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# Apolipoprotein E gene polymorphisms in relation to chronic periodontitis, periodontopathic bacteria, and lipid levels

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ABSTRACT

*Objective:* Inflammatory periodontal diseases may be associated with common systemic conditions and, as recently described, alterations in lipid levels in the blood. The aim of this study was to determine the possible effects of apolipoprotein E (*ApoE*) genotypes on the lipid levels in healthy people and patients with chronic periodontitis (CP) in relation to periodontopathic bacteria.

*Design:* This case–control study comprised 469 unrelated subjects. The genomic DNA of 294 patients with CP and 175 healthy/non-periodontitis controls were genotyped, using the real-time polymerase chain reaction (RT-PCR) method, for *ApoE* (rs429358 and rs7412) *gene* polymorphisms. Subgingival bacterial colonization was investigated by the DNA microarray using a periodontal pathogen detection kit and lipid levels were measured in a subgroup of subjects ($N = 275$).

*Results:* There was no evidence for a significant association between *ApoE* gene polymorphisms and CP ($P > 0.05$). Patients with CP had increased levels of total cholesterol and low-density lipoprotein (LDL) compared to controls ($P < 0.05$); however, no significant difference was found for triglyceride and high-density lipoprotein (HDL) levels. *ApoE* gene variability influenced LDL levels marginally ($P = 0.08$) but it did not modify total

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*Abbreviations:* ANOVA, analysis of variance; ApoE, apolipoprotein E; ApoER2, apolipoprotein E receptor 2; CAD, coronary artery disease; CAL, clinical attachment loss; CI, confidence interval; CP, chronic periodontitis; CPITN, Community Periodontal Index of Treatment Needs; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MFA, minor allele frequency; NF-κB, nuclear factor κB; NLR, NOD-like receptor; OR, odds ratio; *P*, *P*-value; $P_{corr}$, *P*-value correction; PD, probing depth; PRR, pattern recognition receptor; RT-PCR, real-time polymerase chain reaction; SD, standard deviation; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; TNF-α, tumour necrosis factor α; TREM-1, triggering receptors expressed on myeloid cells-1; VLDL, very low-density lipoprotein; VLDLR, VLDL receptor; *A.a.*, *Aggregatibacter actinomycetemcomitans*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *T.f.*, *Tannerella forsythia*; *T.d.*, *Treponema denticola*; *P.m.*, *Peptostreptococcus micros*; *F.n.*, *Fusobacterium nucleatum*.

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cholesterol, triglyceride, and HDL levels or the occurrence of periodontal pathogens in subgingival pockets.[23]

*Conclusions:* In the Czech population studied, *ApoE* genetic variations were not associated with susceptibility to CP or the presence of periodontopathic bacteria.



## 1. Introduction

Periodontitis is a chronic infectious disease characterized by a progressive inflammatory response to bacteria in dental plaque, which finally results in periodontal tissue destruction and eventual tooth loss. Evidence suggests that the local inflammation/infection triggers a systemic host response associated with an increased risk of cardiovascular diseases and atherosclerosis.[1] Periodontitis has also been associated with glucose intolerance,[2] endothelial dysfunction,[3] and dyslipidemia and metabolic syndrome.[4]

Apolipoprotein E (ApoE) is key protein, playing many roles in lipid absorption, transport, local homeostasis in the vessel walls, and endothelial function.[5] Although ApoE is most often associated with the onset of atherosclerosis or related to Alzheimer's disease, it may have an influence on other biological processes, such as sepsis and inflammation.[6] ApoE is mainly produced by cells in the liver where this glycoprotein plays a role in lipoprotein binding to specific receptors. However, ApoE molecules are also synthesized in circulating macrophages,[7] and their production may be negatively influenced by cytokines.[8] ApoE signalling via very low-density lipoprotein receptor (VLDLR) or ApoE receptor 2 (ApoER2) promotes macrophage conversion from the proinflammatory (M1) to the anti-inflammatory (M2) phenotype. This effect may represent a novel anti-inflammatory activity of ApoE.[9] The ApoE protein also promotes the Th1 cytokine response by modulating cytokine production. The binding region of ApoE has been shown to have a direct anti-infective activity against various viral and bacterial species.[10]

The *ApoE* gene is localized on chromosome 19 at position q13.2 and comprises three codominant alleles ε2, ε3, and ε4,[11] which are defined by two non-synonymous single-nucleotide polymorphisms (SNPs) in *ApoE* exon 4. Both SNPs (rs429358 and rs7412) are cytosine/thymine (C/T) substitutions changing arginine (Arg) to cysteine (Cys) in the amino acid sequence of ApoE. In the case of SNP rs429358, the T allele is ancestral and corresponds to the presence of Cys, while a gene variant with C (MFA, minor allele frequency; C = 0.149) encodes Arg at position 112 of the ApoE protein. On the other hand, the ancestral allele for SNP rs7412 is variant with C and then the T allele with MAF 0.074 is responsible for the presence of Cys in the ApoE amino acid sequence at position 158. The allelic compositions of the haplotypes are TT for ε2, TC for the most frequent allele ε3, and CC for ε4, with corresponding protein isoforms 112Cys/158Cys for ApoE2, 112Cys/158Arg for ApoE3 and 112Arg/158Arg for ApoE4. Variability in the *ApoE* gene has functional effects on lipoprotein metabolism mediated through the hepatic binding, uptake, and catabolism of chylomicrons, chylomicron remnants, VLDL, and high-density lipoprotein (HDL) cholesterol subspecies. Allelic variation in *ApoE* is consistently associated with plasma concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, and apo B (the major protein of LDL, VLDL, and chylomicrons). Specifically, *Apoε2* exhibits reduced binding to the receptors, and it contributes to the accumulation of remnant particles in plasma derived from the partial catabolism of triglyceride-rich lipoproteins. On the other hand, *Apoε4* shows increased binding to the receptors leading to increased metabolism of triglyceride-rich lipoprotein and reduced triglyceride level in plasma.[12] Compared to the Apoε3/ε3 group, carriers of the ε2 allele tend to have increased levels of ApoE and decreased levels of total and LDL cholesterol,[13,14] whereas ε4 carriers tend to exhibit decreased levels of ApoE and increased levels of total and LDL cholesterol.[15] E2 and E3 isoforms prefer binding to HDL, while E4 prefers VLDL.[16] These biochemical differences may be responsible for the association of the ApoE isoforms with various pathologic processes. E4 isoform is associated with an increased risk of coronary heart disease[17] and Alzheimer's disease.[18] By contrast, the E2 isoform, which may have a protective character in the case of Alzheimer's disease, atherosclerosis,[19] and chronic HCV (hepatitis C virus) infection,[20] is associated with familial hyperlipoproteinemia type III[21] and the risk of type 2 diabetes mellitus.[22]

However, to date, no study analyzing *ApoE* gene polymorphisms in patients with periodontitis has been performed. Considering the results of previous studies describing changes in concentrations of lipids in patients with periodontitis[23–30] and the fact that hyperlipidemia can impair the immune response to *Porphyromonas gingivalis*,[31] the aim of this study was to determine possible effects of *ApoE* genotypes on the lipid levels in healthy people and patients with chronic periodontitis (CP) in relation to periodontopathic bacteria.

## 2. Materials and methods

### 2.1. *Study population*

All patients were recruited from the patient pool of the Periodontology Department, Clinic of Stomatology, St. Anne's Faculty Hospital Brno, from 2005 to 2013. They had at least 20 remaining teeth and they were of good general health. The exclusion criteria included a history of cardiovascular disorders (such as coronary artery diseases (CADs) or hypertension), diabetes mellitus, malignant diseases, immunodeficiency, current pregnancy, or lactation. The control group consisted of unrelated subjects who did not have a clinical history of periodontal disease. Controls were selected randomly during the same period as patients and matched for age, gender, and smoking status. Similar to patients, all controls had at least 20

remaining teeth and they were of good general health. The exclusion criteria were the same as those applied for patients with periodontitis.

The study was performed with the approval of the Committees for Ethics of the Medical Faculty, Masaryk University Brno and St. Anne's Faculty Hospital. Written informed consent was obtained from all participants, in line with the Helsinki declaration before inclusion in the study.

### 2.2. Case–control study

A total of 469 unrelated Caucasian subjects of exclusively Czech ethnicity from the region of South Moravia were included in this case–control association study. The diagnosis of non-periodontitis/periodontitis was based on detailed clinical examination, medical and dental history, and radiographic assessment. Probing depth (PD) and clinical attachment loss (CAL) were examined by a UNC-15 periodontal probe from six sites on every tooth present. The loss of the alveolar bone was determined radiographically. All patients with generalized CP ($N = 294$) fulfilled the diagnostic criteria defined according to CAL levels by the International Workshop for the Classification of Periodontal Diseases and Conditions for Chronic Periodontitis.[32] The inclusion criteria for patients suffering from generalized CP were as follows: $\geq$30% of the teeth were affected and PD of $\geq$4 mm. Controls (healthy/non-periodontitis, $N = 175$) were screened using a World Health Organization (WHO) probe and the CPITN (Community Periodontal Index of Treatment Needs) was assessed; the values of the CPITN index in controls were $<3$. Phenotyping was performed by two experienced periodontologists (J.V. and H.P.).

In order to adjust for the effect of a history of smoking on periodontal disease, the subjects (patients and controls) were classified into the following groups: subjects who never smoked (referred to as nonsmokers) and subjects who were former smokers or current smokers (referred to as smokers).

### 2.3. Estimation of lipid levels

Venous blood (5 ml) was collected in a plain test tube and serum was separated. The levels of the total cholesterol, triglycerides, LDL, and HDL were estimated using a semiautomatic analyzer and a commercial machine Modular (Roche).

### 2.4. Genetic analysis

#### 2.4.1. DNA-microarray analysis of oral pathogens

Subgingival bacterial colonization (*Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Treponema denticola*, *Peptostreptococcus micros*, and *Fusobacterium nucleatum*) in subgingival pockets was investigated by the DNA microarray using a periodontal pathogen detection kit (Protean Ltd., Ceske Budejovice, Czech Republic) in a subgroup of randomly selected subjects ($N = 164$ for patients with CP, and $N = 111$ for controls) before subgingival scaling. In patients with periodontitis, microbial samples were collected from the deepest pockets (and from the deepest sulcus in healthy subjects) of each quadrant by inserting a sterile paper point into a base of the pocket for 20 s. Bacterial plaque samples from each individual were pooled in one tube. This test determined the individual pathogens semiquantitatively as follows: (–) undetected, which corresponds to the number of bacteria $<10^3$; (+) slightly positive corresponding to the number of bacteria $10^3$–$10^4$; (++) positive corresponding to the number of bacteria $10^4$–$10^5$; and (+++) strongly positive, with the number of bacteria $>10^5$.

#### 2.4.2. Isolation of genomic DNA

DNA for genetic analysis was extracted from the peripheral blood leukocytes using standard phenol/chloroform procedures with proteinase K.[33]

#### 2.4.3. Real-time polymerase chain reaction

Two SNPs in exon 4 at the positions rs429358C/T (112C/R) and rs7412C/T (158C/R) were analyzed using the real-time polymerase chain reaction (RT-PCR) method monitored by SYBR® Green.[34] This method was optimized due to the formation of dimers, which interfered with the analysis as described previously.[19] SDS version 1.2.3 software was used to analyze real-time and end-point fluorescence data. Genotyping was performed by one investigator (P.B.L.) unaware of the phenotype.

### 2.5. Statistical analysis

Statistical analysis was performed using the statistical package Statistica v. 10 (StatSoft Inc., USA). The $\chi^2$ test and Fisher's exact test were used to assess case–control differences in distribution of *ApoE* genotypes and alleles. To compare independent groups, one-way analysis of variance (ANOVA) and Kruskal–Wallis tests were performed to compare continuous variables and Pearson $\chi^2$ analysis was used for the comparison of categorical variables. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated as the strength of association between genotypes and alleles and CP. A $P$-value $<0.05$ was considered statistically significant. Where appropriate, the Bonferroni correction was used to adjust the level according to the number of independent comparisons to the overall value of 0.05. Adjusted $P$ values for particular analyses are denoted as $P_{corr}$.

The estimation was performed using Power and Precision software based on a calculation algorithm for logistic regression with dichotomous categorical predictors.[35]

## 3. Results

The demographic data of the study population are shown in Table 1. The mean ages for patients with generalized CP and

**Table 1 – Demographic characteristics of the Czech study population.**

| Characteristics | Controls | Patients with CP |
|---|---|---|
| Number of subjects | 175 | 294 |
| Mean age (years ± SD) | 43.7 ± 9.8 | 50.1 ± 8.6 |
| Age range (years) | 35–55 | 40–60 |
| Sex ratio (male/female) | 90/85 | 145/149 |
| Percentage of smokers | 27.8 | 30.4 |

CP, chronic periodontitis; SD, standard deviation.

**Table 2 – Distribution of *ApoE* genotypes and alleles between patients with CP and controls.**

| | Genotypes | | | | | | Alleles | | |
|---|---|---|---|---|---|---|---|---|---|
| | ε2/ε2 | ε2/ε3 | ε2/ε4 | ε3/ε3 | ε3/ε4 | ε4/ε4 | ε2 | ε3 | ε4 |
| Controls (N = 175) | 3 (1.7) | 14 (8.0) | 2 (1.1) | 126 (72.0) | 26 (14.9) | 4 (2.3) | 22 (6.3) | 292 (83.4) | 36 (10.3) |
| Patients with CP (N = 294) | 3 (1.0) | 36 (12.2) | 4 (1.4) | 201 (68.4) | 46 (15.6) | 4 (1.4) | 46 (7.8) | 484 (82.3) | 58 (9.9) |

*ApoE*, apolipoprotein E; CP, chronic periodontitis.

healthy subjects were similar. There was no significant difference between patients with periodontitis and controls regarding the male/female ratio and mean percentage of smokers ($P > 0.05$). As the controls were age/gender matched, the lack of demographic difference is an expected result. The study was optimized to keep 80% power (with $\alpha < 0.05$) to detect OR in the range of 1.9–2.2 as statistically significant, for alleles/genotypes with at least 15% frequency.

The distribution of *ApoE* genotypes is shown in Table 2. The frequencies of *ApoE* genotypes in the healthy and periodontitis groups were found in accordance with those expected by the Hardy–Weinberg equilibrium ($P > 0.05$). As expected, ε3 was the most prevalent *ApoE* allele (82.9%), with a near-equal representation of the ε2 and ε4 alleles (7.1% and 10.1%, respectively). The ε3/ε3 genotype was the most common (70.2%) and ε2/ε2 was the least prevalent genotype (1.4%). No association was observed between *ApoE* alleles or genotypes and periodontitis susceptibility in the Czech population, as the frequency distributions were similar between the patients with CP and controls ($P > 0.05$, Table 2). In addition, when the group of subjects was divided according to smoking status, no difference in the frequencies of alleles or genotypes of *ApoE* variants was detected (data not shown).

The comparison of lipid profiles (Table 3) revealed that the total plasma cholesterol and LDL levels were significantly higher in patients with CP than in controls ($P < 0.05$), whereas no significant difference was found between triglyceride and HDL levels. The analysis of associations between *ApoE* polymorphisms and the lipid levels by Kruskal–Wallis ANOVA showed that *ApoE* gene variability affected the LDL levels marginally (although statistically insignificantly) ($P = 0.08$, Fig. 1); conversely, the total cholesterol, triglyceride, and HDL levels were not affected by this polymorphism in our study. The highest LDL levels were detected in persons with ε3/ε4 genotype (median = 3.67 mmol/l); lower levels were found in the carriers of ε2/ε3 genotypes (median = 2.84 mmol/l) and the lowest in the presence of the ε3/ε3 genotype combination (median = 2.34 mmol/l, Fig. 1). Genotypes ε2/ε2, ε2/ε4, and ε4/ε4 in association with the lipid levels were not assessed due to a low number of the individuals with these variants. No statistically significant difference in relationship to *ApoE* gene variability and LDL levels between the controls and patients with periodontitis was found ($P > 0.05$, data not shown).

Possible links between the genetic variant of *ApoE* and microbiological colonization (occurrence of periodontal bacteria in subgingival pockets, including *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, *T. denticola*, *P. micros*, and *F. nucleatum*) were assessed in the subgroups of patients with generalized CP (N = 164) and controls (N = 111). We found significant differences in the occurrence of periodontal pathogens between both groups (from $P < 0.00001$ to $P < 0.05$, data not shown); however, no significant association between *ApoE* polymorphisms and these pathogens was observed in healthy and/or periodontitis groups after correction for multiple comparisons (Table 4).

**Table 3 – Lipid levels in controls and patients with CP.**

| Parameter | Controls (mean mmol/l ± SD) | Patients with CP (mean mmol/l ± SD) | P-value |
|---|---|---|---|
| Total cholesterol | 4.94 ± 0.67 | 5.56 ± 0.89 | **0.038** |
| Triglycerides | 1.67 ± 1.13 | 1.52 ± 0.88 | 0.663 |
| LDL | 2.12 ± 0.44 | 2.66 ± 0.86 | **0.044** |
| HDL | 1.29 ± 0.38 | 1.34 ± 0.36 | 0.688 |

CP, chronic periodontitis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.
Bold values mean that P-values are below 0.05 ($P < 0.05$).

## 4. Discussion

Periodontitis is a chronic inflammation of the supporting tissues of the teeth affecting approximately 30–50% of the population.[36] It was hypothesized that episodes of bacteremia originating from inflammatory periodontal lesions were the cause of the changes in systemic circulation.[37] Periodontitis

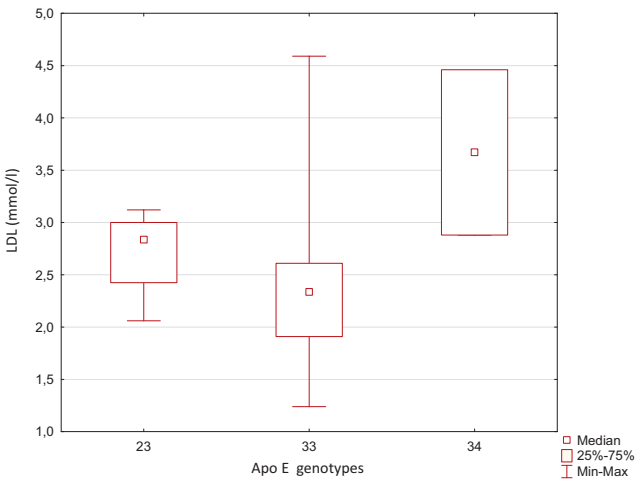


**Fig. 1 – *ApoE* genotypes in relation to LDL levels. *ApoE*, apolipoprotein E; LDL, low-density lipoprotein.**

**Table 4 – Association of the individual *ApoE* genotype distributions and the occurrence of periodontal pathogens in subgroups of subjects with generalized CP (N = 164) and healthy controls (N = 111).**

| Positive findings | *F.n.* (%) | *A.a.* (%) | *P.g.* (%) | *T.f.* (%) | *T.d.* (%) | *P.m.* (%) | *P.i.* (%) |
|---|---|---|---|---|---|---|---|
| Controls *ApoE* | | | | | | | |
| ε2/ε3 (N = 17) | 94.1 | 23.5 | 76.5 | 76.5 | 64.7 | 52.9 | 64.7 |
| ε3/ε3(N = 73) | 91.8 | 26.0 | 58.9 | 83.6 | 65.8 | 78.1 | 53.4 |
| ε3/ε4 (N = 21) | 95.2 | 38.1 | 52.4 | 66.7 | 61.9 | 90.5 | 71.4 |
| P ($P_{corr}$) | 0.84 | 0.50 | 0.29 | 0.23 | 0.95 | **0.02** (0.28) | 0.29 |
| CP *ApoE* | | | | | | | |
| ε2/ε3 (N = 17) | 88.2 | 5.9 | 41.2 | 88.2 | 64.7 | 76.5 | 58.8 |
| ε3/ε3 (N = 121) | 98.3 | 33.1 | 55.4 | 79.3 | 57.9 | 76.0 | 50.4 |
| ε3/ε4 (N = 26) | 96.2 | 30.8 | 57.7 | 73.1 | 57.7 | 73.1 | 57.7 |
| P | 0.07 | 0.07 | 0.51 | 0.49 | 0.86 | 0.95 | 0.68 |

*ApoE*, apolipoprotein E; CP, chronic periodontitis; P, statistical significance by $\chi^2$ test (comparison of a number of negative and positive findings in patients with different ApoE genotypes); $P_{corr}$, correction for multiple comparison by Bonferroni method.
*A.a.*, *Aggregatibacter actinomycetemcomitans*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *T.f.*, *Tannerella forsythia*; *T.d.*, *Treponema denticola*; *P.m.*, *Peptostreptococcus micros*; *F.n.*, *Fusobacterium nucleatum*.
Bold values mean that P-values are below 0.05 ($P < 0.05$).

may be associated with a number of systemic diseases and pathological conditions including hyperlipidemia.[38] The amino acid exchanges at positions 112 and 158 in ApoE result in three common isoforms, which differ in protein functionality. Therefore, we investigated the relationship between *ApoE* genotypes, lipid levels, presence of periodontal pathogens, and CP in the Czech population.

We found increased levels of serum cholesterol and LDL in patients with CP, similar to a very recent study conducted on the Indian population.[39] The association of serum LDL cholesterol level with periodontitis was also observed among patients visiting a tertiary-care hospital in Nepal.[27] No significant increase in serum triglyceride level and a decrease in HDL levels were observed in Czech patients with CP in contrast to other studies.[28,30] Some studies also reported decreased HDL concentrations in patients with CP[40,41] or association between periodontal disease and elevated levels of plasma triglycerides.[42] In addition, the correlation between periodontal disease and dyslipidemia has also been observed in a variety of animal models. The ApoE-deficient (−/−) mice exhibited hyperlipidemia under a normal diet.[43] Although no difference in serum cholesterol was noted, lipid accumulation was observed in the aortas of New Zealand white rabbits with periodontitis induced in response to ligature and application of *P. gingivalis*.[44] Finally, increased serum cholesterol level but no change in triglyceride levels in ligature-induced periodontitis were observed in nonhuman primates.[45] Thus, animal studies also support the concept of a correlation between periodontal disease and dyslipidemia.[46] However, other studies suggested no significant relationship between periodontal disease, regardless of its intensity, and blood lipid levels in systematic healthy subjects.[47] Some studies reported a positive association between periodontal pockets and serum cholesterol only in males but not in females[48] or a relationship between the presence of periodontal pockets of >4 mm and increased triglyceride level and reduced HDL level only in obese subjects.[49] A variety of confounders (type of study, disease definition, associated diseases, inclusion and exclusion criteria, etc.) can thus influence the interpretation of studies correlating dyslipidemia with periodontitis.

Although changes in systemic lipid metabolism have been observed for a long time, the mechanism underlying the correlation between periodontal disease and dyslipidemia still remains unclear. However, a recent study by Lei et al. suggested a possible mechanism by which hyperlipidemia could modify the risk of periodontitis.[31] The authors found that long-term hyperlipidemia impaired the immune response to *P. gingivalis* challenge by altering the expression of pattern recognition receptors (PRRs) in macrophages, leading to an inhibited cytokine network response and disrupted host capability to clear periodontal pathogens, thus possibly resulting in more severe periodontal bone loss.[31,50] In addition, increased serum lipid level alters the cholesterol component in the cell membrane and cytoplasma of macrophages, which might further affect PRR expression on the cell membrane upon *P. gingivalis* challenge leading to altered inflammatory response to bacterial infection in the hyperlipidemic host.[31]

In contrast to the associations of periodontitis with lipid levels, we did not find any significant relationship between *ApoE* variants and lipid profiles or the presence of periodontal pathogens in Czech patients with CP. The present study showed that variability in the *ApoE* gene influenced LDL levels marginally (P = 0.08), but it did not modify total cholesterol, triglyceride, and HDL levels or the occurrence of periodontal pathogens in subgingival pockets. Our results cannot be compared with any other study as this is the first study on *ApoE* gene polymorphisms in CP. However, the association of *ApoE* variants with lipid levels has been described in many studies.[13,14] The "ancestral" ε4 allele has been associated with elevated levels of total cholesterol and LDL,[51] and thus with atherosclerosis and CAD risk. In our study, subjects with the Apoε3/ε4 genotype had on average higher LDL levels compared to those with Apoε3/ε3 genotype; the latter, however, exhibited an extremely high variance of the measured values, which is very likely the reason of the insignificant (only marginal) association between the *ApoE* gene and LDL. The influence of *ApoE* polymorphisms on CAD can be explained by a differential effect on the lipid spectrum, as proven by a large prospective study.[15] However, lipid-independent mechanisms can have a great influence and may be important in

inflammatory diseases, such as periodontitis. The anti-inflammatory effect of *ApoE* alleles decreases in the sequence $\varepsilon 2 > \varepsilon 3 > \varepsilon 4$ according to Jofre-Monseny and colleagues.[52] Our study did not confirm this finding as no significant difference in allele frequencies between patients with CP and healthy controls was detected. Therefore, we presume that the different LDL levels can be determined by other factors, and not only by variability in the *ApoE* gene.

The main limitation of the present study is the case–control approach used, as it is generally vulnerable to population stratification. The present sample, however, is exclusively of Czech Caucasian origin, restricted to a limited geographical area. As all the patients in the current study are native Czechs, the results may be generalized only to white Caucasian subjects. The next complicating factor in the study of isolated locus (such as *ApoE*) is the multifactorial etiopathogenesis of periodontitis, in which interactions between several genes/gene variants and environmental factors play a role and potentially affect the observed phenotype. To our knowledge, this study is the first to analyze associations between polymorphisms in *ApoE*, baseline lipid levels, and the presence of periodontopathic bacteria in patients with periodontitis. The sample size was large enough to detect the association with an acceptable level of significance. In the current study, we did not find any significant association between *ApoE* polymorphism and susceptibility to and severity of CP or the presence of periodontopathic bacteria, but we confirmed the finding of high levels of total cholesterol and LDL in patients with periodontitis. However, the results of the study need to be proven in an independent cohort.

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## Competing interest

The authors declare that they have no conflict of interest.

## Ethical approval

The study was performed with the approval of the Committees for Ethics of the Medical Faculty, Masaryk University Brno and St. Anne's Faculty Hospital. Written informed consent was obtained from all participants, in line with the Helsinki declaration before inclusion in the study.

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