Apolipoprotein E, periodontal disease and the risk for atherosclerosis: a review

L.C. Pereira[1], J.C.R, Nascimento[1], J.M.C., Rêgo[1], K.M., Canuto[1], M.E., Crespo-Lopez[4], J.I., Alvarez Leite[3], A. Baysan[2], R.B., Oriá[1,*]

[1]Laboratory of the Biology of Tissue Healing, Ontogeny and Nutrition, Department of Morphology and Institute of Biomedicine, School of Medicine, Federal University of Ceara, Fortaleza, CE, Brazil

[2]Institute of Dentistry, Barts and the London, School of Medicine and Dentistry, Queen Mary University, London, UK.

[3]Institute of Biological Sciences, Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[4]Laboratory of Molecular Pharmacology, Institute of Biological Sciences, Federal University of Para, Belém, PA, Brazil.

***Corresponding author:**

Reinado B. Oriá, PhD

Institute of Biomedicine, School of Medicine, Federal University of Ceara

Rua Cel. Nunes de Melo, 1315 – Rodolfo Teófilo

Fortaleza, Ceará, Brazil, 60430-270

Phone: +55 85 3366-8239 / Fax: +55 85 3366-8445

Email: rbo5u@virginia.edu; reinaldo70.oria@gmail.com

Running head: ApoE, chronic periodontitis and atherosclerosis

Highlights

- **Accumulating evidence has demonstrated a relationship between chronic periodontal diseases and atherosclerosis**
- **Periodontal pathogens have been associated with atherosclerosis in apoE knockout mice.**
- **ApoE4 may be a candidate antagonistic pleiotropy gene in periodontal diseases.**

ABSTRACT

The association between cardiovascular and periodontal diseases is characterized by chronic inflammatory processes, with a high prevalence worldwide and complex genetic-environment interactions. Although apolipoprotein E4 (ApoE4), one of the isoforms coded by a polymorphic APOE gene, has been widely recognized as a risk factor for cardiovascular diseases and as an immunoinflammatory factor, but less is known regarding how ApoE4 affects atherosclerosis in periodontitis patients. The aim of this review was to investigate the potential underlying mechanisms related to APOE4 that could increase the risk of periodontal disease and, ultimately, of atherosclerosis. There have only been a few studies addressing apoE polymorphisms in patients with chronic periodontitis. To date, no studies have been performed that have assessed how ApoE4 affects atherosclerotic disease in chronic periodontitis patients. Although clinical studies are warranted, experimental studies have consistently documented the presence of periodontal pathogens, which are usually found in the oral cavity and saliva, in the atherosclerotic plaques of ApoE-deficient mice. In addition, in this review, the potential role of the APOE4 allele as an example of antagonistic pleiotropy during human evolution and its relation to oral health is discussed.

Keywords: Apolipoprotein E, periodontal disease, oral microbiota, inflammation, atherosclerosis

Correspondence

Reinaldo B. Oriá, Institute of Biomedicine and Department of Morphology, School of Medicine, Federal University of Ceara. Rua Coronel Nunes de Melo, 1315, CEP: 60430-270, Fortaleza, CE, Brazil. Email: oria@ufc.br; Phone: +55 08533668239

1. Introduction

Periodontal disease (PD) is a multifactorial, chronic, inflammatory disease, associated with bacterial plaque, gingival bleeding, edema and increased crevicular fluid formation that results from endotoxin bacteria-driven host immune responses. PD is characterized by the inflammation and destruction of tooth-supporting connective tissues in response to subgingival infection by various periodontal pathogens (Kinane, Stathopoulou, & Papapanou, 2017). Periodontitis pathophysiology is associated with a myriad of pro-inflammatory cytokines, skewed towards Th1/Th17 responses (de Vries, Andreotta, Loos, & Nicu, 2017) and induced osteolysis (Goncalves, Evangelista, da Silva et al., 2014), the latter of which has been found to be reduced by simvastatin treatment (a cholesterol-lowering drug) (Dalcico, de Menezes, Deocleciano et al., 2013).

Due to its chronic inflammatory etiology, a potential link between the occurrence of periodontal disease and atherosclerosis has been investigated (Cardoso, Reis, & Manzanares-Cespedes, 2018). Pioneer studies from Mattila and colleagues have suggested associations between myocardial infarction and poor oral health, which could be facilitated by chronic low grade bacterial infections (such as dental caries and chronic periodontitis) (Mattila, Nieminen, Valtonen et al., 1989). However, the determination of a causative effect has been hampered because patients with chronic illnesses (including metabolic syndrome and diabetes) are also likely to be affected by periodontitis. However, in the studies reported by Mattila, poor oral health was a predictor of myocardial infarction, even after adjusting for age, social class, hypertension, serum lipid and lipoprotein concentrations, smoking, the presence of diabetes, and serum C peptide concentrations (Mattila, Nieminen, Valtonen et al., 1989).

The complications associated with atherosclerosis and metabolic syndrome are recognized as worrisome public health problems in developing countries and may especially affect populations with nutritional and immunoinflammatory disorders, with the likelihood of increased fatalities (DeBoer, Lima, Oria et al., 2012; Teo & Dokainish, 2017). The systemic inflammatory processes (even those that are low-grade) induced by chronic periodontal

disease (Cardoso, Reis, & Manzanares-Cespedes, 2018) may be related to peripheral arterial (and endothelial) inflammatory conditions (Calapkorur, Alkan, Tasdemir et al., 2017), which may further elevate the risks of atherosclerosis, coronary disease, myocardial infarction, and death.

Cardiovascular diseases (CVD) and periodontal disease share genetic bases of susceptibility and important behavioral components, such as diet, plaque control and smoking-related habits. Studies have shown that both conditions increase with age, lower socioeconomic strata and poor educational background, especially for males, the diabetic population, and individuals undergoing psychological stress or who have a significant genetic predisposition (Nazir, 2017).

Apolipoprotein E (ApoE=protein; APOE=gene) is a glycoprotein synthesized in the liver that plays a key role in the catabolism of triglycerides and cholesterol. In addition, ApoE plays a pivotal role in different inflammatory conditions (Azevedo, Bolick, Roche et al., 2014; Azevedo, Oliveira, Oliveira et al., 2012). In animal models, an ApoE deficiency can lead to increased lipid levels in the bloodstream, even under chow diet conditions. In addition, APOE null mice have been extensively used as animal models for atherosclerosis, as these mice accumulate atheroma in the aortae when chronically fed with cholesterol-enriched diets (Getz & Reardon, 2016).

In this review, recent findings were discussed, highlighting how different ApoE isotypes may affect atherosclerosis in patients with periodontal disease, and discuss about the use of APOE knockout mice as tool for understanding how oral pathogens can be translocated and their effects on the atherosclerotic plaque. Finally, it was speculated that the APOE4 allele may play a potential role as an example of antagonistic pleiotropy during human evolution in relation to oral health. Antagonistic pleiotropy is an evolutionary theory (Gaillard & Lemaitre, 2017) that postulates that certain genes or alleles may differentially impact fitness during the lifespan of an organism (Tuminello & Han, 2011).

2. Periodontal and cardiovascular diseases

Teeth are supported by a connective tissue attachment apparatus (periodontal ligament), which is partly inserted into the outer layer of the root surface (root cementum) and partly into the bone of the maxillary or mandibular alveolar processes, and, to a lesser extent, by the gingival tissues that surround the teeth (Cho & Garant, 2000).

In periodontal disease, inflammatory processes induced by microbes result in the formation of a periodontal pocket with the apical migration of both the gingival attachment to the root surface and the plaque biofilm, gingival recession and alveolar bone resorption (Hajishengallis & Sahingur, 2014).

According to the WHO, severe periodontal disease affects 15% to 20% of the middle-aged global population (35–44 years of age) and may cause 5% to 15% of total tooth loss. Approximately 50% of periodontal diseases have been attributed to genetic variance (Rajendra Santosh, Ogle, Williams, & Woodbine, 2017).

Atherosclerosis or atherosclerotic vascular disease is a chronic inflammatory process characterized by the progressive accumulation of atheroma plaques in the vessel endothelium, which originate from inflammatory responses and immune system activation (Nguyen, Kim, Quan et al., 2015).

Oral infections may interfere with vascular, endothelial, and smooth muscle cell biology, which could cause predisposition to atherosclerosis (Chistiakov, Orekhov, & Bobryshev, 2016). Oral pathogen-related inflammation can promote pro-coagulant effects, followed by greater scavenger receptor (SR) A and CD36 expression and activity by macrophages. (Baer, Huang, & Gibson, III, 2009) The uptake of cholesterol-rich LDL was also related to the increased expression of cell-adhesion molecules (such as integrin CD49c (α3-subunit) in monocytes) (Escate, Padro, & Badimon, 2016). In addition, smooth muscle cell proliferation, migration and anti-apoptosis machinery can be upregulated after exposure to certain oral pathogens, such as *Porphyromonas gingivalis*, which are known to induce pro-inflammatory cytokines in periodontal tissues (Naruishi & Nagata, 2018; Teles & Wang, 2011). Furthermore, oral pathogens may exert pro-atherosclerotic effects by activating the

uptake of oxidized LDL (LDLox) and the production of pro-inflammatory cytokines (such as TNF, IL-6 and IL1-β) and transforming macrophages in "foam cells". The migration of other leukocyte subtypes (such as lymphocytes) contributes to the increased inflammatory status and leads to atherosclerotic plaque instability and rupture (Nguyen, Kim, Quan et al., 2015).

Chronic inflammatory processes driven by the periodontal biofilm may be facilitated by complex host-pathogen interactions (e.g., worsened by immunosuppression and pathogenic virulence). Multiple risk factors, such as age, CVD familial history, dyslipidemia, smoking, systemic arterial hypertension, sedentary lifestyle, diabetes mellitus and being overweight may debilitate the host and create an appropriate scenario for a pro-atherogenic effect that is spread by oral pathogens (Chistiakov, Orekhov, & Bobryshev, 2016; Lockhart, Bolger, Papapanou et al., 2012).

The American Heart Association's Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease (1992) reviewed several risk factors and found a strong association between atherosclerotic vascular disease (ASVD) and PD that was independent of known confounders. However, it was not possible to demonstrate causality between these two disorders. Therefore, therapeutic interventions for PD cannot be considered to prevent ASVD-related events at this stage (Lockhart, Bolger, Papapanou et al., 2012). In a robust meta-analysis of 15 observational studies involving 17,330 patients, Zeng et al. (Zeng, Leng, Lam et al., 2016) found that periodontal disease was an independent risk factor for atherosclerosis, although these authors reported substantial statistical heterogeneity in their studies

Ahn and colleagues (Ahn, Shin, Han et al., 2016) studied the association between PD and atherosclerosis in an adult Korean population, using carotid intima-media thickness (cIMT) and ankle-brachial index (ABI) as markers of central arterial and lower extremity stenosis, respectively. The authors observed that periodontitis was associated with subclinical atherosclerosis and peripheral arterial disease after adjusting for age, gender, education level, smoking, drinking, exercise, central obesity, triglycerides, HDL, LDL, hs-CRP, diabetes and hypertension (Ahn, Shin, Han et al., 2016). These results are consistent

with previous studies showing independent associations between periodontitis and both subclinical atherosclerosis (SA) and peripheral arterial disease (PAD), which represent early atherosclerotic vascular changes (Ahn, Shin, Han et al., 2016)

The biological mechanism through which PD contributes to atherosclerosis may involve direct bacterial effects on platelets, autoimmune responses, the bacterial invasion of endothelial cells and the systemic upregulation of pro-inflammatory mediators (Gurav, 2014). Periodontal infection results in a chronic low-grade bacteremia, which induces both local (at the blood vessel wall) and systemic inflammatory cytokine upregulation, such as IL-1β, IL-12, IL-18 and TNF-α, causing the increased expression of adhesion molecules and the release of leukocyte chemotactic chemokines, such as MCP-1/CCL2, by the endothelium (Teles & Wang, 2011). The subsequent chemoattracted leukocyte infiltration and cytokine release into the vessel wall lead to endothelial dysfunction, increasing the likelihood of atherosclerotic plaque formation, which can be detected at the early stages of an atherosclerotic lesion by the thickening of the carotid wall and the decreased patency of arteries in extremities (Ahn, Shin, Han et al., 2016). Hence, longer and more severe chronic periodontitis increases the risks of experiencing an atherogenic effect (Ahn, Shin, Han et al., 2016) in predisposed and at-risk individuals.

In a cohort of 106 patients with a diagnosis of ischemic stroke or transient ischemic attack (TIA) that were screened for periodontal disease, 27 reported recurrent vascular events (16 with TIA or ischemic stroke) at the 24-month follow-up. However, a survey demonstrated that there was an important association between increased aortic arch atheroma (AA) plaque thickness and calcification in patients with advanced PD (Sen, Chung, Duda et al., 2017). Thus, in this study, the authors concluded that, in patients with TIA or ischemic stroke, PD at an advanced stage is associated with an increase in the AA plaque thickness and is therefore, an important risk factor for recurrent vascular events (Sen, Chung, Duda et al., 2017).

3. ApoE, periodontal disease and atherosclerosis

ApoE is a 34 KDa glycoprotein, discovered in 1970, composed of 299 amino acids and synthesized primarily by the liver and exported to the plasma. ApoE is an important component of all lipoproteins, except LDL, and is involved in the redistribution of triglycerides and cholesterol in various tissues (Mahley, 2016). ApoE, similar to other apoproteins, helps to stabilize and solubilize lipoproteins while circulating in the blood. In general, the role of ApoE in lipid metabolism includes maintaining the integrity of the lipoprotein structure and acting as a ligand for lipoprotein receptors (Mahley, 2016).

The APOE gene is polymorphic, with the three most common alleles being the epsilons E2, E3 and E4 (Huebbe & Rimbach, 2017). Point mutations in the codons result in either a cysteine or arginine residue at positions 112 and 158. In the E2-coded ApoE allele, cysteine amino acids are found at both positions; in the E3-coded ApoE allele, there is a cysteine at position 112 and an arginine at position 158; and in the E4-coded ApoE allele, arginine is found at both positions. The frequency of the E2, E3 and E4 alleles vary widely between different populations, although the E3 allele is the most common, followed by E4 and E2 (Mendes-Lana, Pena, Freitas et al., 2007; Riemenschneider, Schwarz, Wagenpfeil et al., 2002). The E4 allele is considered to be the ancestral allele. In addition to these 3 isotypes, there are other rarer ApoE variants, such as E1, E5 and E7 (Gerdes, 2003).

These modifications of the ApoE isoforms are able to differentially influence lipid metabolism. Due to the tertiary structural conformations of the ApoE molecules (E3= cys-112, arg-158; E3= cys-112, arg-158; E4= arg-112, arg-158), distinct binding forces exist for the ApoE isoforms, either with the LDL receptor or with heparan sulfate proteoglycans (HSPGs), which are important for cholesterol metabolism. These interactions affect lipid levels, with the ApoE4 allele being more associated with increased total cholesterol (rather than ApoE3 and ApoE3) and increasing the risk for cardiovascular diseases. Helix 4 of the ApoE structure contains the LDL-receptor binding region (residues 136-150). In the ApoE 3 and 4 isoforms, the arginine at residue 158 builds a salt bridge with the aspartate at residue 154, allowing the arginine at residue 150 to remain in the highly basic region of the receptor

binding site. A diagram of the ApoE isoforms is depicted in **Figure 1**. Conversely, in the ApoE2 isoform, the cysteine at residue 158 does not bind to aspartate and, therefore, aspartate is allowed to bind to the arginine at residue 150, which is external to the binding site. As a consequence, the ApoE2 isoform has the lowest binding affinity to the LDL receptor.

Kesaniemi and colleagues were the first to report that the E4 phenotype is associated with an increased intestinal absorption of cholesterol (Kesaniemi, Ehnholm, & Miettinen, 1987). APOE4 is associated with increased cholesterol, low-density lipoprotein (LDL) and apolipoprotein B levels, effects which can lead to atherosclerosis, hyperlipidemia and cardiovascular diseases (Eichner, Dunn, Perveen et al., 2002). An increase in the level of LDL in the systemic circulation can activate endothelial cells and build an inflammatory response, which culminates in macrophage recruitment and atherosclerosis formation. This process is accelerated by an adjacent inflammatory disease, such as PD (Finch & Morgan, 2007; Huebbe, Nebel, Siegert et al., 2011).

In a meta-analysis, including 4,564 coronary artery disease (CAD) cases and 3,985 controls, Yin et al. (2013) documented an association between the E4 allele and an increase in the CAD risk of a Chinese population, accumulating valid evidence (Yin, Sun, Zhang et al., 2013).

Bergdah and colleagues (2008) reported on dentate and edentulous individuals and related sociodemographic data, including years of education, age and living conditions. These authors found that APOE4 was found significantly more frequently in the edentulous group (Bergdahl, Bergdahl, Nyberg, & Nilsson, 2008). However, in a Chinese case/control study, Gao and colleagues found that individuals expressing APOE-rs429358-CC/CT had decreased odds for generalized aggressive periodontitis when compared with individuals expressing the APOE-rs429358-TT polymorphism, after controlling for age, sex, BMI and smoking status (Gao, Tian, Meng et al., 2015).

These results have raised questions regarding whether genetic factors, such as APOE4, might contribute to an increased risk of developing complex dental diseases that lead to tooth loss, which is an undesirable outcome for any individuals.

Interestingly, vitamin D deficiency has been associated with chronic periodontitis (Abreu, Tatakis, Elias-Boneta et al., 2016). Vitamin D is considered an important immunomodulatory factor, regulating immune responses in the gingival epithelium and influencing the degree of host responses to *P. gingivalis* (De, Fiorentino, Guida et al., 2017; Huebbe, Nebel, Siegert et al., 2011).

In addition, APOE4 has been associated with an improvement in urinary vitamin D reuptake and a more efficient UVB-induced conversion of pro-vitamin D in the skin (Gerdes, 2003). Because APOE4 carriers have been found to have high levels of vitamin D, it can be speculated that APOE4 could protect against periodontal diseases in vitamin D-deficient endemic areas (Huebbe, Nebel, Siegert et al., 2011).

Although the relationships between APOE4 and cardiovascular diseases are better known, the existence of an APOE4 risk factor for stroke is controversial (Tang, Amiesimaka, Harrison et al., 2018); however, this allele has been associated with subclinical atherosclerotic changes (Doliner, Dong, Blanton et al., 2018) and stroke-related mortality (Rajan, Aggarwal, Schneider et al., 2016).

A model of the potential interactions between APOE4, periodontitis and atherosclerosis is shown in **Figure 2**.

4. Oral infection and risk factors for atherosclerosis in animal models

Studies in atherosclerotic-prone mice have suggested that oral pathogens influence the onset and progression of atherosclerosis (Chukkapalli, Rivera, Velsko et al., 2014), as shown by increased lipid accumulation in the arterial wall, macrophage infiltration, atheroma plaques, and inflammatory responses (Kebschull, Demmer, & Papapanou, 2010; Tuomainen, Jauhiainen, Kovanen et al., 2008).

The intraoral inoculation of *Treponema denticola* in 12- and 24-week-old ApoE -/- mice causes chronic oral infection and, consequently, chronic periodontitis, which shows that this pathogen has a strong relationship with periodontal disease and the progression of atherosclerotic disease (Chukkapalli, Rivera, Velsko et al., 2014).

Decreased HDL levels and increased total and LDL cholesterol serum levels, along with systemic inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6), may increase the risk of atherosclerosis (Ferri, Paoletti, & Corsini, 2006; Hermus, Lefrandt, Tio et al., 2010). In the ApoE -/- mouse model, mice subjected to a hyperlipidemic diet and treated with *Fusobacterium nucleatum* showed significantly higher CRP, LDL and IL-6 plasma levels, as well as lower levels of HDL, when compared with controls without *F. nucleatum* administration (Lee, Jun, Kim et al., 2012). However, oral pathogens, such as *P. gingivalis*, altered vascular responsiveness, mediated by alpha-adrenoceptors, which may not only cause periodontitis but also atherosclerosis (Gibson, III, Hong, Chou et al., 2004; Lalla, Lamster, Hofmann et al., 2003; Miyauchi, Maekawa, Aoki et al., 2012; Pereira, Vasquez, Stefanon, & Meyrelles, 2011).

Some authors have indicated that the development of atherosclerosis in ApoE-deficient mice infected with *P. gingivalis* could be prevented and inhibited by the employment of vaccination and human β-defensin-3 (Gibson, III, Hong, Chou et al., 2004; Li, Messas, Batista, Jr. et al., 2002). In another study, using the same experimental model, a significant 40% increase in the area of the atherosclerotic lesion was identified in the aortic sinus when compared with the uninfected group (Lalla, Lamster, Hofmann et al., 2003). The advancement of atherosclerosis was also present in an ApoE-deficient murine model infected with this bacterium (Li, Messas, Batista, Jr. et al., 2002).

The immune response of polybacterial infections (*P. gingivalis, T. denticola, T. forsythia and F. nucleatum*) in ApoE null mouse models is different from those observed for individual (monobacterial) infections (Rivera, Lee, Aneja et al., 2013). Polybacterial infections exhibited synergism, inducing a significant increase in plasma risk factors, such as

oxidized LDL, nitric oxide, altered lipid profiles and the formation of aortic plaques (Chukkapalli, Rivera, Velsko et al., 2014; Velsko, Chukkapalli, Rivera-Kweh et al., 2015).

In another study, using the same mice models and polymicrobial infections, three of these bacterial strains, with the exception of *F. nucleatum*, were found to result in a significant association between PD and atherosclerotic disease, and the presence of a polymicrobial infection by both areas of the aorta was identified in several of the infected mice through the PCR analysis of thoracic and abdominal aorta samples (Cui, Li, Lei et al., 2016; Rivera, Lee, Aneja et al., 2013), demonstrating that periodontitis caused by binding in experimental models may trigger an inflammatory response in the vascular wall and that nonsurgical periodontal treatment during the early stages of atherosclerosis can reduce systemic inflammation, improving the lipid profile (Cui, Li, Lei et al., 2016).

To date, preclinical studies have consistently demonstrated that periodontal pathogens are able to translocate from the oral microbiota to atherosclerotic plaques in ApoE-deficient mice (see **Table 1** for a summary of the revised literature relating periodontitis pathogens and atherosclerotic lesions). However, only three studies have reported on the effects of APOE polymorphisms in individuals with oral diseases and their associations with cardiovascular risk (**Table 2**).

5. APOE4, a candidate gene for antagonistic pleiotropy in oral infections?

Cohort studies in Brazilian shantytown communities with poor hygiene and sanitation have highlighted a protective role for the APOE4 allele in children with a history of heavy diarrhea illnesses during the first years of life in areas endemic for enteric infections and malnutrition (Oria, Patrick, Oria et al., 2010; Oria, Patrick, Zhang et al., 2005). The APOE4 allele was associated with improved cognitive scores (in a subset of children with heavy diarrheal burdens), even though APOE4 is considered to be deleterious and to increase the risk of developing Alzheimer's disease later in life (Kim, Basak, & Holtzman, 2009; Strittmatter, 2001; Yamazaki, Painter, Bu, & Kanekiyo, 2016). These initial studies were supported by findings from the Bolivian Tsimane populations, which are highly exposed to

enteric infections, where APOE4 was associated with lower C-reactive protein (CRP) serum levels (Vasunilashorn, Finch, Crimmins et al., 2011), which is a marker of systemic inflammation, suggesting reduced infection-driven inflammatory responses.

In another study from the same group, non-APOE4 carriers with a high parasite burden (those with high IgE serum levels) showed a reduction in cognitive performance, which was not observed in APOE4 carriers (Trumble, Stieglitz, Blackwell et al., 2017). These studies suggest that APOE4 might display an antagonistic pleiotropy effect, which means it may be helpful early in life during environmental challenges, such as enteric infections and malnutrition (which prevail in nonindustrialized human civilization). This idea suggests that APOE4 is better fitted for traits related to protection against pathogens and improved fertility (van, Koopman, Bodegom et al., 2017a); however, later in life, it is known to be detrimental during aging-related diseases (Finch & Morgan, 2007). This same premise may be applied to oral infections. Precivilized meat hunters were likely afflicted with severe dental diseases (and enteric diseases) and oral microbiota changes (Weyrich, Duchene, Soubrier et al., 2017) but did not live long enough to develop age-related cardiovascular and Alzheimer's diseases. Therefore, it can be speculated that APOE4 might have played a significant role in this protection. The relationships between APOE4 carriers and the oral microbiota and dental diseases, both during early life and with aging, require further investigation, particularly in regard to western diets. In addition, the associations between APOE4, periodontal diseases and Alzheimer's disease still require further research. One study has documented associations between a low number of teeth, a low level of APOE4 and lower delayed word recall scores in elderly patients in Milwaukee, WI, USA (Stein, Kryscio, Desrosiers et al., 2010).

The interactions between chronic cardiovascular disease, Alzheimer's disease and periodontitis still remain an unexplored field, and research initiatives are unfortunately unrepresented in worldwide populations, especially in the developing world. A diagram of these relationships is shown in **Figure 3**.

6. Summary

The direct causality between atherosclerosis and periodontal diseases is challenging to dissect, due to common etiological roots, such as aging, life-style (diet, sedentarism), and immune-inflammatory and genetic factors. Apolipoprotein E4 (APOE4) is an attractive gene because it has been extensively associated with increased risks and worse outcomes for cardiovascular diseases.

In human evolution, APOE4 is considered to be the ancestral APOE allele, and its preservation in our genetic pool may be related to its role in improving infectious diseases, which were ubiquitous in the preindustrialized era (Oria, Patrick, Blackman et al., 2007; Trumble, Stieglitz, Blackwell et al., 2017). Therefore, the occurrence of APOE4 might be an example of antagonistic pleiotropy, as this gene may be protective under heavy burdens of enteric infections and an adverse environment (by raising innate immunity and inflammation (Gale, Gao, Mikacenic et al., 2014)) early in life (Oria, Patrick, Zhang et al., 2005; van, Koopman, Bodegom et al., 2017a), while being deleterious during aging, contributing to atherosclerosis if one lives long enough in western societies. The antagonistic pleiotropy of genes related to worse CAD outcomes (such as APOE4) are designed for adverse environments during human evolution, which have been reported previously (Corbett, Courtiol, Lummaa et al., 2018; van, Koopman, Bodegom et al., 2017b).

Early human populations were also often afflicted with chronic dental diseases and related-oral infections (in the preantimicrobial therapy era), for which APOE4 would be potentially protective (this unexplored area deserves attention in future studies); however, with this trade-off, individuals become more prone to later cardiovascular diseases if one lives long enough, as we have observed in modern times in western societies. The lack of long-term cohorts and clinical studies examining APOE polymorphisms, especially in deprived countries, have hampered the understanding of how APOE4 affects oral pathogens and related inflammatory responses and how these affects could be associated with atherosclerotic plaques. Studies performed in both clinical settings and using APOE4 target-replacement mice are required. Long-term cohort studies with larger sample numbers in

developing countries and humanized APOE4 knock-in mice are warranted to evaluate the causal association between APOE4, PD and atherosclerosis, especially when considering the periodontal biofilm and its potential atherogenic effects.

In addition, currently, clinicians aim for minimally invasive strategies to manage dental diseases. Over the years, when older patients enter a restorative cycle, as a consequence of a series of traditionally invasive approaches, and present a high disease burden, early detection and prevention are the keys to managing dental diseases. APOE4 could be a marker for dental diseases and for chronic systematic diseases. Therefore, further laboratory and clinical studies are required to investigate these effects.

Conflicts of Interest

The authors declare no conflict of interests regarding the publication of this paper.

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