Journal of
PERIODONTAL RESEARCH

J Periodont Res 2015; 50: 509–518

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12237

Associations of apolipoprotein E and low-density lipoprotein receptor-related protein 5 polymorphisms with dyslipidemia and generalized aggressive periodontitis in a Chinese population

H. Gao*, Y. Tian*, H. Meng, J. Hou, L. Xu, L. Zhang, D. Shi, R. Lu, X. Feng, X. Wang, Z. Chen
Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China

Gao H, Tian Y, Meng H, Hou J, Xu L, Zhang L, Shi D, Lu R, Feng X, Wang X, Chen Z. Associations of apolipoprotein E and low-density lipoprotein receptor-related protein 5 polymorphisms with dyslipidemia and generalized aggressive periodontitis in a Chinese population. J Periodont Res 2015; 50: 509–518. © 2014 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd

Background and Objective: Dyslipidemia is associated with aggressive periodontitis, a condition characterized by the rapid destruction of the periodontium. Apolipoprotein E (APOE) and low-density lipoprotein receptor-related protein 5 (LRP5) are involved in immunomodulation and inflammatory activity. We evaluated the association of LRP5 and APOE polymorphisms with serum lipid concentrations and generalized aggressive periodontitis within a Chinese population.

Material and Methods: Mean serum lipid concentrations were compared across LRP5 and APOE polymorphisms, among cases (n = 185) and controls (n = 138). Multivariable logistic regression was used to evaluate the independent and combined associations of LRP5 and APOE polymorphisms with generalized aggressive periodontitis.

Results: Compared with controls, individuals with generalized aggressive periodontitis exhibited significantly lower serum total cholesterol (TC) and lower high-density lipoprotein cholesterol (HDL-c). Individuals with LRP5 polymorphisms (rs682429-AA or rs312016-GG) exhibited higher TC, higher HDL-c and decreased odds for generalized aggressive periodontitis. Haplotype (A-G), determined by rs682429 and rs312016, was also associated with decreased odds for generalized aggressive periodontitis. Furthermore, individuals with the combined polymorphisms (LRP5-rs682429-AA and APOE-rs429358-CC/CT) had higher levels of low-density lipoprotein cholesterol, higher levels of TC and decreased odds for generalized aggressive periodontitis.

Huanxin Meng, BDS, MS, PhD, Peking University School and Hospital of Stomatology, 22, Zhongguancun Nandajie, Hai Dian District, Beijing 100081, China
Tel: +86 10 82195522
Fax: +86 10 62173402
e-mail: kqhxmeng@bjmu.edu.cn

*These authors contributed equally to this study.

Key words: apolipoprotein E; generalized aggressive periodontitis; low-density lipoprotein receptor-related protein 5; serum lipid; single nucleotide polymorphisms

Accepted for publication August 23, 2014

Conclusion: Independently or combined with APOE, LRP5 polymorphisms may lead to dyslipidemia and are associated with generalized aggressive periodontitis. Dyslipidemia may be a risk indicator for generalized aggressive periodontitis in the Chinese population. Furthermore, two LRP5 polymorphisms (rs682429 and rs312016) might be useful for identifying subjects at higher risk of generalized aggressive periodontitis.

Aggressive periodontitis is an uncommon and severe form of periodontitis, characterized by the rapid destruction of the periodontium in otherwise healthy adolescents and young adults (1). Although microbes are often cited as the primary causative agent of aggressive periodontitis, the manifestation and progression of the disease results from the complex interactions between oral bacteria, the host response and behavioral factors. It has also been suggested that genetic factors seem to play an important role in predisposing individuals to periodontitis, which is supported by several genetic segregation analyses of families and a twin study of chronic periodontitis (2,3).

Evidence is building that hyperlipidemia is a risk indicator for periodontal disease (4–6). Studies have found that severe periodontitis is associated with lower levels of high-density lipoprotein cholesterol (HDL-c), higher levels of low-density lipoprotein cholesterol (LDL-c) and significantly higher levels of plasma triglycerides (7–9). However, contradictory findings have also been reported (10–12).

Nutrition is also an important risk factor for periodontitis, as poor nutrition may inhibit the immune response (13). Specifically, foods high in dietary cholesterol or fatty acids may inhibit the immune response and accelerate the bactericidal effect on *Porphyromonas gingivalis* (14). Although the importance of nutrition on the immune function has been established, it remains unclear whether abnormalities in lipid metabolism or conditions related to dyslipidemia lead to periodontal disease or if periodontal disease leads to impaired lipid metabolism (15).

Apolipoprotein E (APOE) plays an important role in the uptake of lipids. APOE binds to cholesterol to form a highly hydrophilic lipoprotein, allowing efficient transportation in the blood (16), and mediates the binding between the lipoprotein and the low-density lipoprotein receptor (17). Of all the different APOE isoforms, the APOE ε4 isoform is that which is most strongly associated with higher lipid levels and increased risk of cardiovascular disease (18). Furthermore, APOE is involved in immunomodulation and inflammatory activity (19); APOE polymorphisms have been associated with a range of inflammatory and autoimmune diseases and disorders, including Alzheimer's disease (20), diabetic nephropathy (21), severe hepatitis C infection (22), high levels of C-reactive protein (23) and rheumatoid arthritis (16).

Similarly, low-density lipoprotein receptor-related protein 5 (LRP5) plays a regulatory role in cholesterol metabolism (24) and is involved in the clearance of APOE-containing lipoproteins (25). Recent studies have shown that APOE/LRP5 double-knockout mice exhibited higher plasma cholesterol levels that were 60% higher than the levels among APOE knockout mice (26,27). Furthermore, LRP5 is also a co-receptor of Wnt/β-catenin signaling (28), a mechanism that might be involved in the development of periodontitis (29). LRP5 polymorphisms have been associated with cholesterol level (30), bone mineral density (31), osteoporosis (32), diabetes mellitus (33), obesity (34), bone fracture (35), Alzheimer's disease (36) and tumors (28,37,38). As a result of the established relationship between periodontitis and osteoporosis, diabetes mellitus and obesity (15,38), we suspect that LRP5 polymorphisms might also be associated with periodontitis.

As far as the researchers are aware, no published studies have evaluated the independent or combined association of APOE and LRP5 polymorphisms with generalized aggressive periodontitis. The purpose of the current study is two-fold: (i) to evaluate the relationship between the APOE and LRP5 polymorphisms and serum lipid concentrations; and (ii) to evaluate the independent and combined association of APOE (rs429358, rs7412) and LRP5 (rs312016, rs682429) polymorphisms with generalized aggressive periodontitis in a Chinese population.

Material and methods

Study population

A total of 323 unrelated individuals of Chinese ethnicity volunteered to participate in this case–control study. Each case received a full periodontal examination and a set of full-mouth radiographs were obtained. Two senior, experienced periodontists, who were calibrated, carried out all clinical examinations and diagnosis. The kappa value of intrarater agreement for the diagnosis of generalized aggressive periodontitis was estimated for 50 individuals, whilst the kappa value for inter-rater agreement for the diagnosis of generalized aggressive periodontitis was estimated for 20 individuals. The kappa values for intrarater/inter-rater agreement were 0.98 and 0.95, respectively. Patients with generalized aggressive periodontitis (cases) ($n = 185$) were recruited from the Clinic of Periodontology Department, Peking University School and Hospital of Stomatology, from July 2001 to December 2012. We used the 1999 International Classification of Periodontal Diseases and

Conditions (1) as the diagnostic criteria for generalized aggressive periodontitis, which includes all of the following characteristics: (i) under 35 years of age; (ii) at least six teeth (at least three of which were not first molars or incisors) with a probing depth of ≥ 5 mm and clinical attachment loss of ≥ 3 mm; (iii) no periodontal treatments within the past 12 mo; (iv) female cases were not pregnant or lactating; and (v) clinically healthy, except for the presence of periodontitis. The patients with generalized aggressive periodontitis had 22.80 ± 4.91 (range, 10–28) teeth that met the inclusion criterion. Eight cases over 35 years of age were also included because of their diagnosis of aggressive periodontitis when younger than 35 years of age.

Controls (n = 138) were selected from individuals undergoing regular physical check-ups. To be considered a control, the individual must not have had any previous or existing clinical evidence of periodontitis (e.g. probing depth ≤ 3 mm; clinical attachment distances ≤ 1 mm from the cemento–enamel junction; and < 10% of sites with a bleeding index of ≥ 2) or a familial history of severe periodontitis or a known systemic disorder (e.g. atherosclerosis, diabetes, rheumatoid arthritis) that could affect the periodontal conditions. Smoking status was assessed by questionnaires. Height and body weight were measured and body mass index (BMI) was calculated accordingly.

Isolation of genomic DNA and blood examination

A peripheral blood sample, divided into two tubes, was obtained through venipuncture between 8:00 h and 10:00 h, following a fasting period. The tube containing EDTA was used for genomic DNA isolation; the other tube was used for measurement of serum lipid concentrations and fasting serum glucose. Genomic DNA was extracted from each sample using a blood DNA mini kit (Watson Biotechnologies, Inc., Shanghai, China), following the manufacturer's instructions. Serum lipid concentrations, including total cholesterol (TC), triglyceride, HDL-c and LDL-c levels, were measured using a biochemical analyzer (Hitachi 7060; Hitachi, Tokyo, Japan); fasting serum glucose levels were also measured using a biochemical analyzer.

Genotyping

The SEQUENOM MassARRAY matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry platform was used to genotype the LRP5 (rs682429, rs312016) and APOE (rs429358, rs7412) polymorphisms (Sequenom, San Diego, CA, USA). Primers for the PCR and single base extension were designed using the ASSAY DESIGNER software package (Sequenom).

Multiplex PCR was performed in 5-μL volumes containing 0.5 units of HotStar Taq polymerase (Qiagen, Hilden, Germany), 10 ng of whole-genome-amplified genomic DNA, 5 pmol of each primer and 5 μmol of deoxynucleotide triphosphates (dNTPs). Thermocycling was carried out at 94°C for 15 min followed by 45 cycles each at 94°C for 20 s, 56°C for 30 s and 72°C for 1 min, followed by a final incubation at 72°C for 3 min.

Unincorporated dNTPs were deactivated using 0.3 units of shrimp alkaline phosphatase (Sequenom) followed by primer extension using 5.6 pmol of each primer-extension probe, 50 μmol of the appropriate dNTP/dideoxynucleotide triphosphate (ddNTP) combination, and 0.5 units of iPLEX enzyme (Sequenom). The extension reactions were carried out in the following order: one cycle at 94°C for 30 s; one cycle at 94°C for 5 s; five cycles at 52°C for 5 s; and five cycles at 80°C for 5 s; followed by a final incubation at 72°C for 3 min. A cation exchange resin was used to remove residual salt from the reactions. Purified primer-extension reaction products were spotted onto a 384-well spectroCHIP by a MassARRAY Nanodispenser and determined by MALDI-TOF mass spectrometry.

Genotype calling was performed in real time with MASSARRAY RT software version 3.0.0.4 (Sequenom, San Diego, CA, USA) and analyzed using MASSARRAY TYPER software version 3.4 (Sequenom). As a quality check of our data, we sequenced more than 5% of the samples (eight cases and seven controls) using an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA); the genotype concordance rate between duplicate samples was 100%.

Statistical analyses

Preliminary analysis explored differences in participant characteristics between the case group and control using t-tests and ANOVA for continuous variables and chi-square tests for categorical variables. Multiple linear regression was used to compare the serum lipid concentration between the case group and the control group and among subjects with different genotypes after adjusting for major confounders (age, sex, BMI and smoking status). In order to establish genetic inference of our data, Hardy–Weinberg equilibrium (HWE) testing was carried out for the LRP5 (rs312016, rs682429) and APOE (rs429358, rs7412) polymorphisms.

Multivariate logistic regression analysis was performed to test the association between LRP5 polymorphisms, APOE polymorphisms and aggressive periodontitis, after adjusting for major covariates (age, sex, BMI and smoking status). Pairwise linkage disequilibrium values (D′ and r^2) were calculated using HAPLOVIEW version 4.1 (Broad Institute, Cambridge, MA, USA) for markers in LRP5. The statistical power was calculated using PASS version 11.0 (NCSS, LLC., Kaysville, UT, USA).

Prior to statistical analysis, variables of a skewed distribution were logarithmically transformed to meet the assumption of normality; estimates were subsequently back-transformed for presentation in the tables. A type I error level was set a priori at 0.05. Preliminary results were presented in terms of mean ± SD or interquartile ranges for variables that did not meet the assumption of normality. Categorical variables were presented as counts and percentages. Results were presented in terms of adjusted odds ratios

Table 1. Demographic characteristics of the aggressive periodontitis cases and controls

Variable	Cases (patients with generalizedaggressive periodontitis) (n = 185)[a]	Controls (n = 138)[a]	p-value
Age (years)			
Mean ± SD	27.4 ± 5.1	28.6 ± 7.2	0.095[b]
Range	14–48	20–50	
Sex			
Male	75 (40.5)	56 (40.6)	
Female	110 (59.5)	82 (59.4)	0.994[c]
Smoking status			
Nonsmokers	164 (88.6)	135 (97.8)	
Current smokers	21 (11.4)	3 (2.2)	0.002[c]
BMI			
18.5–24.9	127 (68.6)	119 (86.2)	
< 18.5	23 (12.4)	11 (14.5)	
≥ 25.0	35 (18.9)	8 (5.8)	0.001[c]

BMI, body mass index.
[a]Data are presented in terms of mean ± SD or total n (%).
[b]Comparison of age between cases (patients with generalized aggressive periodontitis) and controls, performed using a two sample t-test.
[c]Comparison of sex, smoking status and BMI between cases (patients with generalized aggressive periodontitis) and controls, performed using a chi-square test.

(AORs) and 95% confidence intervals (CIs). Analyses were performed using SPSS version 17.0 (IBM Co., Armonk, NY, USA).

Ethical considerations

This study received ethical approval from the Ethics Committee of Peking University Health Science Center. Written informed consent was obtained from all the participants, in accordance with the Declaration of Helsinki.

Results

Demographic characteristics of the cases with generalized aggressive periodontitis and the controls are presented in Table 1. No significant difference of age and sex was detected by an independent t-test and the chi-square test. The proportion of smoking adults was significantly higher among the cases than the controls (proportions 11.4% vs. 2.2%, respectively; p = 0.002). The proportion of individuals with a BMI of ≥ 25.0 was significantly higher among the cases than the controls (proportions 18.9% vs. 5.8%, respectively; p = 0.001).

The mean serum lipid concentrations, fasting serum glucose and periodontal parameters among cases with generalized aggressive periodontitis and controls are displayed in Table 2. The mean values of the periodontal parameters were higher among the cases than the controls.

Compared with controls, cases exhibited significantly lower TC, reduced HDL-c and higher fasting serum glucose levels after adjusting for age, sex, BMI and smoking status (TC: 163.38 ± 32.91 mg/dL vs. 171.95 ± 28.65 mg/dL, p = 0.021; HDL-c: 57.82 ± 12.15 mg/dL vs. 63.04 ± 15.45 mg/dL, p = 0.015; fasting blood glucose: 94.01 ± 9.54 mg/dL vs. 88.48 ± 9.14 mg/dL, p < 0.001). Cases with generalized aggressive periodontitis tended to have a higher serum triglyceride level compared with controls (80.70 ± 1.63 mg/dL vs. 72.33 ± 1.57 mg/dL, p = 0.059).

For all the single-nucleotide polymorphisms (SNPs), genotype call rates were > 98% and minor allele frequencies were > 0.01. No evidence of deviation from Hardy–Weinberg equilibrium was found for all investigated SNPs (p > 0.05). Two SNPs in LRP5 were in strong linkage disequilibrium (D' = 0.99, r^2 = 0.96). Our sample size provided the power of 0.86, 0.86, 0.80 and 0.74 to detect an effective size of odds ratio = 0.4 for the rs682429, rs312016, rs429358 and rs7412, respectively.

There were significant differences in the distributions of LRP5 and APOE polymorphisms between cases with generalized aggressive periodontitis and controls, after adjusting for age, sex, BMI and smoking status in the multivariate logistic regression model (Table 3). Individuals with the LRP5-rs312016-GG genotype had decreased odds (AOR = 0.44, 95% CI: 0.23–0.83) for generalized aggressive

Table 2. Mean serum lipid concentrations and periodontal parameters according to generalized aggressive periodontitis status

Characteristics	Cases (patients withgeneralized aggressive periodontitis$N1$	Controls$N2$	p-value	p-value[a]
TC (mg/dL)	163.38 ± 32.91, 179	171.95 ± 28.65, 96	0.032[b]	0.021
LDL-c (mg/dL)	90.61 ± 28.76, 149	95.46 ± 22.34, 78	0.163[b]	0.306
HDL-c (mg/dL)	57.82 ± 12.15, 149	63.04 ± 15.45, 78	0.011[b]	0.015
TG (mg/dL)	80.70 ± 1.63, 180	72.33 ± 1.57, 96	0.069[b]	0.059
Glucose (mg/dL)	94.01 ± 9.54, 181	88.48 ± 9.14, 98	< 0.001[b]	< 0.001
Mean PD (mm)	4.88 ± 1.00, 181	1.84 ± 0.41, 135	< 0.001[c]	NA
Mean BI	3.51 ± 0.46, 169	1.09 ± 0.34, 134	< 0.001[c]	NA

Data are presented as Mean ± SD, n.
BI, bleeding index; BMI, body mass index; HDL-c, high-density lipoprotein cholesterol, LDL-c, low-density lipoprotein cholesterol; $N1$, number of cases; $N2$, number of controls; NA, not accessible; PD, probing depth; TC, total cholesterol; TG, triglycerides.
[a]Adjusted for sex (male, female), age (tertile), smoking status (no, yes) and BMI (BMI < 24.9, BMI ≥ 25.0).
[b]Comparison of mean serum lipid concentrations, metabolic markers between generalized aggressive periodontitis cases and controls performed using an independent t-test.
[c]Comparison of mean bleeding index and probing depth between generalized aggressive periodontitis cases and controls performed using a Mann–Whitney U-test.

Table 3. Associations of low-density lipoprotein receptor-related protein 5 (LRP5) polymorphisms with generalized aggressive periodontitis

SNP	Cases (patients with generalized aggressive periodontitis)[a]	Controls[a]	Crude OR (95% CI)	AOR[b] (95% CI)
rs312016				
AA/AG	159 (85.9)	103 (75.2)	1 [reference]	1 [reference]
GG	26 (14.1)	34 (24.8)	0.50 (0.28–0.87)*	0.44 (0.23–0.83)*
rs682429				
GG/AG	160 (86.5)	104 (75.4)	1 [reference]	1 [reference]
AA	25 (13.5)	34 (24.6)	0.48 (0.27–0.45)*	0.42 (0.22–0.81)*
rs429358				
TT	159 (87.8)	112 (81.2)	1 [reference]	1 [reference]
CC/CT	22 (12.2)	26 (18.8)	0.60 (0.32–1.11)	0.49 (0.24–0.99)*
rs7412				
CC	155 (84.7)	112 (83.0)	1 [reference]	1 [reference]
TT/CT	28 (15.3)	23 (17.0)	0.88 (0.48–1.61)	0.97 (0.50–1.88)

AOR, adjusted odds ratio; OR, odds ratio; SNP, single-nucleotide polymorphism.
*Significant at $p = 0.05$.
[a]Values represent *n* (%).
[b]Adjusted for sex (male, female), age (tertile), smoking status (no, yes) and body mass index (BMI) (BMI < 24.9, BMI ≥ 25.0).

periodontitis compared with individuals with the LRP5-rs312016-AA/AG genotype, after adjusting for age, sex, BMI and smoking status. Individuals with the LRP5-rs682429-AA polymorphism had decreased odds (AOR = 0.42, 95% CI: 0.22–0.81) for generalized aggressive periodontitis compared with individuals with the LRP5-rs682429-GG/AG polymorphism, after adjusting for age, sex, BMI and smoking status. Individuals with APOE-rs429358-CC/CT had decreased odds (AOR = 0.49, 95% CI: 0.24–0.99) for generalized aggressive periodontitis compared with individuals with the APOE-rs429358-TT polymorphism, after adjusting for age, sex, BMI and smoking status.

Table 4 demonstrates that individuals with the LRP5 haplotype (rs682429-rs312016: A–G) had decreased odds for generalized aggressive periodontitis compared with individuals with the LRP5 haplotype (rs682429-rs312016: G–A) after adjusting for age, sex and smoking status (AOR = 0.59, 95% CI: 0.41–0.84).

Individuals with the combined genotype (LRP5-rs682429-AA + APOE-rs429358-CC/CT and LRP5-rs682429-AA + APOE-r429358-TT) had decreased odds of generalized aggressive periodontitis compared with individuals with the LRP5-rs682429-GG/AG genotype and the APOE-rs429358-TT genotype, after adjusting for age, sex, BMI and smoking status (AOR = 0.15, 95% CI: 0.03–0.92; AOR = 0.45, 95% CI: 0.22–0.92, respectively).

Table 4. Distribution of low-density lipoprotein receptor-related protein 5 (LRP5) haplotype (rs682429-rs312016) frequencies and odds ratios (ORs) for generalized aggressive periodontitis

Haplotype	Cases (patients with generalized aggressive periodontitis)[a]	Controls[a]	Crude OR (95% CI)	AOR[b] (95% CI)
Total	365	273		
G–A	226 (61.9)	140 (51.3)	1 [reference]	1 [reference]
A–G	139 (38.1)	133 (48.7)	0.65 (0.47–0.89)*	0.59 (0.41–0.84)*

AOR, adjusted odds ratio.
*Significant at $p = 0.05$.
[a]Values represent *n* (%).
[b]Adjusted for sex (male, female), age (tertile), smoking status (no, yes) and body mass index (BMI) (BMI < 24.9, BMI ≥ 25.0).

Figure 1 illustrates the differences in mean serum lipid concentrations between individuals with different LRP5 genotypes (LRP5-rs682429-AA vs. GG/AG; LRP5-rs312016- GG vs. AA/AG), among cases with generalized aggressive periodontitis and controls. In general, individuals with LRP5-rs682429-AA or LRP5-rs312016-GG exhibited higher TC and HDL-c levels than did those with LRP5-rs682429-GG/AG or LRP5-rs312016-AA/AG polymorphisms.

Figure 2 illustrates the differences in mean serum lipid concentrations between individuals with different combined genotypes among cases with generalized aggressive periodontitis and controls. Individuals with the combined genotype of LRP5-rs682429-AA and APOE-rs429358-CC/CT exhibited significantly higher TC and LDL-c levels compared with individuals with the LRP5-rs682429-GG/AG genotype and the APOE-rs429358-TT genotype.

Discussion

Four important findings emerged from our study: (i) compared with controls, individuals with generalized aggressive periodontitis exhibited significantly lower serum TC and lower HDL-c; (ii) individuals with LRP5 SNPs (rs682429-AA or rs312016-GG) exhibited higher TC, higher HDL-c and decreased odds for generalized aggressive periodontitis; (iii) individuals with combined polymorphisms (LRP5-rs682429-AA and APOE-rs429358-CC/CT) had higher serum LDL-c and TC levels, as well as decreased odds for generalized aggressive periodontitis; and (iv) individuals with the LRP5 haplotype (rs682429-rs312016: A–G) had decreased odds for generalized aggressive periodontitis. Our findings build on previous lipid research and contribute to the limited body of knowledge of serum lipid concentrations and the LRP5 and APOE polymorphisms.

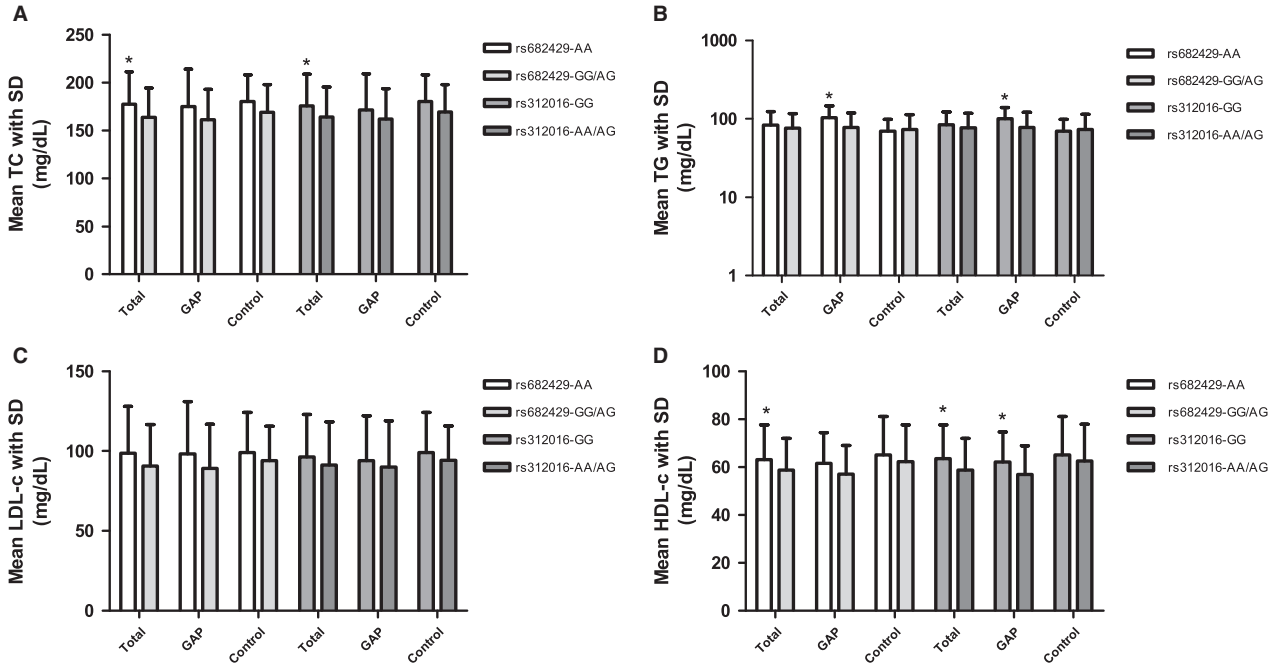

Fig. 1. Comparison of serum lipid concentrations between individuals with different genotypes (rs682429-AA vs. GG/AG; rs312016-GG vs. AA/AG), among cases (patients with generalized aggressive periodontitis) and controls. Serum lipid concentrations of total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein cholesterol (LDL-c) (C) and high-density lipoprotein cholesterol (HDL-c) (D) were compared between individuals with different genotypes (rs682429-AA vs. GG/AG; rs312016-GG vs. AA/AG, respectively) among generalized aggressive periodontitis cases and controls after adjusting for sex (male, female), age (tertile), smoking status (no, yes) and body mass index (BMI) (BMI < 24.9, BMI ≥ 25.0). Results are displayed in terms of means ± SD; statistical significance was determined using multiple linear regression (*$p < 0.05$). GAP, generalized aggressive periodontitis.

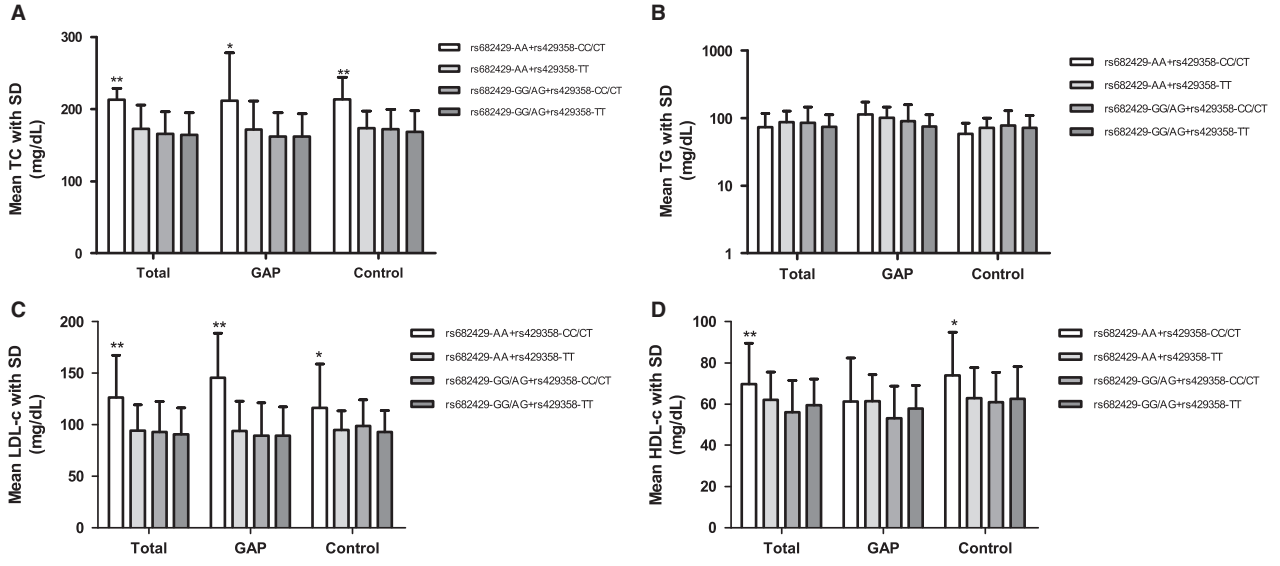

Fig. 2. Comparison of serum lipid concentrations between individuals with different combined genotypes (rs682429-AA+rs429358-CC/CT vs. rs682429-AA+rs429358- TT vs. rs682429-GG/AG+rs429358-CC/CT vs. rs682429-GG/AG+rs429358-TT), among cases (patients with generalized aggressive periodontitis) and controls. Serum lipid concentrations of total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein cholesterol (LDL-c) (C) and high-density lipoprotein cholesterol (HDL-c) (D) were compared between individuals with different combined genotypes (rs682429-AA+rs429358-CC/CT vs. rs682429-AA+rs429358-TT vs. rs682429-GG/AG+rs429358 -CC/CT vs. rs682429- GG/AG+rs429358-TT) among generalized aggressive periodontitis cases and controls after adjusting for sex (male, female), age (tertile), smoking status (no, yes) and body mass index (BMI) (BMI < 24.9, BMI ≥ 25.0). Results are displayed in terms of means ± SD; statistical significance was determined using multiple linear regression (*$p < 0.05$, **$p < 0.01$).

Inflammation and infection are two processes that frequently induce dyslipidemia (39). Biomarkers of inflammation, including tumor necrosis factor, interleukin-2 and interferon-gamma, have been shown to increase serum triglyceride levels and decrease serum cholesterol (40). Additionally, lipopolysaccharide injection (41) and severe sepsis (42) reduce LDL-c and HDL-c levels. Dyslipidemia is often observed among individuals with chronic inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus (43).

Despite this growing understanding of chronic inflammatory conditions and dyslipidemia, the relationship between periodontitis and dyslipidemia is still unclear. Several epidemiological studies have shown the association between hyperlipidemia and periodontal disease. Hyperlipidemia is a risk indicator for periodontal disease (5); specifically, two studies have found higher TC and LDL-c levels among individuals with periodontitis (4,6). However, epidemiological evidence has also presented contradictory findings (10,12). In addition, periodontal treatment plays a beneficial role in lipid metabolism (44,45).

Our findings showed that patients with generalized aggressive periodontitis (the cases) exhibited significantly lower TC and HDL-c levels than controls in a Chinese population. Few studies have evaluated the relationship between dyslipidemia and generalized aggressive periodontitis. Rufail *et al.* (46) observed higher TC and triglyceride levels among individuals with aggressive periodontitis compared with controls, whilst Davis *et al.* (47) reported that levels of inflammatory mediators and serum lipid concentrations were not significantly different among individuals with aggressive periodontitis compared with controls. Our findings are somewhat comparable to results found in a case–control study of chronic periodontitis among a Chinese Han population (48). The discrepancy between our results and those of other studies might be a result of the poor periodontal condition and the severe inflammation in our cases with generalized aggressive periodontitis, as preliminary findings from our study population found systemic inflammation (49–51) among the aggressive periodontitis cases. Additionally, differences in the age and race/ethnicity distributions of these samples could have also contributed to these discrepancies.

In our study, we observed a significant association between two LRP5 SNPs (rs682429, rs312016) or LRP5 haplotypes and generalized aggressive periodontitis, after adjusting for age, sex and smoking status. Considering the functional interaction between APOE and LRP5 genotypes, we attempted to elucidate the relationship between combined polymorphisms and generalized aggressive periodontitis. We observed a significant combined association between APOE and LRP5 polymorphisms and generalized aggressive periodontitis, after adjusting for age, sex, smoking status and BMI; this finding suggests that the APOE and LRP5 polymorphisms may play a critical role in generalized aggressive periodontitis. APOE has been shown to play an important role in immune responses (19,52) and in the presentation of lipid antigens to immune cells (53), whilst LRP5 is a co-receptor of Wnt/β-catenin signaling that has been reported to mediate leukocyte-inflammatory responses (54).

Additionally, we observed higher levels of TC and HDL-c levels among individuals with the LRP5-rs682429 AA or LRP5-rs312016 GG polymorphisms. A significant association between the LRP5-rs3736228 (30) and LRP5-A1330V (31) polymorphisms and hypercholesterolemia has previously been observed. Although the loci we selected were different from those in the above two studies, our results were consistent with their findings, further suggesting that the LRP5 polymorphism is significantly associated with cholesterol levels. The mechanism of action linking LRP5 and generalized aggressive periodontitis probably involves a decrease in hepatic clearance of chylomicron remnants (26).

Indeed, there is an abundance of research supporting the association of APOE polymorphisms with serum lipid concentrations across various study populations (55,56), including Chinese populations (57–59). Earlier studies showed that APOE ε2 reduced the levels of TC and LDL-c, whilst APOE ε4 increased the levels of TC and LDL-c (60). Recently, a meta-analysis revealed that there was approximately linear relationships of APOE polymorphisms (when ordered ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, ε4/ε4) with levels of LDL-c and TC and with coronary risk (18). Our study builds on previous research by confirming the combined association of LRP5-rs682429 and APOE-rs429358 polymorphisms with TC and LDL-c levels among a Chinese population of generalized aggressive periodontitis cases and controls. A recent meta-analysis found that periodontal treatment improved lipid metabolism, especially in those individuals suffering from both periodontitis and comorbidities, such as cardiovascular disease and diabetes mellitus (45). Therefore, periodontitis might precede the occurrence of dyslipidemia.

Strengths and limitations

The strengths of our study include: the novelty of our research question, the consistency in genotyping with previous studies, and the comparability of our results. In general, the polymorphisms and allele frequencies of both APOE and LRP5 were similar to those of previous studies among the Chinese population (61–63). The allele APOE ε2 has a cytosine-to-thymine base-pair substitution in rs7412, whilst in APOE ε4, a thymine is replaced with a cytosine in rs429358 (64). The polymorphisms and allele frequencies of both APOE and LRP5 vary considerably across race/ethnicity. The APOE allele frequencies were similar to that of Japanese (65) and Korean (65) individuals, but were obviously different to those in subjects from other countries, such as Mongolia (66), Malay Aborigines (65), central Africa (65), Brazil (67) and Northern European countries (65). The ε3 allele frequency increased in Chinese individuals, whereas the ε4

allele frequency decreased. In addition, the two SNPs within LRP5 were in strong linkage disequilibrium, which was consistent with a previous study (68). The A allele frequency at rs682429 in the present study was lower than that of US Caucasians (0.481 vs. 0.714), whilst the G allele frequency at rs312016 was lower than that of US Caucasians and Italians (0.485 vs. 0.714 and 0.675, respectively) (34,37,69).

A limitation exists in the relatively small sample size after stratifying according to polymorphisms. Although we observed statistically significant differences in the TC and LDL-c levels across the combined genotype groups, it warrants future investigation with a larger sample size to verify the results of the present study. In addition, the association of LRP5-rs682429, rs312016 polymorphisms and LRP5 haplotypes with aggressive periodontitis need to be replicated in an independent population to confirm the validity.

Implications

Our study contributes to the limited body of knowledge of serum lipid concentrations and the LRP5 and APOE polymorphisms and is among the first to evaluate the independent and combined associations of LRP5 and APOE polymorphisms with aggressive periodontitis. The results from our study suggest that LRP5 and APOE polymorphisms may lead to dyslipidemia and are associated with generalized aggressive periodontitis. The LRP5-rs682429 or LRP5-rs312016 polymorphisms are associated with the levels of TC and HDL-c. The combination of LRP5-rs682429 and APOE-rs429358 polymorphisms is related to the levels of TC and LDL-c. The APOE and LRP5 polymorphisms may partly account for lower TC and HDL-c levels, suggesting that dyslipidemia may be a risk indicator for generalized aggressive periodontitis in the Chinese population. Furthermore, two LRP5 SNPs (rs682429, rs312016) might be useful for identifying individuals at higher risk of generalized aggressive periodontitis.

Acknowledgements

We acknowledge that this work was financially supported by the following organizations: the National Natural Science Foundations of China, Beijing, China (30471882, 30973319, 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.

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