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## **Apolipoprotein E related Co-Morbidities and Alzheimer's disease**

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Running Title: Apoε Co-Morbidities

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## **Abstract**

The primary goal of advancement in clinical services is to provide a health care system that enhances an individual's quality of life. Incidence of diabetes mellitus, cardiovascular disease and associated dementia coupled with the advancing age of the population, have led to an increase in the worldwide challenge to the healthcare system. In order to overcome these challenges prior knowledge of common, reliable risk factors and their effectors is essential. The oral health constitutes one such relatively unexplored but indispensable risk factor for aforementioned co-morbidities, in the form of poor oral hygiene and tooth loss during aging. Behavioural traits such as low education, smoking, poor diet, neglect of oral health, lack of exercise, and hypertension are few of the risk factors that are shared commonly amongst these conditions. In addition, common genetic susceptibility traits such as the apolipoprotein  $\epsilon$  gene, together with an individual's life style can also influence the development of co-morbidities such as periodontitis, atherosclerosis/stroke, diabetes, and Alzheimer's disease. This review specifically addresses the susceptibility of apolipoprotein  $\epsilon$  gene allele 4 as the plausible commonality for the etiology of co-morbidities that eventually result from periodontal diseases and ultimately progress to dementia.

**Key words:** Co-morbidities, periodontitis, apolipoprotein, dyslipidemia, atherosclerosis, Alzheimer's disease

## Introduction

### *The concept of successful aging*

Successful aging describes optimisation of life expectancy while minimising physical and mental deterioration and disability. Such a state would be characterised by good health; and high levels of independent performance and cognitive functioning [1]. Absence of disease would include chronic diseases such as periodontitis (PD), cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), and Alzheimer's disease (AD) all of which have an impact on an individual's longevity and quality of life. Even if PD was manifested by an individual, but the disease process was controlled by regular dental prophylaxis, 'successful aging' would still be measured by having retained a greater number of an individual's own teeth [2, 3]. Interestingly, retention of teeth has been positively associated with higher cognitive functioning in the elderly [4]. Further support comes from longevity in the very elderly subjects, referred to as the centenarians, who appear to bypass dementia [5-7] by circumventing other conditions such as diabetes and CVD [8] supporting the potential association of multiple-co-morbidities in the development of dementia.

According to the focal infection theory [9, 10] the polymicrobial dysbiosis of PD [11] and the subsequent host's immune responses [12] are the pivotal factors that bind the eclectic mix of conditions ranging from the oral condition and T2DM, to inflammatory pathologies including vascular disease(s) and AD. The apolipoprotein gene allele 4 (apoε4) is a susceptibility gene, the inheritance of which not only predisposes individuals to infections [13] that initiate inflammation, but also cause disturbances in their lipid metabolism resulting in dyslipidaemia [14]. However, our own vision of how a risk factor such as an infection may lead to co-morbid states is illustrated in figure 1.

The apoε4 has recently been implicated in the aggressive form of periodontitis [15] and in a more aggressive onset of AD [16-18]. Specific microbes such as *Aggregatibacter actinomycetemcomitans* [A. *actinomycetemcomitans*] is associated with localised aggressive periodontitis in children and teenagers [19, 20]. The age of onset with plausible genetic factors and the host's immune response predisposing an individual to

early (aggressive) or late (chronic) onset PD [21, 22] suggests that both forms of periodontitis may eventually become recognised as one disease entity [23].

Thus, apoε4 with an environmental risk factor such as an infection, and/or a fatty diet, combined with smoking and sedentary life style, will likely enhance its biological function in favour of disease outcome. Given that the apoε4 is linked to several diseases such as PD, T2DM, CVD and AD [15-17, 24-28] all of which demonstrate an element of inflammation and dyslipidaemia in their pathogenesis [29-33], further supports the role for infections [25] as a dominant environmental response modifier of disease states. With the growing interest in co-morbid states as well as with the possible association between PD via infections and life style behaviours, it is of interest to explore apoε and its allelic variants further.

### **Apolipoprotein E**

Apolipoprotein E (apoε) is a 34 kDa plasma lipoprotein and its gene is located on the long arm of chromosome 19 (q) at position 13.2 [16, 17]. The protein structure of apoε shows two structural domains [34] which are held together with a hinge region [35] in the three human allelic (ε2, ε3, and ε4) variants [36] figure 2. The proteins of these allelic variants differ by virtue of two amino acid substitutions at the 61 and 112 amino acid positions. For example, ε2 has cysteines (Cys-61 and Cys-112); ε3 has Arg-61 and Cys-112 and ε4 has arginine (Arg-61 and Arg-112) at both positions [34]. The amino acid Cys-112 in both ε2 and ε3 preferentially bind high density lipoproteins (HDL) whereas Arg-112 in ε4 preferentially binds the very low-density lipoprotein (VLDL) lipoproteins [34]. The amino acid change in ε4 considerably alters its structure with impact on its domain interaction (Fig. 2) and subsequently function in favour of diseases [34, 37, 38] associated with an element of dyslipidaemia in their pathogenesis.

Briefly, dietary fat is converted into fatty acids largely by the various lipase enzymes aiding their digestion [39]. The simplified fatty acids are eventually absorbed by the intestinal mucosa and released into the blood stream in the form of chylomicrons [39]. These plasma lipoproteins based on their relative content of cholesterol and triglycerides are classified into four major classes such as chylomicrons, VLDL, low density

lipoproteins (LDL) and HDL. Apoε within the blood plasma acts as a form of transport for phospholipids and the nonpolar lipids such as cholesterol and triglycerides to remote body locations. Any surplus lipids are returned to the liver where they undergo several biochemical reactions for either storage as adipose tissue or conversion into vitamin D and appropriate hormones [40]. Any surplus LDL over and above its storage capacity in the blood stream is deemed harmful as it initiates atherosclerosis [41].

Apoε is abundantly synthesized by the hepatocytes in the liver [36] and in the brain, predominantly by astrocytes for local needs [42, 43]. Whilst ε2 appears to be rarely inherited, it is associated with the genetic disorder known as type III hyperlipoproteinaemia. Apoε3 is the most common isoform found in humans [44] and is considered to be the normal form [38]. Apoε4 appears to be associated with the metabolic disorder T2DM [27] and various inflammatory pathologies including aggressive periodontitis [15]; CVD [24, 26]; and AD [16, 17, 25, 28]. Apoε4 may therefore, be interfering with the phenomenon described as ‘successful ageing’ processes [1] via dyslipidaemia and behavioural traits. The main focus of this review is to envisage the plausible common risk from apoε4 in these aforementioned co-morbid states, from periodontitis to AD in relation to oral pathobionts.

## **Periodontal disease**

PD is a polymicrobial dysbiotic inflammatory disease of the tooth supporting structures, characterised by the destruction of the gingival connective tissue attachment to the root surface and adjacent alveolar bone. Over 700 different bacterial species have been identified in the oral cavity of humans, 400 of these are from the subgingival sulcus [45]. Of the 400 phylotypes of subgingival microbiota, PD involves interaction of specific bacteria; *A. actinomycetemcomitans*, *Porphyromonas gingivalis* [*P. gingivalis*], *Treponema denticola* [*T. denticola*] and *Tannerella forsythia* [*T. forsythia*] [11, 46] and are considered major contributors of human periodontal disease(s) [11, 47].

Disease progression depends on the host’s inflammatory and immune responses to the pathogens [12]. As a consequence of host-pathogen interaction, low grade inflammatory mediators are continuously being

released [48] and these locally breach the periodontal pocket integrity exposing vascular channels to a flow of inflammatory mediator rich sustenance, favourable for the exponential growth of subgingival microflora. Destruction of host gingival tissues is the consequence of this exposure [49, 50]. Incidence of transient bacteraemia following chewing, tooth brushing and scaling in individuals with periodontal inflammation [51], enabling oral bacteria and bacterial components hematogenous to several systemic organs. Poor oral hygiene, and genetic susceptibility with apoe and low-density lipoprotein receptor-related protein 5 (LRP5) polymorphisms and in the neuropeptide Y (NPY) gene in aggressive periodontitis in the susceptible male host (whereas it is downregulated in female subjects) have been identified suggesting a sex-specific effect of genetic variation of NPY on PD [52]. Genetic polymorphisms would appear to be a risk factor in developing PD, which subsequently associates with remote organ metabolic states such as diabetes [53], and inflammatory pathologies such as vascular disease(s) [54, 55], and AD [56-61], and others that are out of the scope of this review.

Nutrition plays an important role in the development of PD. Poor nutrition, specifically foods high in dietary cholesterol or fatty acids inhibit the immune system [62]. However, it remains unclear whether it is abnormal lipid metabolism or dyslipidaemia that leads to PD or PD leads to impaired lipid metabolism [63]. Dyslipidaemia frequently results from infections that initiate release of inflammatory mediators in the form of cytokines including tumour necrosis factor-alpha (TNF- $\alpha$ ), interleukin-2 (IL-2) and interferon-gamma (IFN- $\gamma$ ) that increase serum triglyceride levels and suppress fatty acid oxidation [14, 64]. Our own vision of how cytokines from periodontal infection in the susceptible host may lead to disturbances in lipid metabolism is illustrated in figure 3.

The case-control study of Gao et al. [15] described four important findings in relation to LRP and dyslipidaemia in the Chinese PD patients. These are; individuals with generalised aggressive periodontitis showed significantly lower total cholesterol and lower HDL than controls; and individuals with LRP5 SNPs (rs682429-AA or rs312016-GG) showed higher total cholesterol, higher HDL and decreased odds for aggressive periodontitis; and individuals with combined polymorphisms (LRP5-rs682429-AA and APOE-rs429358-CC/CT) had high serum LDL and total cholesterol and decreased odds for aggressive periodontitis;

and individuals with LRP5 haplotype (rs682429-rs312016:A-G) had decreased odds for aggressive periodontitis.

LRP5 is a co-receptor of the Wnt/ $\beta$ -catenin signalling cascade [65] that in health affords protection to the individual from vascular diseases as demonstrated in apoE and LRP5 double gene knockout (ApoE<sup>-/-</sup> LRP5<sup>-/-</sup>) mice [66]. Since LRP5 polymorphisms are also being discovered in aggressive PD, this implies that these polymorphisms are contributing to loss of gene function, and thereby predispose individuals to periodontitis [15]. A plausible mechanism is via association of PD with lower levels of HDL cholesterol, higher levels of both LDL cholesterol and plasma triglycerides [15, 67-69].

Hyperlipidaemia, specifically higher total cholesterol and LDL levels, have been reported with periodontitis experimentally, but epidemiological findings have so far contradicted this finding [69]. As periodontal treatment is known to have a beneficial role on lipid metabolism and supports their intricate association, a plausible confounding factor in Machado et al. [69] study may reflect a mixed population of individuals taking part; who regularly receive dental treatment alongside those who rarely visit the dentist.

### **Periodontal disease in ApoE<sup>-/-</sup> mice**

There has been heightened interest in the use of ApoE<sup>-/-</sup> mice as a model to investigate the association between PD and atherosclerosis and hence it is vital to obtain an understanding of the role of periodontitis and its inflammatory mediators. PD is classically initiated by the colonization/infection of the periodontal pathogens via the oral route, to this end, various researchers have investigated the effects of oral infection of ApoE<sup>-/-</sup> mice with periodontal pathobionts (*P. gingivalis*, *T. denticola*, *T. forsythia*, *Fusobacterium nucleatum*) [*F. nucleatum*] [70-73], both as a polymicrobial infection and as mono-infections [71-74]. These studies have demonstrated bacterial colonization and progression of PD in the ApoE<sup>-/-</sup> mouse model (bacterial invasion, gingival inflammation, apical migration of junctional epithelium, alveolar bone resorption, and intra-bony defects). By comparing control to infected mice, a significantly elevated IgG response to *P. gingivalis* and *T. denticola* and *T. forsythia* mono-infections as well as in the polymicrobial infections was recorded [70-72]. The

humoral response generated in all of the infected groups, provides further evidence of a stable response to PD pathogens as well as manifestation of chronic inflammation [70-72]. This primary environmental risk factor (infection) has the potential for pathogenic interplay in the hetero/homozygous apoε4 genotype via initiation of an intrinsic cascade of risk factors (infection>inflammation) for dyslipidaemia. Another common feature of all the mono and polyinfected experiments in ApoE<sup>-/-</sup> mice [71, 75] was the abundant expression of NPY gene in vascular tissues [75]. This suggests an intricate relationship of NPY gene and chronic infections with possible manifestation for the development of insulin resistance as discussed below.

### **Type 2 diabetes mellitus**

Type 2 diabetes mellitus (T2DM) is a metabolic disorder diagnosed in adulthood [76]. It is associated with obesity and is caused by an inefficiency or resistance of the cells to utilise insulin, resulting in a slow but an excess accumulation of sugar in the blood [76]. Insulin resistance reduces glucose tolerance, especially in adipocytes and muscle cells, where the uptake of glucose is insulin dependent. This results in accumulation of glucose in the circulation and a hyperglycaemic state [76], and a homeostatic and systemic imbalance, which is detrimental to health [76]. Increasing evidence supports a bidirectional relationship between T2DM and PD [77]. The hyperglycaemia associated with diabetes results in an increased deposition of advanced glycation end products (AGEs), which bind to neutrophils inhibiting their normal activity [78]. In addition, AGE products activate its receptor (RAGE) which further alters normal macrophage function [78]. These factors subsequently result in an uncontrolled production of proinflammatory cytokines which eventually cause dyslipidaemia as well as increased vascular permeability, collagen fibre break down, destruction of connective tissue, and bone [78, 79]. This may be another mechanism that increases the risk of the diabetic patient to the development of PD.

The ε4 variant of apoε gene also appears to be associated with T2DM as demonstrated by Alharbi et al., [27] in a Saudi population. The differences between T2DM patients and controls for the homozygous ε4 [E4/E4: OR, 4.39 (95% CI: 2.16-8.92); p=0.0001] were shown to be significant. Since patients with this

hetero/homozygous apoε4 genotype are predisposed to infections [13] generally and to oral pathobionts due to the bi-directional relationship of PD with diabetes [77], a chronic inflammatory (cytokines) state in the insulin resistant patient is likely. In addition, NPY is upregulated following PD infection as demonstrated by Chukkapalli et al. [75] in ApoE<sup>-/-</sup> mice. This is significant as NPY during health modulates a multitude of hypothalamus pituitary adrenal (HPA) axis functions via cortisol release including appetite regulation [80], learning and memory [81, 82], mood [83] and neuroprotection [84]. The HPA axis helps to maintain a sustained stress response if the brain continues to sense that a threat, such as an infection, is present in the body. In response, the hypothalamus secretes corticotropin-releasing hormone, which stimulates the pituitary gland to release adrenocorticotropic hormone, and signals to the adrenal glands to increase the levels of circulating cortisol in the blood. Cortisol helps the body to access the resources needed for a sustained response to threat such as maintaining high levels of blood glucose. The individuals having inherited the heterozygous or homozygous apoε4 allelic variant metabolise glucose at a lower rate than those with apoε2 and apoε3 [85]; and the inflammatory mediators contribute to insulin resistance and disturbance in lipid and glucose metabolism [79]. As a result, the function of various tissues and cells, including adipocytes, hepatocytes, muscle and endothelial cells are affected and impaired, which then leads to other chronic metabolic disease states including obesity, CVD, stroke and AD.

### **Insulin resistant T2DM in ApoE<sup>-/-</sup> mice**

ApoE<sup>-/-</sup> mice have not been used as a model for inducing insulin resistance T2DM. Nevertheless, there is a suggestion of an emerging role of NPY gene that may be of relevance to this metabolic syndrome via its effect on the HPA activity as demonstrated by ApoE<sup>-/-</sup> mice following an oral infection [75].

### **Cardiovascular disease**

Cardiovascular disease(s) is characterised by the process of atherosclerosis within blood vessels [86]. It can lead to myocardial infarction, stroke, or peripheral arterial disease according to where it manifests within the

coronary artery tree, cerebral arteries and/or peripheral arteries [86]. Inflammation and inflammatory processes leading to dyslipidaemia in the vessel wall are major contributors of atherosclerosis [87]. Cardiovascular risk factors show overlapping features with other inflammatory pathologies such as PD and vascular dementias encompassing both lifestyle and genetic factors. These include hypertension, diabetes, dyslipidaemia, smoking and others [88]. The LDL receptor protein mutations and the apoE gene are known genetic susceptibility genes in coronary heart disease [89, 90]. Patients with poorly controlled PD show high levels of circulating C-reactive protein (CRP) and fibrinogen levels in their serum [91, 92]. Since CRP is a predictor of heart disease, its rise during episodes of poor dental hygiene is currently the strongest link between PD and atherosclerotic vascular disease (ASVD). Translocation of oral pathogens into the main arterial vessels is reported by many investigators using sensitive polymerase chain reaction (PCR) and sequencing alongside the fluorescence *in-situ* hybridisation (FISH) technique [93-97]. These include *P. gingivalis* and *T. denticola* located within the walls of human coronary artery and atheromatous plaque lesions [93-97]. However these studies remain qualitative as it would take a considerable sample size to determine if there is any statistical significance in these findings.

### **Experimental periodontitis in ApoE<sup>-/-</sup> mice initiate systemic disease pathology**

Previous studies have defined the underlying concepts behind the potential causal-association between microbial agents and atherosclerosis, based on the exacerbation of a chronic inflammatory response largely mediated by bacteria. Recent studies exploring the susceptibility of ApoE<sup>-/-</sup> mice to atheroma formation with mono-infections or as polymicrobial infections demonstrated that oral, metabolically active, pathogens are able to initiate and sustain atherosclerotic lesions in the aorta [70-72, 74, 75, 98]. Furthermore, Hayashi et al. [98] reported that *P. gingivalis* exposure results in an increase of atherosclerotic plaque accumulation in the innominate artery, which is associated with the accumulation of lipids and macrophages closely mimicking the pathology of the human atherosclerosis.

## Alzheimer's disease

Alzheimer's disease (AD), is a neurodegenerative condition characterised by an irreversible memory deficit. The main neuropathological hallmark proteins are amyloid beta ( $A\beta$ ) and the hyperphosphorylated microtubule associated intra-neuronal neurofibrillary tangles (NFTs), both of which are critical to AD post-mortem diagnosis [99]. There are two main forms of AD, the rarer inherited form and the more prevalent, late-onset form. The familial form is characterised by missense mutations in three genes; the amyloid precursor protein (APP), located on chromosome 21, and the presenilin 1 (PSEN 1) and 2 (PSEN 2) genes located on chromosomes 14 and 1 respectively and are all related to enhanced  $A\beta$  deposition. Mutations in the tau gene have been identified in familial forms of frontotemporal dementias linked to chromosome 17 [100] but not in AD that are directly attributed with the NFT lesion. For both forms of AD, there are two major but common risk factors namely advancing age and the apo $\epsilon$ 4 susceptibility gene [16, 17].

Investigating the pathological interactions of mutated genes revealed that the insoluble, fibrillary  $A\beta$  plaques are a break down product of the APP gene proteases known as  $\alpha$ ,  $\beta$  and  $\gamma$  secretases [101]. These proteases are the translational products of PSEN 1 and 2 genes, and the cleavage sites of their substrate (APP) are well documented by numerous reviews [101-103]. In essence, the  $\alpha$  secretase cleavage of APP protein confers little pathogenicity; whereas; depending on the cleavage site of APP protein by the  $\beta$  and  $\gamma$  secretase enzyme(s), two major species of fibrillary  $A\beta_{(40/42)}$  are deposited in AD brains. Of these  $A\beta_{42}$  is regarded as the pathogenic form due to its association with neuritic plaques, which are composed of degenerating nerve tissues with a tight core made up of  $A\beta_{42}$  fibrils [104]. The toxicity of  $A\beta_{42}$  fibrils can be explained by their antimicrobial properties [105]. In the brain,  $A\beta$  fibrils play a role as immune modulators of the innate immune system potentiating activation of the complement cascade [106]. Since neurons are vulnerable to complement mediated lysis [107], the neurites on the periphery of  $A\beta_{42}$  deposits represent debris of dead neurons whilst glia [108] continue, albeit in vain, to synthesize inflammatory components for their clearance.

Despite the generally accepted toxicity of the fibrillary  $A\beta_{42}$ , Braak and Braak, [109] questioned its correlation with progressive cognitive decline in AD cases. To this end, researchers examining

how amyloid fibrils form, led to the simultaneous publication of papers from two laboratories reporting the discovery of 'protofibrils' [110, 111]. Continued work by others has revealed progressively smaller neurotoxic assemblies known as 'oligomers', which appear more toxic than fibrils alone [110-116]. Among these is the soluble form of A $\beta$ \*56 which has been shown to be negatively associated with cognitive decline in an APP transgenic mouse model [117] and when injected into the rat brain [118]. Consequently, the original amyloid hypothesis of Hardy and Selkoe [113] has been modified to the 'A $\beta$  oligomer hypothesis' as originally termed by Ono et al. [115]. Since both insoluble A $\beta$  plaques and NFT lesions are essential for the definitive diagnosis of AD, the weakness of the amyloid hypothesis remains in demonstrating the association of A $\beta$  with many other pathogenic domains of this specific neurodegenerative condition.

The apo $\epsilon$ 4 has so far emerged as the most significant risk factor for both the familial and late-onset forms of AD associating with almost every pathogenic domain as well as an aggressive disease form with an earlier age of onset [16, 17, 119]. Apo $\epsilon$ 4 binds A $\beta$  at the 244-272 residue site (C-terminal residues on apo $\epsilon$ 4) [17, 44]. It has recently been demonstrated that the N-terminal residues of apo $\epsilon$ 4 bind to NFTs [103] highlighting the important role of this protein in both AD and in the two main pathological lesions (A $\beta$  and NFT's) thereby gaining support for its association in AD proteostasis. Furthermore, the full length apo $\epsilon$ 4 is prone to proteolytic cleavage at the C-terminus (methionine 272 or serine 268) that produces a 29 kDa fragment and again at the N-terminal resulting in fragments of 14-20 kDa [38]. The partial proteolytic cleavage of apo $\epsilon$ 4 at the hinge region that holds the two domains together [35] by the yet unidentified proteases has two direct implications in the brain; first the generation of two toxic fragments and second, reduced levels of the whole (apo $\epsilon$ ) protein [38]. The reduced levels of apo $\epsilon$ 4 is unable to maintain adequate lipid homeostasis in the aging brain due to its rapid clearance [120, 121] and its decreased binding to A $\beta$  contributes to amyloid accumulation in AD [44, 122-124] possibly resulting as a form of dyslipidaemia.

Infections and inflammation induce dyslipidaemia [14], and AD pathogenesis is not complete without documenting chronic peripheral infections [61]. These include *Chlamydomphila pneumoniae* [*C. pneumoniae*], *T. denticola*, *P. gingivalis* which are also found in atheroma plaque tissues [93-97] and in AD brains [56, 60, 125],

herpes simplex virus type I [126], and several species of spirochetes of which the well cited ones are *T. denticola* [56] and *Borrelia burgdorferi* [127]. Although the exact aetiological agent(s) responsible the late-onset AD (LOAD) remain elusive, spirochetes appear as highly plausible candidates as exemplified by the condition long-standing, stationary or atrophic form of general paresis, which is caused by *Treponema pallidum* [*T. pallidum*] infection. The atrophic form of general paresis has recently become accepted as an example of a chronic bacterial infection leading to dementia, reproducing the neuropathological hallmarks of AD [128]. More recent reports relating infections to a causative role in the onset of dementia are supported by Kamer et al. [129] suggesting that mild periodontitis is associated with higher brain amyloid load in normal elderly subjects in the hippocampus.

Dementia can result from infections with AD hallmark proteostasis [128] and from infection and inflammation alone as exemplified by HIV-dementia [130]. The introduction of successful antiretroviral medication has led to people with HIV infection living longer. This is introducing an aging group suffering sustained HIV-associated immune activation and chronic inflammation which is thought to be, at least in part, responsible for the increased comorbid chronic disease that this group experiences. HIV positive subjects show increased prevalence of CVD, hypertension, renal disease, diabetes and osteoporosis compared to controls [131] and develop HIV-dementia [130].

### **Experimental periodontitis in ApoE<sup>-/-</sup> mice initiate inflammatory pathology in the brain**

The downstream effects of *P. gingivalis* mono-infection in ApoE<sup>-/-</sup> mice was recently reported by Poole et al. [132] in which they reported the translocation of this PD pathogen from the oral cavity into the brain tissue likely via the haematogenous route; although other pathways for its translocation are also possible [133]. Examination of the brain tissue highlighted the brains own inflammatory cells (microglia and astrocytes) were activated and neurons were being attacked by excessive complement activation supporting ongoing intracerebral inflammation in the absence of AD hallmark proteins [132]. For the relevance of finding *P. gingivalis* in the ApoE<sup>-/-</sup> mice brains, the reader is directed to another review article published elsewhere [134].

## **Apoε4 as the plausible commonality for the etiology of co-morbidities**

All of the above mentioned conditions share at least one common genetic susceptibility the ε4 allelic variant, and the common life style and behavioural traits [15, 16, 24, 27]. They all show an association with peripheral infections directly or indirectly [11, 31, 56, 60, 135], inflammation [12, 31, 135, 136] and dyslipidaemia [29, 30, 32, 33].

In view of the apparent relationship between successful aging and apoε alleles it was of interest to explore the association between aging and retention of natural teeth [4]. When apoε allele frequencies were analysed and compared between groups of edentulous and dentate human subjects, the edentulous group showed a significantly higher frequency of the apoε4 allele [4]; but the limitations with this study were that it is unknown whether possessing the apoε4 allele made an individual more susceptible to periodontitis specifically, or to disease in general and tooth loss was a consequence of an overall deterioration in health.

Borilova Linhartova et al. [32] investigated the association between PD and the apoε4 allele in a case-control study using genomic DNA in which they reported that apoε gene variability was not significantly different between the two groups examined (chronic PD sufferers and those without PD); although those with chronic PD demonstrated increased total cholesterol and LDLs compared to controls [32]. In addition, no significant differences were found between groups for triglyceride and HDL levels [32]. Although environmental influences such as smoking, age and gender, socioeconomic factors, obesity, diabetes and family history are known to associate with PD [137-139], the genetic links on the whole, are only now being documented. It is generally recognised that the aggressive forms of PD have a stronger genetic association [15, 22] than the chronic form. However, the research by Gao et al. [15] demonstrated an association between the apoε gene and LRP5 polymorphism in the aggressive form of PD, which along with this genetic risk factor strengthens the periodontal association with the emerging cardiovascular and AD pathologies.

Literature supports the presence of groups of individuals who are destined to suffer, as in, familial forms of disease. These can be excluded from those who inherit susceptibility genes. In addition to apoε4

commonality for the etiology of co-morbidities, there are individuals with other common susceptibility traits for PD. These account for approximately 50% genetic variance with polymorphisms in inflammatory mediator gene regions such as IL-1, IgG Fc receptor, and TNF- $\alpha$ . Polymorphisms in IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , complement component 1(q subcomponent, A chain) genotypes are reported in periodontitis [140-143]. Additionally, IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ ,  $\alpha$ 2-macroglobulin (also known as LDL receptor related protein or LRP), and alpha1anti-chymotrypsin, complement receptor 1 (CR1) and clusterin are not only all upregulated but also show polymorphic associations in AD cases [144-147] suggesting common inflammatory gene susceptibility profiles in the expression of PD to AD likely contributing to dyslipidaemia. As the susceptibility gene clearly requires an environmental risk factor for the expression of disease, avoidance of risk would be one therapeutic solution. For example, it is documented that not everyone with the hetero/homogeneous inheritance of apo $\epsilon$ 4 will result in manifesting diabetes, vascular diseases and AD [8, 148], and if this risk factor is an oral infection, (56, 60, 61) as supported by the ApoE<sup>-/-</sup> mice induced with PD studies [70-73, 75, 132], then there is a therapeutic window for the related co-morbid states to modify the course of disease by adoption of healthy lifestyles and promotion of awareness about important early warning signs of serious health conditions by regular dental visits.

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### **Conflict of Interests**

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

## Legends and Figures

Figure 1

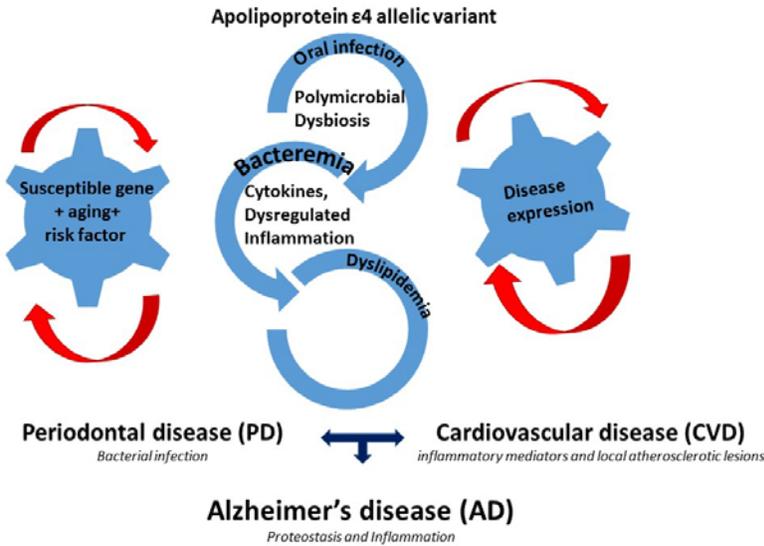


Figure 1. Schematic illustration of the intricate cascade of interaction between apo $\epsilon$ 4 and environmental risk factor such as an oral infection from PD. Following poor oral hygiene the gingivae can bleed and allow access of periodontal bacteria to the systemic circulation where immune cells survey entry of noxious agents. Upon recognizing pathogenic bacteria, these immune cells release inflammatory mediators (cytokines) to combat infection but as the pathobionts have strategies of their own to evade the immune surveillance they remain viable. At the acute phase of infection, disturbances in the lipid metabolism take place in the form of dyslipidaemia. If the lipid imbalance is sustained, during aging, the dyslipidaemia can augment disease pathogenesis including atherosclerosis, cerebrovascular disease and AD.

Figure 2

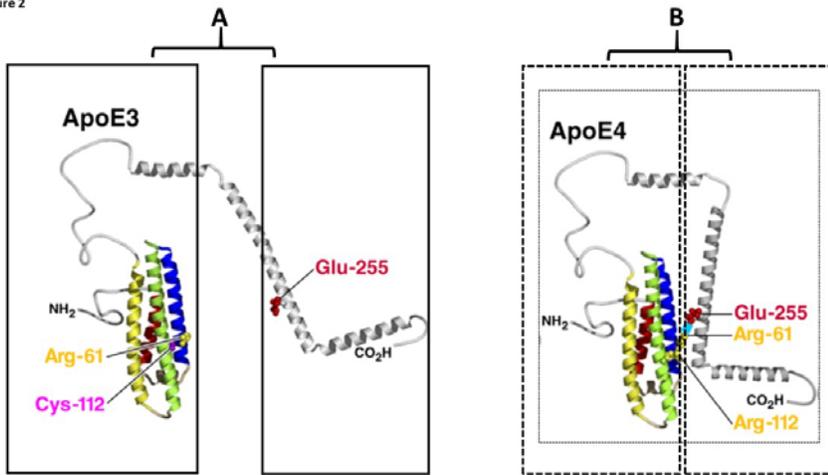


Figure 2. Models of apoε3 and apoε4 adapted from Mahley and Huang [149]. The rectangular boxes show the differences in the molecule in respect to amino acid changes in the two allelic variants. The space between the two boxes (solid lines) in A is greater than in B (boxes with broken lines) where domain interaction appears restricted.

Figure 3

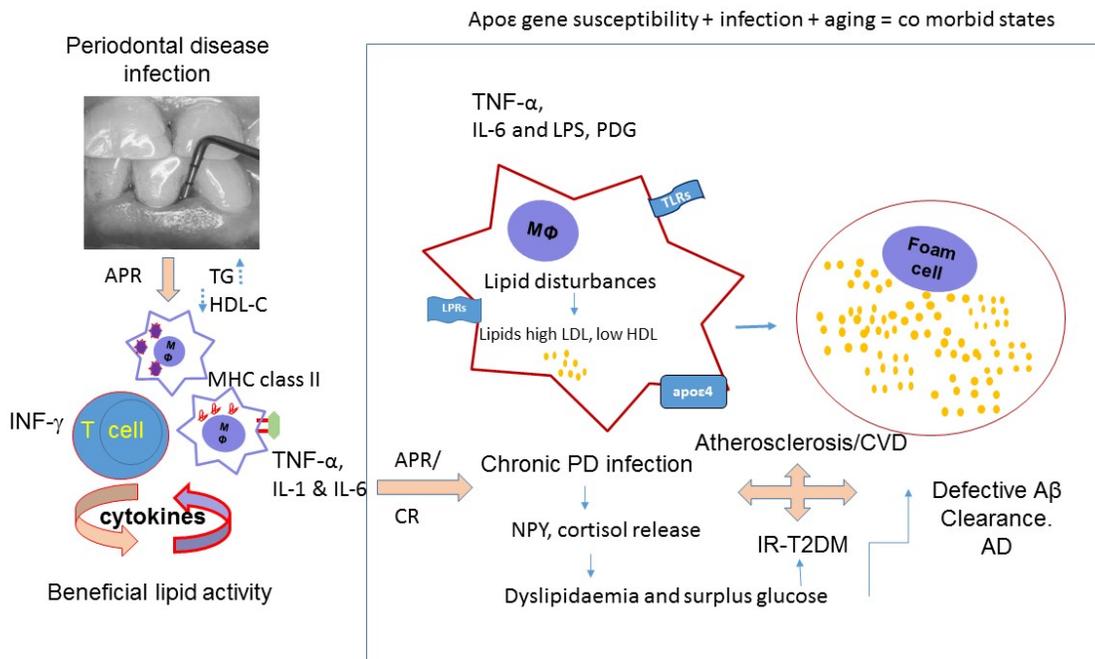


Figure 3. Pictorial illustration of interaction between apoε4 with its infectious risk factor resulting from PD. Following poor oral hygiene, there is local inflammation in the gingivae and in the systemic circulation. The

blood borne immune cells at both tissue sites and in the systemic channels release inflammatory mediators (cytokines = APC or acute phase response) to prevent spread of infection. At the same time disturbances in the lipid metabolism take place thereby the balance of LDL and cholesterol becomes “tilted” leading to higher HDL (dyslipidaemia). If the lipid imbalance is sustained for longer time, that can augment disease pathogenesis such as atherosclerosis and other co-morbidities. Abbreviations: TG = triglycerides, CR = chronic response, M $\phi$  = macrophage, LPS = lipopolysaccharide, PDG = peptidoglycan, TLRs = toll like receptors, LPRs = low density lipoprotein receptors, NPY = neuropeptide Y, IR = insulin resistant, A $\beta$  = beta-amyloid. Cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-1 and IL-6) and Lipids (HDL-C, LDL, HDL) and diseases (PD, CVD, T2DM, AD) are abbreviated as in main body text.

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