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Association of *CYP1A1* rs1048943 variant with aggressive periodontitis and its interaction with hyperlipidemia on the periodontal status

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Background and objective: *CYP1A1* rs1048943 polymorphism was reported to be correlated with periodontitis; however, its association with aggressive periodontitis (AgP) has not yet been investigated. The aim of the study was to investigate the association between the *CYP1A1* gene rs1048943 variant with generalized aggressive periodontitis (GAgP) and platelet activation and analyse whether its interaction with hyperlipidemia affects periodontal status in a Chinese population.

Methods: A case-control study of 224 GAgP patients and 139 healthy controls was conducted. The clinical parameters of probing depth (PD), attachment loss (AL) and bleeding index (BI) were recorded. Platelet count (PLT), platelet distribution width (PDW), platelet large cell ratio (PLCR), mean platelet volume (MPV), serum total cholesterol (TC), triacylglycerol (TG), high and low-density lipoprotein (HDL and LDL) were also measured. The *CYP1A1* rs1048943 SNP was genotyped by time-of-flight mass spectrometry. Logistic and linear regression models were used to measure correlation.

Results: The *CYP1A1* rs1048943 AG/GG genotype was associated with GAgP (OR = 1.56, 95%CI: 1.01, 2.42), PD, AL and decreased PDW, PLCR and MPV after adjustment for covariates. Gene-lipid interactions were found between *CYP1A1* rs1048943 and HDL for PD ($P_{interaction}$ = 0.0033), BI ($P_{interaction}$ = 0.0311) and AL ($P_{interaction}$ = 0.0141) and between *CYP1A1* rs1048943 and LDL for PD ($P_{interaction}$ = 0.013) among patients with GAgP.

Conclusion: The G allele of the *CYP1A1* rs1048943 gene was associated with GAgP, periodontal status and platelet-related inflammation status in a Chinese population. Hyperlipidemia could modulate the effect of *CYP1A1* rs1048943 on the periodontal status of GAgP.

KEYWORDS
CYP1A1, GAgP, gene-environment interaction, hyperlipidemia, platelets

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Xian'e Wang and Wenjing Li are the co-first authors of this article.

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1 | INTRODUCTION

Aggressive periodontitis (AgP) is characterized by early onset, rapid attachment loss, bone destruction and a propensity for tooth-loss. It is a multifactorial complex disease, not only influenced by genotype, including gene-gene interactions and environmental factors, such as smoking status, plaque control, socio-economic status and diabetes, but also by interactions between genotype and environment (G×E).[1,2] It is also a disease associated with systemic diseases, such as chronic obstructive pulmonary disease, chronic kidney disease, pneumonia and rheumatoid arthritis.[3] Cardiovascular disease (CVD) also has strong causative connections with AgP, including direct and indirect influences, and the two conditions share many risk factors such as smoking, obesity, genetics and a history of dyslipidemia.[4]

The cytochrome P450 (CYP) is a super family of cysteinato-heme enzymes that are key mediators of oxidative transformation of exogenous molecules.[5] As a member of CYP, the cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) gene is expressed in the vascular endothelium, where it metabolizes arachidonic acid (AA) into 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs). These two metabolites play critical roles in the regulation of CVD.[6] CYP1A1 gene rs1048943 (Ile^{462}Val, A4889G) polymorphism is an A-to-G transition in exon 7, resulting in the replacement of isoleucine (Ile) by valine (Val).[7] Variant genotypes of CYP1A1 Ile/Val polymorphism were observed to exhibit a significant increase in enzyme activity associated with an increase in CYP1A1 mRNA expression compared to controls with the wild-type genotype.[8] Cytochrome P450 enzymes were reported to be the central players in CVD,[6] and CYP1A1 rs1048943 polymorphism was associated with CVD in Chinese Han and Uygur populations.[9] To date, there has been only one study on the relationship between CYP1A1 rs1048943 polymorphism and the risk of periodontitis, which showed an increased risk of periodontitis (OR = 2.3) for this SNP in a Korean population with 115 periodontitis patients and 126 healthy controls.[10] However, the association of the CYP1A1 rs1048943 variant with AgP has not yet been investigated, let alone its influence on periodontal status, such as probing depth (PD), bleeding index (BI) and clinical attachment loss (CAL).

It has been reported that platelet activation was associated with an increased risk of periodontitis.[11] Mean platelet volume (MPV) is used an inflammatory marker in patients with severe periodontitis. MPV is an important index of platelet activity, production rate and stimulation.[12] In addition, Zhan et al[13] detected platelet-endothelium and platelet-leucocyte interactions in inflamed gingivae and concluded that platelets played a role in inflammatory immune responses in generalized aggressive periodontitis (GAgP). 20-HETE is one of the metabolites of AA, is related to the CYP1A1 pathway,[6] and is also a platelet activator and vasoconstrictor.[14] Since the CYP1A1 mRNA and protein have been identified in human platelets,[14] there is a possible association between CYP1A1 gene polymorphism and platelet activity, which has yet to be studied.

It is well known that smoking status is considered an environmental risk factor that can interact with susceptible genes to increase the risk of periodontitis.[10,15] However, other environmental factors which interact with susceptible periodontitis-associated genes have not yet been investigated. Hyperlipidemia is not only the most important risk factor for CVD,[16] but is also a factor closely associated with the risk of periodontitis.[17-19] CYP1A1 gene polymorphisms have been reported to influence blood lipids in concert with diet,[20] indicating a possible biological pathway between CYP1A1 gene variants and blood lipids. Therefore, whether there is a gene-environment interaction between the CYP1A1 rs1048943 gene polymorphism and hyperlipidemia influencing the periodontal status and platelet-related inflammation of AgP is of great importance for exploring the shared mechanisms of periodontitis and CVD.

Thus, our hypothesis is that CYP1A1 rs1048943 gene polymorphism not only influences GAgP, but also platelet activation. Furthermore, CYP1A1 rs1048943 may interact with hyperlipidemia to influence periodontal parameters. The aim of our study was to investigate the association between the CYP1A1 rs1048943 variant with GAgP and platelet activation and analyse whether its interaction with hyperlipidemia affects periodontal status in a Chinese population.

2 | MATERIAL AND METHODS

2.1 | Subject population

The present study is a case-control study. Two hundred and twenty-four Chinese patients with GAgP were enrolled from the patients of the Department of Periodontology at the Peking University School and Hospital of Stomatology over a period of 15 years from 1 July 2001 to 26 June 2015. The control group comprised 139 periodontally healthy volunteers recruited from the staff and students of the Peking University School and Hospital of Stomatology.

The diagnosis of GAgP was based on the following clinical and radiographic criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions.[21]

Inclusion criteria for GAgP patients:

- were systematically healthy with the onset of periodontal destruction before they were 35 years old;
- at least eight teeth with PD > 5 mm and AL > 3 mm and at least three of which were not first molars or incisors;
- clinical diagnosis was confirmed by evidence of inter-proximal bone loss on full-mouth periapical radiographs.

Inclusion criteria for healthy controls:

- less than 36 years old;
- with PD ≤ 3 mm;
- no obvious AL;
- less than 10% of sites with a bleeding index (BI) ≥ 2, and no sites with a BI > 4.

TABLE 1 The characteristics, periodontal status of GAgP patients and healthy controls

Group	Control group	GAgP group	P-value
N	139	224	
Age	27.15 ± 4.26	27.33 ± 4.60	0.718
Gender/male	53 (38.13%)	85 (37.95%)	0.972
PLI	2.41 ± 0.55	2.40 ± 0.50	0.801
Mean PD (mm)	1.80 ± 0.48	4.87 ± 0.95	<0.001*
Mean BI	1.12 ± 0.35	3.61 ± 0.45	<0.001*
Mean CAL (mm)	-	4.28 ± 1.22	-
PD ≥ 5 mm%	-	55.83 ± 19.51	-
BI ≥ 3%	-	91.48 ± 16.69	-
CAL ≥ 5 mm%	-	41.45 ± 22.32	-

Data were presented as Mean ± SD/N (%); comparison of age between GAgP group and control group was performed using Student's t test; comparison of gender between two groups was performed using a chi-square test. Comparison of PLI, PD and BI between two groups was performed using a Mann-Whitney U test. BI, bleeding index; CAL, clinical attachment loss; GAgP, generalized aggressive periodontitis; PLI, plaque index; PD, probing depth.
*$P < 0.05$, statistical significance.

Exclusion criteria:

- over 35 years old;
- a history of smoking;
- chronic use of non-steroid anti-inflammatory drugs or use of antibiotics within 3 months of the study visit;
- periodontal treatment within the previous 6 months;
- pregnancy or systemic diseases, such as diabetes mellitus, hypertension and cardiovascular disease.

This study was approved by the ethics committee of Peking University Health Science Center. All of the participants provided informed written consent when enrolled into the study.

2.2 | Clinical parameters assessment

All participants were evaluated clinically for the following periodontal parameters. Probing depth (PD) and attachment loss (AL) were measured throughout the entire mouth excluding 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)[22] was recorded 30 seconds after probing, and the most severe sites on the buccal (labial) side and lingual (palatal) side were recorded. Additionally, full-mouth periapical radiographs were taken to determine the diagnosis of GAgP. All clinical periodontal parameters were recorded by two skilled periodontal specialists (Dong Shi and Li Xu), and calibration was performed on 10 patients with GAgP. The consistency of replicated measurements of PD and AL for each examiner (intra-calibration) and between the pair of periodontal specialists (inter-examiner calibration) was recorded.

Of the replicated measurements for each examiner, 97.0% (Dong Shi) and 95.8% (Li Xu) of PD measurements were within 1 mm, and 91.5% (Dong Shi) and 93.2% (Li Xu) of AL measurements were within 1 mm. Of the paired measurements between the two examiners (Dong Shi vs Li Xu), 93.5% of the PD and 90.5% of the AL measurements were within 1 mm.

2.3 | Blood examination

A peripheral blood sample was obtained from each fasted examinee by venipuncture between 8:00 a.m. and 10:00 a.m. The blood sample was divided into two tubes. One contained EDTA and was used for genomic DNA isolation while the other did not and was used for the measurement of serum protein parameters. Platelet activity-related index, including platelet count (PLT), platelet distribution width (PDW), platelet large cell ratio (PLCR) and mean platelet volume (MPV), was measured using a biochemical analyzer (Hitachi 7060; Hitachi, Tokyo, Japan).

2.4 | DNA extraction and genotyping

Genomic DNA was extracted from the blood sample containing EDTA using a blood DNA mini kit (Watson Biotechnologies, Inc., Shanghai, China) according to the manufacturer's protocol. SEQUENOM MassARRAY matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was used to genotype *CYP1A1* rs1048943 polymorphism (Sequenom; San Diego, CA). The protocol for genotyping was described in the previous study.[17]

Primers for the polymerase chain reaction and single base extension were designed using the Assay Designer software package (Sequenom). The primers were as follows:

Forward-ACGTTGGATGTGGGCAAGCGGAAGTGTATC
Reverse-ACGTTGGATGAATTCCACCCGTTGCAGCAG

All genotyping was performed blind with respect to clinical diagnosis by a single investigator.

2.5 | Statistical analysis

Continuous variables are presented as mean ± SD, and categorical variables are presented as N (%). Logistic regression models were used to analyse the association between the *CYP1A1* rs1048943 variant with GAgP, and linear regression models were used to analyse the association of *CYP1A1* rs1048943 polymorphism with periodontal status and platelet-related inflammation in GAgP patients after adjusting for age and gender. The interaction between the *CYP1A1* rs1048943 variant and blood lipids (HDL, LDL, TG and TC) on periodontal parameters and platelet activity-related index (PDW, PLCR and MPV) was examined by likelihood ratio tests. Linear regression models were used to estimate adjusted coefficient (β) and 95% confidence intervals (CI) for the association of blood lipids (HDL, LDL, TG and TC) interactions with *CYP1A1* rs1048943 on clinical periodontal parameters, such as PD, BI and

TABLE 2 Logistic regression analysis for the relationship between genotypes of CYP1A1 rs1048943 and risk of GAgP

Exposure	Control group	GAgP group	Non-adjusted OR (95%CI)	Adjust I OR (95%CI)
CYP1A1 rs1048943				
AA	91 (65.47%)	123 (54.91%)	Ref.	Ref.
AG	47 (33.81%)	82 (36.61%)	1.29 (0.82, 2.02)	1.26 (0.80, 1.98)
GG	1 (0.72%)	19 (8.48%)	14.06 (1.85, 106.92) *	14.44 (1.90, 110.00) *
CYP1A1 rs1048943				
AA	91 (65.47%)	123 (54.91%)	Ref.	Ref.
AG+GG	48 (34.53%)	101 (45.09%)	1.56 (1.00, 2.41) *	1.56 (1.01, 2.42) *

Data were presented as OR (95%CI); adjust I model adjusts for age and gender.
*$P < 0.05$, statistical significance.

AL, and platelet activity-related index, such as PDW, PLCR and MPV in GAgP patients adjusted for age and gender. The sample size for CYP1A1 rs1048943 genotype and allele frequencies was calculated in a dichotomous pattern ($\alpha = 0.05$, power = 0.8, p0 = 0.08, p1 = 0.01, m = 0.5) showing that 181 cases and 91 controls were needed. The reference value was that of genotype frequency. The software Power and Sample Size Calculation was used to calculate the sample size. (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).

A two-tailed $P < 0.05$ was considered to be statistically significant in all analyses. Statistical analyses were performed with R (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc. Boston MA).

3 | RESULTS

The characteristics and clinical periodontal parameters of GAgP patients and healthy controls are depicted in Table 1. The mean PD and BI were significantly higher in the GAgP group ($P < 0.05$). There was no significant difference in age, gender distribution and PLI between the two groups. The mean ± SD of CAL, per cent PD ≥ 5 mm, per cent BI ≥ 3 and per cent CAL ≥ 5 mm in GAgP patients were 4.28 ± 1.22 mm, 55.83 ± 19.51 mm, 91.48 ± 16.69% and 41.45 ± 22.32%, respectively.

Logistic regression analysis of the relationship between CYP1A1 gene rs1048943 polymorphism and GAgP is presented in Table 2. The GG genotype was associated with increased susceptibility to GAgP compared to those with the AA genotype after adjusting for age and gender (AOR = 14.44, 95%CI: 1.90, 100.00). But no increased susceptibility was found for individuals with the AG genotype compared to the AA genotype. After adjusting for age and gender, it was observed that individuals with the G allele had an increased susceptibility to GAgP compared to those without (AOR = 1.56, 95% CI: 1.01, 2.42).

Table 3 shows linear regressions of different CYP1A1 rs1048943 genotypes for clinical periodontal parameters and platelet activity-related index in GAgP patients. For the periodontal parameters, the CYP1A1 rs1048943 AG/GG genotype was associated with increased PD ($\beta = 0.35$, 95% CI: 0.11, 0.59), increased CAL ($\beta = 0.42$, 95% CI: 0.11, 0.73), increased percentage of PD ≥ 5 mm ($\beta = 5.18$, 95% CI: 0.09, 10.28) and increased percentage of CAL ≥ 5 mm ($\beta = 6.43$, 95% CI: 0.75, 12.11) compared to the AA homozygotes after adjusting for age and gender. No significant difference was found for BI or per cent BI ≥ 3 between the different genotypes. When examining platelet activity-related indexes, the CYP1A1 rs1048943 AG/GG genotype was associated with decreased PDW ($\beta = -0.70$, 95% CI: −1.32, −0.09), decreased PLCR ($\beta = -0.04$, 95% CI: −0.06, −0.01) and decreased MPV ($\beta = -0.55$, 95% CI: −0.95, −0.14) compared with the AA homozygotes after adjusting for covariates. There was a marginally significant difference for PLT ($\beta = 12.23$, 95% CI: −1.83, −26.29).

Analyses of the effects of CYP1A1 rs1048943-lipids (HDL-c, LDL-c, TC and TG) interaction on clinical periodontal parameters (mean PD, BI and AL) among patients with GAgP are depicted in Figures 1-4. Statistically significant effects for gene-lipids interactions were found between CYP1A1 rs1048943 and HDL for mean PD ($P_{interaction} = 0.0033$), BI ($P_{interaction} = 0.0311$) and AL ($P_{interaction} = 0.0141$) and between CYP1A1 rs1048943 and LDL for mean PD ($P_{interaction} = 0.013$) among patients with GAgP after adjusting for age and gender (Figures 1 and 2). CYP1A1 rs1048943 was associated with increased mean PD ($\beta = 1.64$, 95% CI: 0.72, 2.55), mean BI ($\beta = 0.48$, 95% CI: 0.02, 0.94) and mean AL ($\beta = 1.57$, 95% CI: 0.33, 2.80) for GAgP given an AG/GG genotype combined with HDL < 1.0 mmol/L compared to an AA genotype and HDL > 1.0 mmol/L (Figure 1). CYP1A1 rs1048943 was associated with increased mean PD ($\beta = 1.30$, 95% CI: 0.67, 1.94), mean BI ($\beta = 0.33$, 95% CI: 0.01, 0.65) and mean AL ($\beta = 1.18$, 95% CI: 0.31, 2.04) for GAgP given an AG/GG genotype combined with LDL > 3.4 mmol/L compared to an AA genotype and LDL < 3.4 mmol/L (Figure 2) No significant interactions were found between CYP1A1 rs1048943 and TC/TG. But CYP1A1 rs1048943 was associated with increased mean PD ($\beta = 0.93$, 95% CI: 0.34, 1.52) and mean AL ($\beta = 1.17$, 95% CI: 0.38, 1.97) for GAgP given an AG/GG genotype combined with TG ≥ 1.7 mmol/L compared to an AA genotype and TG < 1.7 mmol/L. (Figure 3) CYP1A1 rs1048943 was associated with increased mean PD ($\beta = 0.93$, 95% CI: 0.34, 1.52) and mean AL ($\beta = 1.17$, 95% CI: 0.38, 1.97) for GAgP given an AG/GG genotype combined with TC ≥ 5.2 mmol/L compared to an AA genotype and TC < 5.2 mmol/L

TABLE 3 Linear regression for the periodontal status and platelets activity-related index in different genotypes of CYP1A1 rs1048943 in GAgP patients

Outcome	Exposure	Mean ± SD	Non-adjusted β (95%CI)	Adjust I β (95%CI)
Mean PD (mm)	CYP1A1 rs1048943			
	AA	4.70 ± 0.85	Ref.	Ref.
	AG+GG	5.08 ± 1.03	0.38 (0.14, 0.63) *	0.35 (0.11, 0.59) *
Mean BI	CYP1A1 rs1048943			
	AA	3.58 ± 0.44	Ref.	Ref.
	AG+GG	3.65 ± 0.46	0.07 (−0.05, 0.19)	0.07 (−0.05, 0.19)
Mean AL (mm)	CYP1A1 rs1048943			
	AA	4.09 ± 1.15	Ref.	Ref.
	AG+GG	4.51 ± 1.28	0.42 (0.10, 0.74) *	0.42 (0.11, 0.73) *
PD ≥ 5 mm%	CYP1A1 rs1048943			
	AA	53.42 ± 18.78	Ref.	Ref.
	AG+GG	58.77 ± 20.07	5.35 (0.25, 10.45) *	5.18 (0.09, 10.28) *
BI ≥ 3%	CYP1A1 rs1048943			
	AA	90.70 ± 16.62	Ref.	Ref.
	AG+GG	92.44 ± 16.81	1.74 (−2.65, 6.14)	1.71 (−2.73, 6.16)
AL ≥ 5 mm%	CYP1A1 rs1048943			
	AA	38.49 ± 21.94	Ref.	Ref.
	AG+GG	45.06 ± 22.35	6.58 (0.75, 12.40) *	6.43 (0.75, 12.11) *
PLT (10^9/L)	CYP1A1 rs1048943			
	AA	224.91 ± 53.52	Ref.	Ref.
	AG+GG	238.47 ± 45.71	13.56 (−0.42, 27.53)	12.23 (−1.83, 26.29)
PDW (%)	CYP1A1 rs1048943			
	AA	12.23 ± 2.60	Ref.	Ref.
	AG+GG	11.48 ± 1.55	−0.74 (−1.35, −0.13) *	−0.70 (−1.32, −0.09) *
PLCR (%)	CYP1A1 rs1048943			
	AA	0.26 ± 0.11	Ref.	Ref.
	AG+GG	0.22 ± 0.08	−0.04 (−0.06, −0.01) *	−0.04 (−0.06, −0.01) *
MPV(fL)	CYP1A1 rs1048943			
	AA	9.95 ± 1.50	Ref.	Ref.
	AG+GG	9.37 ± 1.37	−0.57 (−0.98, −0.17) *	−0.55 (−0.95, −0.14) *

Data were presented as Mean ± SD and β (95%CI); AL, attachment loss; BI, bleeding index; MPV, mean platelet volume; PD, probing depth; PDW, platelet distribution width; PLCR, platelet large cell ratio; PLT, platelet count. Linear regression was used to analyse the relationship between genotypes of CYP1A1 RS1048943 and clinical periodontal parameters or platelets activity-related index. Adjust I model adjusts for age and gender.
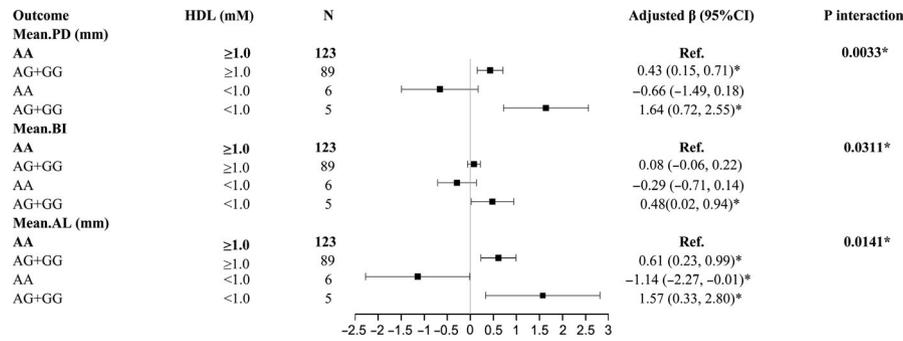*$P < 0.05$, statistical significance.

(Figure 4). There was no significant interaction between *CYP1A1* rs1048943 variants and platelet activity-related indexes including PLT, PDW, PLCR and MPV (not shown).

4 | DISCUSSION

In the present study, a significant association between the *CYP1A1* gene rs1048943 polymorphism and GAgP has been demonstrated after adjusting for covariates among Chinese GAgP patients. Furthermore, patients with the G allele had worse periodontal

status, such as PD and AL. This is consistent with the previous study that found that *CYP1A1* variants increased susceptibility to periodontitis.[10] Possible mechanisms for the association between the rs1048943 variant and GAgP include the following. *CYP1A1* is a member of the cytochrome P450 monooxygenase superfamily, and metabolites of *CYP1A1* could cause cellar injury and induce a reactive inflammatory response.[3] Polymorphisms of *CYP1A1* were associated with enhanced enzyme catalytic activities.[8] When *CYP1A1* rs1048943 changed from A to G, the encoded amino acid of isoleucine became valine,[7] which resulted in increased activity of *CYP1A1*,[23,24] thus leading to an increased susceptibility

Outcome	HDL (mM)	N		Adjusted β (95%CI)	P interaction
Mean.PD (mm)					
AA	≥1.0	123		Ref.	0.0033*
AG+GG	≥1.0	89		0.43 (0.15, 0.71)*	
AA	<1.0	6		−0.66 (−1.49, 0.18)	
AG+GG	<1.0	5		1.64 (0.72, 2.55)*	
Mean.BI					
AA	≥1.0	123		Ref.	0.0311*
AG+GG	≥1.0	89		0.08 (−0.06, 0.22)	
AA	<1.0	6		−0.29 (−0.71, 0.14)	
AG+GG	<1.0	5		0.48 (0.02, 0.94)*	
Mean.AL (mm)					
AA	≥1.0	123		Ref.	0.0141*
AG+GG	≥1.0	89		0.61 (0.23, 0.99)*	
AA	<1.0	6		−1.14 (−2.27, −0.01)*	
AG+GG	<1.0	5		1.57 (0.33, 2.80)*	

−2.5 −2 −1.5 −1 −0.5 0 0.5 1 1.5 2 2.5 3

FIGURE 1 Interactions between polymorphisms of CYP1A1-A2455G and HDL on mean PD, BI and AL in patients with GAgP. PD, probing depth; BI, bleeding index; AL, attachment loss. Multivariable linear regression was used to analyse interactions between polymorphisms of CYP1A1-A2455G and HDL on periodontal parameters in patients with GAgP. Adjust model adjusts for age and gender. *, P < 0.05, statistical significance

Outcome	LDL (mM)	N		Adjusted β (95%CI)	P interaction
Mean.PD (mm)					
AA	<3.4	114		Ref.	0.013*
AG+GG	<3.4	83		0.40 (0.11, 0.69)*	
AA	≥3.4	15		−0.16 (−0.71, 0.39)	
AG+GG	≥3.4	11		1.30 (0.67, 1.94)*	
Mean.BI					
AA	<3.4	114		Ref.	0.5094
AG+GG	<3.4	83		0.10 (−0.05, 0.24)	
AA	≥3.4	15		0.09 (−0.19, 0.37)	
AG+GG	≥3.4	11		0.33 (0.01, 0.65)*	
Mean.AL (mm)					
AA	<3.4	114		Ref.	0.2755
AG+GG	<3.4	83		0.64 (0.24, 1.03)*	
AA	≥3.4	15		−0.10 (−0.85, 0.65)	
AG+GG	≥3.4	11		1.18 (0.31, 2.04)*	

−2.5 −2 −1.5 −1 −0.5 0 0.5 1 1.5 2 2.5 3
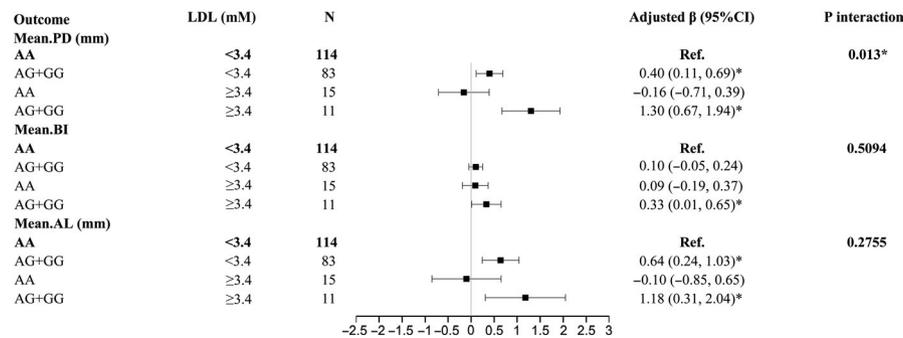
FIGURE 2 Interactions between polymorphisms of CYP1A1-A2455G and LDL on mean PD, BI and AL in patients with GAgP. PD, probing depth; BI, bleeding index; AL, attachment loss. Multivariable linear regression was used to analyse interactions between polymorphisms of CYP1A1-A2455G and LDL on periodontal parameters in patients with GAgP. Adjust model adjusts for age and gender. *, P < 0.05, statistical significance
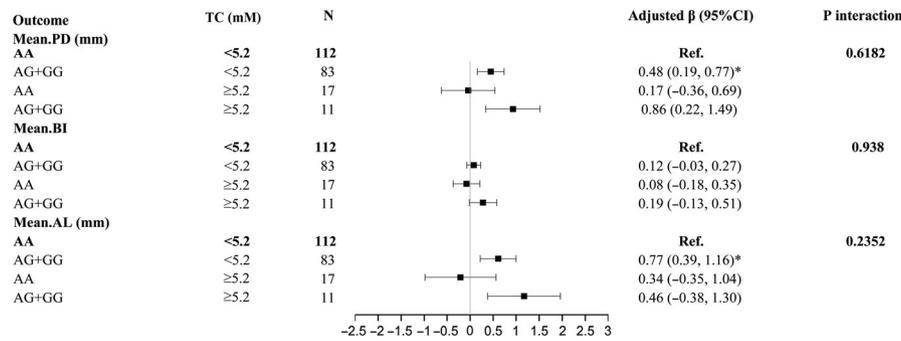
Outcome	TG (mM)	N		Adjusted β (95%CI)	P interaction
Mean.PD (mm)					
AA	<1.7	115		Ref.	0.2178
AG+GG	<1.7	81		0.45 (0.16, 0.75)*	
AA	≥1.7	14		−0.04 (−0.62, 0.53)	
AG+GG	≥1.7	13		0.93 (0.34, 1.52)*	
Mean.BI					
AA	<1.7	115		Ref.	0.1858
AG+GG	<1.7	81		0.08 (−0.07, 0.22)	
AA	≥1.7	14		−0.08 (−0.37, 0.21)	
AG+GG	≥1.7	13		0.28 (−0.02, 0.58)	
Mean.AL (mm)					
AA	<1.7	115		Ref.	0.1754
AG+GG	<1.7	81		0.61 (0.22, 1.01)*	
AA	≥1.7	14		−0.21 (−0.98, 0.56)	
AG+GG	≥1.7	13		1.17 (0.38, 1.97)*	

−2.5 −2 −1.5 −1 −0.5 0 0.5 1 1.5 2 2.5 3
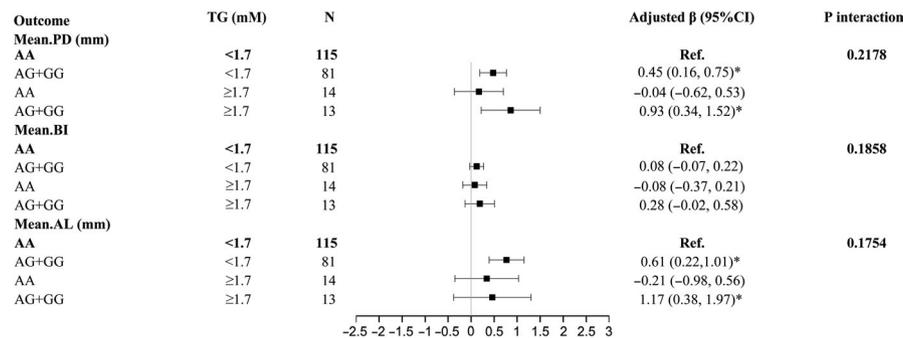
FIGURE 3 Interactions between polymorphisms of CYP1A1-A2455G and TG on mean PD, BI and AL in patients with GAgP. PD, probing depth; BI, bleeding index; AL, attachment loss. Multivariable linear regression was used to analyse interactions between polymorphisms of CYP1A1-A2455G and TG on periodontal parameters in patients with GAgP. Adjust model adjusts for age and gender. *, P < 0.05, statistical significance

to GAgP and the destruction of the periodontium. On the other hand, it has been demonstrated that CYP1A1 gene polymorphisms had biologic consequences on oestrogen, which is critical to bone health.[25] Oestrogen metabolism has been reported to be an important determinant of bone mass. In addition, oestrogen has also been demonstrated to play a significant role in modulating periodontal tissue responses to LPS, exerting its bone-sparing effects on periodontal tissues via altering the expression of inflammatory cytokines in hPDL cells.[26] Therefore, we speculated that CYP1A1 gene polymorphisms affect the susceptibility to GAgP and its periodontal status via increasing the activity of CYP1A1 and oestrogen-related bone metabolism.

Furthermore, the present study demonstrated, for the first time, that CYP1A1 gene rs1048943 polymorphism was associated with platelet-related inflammation in GAgP patients. The study found that the allele of CYP1A1 rs1048943 was inversely correlated with

Outcome	TC (mM)	N		Adjusted β (95%CI)	P interaction
Mean.PD (mm)					
AA	<5.2	112		Ref.	0.6182
AG+GG	<5.2	83		0.48 (0.19, 0.77)*	
AA	≥5.2	17		0.17 (−0.36, 0.69)	
AG+GG	≥5.2	11		0.86 (0.22, 1.49)	
Mean.BI					
AA	<5.2	112		Ref.	0.938
AG+GG	<5.2	83		0.12 (−0.03, 0.27)	
AA	≥5.2	17		0.08 (−0.18, 0.35)	
AG+GG	≥5.2	11		0.19 (−0.13, 0.51)	
Mean.AL (mm)					
AA	<5.2	112		Ref.	0.2352
AG+GG	<5.2	83		0.77 (0.39, 1.16)*	
AA	≥5.2	17		0.34 (−0.35, 1.04)	
AG+GG	≥5.2	11		0.46 (−0.38, 1.30)	

FIGURE 4 Interactions between polymorphisms of CYP1A1-A2455G and TC on mean PD, BI and AL in patients with GAgP. PD, probing depth; BI, bleeding index; AL, attachment loss. Multivariable linear regression was used to analyse interactions between polymorphisms of CYP1A1-A2455G and TC on periodontal parameters in patients with GAgP. Adjust model adjusts for age and gender. *, $P < 0.05$, statistical significance

PDW, PLCR and MPV, which supported our hypothesis of the correlation between *CYP1A1* rs1048943 and the susceptibility to GAgP. We propose the following possible mechanism for the relationship between the *CYP1A1* rs1048943 gene polymorphism and the decrease of MPV in GAgP patients. *CYP1A1* mRNA expression was detected in the gingiva,[27] and its mRNA and protein are expressed in platelets.[14] 20-HETE is produced by *CYP1A1* during the metabolism of arachidonic acid (AA), and it is also a platelet activator and vasoconstrictor.[14] MPV is an important index of platelet activity that reflects platelet stimulation and rate of production rate. It has also been demonstrated to be an inflammatory marker of severe periodontitis[12] and play a role in the inflammatory immune responses in GAgP.[13] Periodontal infection increased the quantity of circulating platelets causing a mass of highly reactive large-sized platelets to migrate to inflammatory sites where they were consumed.[28] Therefore, we speculated that the *CYP1A1* rs1048943 gene polymorphism may change platelet activity by increasing inflammatory level or influencing metabolites by altering mRNA and protein levels and the metabolites of *CYP1A1*.

More importantly, our study demonstrated for the first time that hyperlipidemia, as a new environmental risk factor, modified the effects of *CYP1A1* rs1048943 variants on the periodontal status of GAgP patients. Interestingly, while hyperlipidemia was not associated with periodontal status in the present study, it significantly enhanced the influence of *CYP1A1* rs1048943 variants on the periodontal parameters, including PD and AL. There was also an interaction between *CYP1A1* rs1048943 variants and hyperlipidemia in patients with GAgP, suggesting that hyperlipidemia increased the effect of *CYP1A1* rs1048943 variant on periodontal destruction. It is well known that AgP is a multifactorial disease, not only influenced by the independent effects of genes and the environment, but even more so by the interactions between them.[1,2] As early as 1990, Ruth Ottman described five models of interaction between gene and environment.[29] The interaction in the present study is model C where environmental factors are not associated with the outcome of the disease. However, genetic susceptibility to the disease is increased when exposed to the environmental factor. In this study, we demonstrated the interaction between *CYP1A1* rs1048943- and HDL-c/

LDL-c-related hyperlipidemia on periodontal status in patients with GAgP. HDL is "good" cholesterol that previous studies have shown can modulate cholesterol bioavailability in the lipid rafts, membrane microdomains enriched in glycosphingolipids and cholesterol, is evolutionarily conserved and affects the properties of cells involved in the innate and adaptive immune response, tuning inflammatory response and antigen presentation functions in macrophages as well as B- and T-cell activation. Sphingosine-1 phosphate (S1P), a major active sphingolipid carried by HDL, is also of relevance in the pathogenesis of several immuno-inflammatory disorders through the modulation of macrophage and lymphocyte functions.[30] As periodontitis is closely related to the innate and adaptive immune response, we speculate that HDL-c levels below the normal range affect the metabolic enzyme system and stimulate the expression of cytochrome P450 aggravating oxidative damage and leading to accelerated periodontal destruction. LDL is known as "bad" cholesterol that is normally found in oxidizing conditions; the oxidation of LDL is associated with injury of vascular endothelial cell function. Cytochrome P450 is expressed in endothelial cells, so we speculate that oxidized LDL would probably influence the expression of *CYP1A1* and the activity of the enzyme by damaging endothelial cells, and then collaboratively damage periodontal tissue. Therefore, dyslipidemia, especially for HDL and LDL, interacting with *CYP1A1* gene polymorphisms, associated with periodontal destruction via the influence the *CYP1A1* enzymes.

This study has several strengths. The results not only demonstrated that *CYP1A1* gene rs1048943 polymorphism is associated with GAgP and periodontal status, but also that there is a correlation between *CYP1A1* rs1048943 and platelet-related inflammation, which strongly indicates that *CYP1A1* rs1048943 is a genetic contributor to GAgP. Since *CYP1A1* rs1048943 is demonstrated to contribute to CVD,[9] this indicates that gene polyploidy of CYP1A1 contributes to both GAgP and CVD. These results contribute to knowledge of the shared genetic risk factors of two conditions. Furthermore, this study found that hyperlipidemia enhanced the effect of *CYP1A1* gene rs1048943 polymorphism on periodontal status, so that patients with a history of hyperlipidemia require additional attention. Moreover, considering that hyperlipidemia is also

a risk factor for CVD,[16] the gene-environment interaction between the *CYP1A1* gene and hyperlipidemia provides a valuable avenue for exploring the shared genetic and environmental risk factors of the two conditions. Given these results, medical evaluation of AgP patients should include a blood lipid profile, especially for HDL and LDL. This study also has several limitations. It is a population-based case-control study and so fails to avoid selective bias due to genetic heterogeneity. Furthermore, the cross-sectional design makes it impossible to determine the direction of causality. In addition, this study identified a statistical interaction, but determining whether there is a biological interaction requires further basic experimental research. Moreover, we cannot exclude the possibility that the healthy control subjects included in this study younger than 35 years of age may develop chronic periodontitis later in life. Last but not least, only one locus of *CYP1A1* gene was examined in this study. Other loci will be studied by our group in the future.

In conclusion, the present study demonstrated that individuals with a GG/AG *CYP1A1* rs1048943 genotype had increased susceptibility to GAgP when compared to individuals with an AA genotype. Furthermore, patients with the GG/AG genotypes showed worse periodontal status and platelet-related inflammation. More importantly, a novel gene-environment interaction between *CYP1A1* rs1048943 and dyslipidemia was found to effect periodontal status. These findings not only provide new strategies and recommendations for the future treatment and mechanisms of AgP pathogenesis, but also provide new evidence that periodontitis and CVD share genetic and environmental risk factors.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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