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Porphyromonas gingivalis is a strong risk factor for Alzheimer's disease

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ABSTRACT

Porphyromonas gingivalis (*P. gingivalis*) is one of the several important bacterial pathogens associated with the sporadic Alzheimer's disease (AD). Different serotypes are either capsulated or are non-capsulated. It has been demonstrated that *P. gingivalis* (non-capsulated) can reproduce the neurodegenerative AD-like changes *in vitro*, and a capsular *P. gingivalis* (strain W83) could reproduce the cardinal hallmark lesions of AD in a wild-type mouse model. All *P. gingivalis* forms express proteolytically active proteases that enable cleavage of the amyloid precursor protein (APP) and tau resulting in the formation of amyloid-beta and neurofibrillary tangles (NFTs). Tau is an established substrate for gingipains, which can cleave tau into various peptides. Some of the *P. gingivalis* fragmented tau protein peptides contain "VQIINK" and **"**VQIVYK" hexapeptide motifs which map to the flanking regions of the microtubule binding domains and are also found in paired helical filaments that form NFTs. *P. gingivalis* can induce peripheral inflammation in periodontitis and can also initiate signaling pathways that activate kinases, which in turn, phosphorylate neuronal tau. Periodontal disease related inflammation has metabolic implications for an individual's peripheral and brain health as patients suffering from generalized periodontitis often have related co-morbidities and are "at risk" of developing AD. The aim here is to discuss the role of *P. gingivalis* behind such associations with the backdrop of huge efforts to test *P. gingivalis* virulence factors clinically (GAIN Trial: Phase 2/3 Study of COR388 in Subjects with AD) with inhibitors, which may lead to an intervention by reducing the pathogenic bacterial load.

KEYWORDS: Alzheimer's disease, gingipains, *Porphyromonas gingivalis*, periodontal disease

Alzheimer's disease and its association with periodontal disease

The presence of extra neuronal amyloid (Aβ) plaques and intra-neuronal neurofibrillary tangles (NFTs) in the brain, together with defined clinical signs of cognitive deficit, form the basis of the diagnostic criteria for AD at autopsy [1, 2]. The origins and the roles of Aβ plaques and NFTs appear quite distinct but together they lead to neurodegeneration. The hippocampus contains abundant intra-neuronal NFTs composed of abnormally phosphorylated tau protein. According to Braak staging of AD; the NFTs spread in the brain in a defined distribution, which allows correlations to be made with the clinical stages [3]. Early involvement of tau pathology has been described in the subcortical nuclei such as the locus coeruleus and the pons anatomical areas of the brainstem [3, 4]. This anatomical site of the brain releases norepinephrine in response to emotional and stress related factors. The locus coeruleus also controls the attention and alertness of an individual, so any adverse change in its homeostasis could affect behaviour and mood. Notably, the trigeminal ganglion is located adjacent to the locus coeruleus of the brainstem [5]. It follows that the brainstem and the periodontium communicate via the trigeminal nerve because tooth related pain is registered in the brain. In support, the neurons of the trigeminal nerve are known to be distributed within the periodontal ligament [13]. This provides an important link between periodontitis and the areas of the brain that are affected early in the progression of AD pathology.

Periodontitis is a chronic inflammatory disease which damages the tooth supporting tissues i.e. gingivae, periodontal ligament and alveolar bone [6, 7]. Bacteria which can instigate changes in a normally symbiotic microbial community to a dysbiotic state are termed ‘keystone pathogens’. *Porphyromonas gingivalis* (*P. gingivalis*), is a Gram negative coccobacillus shaped bacterium which has long been established as a keystone pathogen for periodontitis [6, 7]. This oral commensal becomes pathogenic and can exert its virulence via its endo/exotoxins (surface membrane lipopolysaccharide or LPS, and gingipains) and capsular polysaccharides that allow this bacterium to induce chemokine paralysis of the host and evade immune

recognition, even in low abundance. The compromised host innate and adaptive immune responses may be inadequate to control the inflammophilic microbiota and paradoxically, can contribute to connective tissue damage and inflammatory bone loss [8]. Oral microbes can enter the systemic circulation during transient episodes of bacteraemia which can occur with daily oral hygiene activities and dental interventions [9]. Periodontal pathogens including *P. gingivalis* have been detected at disparate sites such as the atherosclerotic plaque [10] and AD brains [11, 12]. Pertinent to this, it has been demonstrated in AD transgenic mice that extraction of molar teeth generated release of the cytotoxic Aβ, and triggered neurodegeneration in the locus coeruleus via its connection with the trigeminal nerve pathway connecting the periodontium [13]. This may provide an explanation for the underappreciated concepts such as missing molar teeth and their contribution to neuronal loss in AD [14-17].

This review will cover some salient aspects of *P. gingivalis*, the keystone periodontal disease pathogen, that makes this bacterium "important" as a strong risk factor for developing AD pathophysiology with an emphasis on the pathways that produce hallmark pathology. This is plausible as we now have a better understanding of the *P. gingivalis* secreted exotoxin known as gingipains that expresses cathepsin B proteolytic enzymatic activity, which enables cleavage of amyloid precursor protein (APP), to form Aβ plaques. Furthermore, gingipains has the potential to disturb tau homeostasis by hyperphosphorylating serine and threonine residues via inflammatory signaling that activate glycogen synthase kinase-3beta (GSK-3β). In addition, proteolytically active gingipains can hydrolyse tau protein to release fragments [12] with the "VQIINK" and **"**VQIVYK" hexapeptide motifs that are present in the paired helical filaments that constitute the NFT lesion. These are significant recent leaps in scientific advances in order to understand the risk factor involvement of periodontal disease with the development of AD.

What makes *P. gingivalis* a risk factor for AD?

Inflammation is widely thought to contribute to the cognitive decline in AD [18]. However, peripheral episodes of inflammation and/or microbial access to the brain and their impact on triggering cerebral inflammation have largely been ignored. The pathways for microbial access to the brain are many [19] including a leaky blood-brain barrier (BBB). *P. gingivalis* can trigger the peripheral and cerebral immune responses. Periodontitis can exert its influence indirectly by sustaining peripheral inflammation. This together with defective susceptibility genes that normally help with clearance of waste from the brain, can prime microglial cells into a pro-inflammatory phenotype [20].

Other major risk factors for AD are comorbid in the presence of the *Apolipoprotein E, allele 4* (*APOE ϵ4*) susceptibility gene inheritance [21]. One effect is related to the decline of cerebral blood flow across the lifespan of an individual during normal aging but this effect is greater in subjects with *APOE ϵ4* susceptibility gene inheritance [22]. Lack of an adequate systemic blood flow to the brain in older individuals and those suffering from dementia harbouring the *APOE ϵ4* susceptibility gene can precipitate cerebrovascular pathologies such as stroke, small vessel arteriosclerosis and others [23-25]. In addition, middle aged hypertension and diabetes also increase the risk of AD [26], this risk may be heightened in the presence of *P. gingivalis* oral infection due to severe periodontitis, which has metabolic implications for an individual's peripheral and brain health [27, 28]. Meta-analysis has shown that there is a 250% higher risk for incident dementia in persons with both *APOE ϵ4* inheritance and diabetes than those without; and a 35% higher risk for those with *APOE ϵ4* alone [29]. Figure 1 illustrates the shared risk factors of periodontal disease and AD, which includes the genetic components, comorbidities and environmental factors. This further supports the plausibility of *P. gingivalis* as a risk factor in itself.

In addition, the BBB function becomes inadequate during normal aging and in cognitively impaired individuals carrying the *APOE ϵ4* genetic susceptibility [30-32]. A disrupted BBB

during aging and dementia [33] can potentially facilitate the passage of *P. gingivalis* from the systemic circulation into the brain. The scientific rationale for this statement comes from observations made from *P. gingivalis* mono-infected apolipoprotein null mice models of periodontitis for AD pathophysiology. *P. gingivalis* from the oral cavity accessed the brain and the BBB appeared significantly damaged [34-36]. A mechanistic pathway for bacterial entry into the brain is suggested to be via proteolytically active gingipains (the extracellular cysteine proteinases of *P. gingivalis* strain W83) breaking down the epithelial transmembrane proteins, E-cadherin, β_1 integrin and occludin, thereby disrupting the tight junctions between capillary endothelial cells. This increases the permeability of the BBB [37]; and in addition, systemic proinflammatory cytokines also can potentially disrupt the BBB [38]. It is assumed that when tight junction integrity is sufficiently disturbed, passage of bacteria into the brain is likely to take place. In supporting the adverse effects of the cytokine on the BBB integrity, Vernal et al. [39] have reported that capsular serotype K1 and K2 *P. gingivalis* strains outside the central nervous system induce an enhanced secretion of pro-inflammatory interleukin cytokines (IL)-1β, IL-4, IL-6, IL-10, IL-17, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α in macrophages and dendritic cells, implying capsular *P. gingivalis* strains have the armoury for potentiating BBB permeability through cytokine liberation. Encapsulated bacterial infections for example, *P. gingivalis* W83, harbours an additional virulence factor in the form of the capsular polysaccharide or A-lipopolysaccharide (A-LPS) which is different from the lipopolysaccharide (LPS) of the outer membrane of non-encapsulated bacteria for example *P. gingivalis* (strain ATCC 33277) [see 40]. The A-LPS in the capsulated *P. gingivalis* (W83) plays a vital role in this bacterium's virulence development by inducing proinflammatory cytokine paralysis [41] as it is connected to the post-translational additions of Arg-gingipains that make the bacterium more virulent [42, 43].

Therefore, a person harbouring capsular forms of *P. gingivalis* could have a higher risk of developing AD, compared with those who carry the non-capsulated bacterium. This may be one reason why not all individuals who suffer from periodontal disease also manifest AD [15, 43]. Prevalence studies among the Dutch population identified K6 serotype (capsular) as the most prevalent (23%) [44], whilst, the K5 serotype (capsular) was frequently observed in 25% of the subgingival isolates examined from Indonesian AD subjects [45].

***P. gingivalis* gingipains, the most critical virulence factor**

P. gingivalis produces gingipains R, (RgpA, RgpB) and gingipain K, (Kgp). Gingipains are classified as collagenases and trypsin-like cysteine proteinases [46], and are secreted by all strains (with/without capsule) of *P. gingivalis* [47, 48]. Together they degrade a variety of proteins involved in the immune response [49-52]. Here, our interest is in the trypsin-like cysteine proteinases, secreted by all strains of *P. gingivalis* [47, 48], which specifically cleave peptides at Arginine-Xaa and Lysine-Xaa (Xaa = any amino acid) from the C-terminus. The Arg-specific proteolytic activity is encoded by *rgpA/B* genes while the Lys-specific activity is encoded by the *kgp* gene. Rgp and Kgp are enzymes responsible for the trypsin-like activity associated with *P. gingivalis* and hence are important in the context of tau NFTs [47].

Recent studies have postulated that tau protein (associated with microtubules) and actin are substrates of Kgp gingipain [13, 53]. Neither Lys-gingipain nor Arg-gingipain are inhibited by internal protease inhibitors such as cystatins and α-antichymotrypsin and are therefore, able to diffuse into host tissues. Furthermore, gingipains are thermally stable from 0-45ºC and over a range of pH fluctuations 5.5 to 10.5 [54]. It is not surprising in view of the sensitivity of tau to trypsin [55], that it is a potential substrate for gingipains *in vivo* [12]. This exciting finding is potentially a major breakthrough for therapy based on low molecular weight small-molecules designed to block the toxic effects of the different types of gingipains secreted

by *P. gingivalis*, which are currently in Phase 3 clinical trials. An aspect of the gingipains inhibitor COR388 based therapy is provided elsewhere [56].

P. gingivalis **and its association with neurodegeneration**

AD is a neurodegenerative disease and hence neuronal loss is pivotal to the disease process. To this end, Goto et al. [13], performed surgical extractions of molar teeth in their triple transgenic AD (3xTg-AD) mice and uncovered a neurodegenerative pathway involving the trigeminal distribution within the periodontal ligament to the locus coeruleus. From the locus coeruleus, the tau pathology subsequently spreads to the hippocampus. The loss of molar teeth in AD patients is predominantly associated with periodontitis and in general, tooth loss has been linked to a higher risk of AD [14-17]. *P. gingivalis* (ATCC 33277) has been reported to cause AD-like neurodegeneration in infected neurons derived from induced pluripotent stem cells (iPSC) in an *in-vitro* culture system with persistent expression of active gingipains, resulting in a 25% loss of neurons over three days [57]. The differentiated iPSC neurons can be maintained for months and offer a time efficient *in vitro* analysis of measuring neuronal degeneration compared to examining the years of AD-related neurodegenerative processes in humans. Furthermore, *P. gingivalis* can invade and survive within neurons and generate intra-neuronal gingipains, which are proteolytically active, implying the plausibility of direct neurodegeneration associated with NFT lesion formation in AD taking place [57].

P. gingivalis **infection and periodontitis are linked to deteriorating memory**

The first interventional study with human AD subjects was carried out by Rolim et al. [58] in which they demonstrated a beneficial outcome of dental treatment in AD subjects. The reported benefit to the patients with mild AD was in terms of reduced orofacial pain and an improvement in the mandibular function and periodontal indices. These improvements were maintained until

the last evaluation after six months, and were followed by a reduction in the functional cognitive impairment. Several proof of concept studies carried out in either mice with experimentally induced periodontal disease with an oral infection with *P. gingivalis*, or with the introduction of its LPS have indicated that inflammatory signaling pathways contribute to a clinical phenotype in which impaired learning and memory is observed [59-61]. The inflammatory basis of deteriorating memory is upheld by the results from clinical trials in human AD subjects with periodontitis [62]. For a more comprehensive overview, see Singhrao and Olsen [63].

P. gingivalis **and its association with AD diagnostic lesion formation**

P. gingivalis has the potential to induce inflammation peripherally due to periodontitis [64] and subsequently in the brain via its intracerebral entry or entry of its virulence factors (LPS and gingipains) [65-67]. Gingipains released by *P. gingivalis* which have similar APP-cleaving action as cathepsin B (of the host) [12], interact with the APP signaling pathways (amyloid cascade) to release Aβ [60]. In this process, gingipains together with LPS can proteolytically activate kinases such as GSK-3β which subsequently phosphorylates neuronal tau [57, 66].

Up until now, abnormally phosphorylated tau protein has not featured negatively in the pathophysiology of periodontal disease *per se*. However, Adamowicz et al. [68] implicated the role of GSK-3β in bacterial-induced periodontitis because its inhibition rescued bone loss. Thus, GSK-3β may be influencing phosphorylation of brain tau via immune responses mediated by *P. gingivalis*, in the Ilievski et al. [66] and Haditsch et al. [57] studies. The introduction of phosphorylated tau is an important point, especially since the intraneuronal cytoskeletal alterations precede the formation of amyloid in AD locus coeruleus [69]. This is

interesting when compared with the Tg mouse tooth extractions where cytotoxic Aβ, triggered neurodegeneration in the same brain region (i.e locus coeruleus) [13].

P. gingivalis and the Aβ plaque lesion

The extra neuronal AD plaque is composed of Aβ forming the basis of the "Amyloid Hypothesis" [70]. Aβ is a cleavage product of APP, which is seen in internal vesicular membranes, including the Golgi apparatus and endosomes [63, 71]. Hence, a direct extracellular deposition of soluble/insoluble Aβ as well as an intra-cellular processed soluble Aβ [63, 72, 73] both contribute to the extracellular fibrillary/insoluble (diffuse and neuritic) plaques in AD brains. The APP proteolytic cleavage is completed by the proteolytically active secretase enzymes (α-, β- and γ-secretases). In rare familial AD cases however, there is a mutant form of APP (Swedish double mutation APP K67ØM, N671L) which has a double mutation at the start of the cleavage site of the beta-site of APP cleaving enzyme 1 (BACE -1). BACE-1 has been shown to bind this mutated site with much higher affinity than the wtAPP and thus cleaves to form Aβ much more readily [73]. Notably, the enzyme cathepsin B, which is expressed in secretory vesicles within neurons, is shown, conversely, to bind with high affinity to wtAPP (and not to the Swedish mutant APP) and has been suggested as a likely candidate for production of Aβ in the sporadic form of AD [73]. As described earlier, gingipains, the exotoxin of *P. gingivalis,* also has cathepsin B-like activity [12] and may act to cleave APP [73]. Wu et al. [60] demonstrated the host related cathepsin B to interact with APP to liberate Aβ. In order to determine the intracellular processing of APP by *P. gingivalis*-LPS (Pg-LPS), Wu et al. [60] challenged the wt-APP mice and observed that the host's cathepsin B, together with inflammatory mediators (IL-1β), directed APP proteolysis to release soluble amyloid which they interpreted to be Aβ42 species. Why the host's cathepsin B activity and not the *P. gingivalis* cathepsin B-like activity are acting here is because we postulate that the

highly purified Pg-LPS used by Wu et al. [60] is likely to have been denatured by the purification process leaving the LPS activity intact. Following *P. gingivalis* infections, an increase in peripheral $A\beta_{1\text{-}40/42}$ accumulation within periodontal tissues have been shown in mice models and in the human gingival tissues and human serum thereby potentially adding to the Aβ pool in the AD brain [74-76]. To this end, Zeng et al. [77] identified advanced glycation end products (AGE) as a likely receptor for $A\beta_{1\text{-}40/42}$ in cerebral endothelial cells implying AGE products-receptor are a plausible mediator of cerebrovascular-related Aβ accumulation after *P. gingivalis* infection in their mouse model. This supports the hypothesis that patients harboring the generalized form of periodontal disease may be "at risk" of developing AD via multiple pathways. Key evaluations of prospective and retrospective population based data have shown that chronic periodontal disease that exists for longer than ten years results in a doubling of the risk for the sporadic form of AD [78-80]. Putting this information in perspective, the 10 year lag allows amyloid-beta (Aβ) plaques to reach a plateau in the human host and this is when the host begins to indicate the earliest stage (mild cognitive impairment or MCI or prodromal) of AD. Following this stage, the pathological cascade of progressive AD would take over. This is interesting because the pieces of the jig-saw puzzle that form the picture of AD is beginning to emerge from reported scientific observations regarding the co-morbidity between periodontal disease and AD in some patients. Periodontal disease can start at any age and the time it takes to become chronic may vary from individual to individual. If that individual was vulnerable to manifesting AD later in life, it should be possible to predict the risk age of that individual from the time that their periodontitis became chronic. Taken together, an early detection of disease means patients may benefit from an earlier medical intervention.

"Gingipains" interaction with tau protein: what does this mean for neurofibrillary tangles?

The NFTs represent hyperphosphorylated tau proteins binding to microtubules. Hyperphosphorylation of microtubules is abnormal because normal tau becomes insoluble and subsequently aggregates. This also causes the collapse of microtubules into non–membrane-bound masses of abnormal paired helical and straight filaments (PHF) which are found in the perinuclear cytoplasm of specific neurons. These constitute NFTs [81, 82]. Understanding the formation of the NFT lesion due to bacterial interaction is important as autopsied brains from AD cases have confirmed the presence of the following microbes (Actinomycetes [83, 84], *P. gingivalis* [12, 56], *Helicobacter pylori*,
and *Chlamydia pneumoniae* [85], Herpes Simplex type 1 virus (HSV1) [86], select species of oral/non-oral spirochaetes [87], and select fungi [88]). Furthermore it has been reported that *Bacteroides* species such as *P. gingivalis* are more virulent as a result of mixed infection with *the Actinobacillus actinomycetemcomitans* bacteria facilitating their movement between different organs [89, 90].

Dominy et al. [12] demonstrated tau protein as a substrate for *P. gingivalis* protease gingipains and as a consequence, the resultant tau protein fragments can be released into the brain's parenchymal tissues. Depending on their size (Table 1), these extracellular phosphorylated tau fragments may be directly toxic to other neurons. The smaller sized phosphorylated tau fragments may be taken up by other connecting cells at the synaptic clefts during neurotransmitter uptake, thereby facilitating its spread from neuron to neuron and subsequently spreading pathology.

Tau cleavage by gingipains and its involvement in paired helical filaments

P. gingivalis protease gingipains have been demonstrated to co-localize with microtubules and paired helical filaments (PHF) constituting NFTs in AD brains [12]. As

mentioned earlier, tau protein can be hydrolyzed by gingipains into several fragments both from the N and C termini [12]. Six out of several fragments have “VQIINK” and **“**VQIVYK” hexapeptide common motifs that bind to the microtubule binding domains flanking regions, which support the microtubule filaments (Figure 2, Table 1). This implies that six of the several tau fragments released by gingipains are from the pivotal sites of the functional microtubules and agrees with previously reported observations [12]. Furthermore, PHF filaments also have VQIINK and VQIVYK hexapeptide signatures [see ref 91]. Therefore, tau cleavage at VQIINK and VQIVYK hexapeptides from the regions of considerable functional significance to microtubules “would collapse” the intact microtubule assembly with the said hexapeptide peptide amino acid signatures within tau and PHFs, constituting the NFT lesions [12, 91]. Further research is needed to clarify the role of gingipains fragmented tau peptides in the pathogenesis of AD.

P. gingivalis **phosphorylates tau protein**

Tau protein binds to microtubules and is prone to hyperphosphorylation. Hyperphosphorylation of tau protein signifies a pathological change, which precedes NFT formation. It is believed that phosphorylated tau proteins accumulate within neurons prior to NFT formation [92]. What causes this shift between bound to free pathological tau in neuronal cells is not clear, but *P. gingivalis* enzyme activity hydrolysing this protein as shown by Dominy et al. [12] cannot be ruled out. Haditsch et al. [57] demonstrated an increased tau phosphorylation at two residues (enhanced tau phosphorylation at Thr231and at Ser396) following *P. gingivalis* infection in an iPSC differentiated neuronal cell culture model. Furthermore, the capsular serotype K1 *P. gingivalis* W83 strain has been shown to have the potential to contribute to tau phosphorylation at Ser396 in the *in vivo* wild-type mouse model [66]. These are accepted phosphorylation sites as evaluated previously by Hanger et al. [93].

However, which kinase may be responsible for phosphorylating Ser396 and Thr231 following *P. gingivalis* infection is not clear, but glycogen synthase kinases-3 beta (GSK-3β) is a strong candidate [93]. Recently Liu et al. [94] observed in their *gingivalis*-infected microglial cell model that phosphoinositide 3-kinase (Pi3K)/ protein kinase B1 (Akt) and mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase/ERK pathways were activated. Our study in which purified *P. gingivalis* LPS (Pg-LPS) was applied to a neuroblastoma cell line, *in vitro* cell model also demonstrated that the PI3K/AKT pathways were activated [95]. In addition, Bahar and Singhrao [95] showed GSK-3β and forkhead box class O1 (FOXO1) and NF-kB mRNA expression was also up-regulated and that this was dependent on the MyD88 pathway [95]. The importance here is that GSK-3β is one of the enzymes that can phosphorylate tau residues Ser396 and Thr231 [93] and its activation implicates the onset of inflammatory signaling [95] that are known to be involved in AD pathophysiology [96, 97]. This implies that *P. gingivalis* infection plays an important role in the infected cells where balance of inflammation and inflammation responsive kinases (e.g. GSK-3β) are tipped in favour of phosphorylation, NFT lesion formation and subsequent pathophysiology of AD.

Conclusions

This review has applied a pathobiome concept in substantiating the link between *P. gingivalis* infection and AD lesions. It is clear from the human and proof of concept studies in animal models that whole bacteria and their constituent endo/exotoxins enter the central nervous system. In-situ, this bacterium has a range of enzymes that are shown to interact with APP and tau, deregulating their structure and intracellular processing, with resultant formation of Aβ and PHF respectively. We appreciate that other bacterial, fungal or viral pathogens

implicated in AD may follow different pathways towards AD pathophysiology compared to those described here for *P. gingivalis*.

It is evident that *P. gingivalis* is a potentially significant etiological agent for AD pathophysiology. Whilst generalised periodontitis develops around middle age, the sporadic form of AD is of much later onset. The lag phase between the two comorbidities could provide us with a mechanistic association between the two diseases and a window of opportunity to inhibit the toxic insults of gingipains in patients with periodontitis, and its downstream inflammatory effects such as systemic inflammation contributing to the risk of developing AD or increasing the severity of dementia by hyperphosphorylating tau. Hence, the clinical "GAIN Trial": Phase 2/3 Study of COR388 in Subjects with Alzheimer's disease. (ClinicalTrials.gov Identifier: NCT03823404) is key to testing this hypothesis and advancing the adjunctive management of healthcare landscape in preventing and/or slowing down AD. It is imperative that the oral health component is included as a modifiable risk factor in AD public health messages along with other preventative advice such as keeping active, eating healthily and exercising.

Disclosure statement

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Table 1: Peptides of interest taken from reference 12. Amino acids indicated in green colour are putative phosphorylation sites in the tau protein. * indicates peptides of significance to the PHF tau constituting neurofibrillary tangles that bind to microtubule binding domain (MBD).

Peptide number	Region in Tau N-C termini	Nucleotide/ Peptide
1 N-terminal projection domain	K87-R126	QAAQPHTEIPEGTTAEEAGIGDTP**S**LEDEAAGHVTQAR

Tau(1–165)		
2 Proline rich domain 166-242	R211-R221	**T**P**S**LP**T**PPTR
3* MBD R1/2(274–304)	K259-K290	**267**HQPGGGK**VQIINK**KLDLSNVQ**S**^{184}K**287**
4* MBD-2 R2(274–304)	K28-K290	**275VQIINK**KLDLSNVQ**S**^{184}K**285**
5* MBD-2/3 305–335	K298-K317	**298**HVPGGGS**VQIVYK**PVDLSK**317**
6* MBD-2/3 305–335	K298-K321	**298**HVPGGGS**VQIVYK**PVDLSKVTSK**321**
7* MBD-2/3 305–335	K294-K317	**294**DNIKHVPGGGS**VQIVYK**PVDLSK**317**
8* MBD-2/3 305–335	K294-K321	**294**DNIKHVPGGGS**VQIVYK**PVDLSKVTSK**321**
9 C-terminal domain (368–441)	R406-K438	HL**S**NV**SST**^{414}G**S**^{416}IDMVD**S**PQLA**T**LADEV**S**ALAK

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Figure Legends

Figure 1: The keystone periodontal pathogen, *P. gingivalis* (P.g) can enter the bloodstream during episodes of transient bacteraemia and gain access to the brain via multiple routes including a leaky blood-brain barrier (BBB). The microbial invasion triggers the cerebral immune response resulting in neuroinflammation and in the formation of the two diagnostic lesions of AD. Periodontitis can exert its influence indirectly by sustaining peripheral inflammation (blue dotted arrow). This can affect glial cells by priming them into a pro-inflammatory phenotype. In addition, this could also overload and overwhelm the clearing of toxic neuropeptides from the central nervous system (CNS). The potential causal relationship of periodontitis and AD is further supported by the shared risk factors.

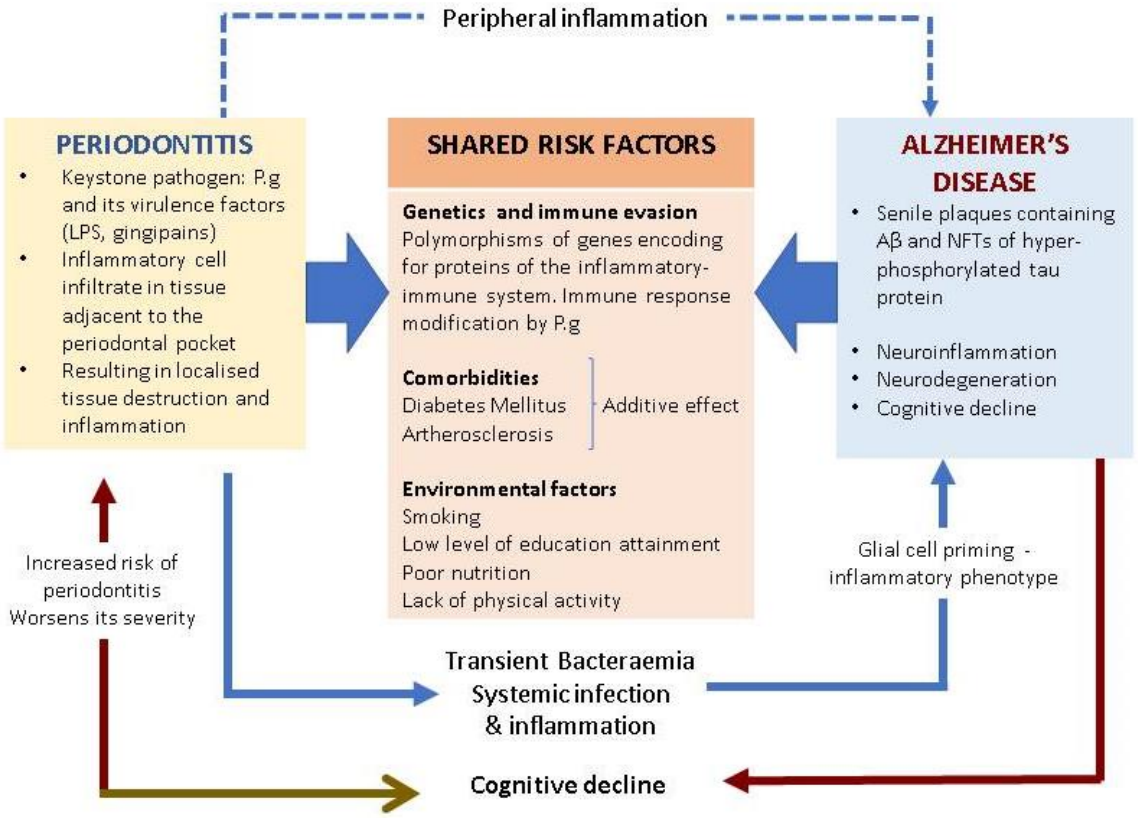

Fig 2. Schematic of tau protein and domain organisation to help understand the position of peptides in Table 1 and the 1-4 microtubule binding domains of Tau to microtubules.