Received: 3 March 2019 | Revised: 15 November 2019 | Accepted: 15 November 2019

DOI: 10.1002/JPER.19-0135

JOURNAL OF Periodontology

ORIGINAL ARTICLE

A novel multi-locus genetic risk score identifies patients with higher risk of generalized aggressive periodontitis

Wenjing Li | **Xian'e Wang** | **Yu Tian** | **Li Xu** | **Li Zhang** | **Dong Shi** | **Xianghui Feng** | **Ruifang Lu** | **Huanxin Meng**

Department of Periodontology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing, China

Correspondence
Prof. Huanxin Meng, Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, 100081, Beijing, China.
Email: kqhxmeng@bjmu.edu.cn

Abstract

Background: Each genetic variant individually explains only a tiny proportion of the genetic variation with insignificant predictive power. The tool of multi-locus genetic risk score (GRS), which aggregates information from multiple genetic variants, has been widely used in many complex diseases but not yet applied to generalized aggressive periodontitis (GAgP).

Methods: A total of 335 GAgP patients and 114 healthy controls were enrolled in the case-control study. The unweighted GRS (uGRS) and weighted GRS (wGRS) were calculated based on significant variants. Logistic regression models were conducted for the GRS-based association analyses on the risk of GAgP. Receiver operating characteristic analysis was performed to compare the discriminatory ability of predictors of GAgP risk.

Results: Four loci were found to be significantly associated with GAgP. They were matrix metalloproteinase 8 rs11225395 (odds ratio [OR] = 1.40, 95% CI: 1.03 to 1.91), epidermal growth factor rs2237051 (OR = 1.41, 95% CI: 1.03 to 1.93), PPAR-a rs4253623 (OR = 1.53, 95% CI: 1.03 to 2.26), and apolipoprotein E rs429358 (OR = 1.79, 95% CI: 1.08 to 2.97). Each additional point of the uGRS/wGRS was associated with a 50%/31% increased risk of developing GAgP (OR = 1.50, 95% CI: 1.21 to 1.85 or OR = 1.31, 95% CI: 1.14 to 1.51, respectively) after adjusting for age, sex, and body mass index (BMI). Participants in the high group of uGRS/wGRS (OR = 2.87, 95% CI: 1.59 to 5.17 or OR = 2.67, 95% CI: 1.46 to 4.88, respectively) and the middle group of uGRS/wGRS (OR = 2.21, 95% CI: 1.29 to 3.78 or OR = 1.88, 95% CI: 1.09 to 3.08, respectively) had an increased risk of GAgP compared with those in the low group of score after adjustment for age, sex, and BMI. The addition of GRS to a model of conventional risk factors improved discrimination by 4.5% (from 0.695 to 0.740, $P = 0.048$).

Conclusions: We demonstrated that the multi-locus GRS based on four significant single nucleotide polymorphisms might be useful to assess genetic predisposition to GAgP. The GRS in combination with conventional risk factors significantly improved

the power of identifying subgroups of Chinese population with a particularly high risk for GAgP.

KEYWORDS
aggressive periodontitis, genetic risk score, SNP

1 | INTRODUCTION

Aggressive periodontitis (AgP) is a rapid and severe progressive form of periodontitis. It occurs in the absence of systemic diseases and is characterized by familial aggregation. AgP is classified into a localized or generalized form of periodontitis. The defining characteristic of generalized aggressive periodontitis (GAgP) is clinical attachment loss (AL) of at least three teeth except for the incisors and first molars according to the 1999 International World Workshop for a Classification of Periodontal Diseases and Condition.[1] Although "aggressive periodontitis" and "chronic periodontitis" are now grouped under a single category "periodontitis" according to the new classification of periodontitis.[2] The latest review of epidemiology of AgP reported that AgP has a low prevalence varying between 0.1% and 0.13% in Europe, 0.13% and 0.8% in North America, 0.32% and 5.5% in South America, 0.13% and 0.86% in Asia, and 3.4% in Africa according to the studies using representative samples.[3] In spite of affecting only a minority of all periodontal patients, GAgP is still perceived a considerable disease because of its severe destruction, which may lead to edentulism early in life.[4] Therefore, it emphasizes the significance of for investigating the etiology of GAgP.

As we all know, GAgP is a multifactorial complex disease,[5] arising from the influences of multiple loci with small individual effects. Although rapidly expanding repository of germline genetic information has been instrumental in discovering disease-associated loci, it is remarkable that, no matter in candidate gene study or Genome-wide association study, nearly all variants discovered so far confer relatively low risk. Each genetic marker individually explains only a tiny proportion of the genetic variation with insignificant predictive power.[6] A genetic risk score (GRS) is an estimate of the cumulative contribution of genetic factors to a specific outcome of interest in an individual that takes into account the risk alleles. This method has been widely used to investigate many diseases, including breast cancer,[7] type 2 diabetes,[8] schizophrenia, other psychiatric disorders,[9,10] and Alzheimer disease.[11,12] The utility of GRS could be categorized into three classes based on the major classes of intervention: GRS-informed therapeutic intervention, GRS-informed disease screening, and GRS-informed life planning. For example, numerous studies have shown that coronary artery disease GRSs are useful, independent of family history, for the identification of some high-risk individuals who receive greater benefit from the initiation of statin therapy.[13–16] Individuals in the top quintile of genetic risk have the ability to offset much of this risk by maintaining optimal lifestyle habits, which reduces their overall risk of disease by nearly half.[14] For breast cancer, if healthy lifestyle choices were preferentially targeted to and used by women in the top decile of genetic risk, an estimated about 20% of all preventable breast cancer cases would be avoided.[11] Therefore, GRS is of great significance to be applied to clinical practice. However, whether GRS could be used to identify high risk of GAgP patients remains unknown. In addition, few studies have investigated the impact of conventional risk factors combined with joint effects of susceptible loci on the risk of GAgP.

Therefore, based on case-control studies and candidate gene studies, we selected some genes and loci which might be associated with GAgP, to identify the susceptibility genes of GAgP in the Chinese population. Then, we used these susceptibility genes to calculate the GRS and analyzed the relationship between GRS and the risk of GAgP. Finally, GRS was added into traditional risk factor models to verify whether it could significantly increase the prediction of identifying high risk of GAgP patients.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The present study is based on a case-control design. A total of 449 unrelated Chinese individuals were enrolled in the study. A total of 335 of them recruited from the Department of Periodontology at the Peking University School and Hospital of Stomatology were affected with GAgP, and 114 of them enrolled from the staffs or students of Peking University School of Stomatology were healthy controls. The flowchart of study population inclusion is shown in Figure 1. The diagnosis of GAgP was based on the clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions.[1]

2.2 | Clinical examination

All participants were evaluated clinically at the first visit. The following clinical parameters were assessed. Probing depth (PD) and AL were measured throughout the entire

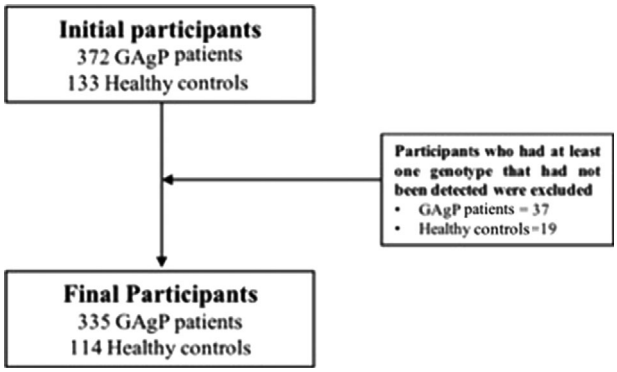

FIGURE 1 The flowchart of study population. GAgP, generalized aggressive periodontitis

mouth apart from for the third molar using a Williams periodontal probe at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal, mid-palatal, and disto-palatal) per tooth. Bleeding index (BI)[17] was recorded in 30 seconds after probing and the most severe sites were recorded in the buccal (labial) side and lingual (palatal) side. Additionally, full-mouth periapical radiographs were taken to determine the diagnosis of GAgP. All the clinical periodontal parameters were recorded by two skilled periodontal specialists (Dong Shi and Li Xu). The calibration was performed on 10 patients with GAgP. The consistency of the replicated measurements of PD and AL for each examiner (intra-calibration) and paired measurements between the pair of two periodontal specialists (inter-examiner calibration) were recorded. Of the replicated measurements for each examiner, 97.0% (Dong Shi) and 95.8% (Li Xu) were within 1 mm for PD; and 91.5% (Dong Shi) and 93.2% (Li Xu) were within 1 mm for AL. Of the paired measurements between the two examiners (Dong Shi versus Li Xu), 93.5% were within 1 mm for PD and 89.8% were within 1 mm for AL.

This study was approved by the human subjects ethics board of Peking University Health Science Center (NO.0313) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Each participant signed the informed consent, completed the questionnaire, received periodontal examination and provided a blood sample when enrolled into the present study.

2.2.1 | Inclusion criteria of GAgP patients

Inclusion criteria for GAgP patients included the following items: aged <36 years; at least six teeth with PD ≥ 5 mm, AL ≥ 3 mm, and radiographic evidence of interproximal bone loss; affected at least three permanent teeth other than first molar and incisors; and familial aggregation which was verified by self-reported family history and periodontal examination from other members of this family.

2.2.2 | Inclusion criteria of healthy controls

Inclusion criteria for healthy controls were: aged <36 years; individuals with PD ≤3 mm; no obvious AL; and the percentage of sites with BI ≥2 below 10%, and with no sites with BI >4.

2.2.3 | Exclusion criteria of participants

Participants were excluded for the following reasons: with conditions of pregnancy or smoking; chronic use of nonsteroidal anti-inflammatory drugs or antibiotics use within 3 months of study visit; periodontal treatment within the previous 6 months; with systemic diseases such as diabetes mellitus, hypertension, and cardiovascular disease, etc.; and with at least one genotype not being detected (if one of the individual genotypes is missing, it is impossible to calculate the GRS).

2.3 | Single nucleotide polymorphism selection and genotyping

There were 54 candidate single nucleotide polymorphisms (SNPs) of 35 genes, which were selected based on the pathogenesis of periodontitis, such as immune inflammation, glycolipid metabolism, cardiovascular disease related genes, bone metabolism, and the growth and development of periodontal tissue (see Table S1 in online *Journal of Periodontology*). Five of these SNPs did not satisfy the Hardy-Weinberg equilibrium (P <0.001), 45 of them were not significantly associated with the risk of GAgP, and finally, four significant SNPs were selected in a GRS calculation.

The whole blood samples were obtained from each fasting examinee by standard venipuncture using EDTA-containing tubes. Genomic DNA was extracted from each sample using a blood DNA mini kit* according to the manufacturer's protocol. SEQUENOM MassARRAY matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry platform was used to genotype the SNPs.† Protocol for genotyping was described in the previous study.[18] All genotyping was performed masked with respect to clinical diagnosis by a single investigator.

2.4 | Genetic risk score computation

The GRS was calculated on the basis of SNPs reaching candidate-gene levels of significance (P <0.05) at univariate SNP association analysis. Four SNPs were detected to be the susceptibility variants of GAgP and then included into the GRS calculation. Two approaches were used to calculate the GRS: a simple risk allele count method (unweighted GRS

* Watson Biotechnologies, Shanghai, China.

† Sequenom; San Diego, CA.

[uGRS]) and a weighted method (weighted GRS [wGRS]). Both methods assumed that each SNP were independently associated with GAgP risk. The uGRS method assumes that each SNP in the panel contributes equally to the risk for GAgP and was calculated by summing the values for each of the SNP. The wGRS was calculated by multiplying each β-coefficient by the number of corresponding risk alleles (zero, one, or two) and then summing the values.[19] For example, two matrix metalloproteinase 8 (MMP8) risk alleles contribute $2 \times 1.40 = 2.80$ to the wGRS.

2.5 | Statistical analysis

Continuous variables were presented as mean ± SD/median (min, max), categorical variables were presented as n (%). Chi-square tests and *t* tests were used for comparisons of means and proportions between GAgP group and healthy controls. Agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested by using a $\chi 2$ goodness-of-fit test for the controls. Pairwise linkage disequilibrium was assessed using Lewontin's D' and r^2 as implemented in *Haploview*.[20] Univariate SNP analysis is performed to detect the associations of the four SNPs between GAgP patients and healthy controls. Generalized multifactor dimensionality reduction[21] was used to analysis the gene-periodontitis interaction. Multivariate logistic regression was performed to estimate the multi-locus GRS on association of GAgP risk. In addition, we tested whether the association between the uGRS/wGRS and risk of GAgP was modified by sex and body mass index (BMI). To measure the discriminative improvement attributable to the GRS, we plotted receiver operating characteristic (ROC) curves and calculated corresponding areas under the curve (AUCs) for a logistic regression model including age, sex, and other factors, with or without wGRS. The power calculation was based on the means and standard deviation of GRS, the end sample size, and odds ratio.

A two-tailed P <0.05 was considered to be statistically significant in all analyses. The statistical analyses were performed with R and EmpowerStats software, X&Y solutions, Boston MA).

3 | RESULTS

3.1 | Characteristics of study participants

Table 1 shows the characteristics and periodontal parameters of study participants. A total of 449 individuals (case: control = 335: 114) were enrolled in the study. Among all the participants, no significant difference was found for age, sex, and BMI between GAgP patients and healthy controls ($P > 0.05$). All clinical periodontal variables (mean values of PD, BI, and AL) were significant higher in GAgP group than that in control group ($P < 0.001$).

TABLE 1 Demographic characteristics and periodontal parameters of GAgP patients and healthy controls

Variables	Healthy control	GAgP group	*P* value
n	114	335	
Age, y	28.89 ± 7.11	27.37 ± 5.23	0.745
Sex/male	46 (40.35%)	137 (40.90%)	0.919
BMI, Kg/m^2	21.17 ± 2.33	22.24 ± 5.55	0.201
Mean PD, mm	1.77 ± 0.47	4.81 ± 1.06	<0.001*
Mean BI	1.08 ± 0.33	3.49 ± 0.53	<0.001*
Mean AL, mm	0.00 ± 0.01	4.39 ± 1.51	<0.001*

Data were presented as mean + SD/n (%).
AL, attachment loss; BI, bleeding index; BMI, body mass index; GAgP, generalized aggressive periodontitis; PD, probing depth.
*P value <0.05.

3.2 | Univariate SNP association analysis

Table 2 shows allele frequencies and odds ratios (ORs) (95% CI) of four significant SNPs on GAgP risk using univariate logistic regression models. The significant loci were MMP8 rs11225395 (OR = 1.40, 95% CI: 1.03 to 1.91), epidermal growth factor (EGF) rs2237051 (OR = 1.41, 95% CI: 1.03 to 1.93), peroxisome proliferator-activated receptor alpha (PPARa) rs4253623 (OR = 1.53, 95% CI: 1.03 to 2.26), and apolipoprotein E (APOE) rs429358 (OR = 1.79, 95% CI: 1.08 to 2.97). These significant loci were selected to the GRS calculation.

3.3 | A multi-locus GRS-based association analysis

As shown in Table 3, the uGRS ranged from 2 to 8. The crude ORs for GAgP were 1.47 (95% CI 1.22 to 1.78) with each additional point of uGRS. The adjusted ORs for GAgP were 1.50 (95% CI 1.21 to 1.85) with each additional point of uGRS, adjusted for age, sex, and BMI. The adjusted model did not change the associations. Each additional point of the uGRS was associated with a 50% increased risk of developing GAgP (OR 1.50 [95% CI 1.21 to 1.85]), adjusted for age, sex, and BMI. The ORs for GAgP risk significantly increased across the groups of the uGRS (P for trend<0.0001). Compared with those in the low score, participants in the high score of the uGRS had an OR of 2.87 (95% CI 1.59 to 5.17), adjusted for confounders. Results were similar when the independent variable was restricted to wGRS. The wGRS ranged from 3.19 to 12.26. The crude ORs for GAgP were 1.30 (95% CI 1.15 to 1.47) with each additional point of wGRS. The adjusted ORs for GAgP were 1.31 (95% CI 1.14 to 1.51) with each additional point of of wGRS, adjusted for age, sex, and BMI. The adjusted model did not change the associations as

JOURNAL OF Periodontology

TABLE 2 The univariate association analysis between single SNP and risk of GAgP

Gene	SNP	Allele	Healthy control	GAgP group	OR (95% CI)	*P* value
MMP8	rs11225395	T	95 (41.67%)	226 (33.73%)	Ref.	
		C	133(58.33 %)	444 (66.27%)	1.40 (1.03 to 1.91)*	0.0312
EGF	rs2237051	G	87 (38.16%)	204 (30.45%)	Ref.	
		A	141 (61.84%)	466 (69.55%)	1.41 (1.03 to 1.93)*	0.0321
PPARa	rs4253623	G	45 (19.74%)	93 (13.88%)	Ref.	
		A	183 (80.26%)	577 (86.12%)	1.53 (1.03 to 2.26)*	0.0351
APOE	rs429358	C	26 (11.40%)	45 (6.72%)	Ref.	
		T	202 (88.60%)	625 (93.28%)	1.79 (1.08 to 2.97)*	0.0251

Data were presented as n (%)/OR (95% CI).

APOE, apolipoprotein E; EGF, epidermal growth factor; MMP8, matrix metalloproteinase 8; PPARa, peroxisome proliferator-activated receptor alpha.

*P value <0.05

TABLE 3 Association between the unweighted and weighted genetic risk score and GAgP risk

Variables	Score range	Healthy control (n = 114)	GAgP group (n=335)	Non-adjusted OR (95% CI)	Adjusted OR† (95% CI)	*P* for trend
uGRS	2 to 8	5.78 ± 1.27	6.30 ± 1.09	1.47 (1.22 to 1.78)*	1.50 (1.21 to 1.85)*	
wGRS	3.19 to 12.26	9.01 ± 1.91	9.79 ± 1.62	1.30 (1.15 to 1.47)*	1.31 (1.14 to 1.51)*	
uGRS group						
Low	2 to 5	47 (41.23%)	71 (21.19%)	Ref.	Ref.	<0.001
Middle	5 to 7	33 (28.95%)	113 (33.73%)	2.27 (1.33 to 3.87)*	2.21 (1.29 to 3.78)*	
High	7 to 8	34 (29.82%)	151 (45.07%)	2.94 (1.74 to 4.96)*	2.87 (1.59 to 5.17)*	
wGRS group						
Low	3.19 to 8.05	50 (43.86%)	87 (25.97%)	Ref.	Ref.	<0.001
Middle	8.05 to 10.86	35 (30.70%)	117 (34.93%)	1.92 (1.15 to 3.21)*	1.88 (1.09 to 3.08)*	
High	10.86 to 12.26	29 (25.44%)	131 (39.10%)	2.60 (1.53 to 4.42)*	2.67 (1.46 to 4.88)*	

uGRS, unweighted genetic risk score; wGRS, weighted genetic risk score.

*P <0.05.

†Adjusted for age (years), sex (male/female), and BMI (Kg/m^2).

well. Each additional point of the wGRS was associated with a 31% increased risk of developing GAgP (OR 1.31 [95% CI 1.14 to 1.51]). The ORs for GAgP risk significantly increased across the groups of the wGRS (P for trend<0.0001). Compared with those in the low score, participants in the high score of the wGRS had an OR of 2.67 (95% CI 1.46 to 4.88). Regarding to the power, we calculated the power of 0.9667 for uGRS and 0.9647 for wGRS.

3.4 | Stratified analyses by sex and BMI

We next examined whether the association between the uGRS/wGRS and GAgP risk varied across subgroups of the population stratified by sex (male, female) and BMI. We observed consistent associations across subgroups of the population stratified by sex (data not shown). There was no significant interaction between the uGRS/wGRS and sex on GAgP risk (all P for interaction >0.05).

3.5 | Prediction of GAgP based on non-genetic factors with or without GRS

The AUC of wGRS model was 0.619 (95% CI 0.5599 to 0.6796), which was similar that of uGRS (0.620, 95% CI 0.5573 to 0.6809), (P = 0.92, see Figure S1 in online *Journal of Periodontology*). Figure 2 shows the ROC curves for the logistic regression model incorporating conventional risk factors (age, sex, BMI, WBC, and albumin/globulin) with or without wGRS. The AUC for the conventional model was

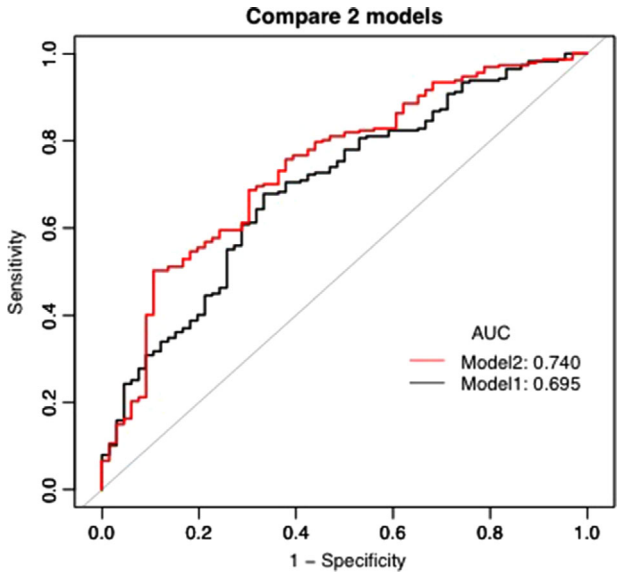

FIGURE 2 Receiver operating characteristic curves for GAgP risk. The curves are based on logistic models incorporating conventional risk factors (age, sex, BMI, WBC, albulin/globulin) with and without wGRS. Model 1: Conventional risk factors (the black curve), including age, sex, BMI, WBC, and albulin/globulin. Model 2: uGRS + Model 1 (the red curve). AUC, area under the curve; uGRS, unweighted genetic risk score. Model 2 had an significant increased AUC of 0.045 than that of Model 1 (from 0.695 to 0.740, $P = 0.048$)

0.695 (CI, 0.624 to 0.767) and significantly increased to 0.740 (CI, 0.672 to 0.808) when the uGRS was added ($P = 0.048$).

4 | DISCUSSION

In the present study, four significant loci, including MMP8 rs11225395, EGF rs2237051, PPAR-a rs4253623, and APOE rs429358, were found to be associated with risk of GAgP. Among them, MMP8 is one of the key mediators of the irreversible tissue destruction in periodontitis.[22] It is expressed in periodontium, and has been reported to be upregulated in the tissues of individuals with inflammation and periodontitis.[23–25] A significant correlation between increased MMP8 levels and periodontal disease severity has been suggested.[26] The polymorphisms of rs11225395 (−799C/T) in the MMP8 gene were studied in 341 patients with CP and 278 unrelated non-periodontitis controls.[27] It was found that the specific haplotype [rs11225395 (−799C/T) and rs2155052 C(+17)] showed association with clinical manifestation of chronic periodontitis in a Czech population. These evidences support our result that MMP8 rs11225395 polymorphisms were associated with GAgP. EGF gene rs2237051 polymorphisms is an A-to-G transition, resulting in the replacement of methionine by isoleucine. Hormia et al. compared the concentration of EGF in saliva of patients with AgP and healthy controls and found that the concentration of EGF in saliva of patients with AgP significantly increased. The secretion rate of EGF in saliva was also rose notably.[28] In addition, EGF at different concentrations can regulate the expression of MMP-1, 3, 7, and 11 in gingival fibroblasts.[29] Therefore, we speculate that EGF gene rs2237051 polymorphisms may influence the risk of GAgP. PPARa-rs4253623 is an activated receptor of an SNP loci. It was reported that PPAR-a could mediate inhibition of nuclear factor (NF)-kB signaling pathways. The del/del genotype of NF-kB was shown to be associated with the occurrence of AgP.[30] Therefore, PPARa-rs4253623 might influence the risk of GAgP by NF-kB signaling pathways. The association between APOE rs429358 and GAgP was reported before. Individuals with the combined polymorphisms of APOE rs429358 with LRP5 rs682429 could influence the risk of GAgP, levels of low-density lipoprotein cholesterol and levels of total cholesterol.[18] Considering that APOE has been shown to play an important role in immune responses[31,32] and in the presentation of lipid antigens to immune cells.[33] Hence, APOE may affect GAgP risk through the immune-related pathway. Together, these four SNPs were associated with risk of GAgP.

Then, these four significant SNPs were selected to calculate the GRS. Although significantly associated with GAgP, each locus confers modest risk limits the clinical utility of each when considered independently. Taken collectively, they provide a global measure of an individual's genetic predisposition to GAgP. We took a conservative approach to creating a GRS by including only loci that reached significance in this candidate gene study. To our knowledge, GRS, which aggregates information from multiple genetic variants, was for the first time used in periodontitis to assess genetic predisposition to GAgP. According to the results, each risk allele (1 point) was associated with an ≈50% increased risk for GAgP. Persons with ≥7 risk alleles (the high score) had a more than two fold increased risk for GAgP compared with those with ≤5 (the low score). To account for the different magnitudes of effect attributable to each SNP, we computed a wGRS by using β-coefficients. These β-coefficients should represent the best estimates of risk available and account for different genotype and environment backgrounds of the populations studied. Each additional point of wGRS was associated with a ≈31% increased risk for GAgP, adjusted for age, sex, and BMI. Individuals' wGRS in the high score (ranged from 10.86 to 12.26) had a greater than two-fold increased risk for GAgP compared with those in the low score (ranged from 3.19 to 8.05). The results of the wGRS were very similar to those of the count GRS score, possibly because the range of risk effects attributable to each of the loci was narrow. However, the GRS which calculate the significant loci was more powerful than the approach of a single locus. According to the Maier et al. study, the multi locus approach prediction accuracy sig-

nificantly increases the prediction accuracy when compared with the single locus approach.[6] Because each genetic marker individually explains only a tiny proportion of the genetic variation with insignificant predictive power, and the GRS is an estimate of the cumulative contribution of genetic factors. Therefore, GRS could be a useful tool to identify a substantial proportion of people with a high genetic risk for GAgP, especially the person in the in the high score. Individuals in the high score should be paid more attention to take preventive measures or early treatment.

In addition, the GRS significantly improved case–control discrimination beyond that afforded by conventional risk factors. The AUC of traditional model which included age, sex, BMI, WBC, and Albulin/globulin was 0.695, and the magnitude of this improvement was good: addition of the GRS increased the AUC by 4.5%. Although GRS on its own explained only a small amount (0.620 and 0.619 for uGRS and wGRS, respectively) of GAgP, a significant novel feature of this study is that taking non-genetic risk factors together with GRS significantly improves the risk assessment of GAgP (the ROC curve increased from 0.695 to 0.740, $P = 0.048$).

Furthermore, the introduction of multi-locus GRS tool association analysis on the risk of GAgP may have significant and clinical implications. First of all, the GRS, which overlaps the genetic variation, greatly improved the predictive power of disease risk when compared with the individual SNPs. In addition, GRS can be a useful tool for both patients and clinicians, simplifying the assessment of the multifactorial nature of periodontitis and incorporating it into clinical practice. This represents an effort to provide a tool for clinicians in their decision-making process to assist them in motivating patients toward healthy behaviors. Regarding the patients, risk scores can be used to induce/motivate behavioral changes to reduce the risk score and corresponding periodontitis risk. The use of the risk score for periodontitis over the patients' follow-up may influence positively the accuracy of periodontal clinical decisions, with a potential impact in the patients' oral health, reducing both the healthcare cost and the need for complex restorations and/or periodontal therapy.

Several limitations in the study are needed to be acknowledged. The case-control study design inevitably brings selected bias. The GRS which captured the combined information based on the candidate study may account for only a small amount of variation of GAgP. In addition, the results which based on the Chinese population remains to be examined whether it could be generalizable to other ethnic groups.

5 | CONCLUSION

We used the multi-locus GRS-based association analysis, for the first time, on the risk of GAgP and found that the GRS was significantly associated with the increased risk of GAgP in Chinese population independent of age, sex, and BMI. Although its discriminatory value is currently limited, a GRS that combines information from multiple genetic variants, in conjunction with known traditional risk factors, might be useful to identify subgroups with a particularly high risk for GAgP to prevent the progression of disease with medical intervention.

ACKNOWLEDGMENTS

The study was funded by the National Natural Science Foundations of China, Beijing, China (30471882, 30973319, and 81271149); the National Key Project of Scientific and Technical Supporting Programs of China, Beijing, China (2007BAI18B02); and the Key program of Clinical Specialty, National Ministry of Health P.R.C., Beijing, China. The authors report no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

Concept/design: Prof. HM and Dr. WL. Data collection: Dr. XW, Dr. YT, Prof. LX, Prof. LZ, Prof. DS, and Prof. RL. Data analysis/interpretation: Dr. WL. Drafting article: Dr. WL. Critical revision of article: Prof. HM.

ORCID

Ruifang Lu https://orcid.org/0000-0002-9581-508X

REFERENCES

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol*. 1999;4:1-6.
2. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(Suppl 1):S173-S182.
3. Susin C, Haas AN, Albandar JM. Epidemiology and demographics of aggressive periodontitis. *Periodontol 2000*. 2014;65:27-45.
4. Guerrero A, Griffiths GS, Nibali L, et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. *J Clin Periodontol*. 2005;32:1096-1107.
5. Stabholz A, Soskolne WA, Shapira L. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. *Periodontol 2000*. 2010;53:138-153.
6. Maier R, Moser G, Chen GB, et al. Joint analysis of psychiatric disorders increases accuracy of risk prediction for schizophrenia, bipolar disorder, and major depressive disorder. *Am J Hum Genet*. 2015;96:283-294.
7. Kuchenbaecker KB, McGuffog L, Barrowdale D, et al. Evaluation of polygenic risk scores for breast and ovarian cancer risk prediction in BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst*. 2017;109.
8. Cornelis MC, Qi L, Zhang C, et al. Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry. *Ann Intern Med*. 2009;150:541-550.

9. International Schizophrenia Consortium, Purcell SM, Wray NR, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748-752.
10. Vassos E, Di Forti M, Coleman J, et al. An examination of polygenic score risk prediction in individuals with first-episode psychosis. *Biol Psychiatry*. 2017;81:470-477.
11. Desikan RS, Fan CC, Wang Y, et al. Genetic assessment of age-associated Alzheimer disease risk: development and validation of a polygenic hazard score. *PLoS Med*. 2017;14:e1002258.
12. Mormino EC, Sperling RA, Holmes AJ, et al. Polygenic risk of Alzheimer disease is associated with early- and late-life processes. *Neurology*. 2016;87:481-488.
13. Mega JL, Stitziel NO, Smith JG, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. *Lancet*. 2015;385:2264-2271.
14. Khera AV, Emdin CA, Drake I, et al. Genetic risk, adherence to a healthy lifestyle, and coronary disease. *N Engl J Med*. 2016;375:2349-2358.
15. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. *Lancet*. 2010;376:1393-1400.
16. Natarajan P, Young R, Stitziel NO, et al. Polygenic risk score identifies subgroup with higher burden of atherosclerosis and greater relative benefit from statin therapy in the primary prevention setting. *Circulation*. 2017;135:2091-2101.
17. Mazza JE NM, Sims TN. Clinical and antimicrobial effect of stannous fluoride on periodontitis. *J Clin Periodontol*. 1981;8(3):203-212.
18. Gao H, Tian Y, Meng H, et al. Associations of apolipoprotein E and low-density lipoprotein receptor-related protein 5 polymorphisms with dyslipidemia and generalized aggressive periodontitis in a Chinese population. *J Periodontal Res*. 2015;50:509-518.
19. Zupancic K, Skok K, Repnik K, Weersma RK, Potocnik U, Skok P. Multi-locus genetic risk score predicts risk for Crohn's disease in Slovenian population. *World J Gastroenterol*. 2016;22:3777-3784.
20. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263-265.
21. Lou XY, Chen GB, Yan L, et al. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. *Am J Hum Genet*. 2007;80:1125-1137.
22. Sorsa T, Tjaderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis*. 2004;10:311-318.
23. Emingil G, Han B, Gurkan A, et al. Matrix metalloproteinase (MMP)-8 and tissue inhibitor of MMP-1 (TIMP-1) gene polymorphisms in generalized aggressive periodontitis: gingival crevicular fluid MMP-8 and TIMP-1 levels and outcome of periodontal therapy. *J Periodontol*. 2014;85:1070-1080.
24. Kubota T, Nomura T, Takahashi T, Hara K. Expression of mRNA for matrix metalloproteinases and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. *Arch Oral Biol*. 1996;41:253-262.
25. Ingman T, Sorsa T, Michaelis J, Konttinen YT. Matrix metalloproteinases-1, -3, and -8 in adult periodontitis in situ. An immunohistochemical study. *Ann N Y Acad Sci*. 1994;732:459-461.
26. Xu L, Yu Z, Lee HM, et al. Characteristics of collagenase-2 from gingival crevicular fluid and peri-implant sulcular fluid in periodontitis and peri-implantitis patients: pilot study. *Acta Odontol Scand*. 2008;66:219-224.
27. Izakovicova Holla L, Hrdlickova B, Vokurka J, Fassmann A. Matrix metalloproteinase 8 (MMP8) gene polymorphisms in chronic periodontitis. *Arch Oral Biol*. 2012;57:188-196.
28. Hormia M, Thesleff I, Perheentupa J, Pesonen K, Saxen L. Increased rate of salivary epidermal growth factor secretion in patients with juvenile periodontitis. *Scand J Dent Res*. 1993;101:138-144.
29. Cury PR, de Araujo VC, Canavez F, Furuse C, Leite KR, de Araujo NS. The effect of epidermal growth factor on matrix metalloproteinases and tissue inhibitors of metalloproteinase gene expression in cultured human gingival fibroblasts. *Arch Oral Biol*. 2007;52:585-590.
30. Schulz S, Hierse L, Altermann W, et al. The del/del genotype of the nuclear factor-kappaB -94ATTG polymorphism and its relation to aggressive periodontitis. *J Periodontal Res*. 2010;45:396-403.
31. Zhang H, Wu LM, Wu J. Cross-talk between apolipoprotein E and cytokines. *Mediators Inflamm*. 2011;2011:949072.
32. Vitek MP, Brown CM, Colton CA. APOE genotype-specific differences in the innate immune response. *Neurobiol Aging*. 2009;30:1350-1360.
33. Mahley RW. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet*. 2000;1:507-537.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Li W, Wang X, Tian Y, et al. A novel multi-locus genetic risk score identifies patients with higher risk of generalized aggressive periodontitis. *J Periodontol*. 2020;91:925–932. https://doi.org/10.1002/JPER.19-0135