and NDM groups using Student's *t*-test (normal distribution) or the Mann-Whitney U test (non-normal distribution) for continuous variables and the Chi-squared (χ^2) analysis for categorical variables (Statistical Package for Social Sciences: SPSS, version 13.0). The Hardy-Weinberg equilibrium (HWE) was performed using SNPstats (available online at http://bioinfo.iconcologia.net/SNPstats). GMDR was used to analyse the gene-periodontitis interaction, cross-validation consistency, the testing balanced accuracy, and the sign test, in order to assess each selected interaction. The logistic regression model was used to examine the effect of the interactions among ADIPOQ (rs1501299) and LEPR (rs1137100) and

Table 1
Probe sequence for two SNPs used for Taqman fluorescence probe analysis.

ID	Gene	Chromosome position (GRCh38.p12)	Alternative Name	Functional Consequence	Allele (Major/ Minor)	Primer sequence
Rs1501299	ADIPOQ	3:186853334	ADIPOQ 276G > T	intron variant	G/T	Forward: 5'-GTCTCTCCATGGCTGACAGT-3' Reverse: 5'GGTGAAGATGGGAAAGGGGA-3' Reverse: 5'-TCTGTGATGAAAGAGGCCAG-3'
Rs1137100	LEPR	1:65570758	LEPR K109R	missense	G/A	Forward, -5′TTT CCA CTG TTG CTT TCG GA3′-; Reverse -5′AAA CTA AAG AAT TTA CTG TTG AAA CAA3′-;

Table 2
General characteristics, serological index and periodontal severity of T2DM patients and non-diabetes controls.

Variables	NDM (n = 129)	T2DM (n = 110)	*p*-value
Age	59.08 ± 10.90	61.85 ± 9.42	0.038*
Gender/Male	57 (44.19%)	51 (46.36%)	0.736
BMI	23.76 ± 3.17	25.48 ± 3.01	< 0.001*
WHR	0.87 ± 0.07	0.92 ± 0.06	< 0.001*
Smoking status			0.176
No	34 (26.36%)	26 (23.64%)	
Yes	95 (73.64%)	84 (76.36%)	
Alcohol consumption			0.097
No	105 (81.40%)	98 (89.09%)	
Yes	24 (18.60%)	12 (10.91%)	
Economy Status			0.012*
< 1000	35 (27.13%)	39 (35.45%)	
1000–2000	64 (49.61%)	61 (55.45%)	
> 2000	30 (23.26%)	10 (9.09%)	
Hypertension			< 0.001*
No	70 (54.26%)	33 (30.00%)	
Yes	59 (45.74%)	77 (70.00%)	
FBG (mmol/L)	5.12 ± 0.41	8.89 ± 3.38	< 0.001*
HDL (mmol/L)	1.581 ± 0.312	1.276 ± 0.281	< 0.001*
LDL (mmol/L)	2.857 ± 0.646	2.708 ± 1.302	0.284
Mean PD (mm)	2.65 ± 0.65	2.78 ± 0.65	0.131
Mean BI	1.97 ± 0.68	2.13 ± 0.58	0.063
Mean CAL (mm)	2.09 ± 1.71	2.83 ± 1.54	< **0.001**
BOP (+) sites	52.37 ± 24.93	55.81 ± 22.40	0.309
Moderate/severe periodontitis (%)	53.6	73	< 0.001*
Missing teeth	5.79 ± 7.61	7.21 ± 8.77	**0.182**

WHR: Waist-to-Hip Ratio; FBG: Fasting blood glucose; HDL: High-density lipoprotein; LDL: low density lipoprotein; BOP: bleeding on probing. PD: probing depth; BI: bleeding index; CAL: clinical attachment loss.
Data were represented as mean ± SD/N (%).
* $p < 0.05$.

moderate/severe periodontitis on the risk of T2DM, odds ratio (OR), and 95% confident interval (95%CI) were calculated with adjustments for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status, and hypertension. The statistical power was calculated using PASS version 11.0 (NCSS, LLC, Kaysville, UT, USA).

A two-tailed P value below 0.05 was considered statistically significant.

3. Results

A total of 239 participants consisting of 108 males and 121 females were enrolled, including 110 T2DM patients and 129 NDM controls. Table 2 depicts the general characteristics, serological indicators, and clinical periodontal parameters in the T2DM patients and NDM subjects, respectively. The mean ± SD of age, BMI, WHR, fasting blood glucose (FBG), high-density lipoprotein (HDL), mean CAL, and the distributions of economic status, hypertension, and moderate/severe periodontitis were significantly different between the T2DM and NDM groups. There was no significant differences for gender, smoking status, alcohol consumption, mean PD, mean BI, Bop (+) sites, and missing teeth.

Table 3 shows the associations of ADIPOQ-rs1501299 and LEPR-rs1137100 with the risk of T2DM. The frequencies of the GG genotype for ADIPOQ-rs1501299 and the frequencies of the GG genotype for LEPR-rs1137100 were significantly higher in the T2DM group than in the NDM group. The logistic regression analysis showed a significantly positive association of the rs1501299-GG genotype and the rs113710-GG genotype with the risk of T2DM after adjusting for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status, and hypertension. Individuals with the rs1501299-GG genotype have a higher risk of T2DM than those with the rs1501299-TT/GT genotype [Adjusted OR (AOR) = 3.42, 95%CI: 1.81–6.46], and those with the rs1137100-GG genotype have a higher risk of T2DM than those with the rs1137100-AA/AG genotype (AOR = 3.16, 95%CI: 1.56–6.39). The prevalence of moderate/severe periodontitis in the T2DM group was significantly higher than in the NDM group. Moderate/severe periodontitis was positively correlated with the risk of T2DM (AOR = 3.67, 95%CI: 1.26–10.71). The sample size provided the power of 0.997, 0.965, and 0.98 for rs1501299, rs1137100, and chronic periodontitis, respectively.

GMDR was employed to screen the best gene-gene and gene-environment interacted combinations after adjusting for covariates (Table 4). The GMDR analysis indicated the best model was a significant three-factor model (p = 0.001). This model involves rs1137100, rs1501299, and moderate/severe periodontitis. The results indicated a potential gene-environment interaction between rs1137100, rs1501299, and moderate/severe periodontitis. Overall, the cross-validation consistency of this model was 10/10, and the testing accuracy was 62.19% (p = 0.0010). A stratified analysis was conducted for rs1137100, rs1501299, and moderate/severe periodontitis using logistic regression. Moderate/severe periodontitis individuals with the rs1501299 GG genotype and the GG rs1137100 genotype had the highest risk of T2DM compared to none/mild periodontitis with rs1501299-TT/GT and rs1137100-AA/GA (AOR = 20.39, 95%CI: 2.64–157.26) after adjusting for covariates (Table 5).

4. Discussion

The present study confirmed that in moderate/severe periodontitis, the variants in ADIPOQ-rs1501299 and LEPR-rs1137100 were associated with the risk of T2DM after adjusting for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status, and hypertension.

Moderate/severe periodontitis is a confirmed risk factor for the occurrence and development of T2DM or its complications. Severe periodontitis at baseline was associated with an increased risk of poor glycaemic control (HbA1c > 9.0%) at follow-up (minimum 2 years), suggesting that severe periodontitis was a risk factor for compromised diabetes management (Taylor et al., 1996b). Diabetes patients had worse periodontal status than non-diabetics (Preshaw et al., 2012). In addition, various studies reported that the prevalence and severity of other diabetes-related organ complications correlated with the severity of periodontitis (Karjalainen, Knuuttila, & von Dickhoff, 1994; Thorstensson, Kuylenstierna, & Hugoson, 1996). The negative impact of periodontitis on changes in HbA1c was reported in a prospective 5-year study of 2973 non-diabetics (Demmer et al., 2010). Our findings showed that diabetes patients had more severe periodontal destruction with higher CAL than non-diabetics, and moderate/severe periodontitis was associated with T2DM risk, which was in line with previous studies. In this study, there was no significant difference in mean PD between the two groups. Considering that the average age of the subjects was approximately 60 in both groups, gingival remission was widespread, so the mean CAL could better reflect the real results.

Numerous studies demonstrated a significant association between ADIPOQ-rs1501299 and T2DM (Cen et al., 2017; Jing, Xueyao, & Linong, 2012; Tu et al., 2014; Wang, Zhu, Xie, & Li, 2015), consistent with what we found in the present study. However, some different results that no significant association occurred between ADIPOQ-rs1501299 and T2DM risk were reported (Goto et al., 2017; Pawlik et al., 2017). The correlation between LEPR-rs1137100 and risk of T2DM were also controversial. Studies reported a relationship between LEPR-rs1137100 and the risk of diabetes (Sun et al., 2010), consistent with our finding. But some research reported no significant correlation between LEPR-rs1137100 and the risk of diabetes (Anghebem-Oliveira et al., 2017; Yang & Niu, 2018). The inconsistent results for an association between ADIPOQ-rs1501299/LEPR-rs1137100 and T2DM risk

Table 3
Distributions of rs1501299, rs1137100 and periodontal condition in T2DM group and NDM group.

		NT2DM	T2DM	Crude OR (95%CI)	Adjusted[#] OR (95%CI)
ADIPOQ, rs1501299	TT + GT	77 (60.16%)	37 (33.64%)	Ref.	Ref.
	GG	51 (39.84%)	73 (66.36%)	2.98 (1.75, 5.06) *	3.42 (1.81, 6.46) *
LEPR rs1137100	AA + AG	46 (36.80%)	23 (21.70%)	Ref.	Ref.
	GG	79 (63.20%)	83 (78.30%)	2.10 (1.17, 3.78) *	3.16 (1.56, 6.39) *
CP	Healthy/mild	33 (33.33%)	8 (9.88%)	Ref	Ref
	Moderate/severe	66 (66.67%)	73 (90.12%)	4.56 (1.97, 10.58) *	3.67 (1.26, 10.71) *

CP: chronic periodontitis.
Data were presented as OR (95%CI).
[#] Adjusted for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status and hypertension.
* $p < 0.05$.

Table 4
Best gene-environment interaction models, as identified by GMDR.

No. of loci considered	Best model	Training Bal. Acc.	Testing Bal. Acc.	Sign test(p)	Cross–validation consistency
With adjustment[#]					
1	rs1501299	0.625	0.6391	8(0.0547)	10/10
2	rs1501299 + Periodontitis	0.6655	0.6456	10(0.0010*)	8/10
3	rs1137100 + rs1501299 + Periodontitis	0.679	0.6547	10(0.0010*)	10/10
Without adjustment					
1	rs1501299	0.6317	0.5918	9(0.0107*)	8/10
2	rs1501299 + Periodontitis	0.6644	0.638	10(0.0010*)	9/10
3	rs1137100 + rs1501299 + Periodontitis	0.6797	0.6219	10(0.0010*)	10/10

P-value based on 500 permutations.
[#] Adjusted for age, gender, BMI, WHR, smoking status, consumption, economic status and hypertension.
* $p < 0.05$.

were probably explained by racial heterogeneity (differences in the genetic mechanisms of different ethnic groups) and selective bias (selection of the controls cannot avoid selective bias due to differences in population genetic background factors). However, we confirmed that ADIPOQ-rs1501299, LEPR-rs1137100, and moderate/severe periodontitis were the risk factors for T2DM in a Chinese population.

T2DM is influenced by genetic and environmental factors, as well as gene-environment interactions. Obesity was reported to be the environmental factor that interacted with rs1805192 located on PPARG influencing the risk of T2DM (Lv et al., 2017). Also, rs662799 on the apolipoprotein A5 gene (APOA5) was suggested to collaborate with dietary fat in the risk of T2DM (Lai et al., 2006). In the present study, for the first time, periodontitis was regarded as an environmental factor that interacts with genes influencing the risk of T2DM, and the gene-periodontitis interaction on the risk of T2DM was detected. A three-factor interaction among ADIPOQ-rs1501299, LEPR-rs1137100, and moderate/severe periodontitis was strongly associated with the risk of T2DM (AOR = 20.39). Moderate/severe periodontitis with the rs1501299-GG and rs1137100-GG genotypes had the highest T2DM risk compared to non/mild periodontitis individuals with the rs1501299-TT/GT and rs1137100-AA/AG genotypes. This observed interaction provides a new idea for the pathogenesis of the association between periodontitis and T2DM. Although the result is first reported, the finding is biologically plausible. Previous studies showed that plasma adiponectin levels were influenced by the polymorphisms of ADIPOQ (Fumeron et al., 2004). Also, the circulating levels of adiponectin are decreased in periodontitis (Zimmermann et al., 2013). Leptin is one of the pro-inflammatory factors of periodontitis (Li, Huang, Liu, Hou, & Meng, 2015; Sun et al., 2011). Furthermore, the serum leptin levels were supposed to mediate the expression of the cell surface leptin receptor (Chan et al., 2002) that is encoded by the LEPR gene. In addition, both the adiponectin and leptin levels were associated with T2DM (Kadowaki et al., 2006; Spranger et al., 2003; Wauters et al., 2003; Welsh et al., 2009). Thus, it is reasonable to believe that adiponectin, leptin, and periodontitis are related to the risk of T2DM. Therefore, the present study suggested that periodontitis could affect the risk of T2DM through the lipid metabolism pathways, possibly with synergistic effects.

Table 5
Effect of the degree of periodontitis combined with different genotypes of rs1501299, rs1137100 on T2DM.

Moderate/severe periodontitis	rs1501299	rs1137100	Crude OR (95% CI)	Adjusted[#] OR (95% CI)
No	TT + GT	AA + AG	Ref.	Ref.
No	TT + GT	GG	0.64 (0.05, 8.62)	0.14 (0.01, 2.57)
No	GG	AA + AG	1.50 (0.22, 10.08)	0.96 (0.11, 8.84)
No	GG	GG	0.90 (0.06, 12.58)	0.46 (0.02, 8.81)
Yes	TT + GT	AA + AG	1.80 (0.26, 12.3)	0.60 (0.06, 6.03)
Yes	TT + GT	GG	3.12 (0.54, 17.97)	1.82 (0.21, 15.76)
Yes	GG	AA + AG	2.90 (0.57, 14.85)	1.50 (0.23, 9.89)
Yes	GG	GG	16.65 (3.09, 89.69)*	20.39 (2.64, 157.26) *

Data were presented as OR (95%CI).
[#] Adjusted for age, gender, BMI, WHR, smoking status, alcohol consumption, economic status and hypertension.
* $p < 0.05$.

The strength of this study was that it was the first to explore the gene-periodontitis interaction on T2DM using the GMDR model. The GMDR model is recommended to be applied to the related study, that is, the effect of periodontitis on T2DM, to find the possible gene-periodontitis interaction. Several limitations of this study should be considered. First, it had a relatively small sample size, although the number of study participants met the requirement for analysis (power value > 0.9). Other larger sample studies in Chinese and other races should be conducted to confirm the present result. In addition, the ADIPOQ gene spans ˜16 kb and consists of three exons (Hivert et al., 2008). In addition to the intron 2 rs1501299, there are other SNPs that could be assessed, which include rs17300539 (−11391 G > A) and rs266729 (-11377 C > G), that could affect ADIPOQ promoter activity (Kyriakou et al., 2008), the exon 2 SNP +45T > G rs2241766 (Melistas et al., 2009), a synonymous polymorphism that has been previously associated with T2DM risk in a Japanese population (Hara et al., 2002). The LEPR gene spans ˜168 kb and consists of 20 exons (Yang & Niu, 2018). In addition to the exon 4 missense SNP rs1137100, there are other missense SNPs that could be assessed, including the exon 6 missense SNP rs1137101 and the exon 14 missense SNP rs8179183 (Yang & Niu, 2018). If multiple SNPs were genotyped for each of the ADIPOQ and LEPR genes, haplotype-based association analyses could be performed for each gene, which could provide greater statistical power than single-SNP analysis to detect the combined effect of multiple variants on disease risk. Furthermore, although the three-factor interaction was statistically significant in the present study, more experimental evidence is needed to verify the possible significant biological pathways among periodontitis and variants in rs1501299 and rs1137100.

5. Conclusion

In conclusion, a significant association between moderate/severe periodontitis and variants in rs1501299 and rs1137100 with increased T2DM risks was found after adjustment of the covariates. In addition, a potential gene-environment interaction between rs1501299, rs1137100, and periodontitis has, for the first time, been demonstrated: that is, moderate/severe periodontitis patients with the rs1501299-GG and rs1137100-GG genotypes have the highest T2DM risk compared to none/mild periodontitis individuals with rs1501299-GT/TT and rs1137100-AA/AG genotypes. This study provided new insights into gene-environment interaction on T2DM.

Conflicts of interest

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