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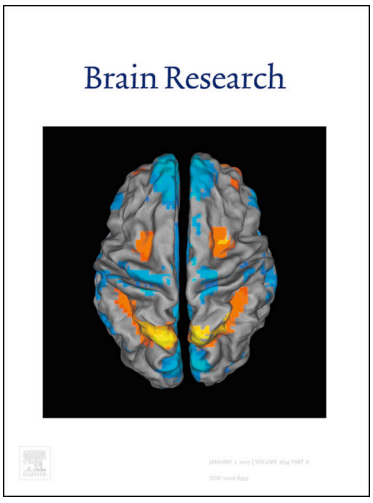

Research report

Endocannabinoid system alterations in Alzheimer's disease: a systematic review of human studies

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Title: Endocannabinoid system alterations in Alzheimer's disease: a systematic review of human studies

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Abstract:

Studies investigating alterations of the endocannabinoid system (ECS) in Alzheimer's disease (AD) in humans have reported inconsistent findings so far. We performed a systematic review of studies examining alterations of the ECS specifically within humans with AD or mild cognitive impairment (MCI), including neuroimaging studies, studies of serum and cerebrospinal fluid biomarkers, and post-mortem studies. We attempted to identify reported changes in the expression and activity of: cannabinoid receptors 1 and 2; anandamide (AEA); 2-arachidonoylglycerol (2-AG); monoacylglycerol lipase (MAGL); fatty acid amide hydrolase (FAAH); and transient receptor potential cation channel V1 (TRPV1). Twenty-two studies were identified for inclusion. Mixed findings were reported for most aspects of the ECS in AD, making it difficult to identify a particular profile of ECS alterations characterising AD. The included studies tended to be small, methodologically heterogeneous, and frequently did not control for important potential confounders, such as pathological progression of

AD. Eight studies correlated ECS alterations with neuropsychometric performance measures, though studies infrequently examined behavioural and neuropsychiatric correlates.

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Keywords:

Alzheimer, endocannabinoid, cannabinoid receptor, anandamide, 2-arachidonoylglycerol, fatty acid amide hydrolase, monoacylglycerol, TRPV1, neurodegeneration

1. Introduction:

Alzheimer's disease (AD) is the most common form of dementia, with a global prevalence of approximately 40 million (Nicholls et al. 2018). The characteristic neuropathology includes the presence of intraneuronal neurofibrillary tangles, extracellular beta-amyloid(Aβ)-rich neuritic plaques, synaptic dysfunction, and glial cytopathology (Chen et al. 2019, Henstridge et al. 2019, Selkoe and Hardy. 2016).

The endocannabinoid system (ECS) is an important regulator of synaptic transmission, synaptic plasticity, cytokine release within the central nervous system (CNS), and may exert neuroprotective effects during neuronal injury (Bisogno & Di Marzo 2010, Cristino et al. 2020). The ECS consists of two primary receptors: cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R). CB1R is one of the most widespread G protein-coupled receptors within the human CNS, and highly expressed in prefrontal cortex, anterior cingulate cortex, hippocampus and striatum (Kano et al. 2009). Contrastingly, CB2R is expressed predominantly within the cellular immune system (namely B-cells, natural killer cells, activated microglia and macrophages) (Galiegue et al. 1995), and less frequently

expressed in healthy neural tissue (with expression identified in human brainstem neurons, and in rodent hippocampus)(Stempel et al. 2016, van Sickle et al. 2005).

The endogenous neurotransmitters of the ECS are referred to as endocannabinoids. The two best-characterised endocannabinoids are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG). 2-AG is expressed at approximately 200-fold greater concentrations than AEA within the CNS, and is a full agonist at CB1R and CB2R, whilst AEA acts as a partial agonist at CB1R and CB2R (Pertwee et al. 2010). A number of additional ligands and receptors have been identified as part of the ECS, including transient receptor potential channel ionotropic receptors, and nuclear receptors (Di Marzo. 2018, Pertwee et al.2010). Endocannabinoids are synthesised on-demand from lipid membrane components, and exert retrograde inhibition of neurotransmitter release from adjacent neurons via CB1R-mediated signalling. Degradative enzymes serve an important role in regulating endocannabinoid activity, with AEA predominantly degraded by fatty acid amide hydrolase (FAAH) and 2-AG being predominantly degraded by monoacylglycerol lipase (MAGL) (Kano et al. 2009).

Various lines of evidence suggest that ECS alterations are associated with AD pathophysiology, and that ECS-targeted pharmacotherapies may have disease-modifying effects. Transgenic mouse models of AD have demonstrated reduced hippocampal expression of CB1R, suggesting specific involvement of CB1R during disease progression (Bedse et al. 2014, Takkinen et al. 2018). CB1R mediated-signalling likely plays a role in reducing excitotoxicity-mediated apoptosis in AD, possibly through inhibiting glutamatergic excitoxicity (Rossi et al. 2015). CB2 agonist treatment appears to moderate microglial recruitment and cytokine release, promote amyloid clearance, and improve cognitive performance in transgenic mouse models of AD (Ehrhart et al. 2005, Ramirez et al. 2005). Additionally, combined phytocannabinoids Δ9-tetrahydrocannabinol and cannabidiol have been shown to inhibit microglial, astrocytic and amyloid-related neuropathological progression, and improve cognitive performance in a mouse model of AD (Aso et al. 2015).

Neuropsychiatric symptoms in AD represent an important and difficult-to-treat aspect of the condition (Ballard et al. 2009, Declerq et al. 2013, Lyketsos et al. 2011), and the ECS has attracted considerable interest as a potential target for novel drug development (Ahmed et al. 2015). Reviews focusing on ECS alterations in AD have reported a number of inconsistent findings reported by investigators (Ahmed et al. 2015, Bedse et al. 2015). Additionally, studies examining ECS alterations in AD rarely include those with mild cognitive impairment (MCI) (Ahmed et al. 2015, Bedse et al. 2015). Those with MCI are an important group to consider, as a proportion will have prodromal AD, which could aid identification of ECS alterations at relatively earlier stages in the AD disease process (Okello et al. 2009, Vos et al. 2013).

We are not aware of any systematic review of ECS alterations in AD that focuses solely on findings from human studies. Our aim was to identify reports of the ECS alterations in human AD or MCI in the following areas:

- CB1R and CB2R expression and functioning
- Expression and availability of anandamide (AEA) and 2-arachidonoyl glycerol (2-AG)
- Expression and activity of the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)
- Expression and activity of transient receptor potential vanilloid receptor 1 (TRPV1)

Secondary outcomes of interest were:

- To identify correlations between ECS alterations and other neuropathological changes in Alzheimer's disease
- To identify correlations between ECS alterations and neurocognitive or neuropsychological deficits in Alzheimer's disease

2. Results:

2.1 Literature search and study selection:

The database search was conducted on 13th March 2018, as summarised in the PRISMA flow chart **(figure 1)**.

2.2 Study characteristics:

22 studies were included, with sample sizes ranging from 4-75 AD cases (456 cases in total), and 2-45 controls (356 controls in total). Two studies assessed participants over multiple time-points. (Grünblatt et al. 2009, Lee et al. 2010) Study characteristics are summarised in **table 1**.

2.3 Study Quality:

No studies used power calculations to pre-determine sample size. One study did not use a control group (Tolon et al. 2009). Studies including MCI patients used recognised MCI diagnostic criteria, but did not report MCI clinical subtypes (Fonteh et al. 2013, González-Domínguez et al. 2016). 12 studies reported 100% of AD cases had either clinically probable or definite diagnoses (Ahmad et al. 2014, Ahmad et al. 2016, Altamura et al. 2015, Benito et al. 2003, Farooqui et al. 1988, Fonteh et al. 2013, Grünblatt et al. 2007, Jung et al. 2012, Lee et al. 2010, Mulder et al. 2011, Solas et al. 2013, Westlake et al. 1994). Risk of selection bias was low with the majority of studies using recognised criteria for AD diagnosis and with adequately described recruitment procedures.

All studies investigating ECS biomarkers used validated techniques, the limitations of which were appropriately discussed, with the exception of one older study (Farooqui et al. 1998). 13 studies adjusted for multiple comparisons (Ahmad et al. 2016, D'Addario et al. 2012, Fonteh et al. 2013, González-Domínguez et al. 2016, Grünblatt et al. 2007, Grünblatt et al. 2009, Halleskog et al. 2011, Manuel et al. 2014, Mulder et al. 2011, Pascual et al. 2014, Ramirez et al. 2005, Tolon et al. 2009, Westlake et al. 1994).

Seven studies controlled for potential confounders (e.g.: presence of inflammatory disease, medication exposure) (Ahmad et al. 2014, Ahmad et al. 2016, D'Addario et al. 2012, Pascual et al.

2014, Mulder et al. 2011, Ramirez et al. 2005, Solas et al. 2013). Correlational analyses tended to be exploratory in nature, though 8 studies used clear hypothesis-driven statistical analyses (Ahmad et al. 2014, Ahmad et al. 2016, D'Addario et al. 2012, González-Domínguez et al. 2016, Koppel et al. 2009, Manuel et al. 2014, Mulder et al. 2011, Ramirez et al. 2005).

A summary of study quality ratings is found in **table 2**. **[Further details on scoring is in the supplementary materials]**

2.4.1 Cannabinoid Receptor 1 (CB1R):

CB1R expression in AD remains ambiguous. Two moderate quality studies reported increased CB1R expression (in the frontal cortex, entorhinal cortex and caudate nucleus) in the earliest stages of AD pathological change (Braak Stage I-II) (Farkas et al. 2012, Manuel et al. 2014). It has been suggested that this early increase in CB1R expression may reflect compensatory upregulation of CB1R in early AD. Three post-mortem studies reported reduced expression of CB1R in later stage (Braak stage V-VI) disease (within the prefrontal cortex, entorhinal cortex, CA3 and CA1 hippocampal subfields, caudate nucleus and putamen) compared to either healthy controls, or those with Braak stage I-II disease (Manuel et al 2014, Ramirez et al. 2005, Solas et al. 2013). However, one group observed increased CB1R expression in prefrontal cortex in Braak Stage V-VI disease compared to controls (though expression was relatively decreased compared to cases at Braak Stages I-IV) (Farkas et al. 2012).

An older autoradiographic study, using ligand [^{3}H]CP55,940, reported reductions in CB1R binding density in the entorhinal cortex, subiculum, CA1 hippocampal subfield, dentate gyrus, substantia nigra pars reticularis, globus pallidus interna, and the caudate nucleus, relative to aged controls (Westlake et al. 1994). It is notable that AD neuropathological progression was not reported, making meaningful interpretation of this finding difficult, given the previous findings suggesting CB1R expression may vary according to the pathological progression of AD (Farkas et al. 2012, Manuel et al. 2014).

Other groups have reported no difference in CB1R expression between AD cases and controls, using a range of methods including PET (Ahmad et al. 2014), immunoblot analyses, autoradiography of frontal cortex samples (Lee et al. 2010), and Western blot analysis with immunofluorescence studies of hippocampal tissue (Mulder et al. 2011). Only one of these studies stratified cases according to Braak staging, and interpreted results in light of this (Mulder et al. 2011). It is notable that the PET study (Ahmed et al.2014) included AD cases with clinically milder disease (evidenced by higher cognitive scoring), which may have contributed to the finding of an apparent lack of difference in CB1R expression, given the possible upregulation of receptors in early disease (Farkas et al. 2012, Manuel et al. 2014).

CB1R mRNA expression did not significantly differ between AD cases and controls, including mRNA isolated from peripheral blood mononuclear cells (PBMCs) (D'Addario et al. 2012, Westlake et al. 1994). CB1R expression on hippocampal neuron presynaptic terminals did not differ from aged controls, suggesting that intracellular trafficking of CB1R is not altered in AD (Mulder et al. 2011). CB1R functioning may be altered in AD, with 2 separate groups reporting a reduction in CB1R-mediated G protein-coupling in hippocampal (Manuel et al. 2011) and frontal cortex neurons (Ramirez et al. 2005). Only one of these studies stratified cases based on the progression of AD, and identified reduced CB1R G protein-coupling in later stages of the disease (Braak V-VI cases) (Manuel et al. 2011). CB1R nitration (a marker of perioxynitrite radical formation) is increased in AD relative to controls, and thought to reflect increased microglial involvement in AD disease progression, though the impact of nitration on CB1R functioning is unclear (Ramirez et al. 2005).

2.4.2 Cannabinoid 2 Receptor (CB2R):

CB2R expression in AD has been identified within the hippocampus, entorhinal cortex, parahippocampus (Benito et al. 2003, Halleskog et al. 2011) and frontal cortex (Ramirez et al. 2005, Solas et al. 2013). CB2R expression positively correlated with Aβ-42 concentration, amyloid plaque burden, levels of hyperphosphorylated tau and neuritic tangles, consistent with the well-documented

finding of activated microglia accumulating in the vicinity of plaque and tangle pathology in AD (Halleskog et al. 2011, Ramirez et al. 2005, Solas et al. 2013).

CB2R expression appears increased within the hippocampus, parahippocampus and prefrontal cortex in Braak stage VI AD compared to controls, which likely reflects increased activated microglial involvement in advanced AD (Halleskog et al. 2011, Solas et al. 2013). One group reported no significant differences in frontal cortex CB2R expression between AD cases and age-matched controls, though AD pathological progression amongst cases was not reported (Ramirez et al. 2005). CB2R nitration is increased in AD compared to controls, though the functional significance of this is unclear (Ramirez et al. 2005). A small ex vivo study (of 4 AD cases) using hippocampal and parahippocampal tissue incubated with the CB2R agonist JWH-015, demonstrated microglial clearance of amyloid plaque occurring via a CB2R-mediated process, and amyloid clearance may be disrupted by co-administration of a CB2R antagonist (Tolon et al. 2009).

CNR2 mRNA expression has been reported to be increased in hippocampal samples in AD in a single postmortem study (Grünblatt et al. 2007). However, attempts to measure peripheral *CNR2* mRNA expression using peripheral blood mononuclear cells (PBMCs) (D'Addario et al. 2012) and serum *CNR2* RNA content (Grünblatt et al. 2009) have failed to show differences between AD cases and controls. Surprisingly, the only PET study that examined CB2R availability in AD has reported reduced uptake of CB2R ligand [^{11}C]NE40 in mild AD compared to healthy volunteers, with no correlation between [^{11}C]NE40 uptake and uptake of amyloid PET ligand [^{11}C]PiB (Ahmad et al. 2016).

2.4.3 Arachidonoylethanolamine (AEA):

Reductions in AEA concentration have been reported within the temporal (Jung et al. 2013) and mid-frontal cortex in AD at post-mortem (Pascual et al. 2014), with one group additionally reporting a reduction in AEA hydrolysis within the frontal cortex (Pascual et al. 2014). These studies were of moderate quality, though only one group examined the relationship between reduced cortical AEA content and neuropathological markers of AD progression (Jung et al. 2013). AEA and *N*-arachidonoyl-

substituted phosphatidylethanolamine (NAPE, a precursor molecule in AEA synthesis) concentrations negatively-correlated with mid-frontal lobe Aβ-42 content in one post-mortem study (without a corresponding correlation observed with hyperphosphorylated tau, amyloid plaque burden or APOEe4 gene carriership) suggesting a specific relationship between the Aβ-42 fibrils and AEA (Jung et al. 2013).

Serum AEA concentration appears unaltered in AD (Altamura et al. 2015, Koppel et al. 2009). The only group that attempted to detect AEA content within the CSF, were unable to detect AEA in either AD, or healthy controls, using high-performance liquid chromatography tandem mass-spectrometry (Koppel et al. 2009). Reduced phosphoethanolamine (PE, a synthetic precursor to AEA) in "nanometer-sized particle" fractions of CSF (a fraction reported to reflect lipid membrane exocytosis within the CNS) has been observed in AD compared to healthy controls. The group unfortunately did not examine AEA concentrations directly, making it difficult to draw firm conclusions about CSF AEA content in AD (Fonteh et al. 2013).

2.4.4 2-Arachidonoylglycerol (2-AG):

At post-mortem, a single study reported that mid-frontal and temporal cortex 2-AG content did not differ between AD and controls (Jung et al. 2012). An older, weaker-quality study reported increased diacylglycerol lipase (DAGL - involved in the synthesis of 2-AG) activity within the hippocampus and nucleus basalis of Meynert in AD (Farooqui et al. 1988). This finding is supported by a more recent and methodologically rigorous study showing increased DAGL expression (particularly the DAGLβ isoform) within hippocampal neurons and local microglia as AD pathology progresses (Mulder et al. 2011). Sites of 2-AG hydrolysis activity may also be altered in AD, with the same group reporting a relative "shift" of hydrolytic activity from neuronal membranes, to the cytosol (Mulder et al. 2011). The authors also used immunofluorescence histochemistry to demonstrate that ABHD6 (a serine hydrolase responsible for approximately 4% of 2-AG hydrolysis) is downregulated amongst neurons staining for markers of hyperphosphorylated tau, and co-localised with amyloid plaque and microglial markers.(Mulder et al.

2011) These findings suggest alterations in production, and trafficking of hydrolytic enzymes involved in 2-AG metabolism, occur in AD. The authors propose that relative increases in 2-AG retrograde signalling may directly lead to synaptic dysfunction in later stages of AD, but notably did not directly examine 2-AG content.

Two studies investigated plasma 2-AG concentration (Altamura et al. 2015, Koppel et al. 2015). The smaller study reported no difference in circulating plasma 2-AG concentration (Koppel et al 2009), whereas the larger (consisting of 41 AD cases), and arguably methodologically-stronger, study identified higher plasma 2-AG concentration in AD compared to age-matched controls (Altamura et al. 2015). CSF 2-AG concentration has been investigated by one group, which reported no difference between AD and age-matched controls (Koppel et al. 2009).

An association between 2-AG and vascular endothelial change has been reported (with higher 2-AG serum concentration associated with leukoaraiosis and a history of ischaemic heart disease), though the findings have only been reported by a single group, and the statistical methods used precluded further examination of the exact nature of this association (Altamura et al. 2015).

2.4.5 Fatty Acid Amide Hydrolase (FAAH):

In one of the only peripheral biomarker studies included, reduced methylation has been observed at the *FAAH* gene locus (corresponding to increased FAAH expression) in peripheral blood mononuclear cells (PBMCs) obtained from AD patients with moderate disease severity (D'Addario et al. 2012). Reduced serum concentrations of oleamide (a substrate of FAAH) has also been reported (González-Domínguez et al. 2016), providing some indirect evidence for peripherally increased FAAH activity in AD, though the study authors notably did not assess FAAH activity directly. Functional assays of FAAH activity have demonstrated increased FAAH activity in AD, both centrally (obtained from entorhinal and parahippocampal cortex, and dentate gyrus post-mortem) (Benito et al. 2003) and peripherally, from PBMCs (D'Addario et al. 2012).

FAAH has been demonstrated to co-localise with beta-amyloid rich plaques and hypertrophic astrocytes, which suggests FAAH expression and activity being dependent on the locational proximity of AD-related neuropathologic change (Benito et al. 2003). It should be noted that small tissue samples were obtained amongst controls, which may have underestimated FAAH activity seen in controls (Benito et al. 2003). Two more recent, methodologically rigorous studies reported mixed results. Reduced FAAH activity within neuronal membrane fractions obtained from the frontal cortex of AD cases was identified by one group (Pascual et al. 2014) (though this study did not provide information on neuropathological progression amongst AD cases), with a separate group finding no difference in FAAH protein expression within AD hippocampal samples compared to controls, including in Braak stage V-VI cases (Mulder et al. 2011).

2.4.6 Monoacylglycerol Lipase (MAGL):

Increased MAGL activity has been reported within the nucleus basalis of Meynert in a modestly-sized study of AD cases post-mortem (10 cases) (Farooqui et al. 1988). This study was published before the ECS had begun to be characterised, so other aspects of the ECS were not examined, and results were not interpreted with reference to the ECS. Additionally, AD cases were diagnosed clinically, with no information on diagnostic criteria used, or information on the extent of pathological progression AD amongst cases, limiting the interpretation of results. A more recent study utilised immunofluorescence staining and Western blotting techniques, and demonstrated an overall positive correlation between MAGL expression and pathological progression of AD in post-mortem hippocampal samples. Quantitative immunofluorescence analysis revealed a more specific relationship between hyperphosporylated tau and MAGL, where intraneuronal hyperphosphorylated tau is associated with a specific reduction in MAGL expression in hippocampal neurons (Mulder et al. 2011). Intraneuronal localisation of MAGL has been reported as being unaltered in AD, predominantly being localised at the presynaptic terminal (Mulder et al. 2011).

Indirect evidence for enhanced peripheral MAGL activity has been suggested by the observation of a reduced circulating concentrations of plasma monopalmitin and monostearin in AD (both of which are substrates for MAGL) (González-Domínguez et al. 2016). Contrastingly, a separate group reported no change in *MAGL* mRNA expression in PBMCs from AD cases, though this group did not directly examine peripheral MAGL activity in AD (D'Addario et al. 2012).

2.4.7 TRPV1 receptor:

A post-mortem study investigating TRPV1 immunoreactivity failed to demonstrate differences in hippocampal TRPV1 expression or binding density in AD (Mulder et al. 2011). *TRPV1* mRNA expression in PBMCs did not significantly differ between AD patients and controls (D'Addario et al. 2012).

2.4.8 ECS and Mild Cognitive Impairment (MCI):

Two of the studies included MCI patients. One group reported that concentrations of CSF glycerophospholipids involved in AEA synthesis - phosphoethanolamine (PE) and n-acylphosphatidylethanolamine (NAPE) – and the synthetic enzyme phospholipase A2, were not significantly altered in MCI compared to healthy controls. Whilst CSF PLA2 activity was reportedly increased compared to controls, this did not reach statistical significance (Fonteh et al. 2013). Serum oleamide (a substrate of FAAH) has been reported to be reduced in a single study, with a less pronounced decrease in serum oleamide observed in AD (possibly suggesting increased FAAH activity in early AD, normalising as the disease progresses) (González-Domínguez et al. 2016). No groups have directly examined FAAH activity or expression in MCI, however.

2.4.9 Neuropsychological correlates of ECS alterations in AD:

Few studies employed the same neuropsychological tests to compare individual aspects of the ECS, which impairs the ability to make direct comparisons of findings between studies.

The Mini Mental State Examination (MMSE) was the most frequently utilised tool for assessing cognition amongst the included studies (Ahmad et al. 2014, Altamura et al. 2015, D'Addario et al.

2012, Jung et al. 2012, Koppel et al. 2009, Lee et al. 2010). A positive correlation between frontal cortical CB1R immunoreactivity with MMSE and Cambridge Cognition Examination (CAMCOG) test performance prior to death has been reported by a single group (Lee et al. 2010). This study had the advantage of having assessed participants at multiple time-points prior to death, and having AD diagnoses confirmed at post-mortem (though CB1R expression was not interpreted in light of AD neuropathologic progression). No similar correlation between frontal cortex CB1R immunoreactivity and MMSE or CAMCOG performance were identified at earlier time-points, and CB1R immunoreactivity within the hippocampus, caudate nucleus or anterior cingulate cortex at post-mortem did not correlate with other either the MMSE or CAMCOG scores (Lee et al. 2010). Mid-frontal or temporal lobe 2-AG or AEA content at post-mortem also failed to correlate with MMSE performance (which was undertaken 10 months prior to death, on average) (Jung et al. 2012).

Reduced methylation at the *FAAH* gene locus (associated with increased FAAH expression) in PBMCs has been associated with poorer cognitive performance (scoring <10 on the MMSE), suggesting that epigenetic changes of ECS biomarkers may be detectable in the later stages of AD (D'Addario et al. 2012). This finding is yet to be replicated, and the significance of epigenetic changes at the *FAAH* gene on cognition in AD remains unclear. Other studies have reported no correlation with CB1R PET ligand [^{18}F]MK-9740 binding (Ahmad et al. 2014), or CSF and plasma 2-AG content (Koppel et al. 2009) and MMSE scores in AD.

Other memory tests utilised included the Rey Auditory Verbal Learning Test (RAVLT), with a single group identifying an association increased 2-AG plasma concentration and improved task-performance (Altamura et al. 2015). No correlation has been identified between RAVLT performance and uptake of PET radioligands [^{18}F]MK-9740 (for CB1R) (Ahmad et al. 2014) and [^{11}C]NE40 (for CB2R) in AD (Ahmad et al. 2016). Task performance on the Boston Naming Test (BNT) has been associated with temporal lobe AEA content at post-mortem in AD (though mid-frontal AEA content did not correlate with task performance). It should be noted that the control group in this particular study

were tested a mean of 45 months prior to death, compared to 10 months prior to death in the AD group (though the groups were age-matched, suggesting the differences were not necessarily due to ageing) (Jung et al. 2012). Uptake of CB2R PET radioligand [^{11}C]NE40 did not correlate with BNT performance (Ahmad et al. 2016). It is notable that uptake of the CB2R PET ligand [^{11}C]NE40 did not correlate with any cognitive test performances, which may suggest a floor-effect, where PET using CB2R radioligands may lack the sufficient resolution to detect potentially very small changes in CB2R availability in AD relative to controls (Ahmad et al. 2016). A single study utilised the Kendrick Digit Copy Test (KDCT), reporting that task performance correlated with and midfrontal AEA content at post-mortem in AD, compared to controls (Jung et al. 2012).

Tests of executive function and fluid intelligence were utilised by some studies. Three groups utilised trail-making tests A and B, though no correlations were reported between task performance and plasma 2-AG, plasma AEA , CSF 2-AG concentration (Koppel et al 2009), temporal and midfrontal cortical 2-AG or AEA content at post-mortem (Jung et al 2012), or regional uptake of CB2R PET radioligand [^{11}C]NE40 (Ahmad et al. 2016). One group utilised Raven's Coloured Progressive Matrices Task, and reported no correlation between plasma 2-AG and task performance (Altamura et al. 2015).

Behavioural aspects of AD were investigated by one group (Solas et al. 2013), where hypophagia (measured using the Present Behavioural Examination) associated with reduced frontal cortex CB1R expression (independent of MMSE scores, Aβ42 expression, or plaque burden).

3 Discussion:

3.1 Methodological issues:

The included studies were methodologically heterogeneous, with aspects of the ECS rarely examined using same methodology across studies, making meaningful comparison between individual studies difficult. Many studies did not analyse results in light of clinical or neuropathological progression of

AD, which limited the conclusions that could be drawn about ECS change and AD progression (Benito et al. 2003, Farooqui et al. 1988, Fonteh et al. 2013, González-Domínguez et al. 2016, Grünblatt et al. 2007, Lee et al. 2010, Pascual et al. 2014,Solas et al. 2013, Tolon et al. 2009, Westlake et al. 1994).

Post-mortem studies varied in terms of neuropathologic criteria used for AD diagnosis, anatomical site sampled, tissue preparation, and post-mortem interval. One study included patients with coexisting neurodegenerative conditions in the AD patient group, which may have confounded their results (Halleskog et al. 2011). 8 studies did not state proportions of "probable" or "definite" AD diagnoses (D'Addario et al. 2012, González-Domínguez et al. 2016, Grünblatt et al. 2009, Koppel et al. 2009, Manuel et al. 2014, Pascual et al. 2014, Ramirez et al. 2005, Tolon et al. 2009). Interpolating ECS alterations underlying performance in neuropsychological test scores in life from post-mortem findings, remains open to a number of potential biases – particularly as the mean time-interval between test and post-mortem was as long as 10 months in some studies (Jung et al. 2012).

Control groups were not always age-matched (Ahmad et al. 2016, Benito et al. 2003, Farkas et al. 2012, Farooqui et al. 1988, Westlake et al. 1994), which is important as ECS functioning undergoes age-related change (Takkinen et al. 2018). Additionally, few studies specifically reported on gender or ethnicity of participants.

No studies undertook power calculations, and the design of the majority of studies was rated as "weak", accordingly. Only 5 studies had sample sizes of ≥30 AD cases (Altamura et al. 2015, D'Addario et al. 2012, González-Domínguez et al. 2016, Jung et al. 2012, Manuel et al. 2014). Adequate powering remains a debated area in PET and post-mortem studies, and there is a lack of consensus regarding minimum sample sizes required for these methods (Doot et al. 2012, Meurs 2016). A number of studies carried out extensive subgroup analyses within AD case cohorts, and while 13 studies included correction for multiple comparisons, the possibility of type I error remains (Manuel et al. 2014, Mulder et al. 2011, Westlake et al. 1994).

No studies that included MCI cases specified clinical subtype (eg: amnestic-type), or utilised amyloid PET imaging for more informed case ascertainment (Fonteh et al. 2013, González-Domínguez et al. 2016).

3.2 Recommendations for future research:

ECS tone and functioning varies according to age, gender and ethnic group, and controlling for these factors as far as possible will help reduce potential bias in future studies (Kantae et al. 2017, Laurikainen et al. 2019). Screening for the presence of inflammatory conditions and anti-inflammatory medication is advisable, as both appear to influence the functioning of the ECS and the endovanilloid system in vitro (Donvito et al. 2017, Fowler 2012, Malek and Starowicz 2016). Cannabis exposure may transiently reduce CB1R availability, so a standardized assessment of participants' cannabis exposure should also be undertaken (Bloomfield et al. 2019).

CB1R expression in AD remains ambiguous, as approximately half of the reports suggest no significant difference compared to controls (Ahmad et al. 2014, Lee et al. 2010, Mulder et al. 2011). A correlation between prefrontal cortex CB1R expression and cognitive function in AD has been suggested (Lee et al. 2010), which is consistent with the reported cognitive benefits associated with CB1R agonist therapy in mouse models of AD (Aso et al. 2015). The literature regarding the role of CB1R in cognition is complex, however, with CB1R agonism having been demonstrated to impair a variety of hippocampal-dependent memory processes (Morena et al. 2014). Prefrontal CB1R activity likely confers a different effect on cognitive performance to hippocampal CB1R activity, with prefrontal CB1R agonism being associated with increases in noradrenergic signalling, which may confer effects on non-memory related cognitive function, such as attention (Oropeza et al. 2005).

Use of PET to identify ECS alterations in AD remains a nascent field. The unexpected finding of CB2R radioligand [^{11}C]NE40 uptake being lower in AD compared to controls (Ahmad et al. 2016) is difficult to make sense of, but may be explained by ligand cross-reactivity with CB1R receptors, or other off-

target activity in controls – a problem increasingly recognized with a number of CB1R and CB2R ligands (Soethoudt et al. 2017).

Some groups utilised functional assays to assess CB1R and CB2R activity, such as cannabinoid receptor-associated G-protein activity assays (Manuel et al. 2014, Ramirez et al. 2005), or by using cannabinoid agonists to assess cannabinoid receptor-mediated downstream effects on AEA hydrolysis (Pascual et al. 2014) and amyloid clearance (Tolon et al. 2009). These methods have the potential to provide a more sophisticated mechanistic understanding of the role of the ECS in AD. It is now recognised that particular CB1R and CB2R ligands appear to show preferences for some specific secondary-messenger signalling pathways over others, so future similar study designs will need to take this finding into account (Soethoudt et al. 2017).

Few studies attempted to correlate ECS alterations with neuropsychiatric features, though one group identified a correlation between reduced prefrontal cortex CB1R expression and hypophagia (Solas et al. 2013). This is perhaps consistent with findings from small trials using Δ9-tetrahydrocannabinol (THC) analogues that have demonstrated improvements in anorexia and circadian rhythm disturbance associated with in dementia (Volicer et al. 1997, Walther et al. 2011, Woodward et al. 2014). Whether other specific neuropsychiatric features of AD such as agitation, apathy or psychosis are characterised by particular ECS alterations remains uncertain.

ECS alterations in psychosis have been the focus of intense research, and there is evidence for some commonalities underlying the neurobiology of both psychosis in schizophrenia, and psychosis in Alzheimer's disease (Reeves et al. 2012). Recently identified ECS-relevant biomarkers for psychosis include increased CSF AEA content (Minichino et al. 2019), and an intronic variant *CNR2* which may moderate propensity to psychotic experiences. (Legge et al. 2019) Koppel et al. had attempted to assess CSF AEA content in AD, but were unable to detect this using high-performance liquid chromatography tandem-mass spectrometry (Koppel et al. 2009). A separate group has managed to detect and quantify CSF AEA content in participants with dementia (their sample consisting of both

AD and vascular subtypes), indicating that it is possible to detect CSF AEA content within this population.(Giuffrida et al. 2004) Though TRPV1 alterations were not identified in AD (Mulder et al. 2011) it is possible that endovanilloid-targeted treatments may show some promise in the treatment of psychosis in AD nevertheless. TRPV1 agonism has been implicated in reducing striatal hyperdopaminergia, suggestive of a novel therapeutic target for psychotic symptoms (Almeida et al. 2014, Tzavara et al. 2006).

Hippocampal MAGL expression has been reported to increase (Farooqui et al. 1988) and "shift" from neuronal to predominantly microglial expression during AD progression, though the exact significance of this is unclear. (Mulder et al. 2011) Interactions between beta amyloid-42, 2-AG and MAGL appears to contribute to hippocampal dysfunction in AD, with aberrant 2-AG-associated depolarisation-induced suppression of inhibition being hypothesised to compound beta amyloid-42-related synaptic dysfunction (Mulder et al. 2011, van der Stelt et al. 2006). More research is required to elucidate the relationship with 2-AG and MAGL in hippocampal dysfunction in AD at a cellular and electrophysiological levels. Novel PET radioligands, such as [^{11}C]SAR127303, may offer a promising tool to explore changes in MAGL in AD in vivo (Yamasaki et al. 2018).

The importance of glial involvement in AD pathology has been increasingly recognised, and ECS-glial interaction occurring in AD requires further characterisation (Hansen et al. 2018, Henstridge et al. 2019). In vitro and in vivo evidence suggests CB2R-mediated mechanisms attenuate microglial activation and release of proinflammatory cytokine tumour necrosis factor(TNF)-α associated with Aβ fibril exposure (López et al. 2018, Ramirez et al. 2005).

Peripheral expression and activity of FAAH may be increased in AD, though the underlying mechanism responsible remains unclear (Benito et al. 2003, D'Addario et al. 2012). Enhanced FAAH activity may contribute to a proinflammatory state, arising from increased downstream production of arachidonic acid and eicosanoid products following the breakdown of AEA(D'Addario et al. 2012). Similarly, rodent models have shown FAAH inhibition (and TRPV1 antagonism) have demonstrated reduced

concentrations of proinflammatory cytokine interleukin-6 within the rodent hippocampus (Henry et al. 2017). The FAAH PET radioligand [^{11}C]CURB (Boileau et al. 2016) may help identify region-specific changes in CNS FAAH availability in AD, and help quantify the effects of FAAH-directed pharmacotherapies in vivo. Though previous trials of FAAH inhibitors in humans have been beset by failure (Di Marzo et al. 2018)**,** compounds such as the dual FAAH-inhibitor and TRPV1 antagonist *N*-arachidonyl serotonin, or combined FAAH- and acetylcholinesterase-inhibitors, represent intriguing avenues for novel drug development in AD treatments (Montanari et al. 2016, Micale et al. 2009).

Improved cognition in AD appears to be associated with both reduced *FAAH* gene expression (D'Addario et al. 2012), and increased AEA content in the midfrontal and temporal cortex, which suggests a potential therapeutic benefit of FAAH inhibition in AD (Jung et al. 2012). However, there is also evidence that cognitive impairment in AD may be exacerbated by FAAH inhibition, as a potential consequence of persistent AEA activity at hippocampal CB1R (Basavarajappa et al. 2014, Goonawardena et al. 2011).

Late-life anxiety is an increasingly-recognised risk factor for the development of AD (Santabárbara et al. 2020). Given the indirect evidence of FAAH activity being increased in both MCI and AD (González-Domínguez et al. 2016), and the apparent anxiolytic effects of FAAH-inhibition (Bedse et al. 2018, , Mayo et al. 2020), it is tempting to speculate on the role AEA and FAAH may play in contributing to anxiety states associated with MCI and AD.

Studies investigating ECS alterations in MCI should attempt to differentiate between MCI clinical subtypes, as the amnestic- and amnestic-dysexecutive subtypes appear more predictive of prodromal AD (Vos et al. 2013, Jung et al. 2020). Biomarker-assisted MCI case ascertainment (including amyloid-retention status on PET, CSF Aβ42/tau content, novel CSF biomarkers such as neurofilament light, and *APOE* ε4 carriership) would allow for more reliable identification of those where MCI is likely to be a manifestation of prodromal AD (Csukly et al. 2016, Okello et al. 2009, Zetterberg et al. 2016).

Post-mortem studies investigating the ECS alterations in AD should attempt to assess for the presence of co-existing non-AD proteinopathies, as their presence could conceivably influence ECS functioning in vivo (Cristino et al. 2020, Nelson et al. 2019).

3.3 Limitations:

The review was not designed to identify publication bias, or the grey literature, and was limited to English-language publications.

This review examined the most well-characterised endocannabinoids, degradative enzymes, and receptors of the ECS. This review did not focus on precursor or synthetic molecules such as *N*-Arachidonoyl-phosphatidylethanolamine and DAG, and focussed solely on TRPV1 as opposed to other receptors in the TRP family (De Petrocellis et al. 2017, Di Marzo 2018, Wood et al. 2015).

Assessing methodologically heterogeneous studies using a single quality assessment does not allow for particularly detailed analysis of the quality of individual studies. The methodological heterogeneity meant that few of the study findings can be meaningfully compared with each other, and meta-analysis was not possible. The limited quality of studies included (with the majority rated "moderate" or "weak" in quality) mean that findings should be interpreted with some caution. Lastly, we cannot exclude the possibility that other relevant studies were conducted since the literature search was conducted.

3.4 Conclusions:

To our knowledge, this is the first systematic review to synthesise findings of ECS alterations in AD from all known human studies, published over a span of over 30 years. The studies are methodologically heterogeneous, and typically of moderate-quality, limiting the interpretations that can be drawn from them. However, the results support the notion that alterations in CB1R, CB2R,

central AEA concentration, MAGL and FAAH activity, occur in AD. ECS biomarkers are amenable to testing in AD, and may represent promising avenues for novel drug development.

CB1R expression appears reduced in hippocampal and parahippocampal areas as AD progresses, though there are mixed findings overall (Farkas et al. 2012, Manuel et al. 2014, Mulder et al. 2011, Ramirez et al. 2005, Solas et al. 2013, Westlake et al. 1994). CB2R expression appears increased in hippocampal and parahippocampal regions as microglial involvement becomes more prominent in the disease process (Benito et al. 2003, Ramirez et al. 2005, Halleskog et al. 2012). Frontal and temporal cortical AEA concentrations may decrease in AD, with limited evidence to link this with cognitive performance in life (Jung et al. 2012). Sites of cellular hydrolysis of 2-AG may be altered in AD, with mixed evidence on whether 2-AG availability changes in AD (Mulder et al. 2011, Jung et al. 2012, Altamura et al. 2015). There is evidence suggesting MAGL expression increases with progression of AD in hippocampal neurons (Farooqui et al. 1988, Mulder et al. 2011). FAAH expression and activity may be increased in AD, and may be able to be detected peripherally (D'Addario et al. 2012, González-Domínguez et al. 2016). Currently, there is no evidence for TRPV1 alterations in AD. A number of studies examined the relations between ECS alterations and cognitive performance, though very few studies examined the relationship between ECS alterations and behavioural or psychiatric symptomatology in AD (Ahmad et al. 2014, Altamura et al. 2015, D'Addario et al. 2012, Jung et al. 2012, Koppel et al. 2009, Lee et al. 2010).

4. Methods and materials:

4.1 Literature search:

We reviewed literature on ECS alterations in AD published within the following: PubMed; Embase; MEDLINE; PsycINFO; and ALOIS, from inception to 2018. The review was registered on the PROSPERO database (CRD42018096249). (Search terms used can be found in the Supplementary Materials).

References were screened for studies suitable for inclusion. Ethical approval was not required for the review.

4.2 Inclusion and exclusion criteria:

English-language publications reporting people diagnosed with AD or MCI (including post-mortem studies with pathologically-confirmed AD, and studies without controls) were included. To make the review more specific to changes in late-onset AD, we excluded studies involving people with Down's syndrome, murine studies, in vitro studies performed on non-human tissue, studies which did not analyse AD or MCI cases separately, or those that did not specify the number of patients with AD or MCI included. Grey-literature publications, conference abstracts and presentations were excluded.

4.3 Data Extraction:

Two review authors (AB and OZ) independently extracted data from studies relating to any of the primary or secondary outcomes of interest. Data was tabulated using Microsoft Excel. Disagreements amongst authors were resolved by discussion and involvement of a third author (SR or RH).

4.4 Quality Assessment:

Study quality was assessed using an adapted EPHPP Quality Assessment Tool for Quantitative Studies (Moher et al. 2009). We anticipated heterogeneous study designs, and the tool was modified to reflect this. The following sections were omitted: (D)Blinding; (E) Data Collection Methods; (F) Withdrawals and Dropouts; (G) Intervention integrity. Selection bias was assessed to ensure that cases were representative of AD and MCI patients. Study design was assessed for the following: sample size, power calculations, whether inclusion/exclusion criteria were clearly defined, and whether sample characteristics (age, sex, post-mortem delay) were reported. ECS system biomarkers of interest were assessed for whether they had been clearly defined by authors, if the biomarkers were investigated using validated techniques, and limitations of the techniques in relation to AD (if relevant) were

discussed within the studies. For secondary outcomes of interest, we assessed if validated techniques or tools were used. Statistical analyses were assessed for appropriateness, whether corrections for multiple comparisons were performed, appropriate correction of confounders took place, whether analyses were hypothesis-driven, and if results were explained in light of statistical adjustments. Scores for individual sections A (selection bias), B (study design), C (ECS biomarker used), D (correlates), E (analysis and confounders) were combined to give ratings of "weak", "medium" and "strong". An overall score for each study was also assigned in accordance with the following criteria: Strong = 0 Weak ratings; Moderate = 1 Weak rating (cannot be from section A); Weak = ≥2 Weak ratings).

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5. References:

1. Ahmad R, Goffin K, Van den Stock J, De Winter F-L, Cleeren E, Bormans G, Tournoy J, Persoons P, Van Laere K, Vandenbulcke M. In vivo type 1 cannabinoid receptor availability in Alzheimer's disease. Euro Neuropsychopharmacology. 2014. 24. pp242-250
2. Ahmad R, Postnov A, Bormans G, Versjipt J, Vandenbulcke M, van Laere K. Decreased in vivo availability of the cannabinoid type 2 receptor in Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2016. 43. pp2219-2227
3. Ahmed AIA, van der Marck MA, van den Elsen GAH, Olde Rikkert MGM. Cannabinoids in late onset Alzheimer's disease. *Clin Pharmacol Ther*. 2015. 97(6). pp597-606
4. Altamura C, Ventriglia M, Martini MG, Montesano D, Errante Y, Piscitelli F et al. Elevation of plasma 2-arachidonoylglycerol levels in Alzheimer's disease patients as a potential protective mechanism against neurodegenerative decline. *J Alzheimers Dis*. 2015. 46. pp497-506
5. Almeida V Peres FF, Levin R, Suiama MA, Calzavara MD, Zuardi AW et al. Effects of cannabinoid and vanilloid drugs on positive and negative symptoms on an animal model of schizophrenia. *Schizophr Res*. 2014. 153(1-3). pp150-159
6. Aso E, Sanchez-Pla A, Vegas-Lozano E, Maldonado R, Ferrer I. Cannabis-based medicine reduced multiple pathological processes in AβPP/PS1 mice. *J Alzheimers Dis*. 2015.43.pp977-991
7. Basavarajappa BS, Nagre NN, Xie S, Subbanna S. Elevation of endogenous anandamide impairs LTP, learning and memory through CB1R signalling in mice. *Hippocampus*. 2014. 24(7).pp808-818
8. Ballard CG, Gauthier S, Cummings JL, Brodaty H, Grossberg GT, Robert P, Lyketsos CG. Management of aggression and agitation in Alzheimer disease. *Nat Rev Neurol*. 2009. 5(5). pp245-55
9. Bedse G, Romano A, Cianci S, Lavecchia AM, Lorenzo P, Elphick MR et al. Altered expression of the CB1 cannabinoid receptor in a triple transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis*. 2014. 40 (3). pp701-12

10. Bedse G, Romano A, Lavecchia AM, Cassano T, Gaetani S. The role of endocannabinoid signalling in the molecular mechanisms of neurodegeneration in Alzheimer's disease. *J Alzheimers Dis*. 2015. 43. pp1115-1136
11. Bedse G, Bluett RJ, Patrick TA, Romness NK, Gaulden AD, Kingsley PJ et al. Therapeutic endocannabinoid augmentation for mood and anxiety disorders: comparing profiling of FAAH, MAGL and dual inhibitors. *Transl Psychiatry*. 2018.8 (1). 92 doi: 10.1038/s41398-018-0141-7
12. Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ, Romero J. Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci*. 2003. 23(35). pp11136-11141
13. Bisogno T, Di Marzo V. Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. *CNS Neurol Disord Drug Targets*. 2010. 9. pp564-73
14. Bloomfield MAP, Hindocha C, Green SF, Wall MB, Lees R, Petrilli, Costello H et al. The neuropharmacology of cannabis: a review of human imaging studies. *Pharmacol Ther*. 2019. 195. pp132-161
15. Boileau I, Mansouri E, Williams B, Le Foll B, Rusjan P, Mizrahi R, Tyndale RF et al. Fatty acid amide hydrolase binding in brain of cannabis users: imaging with the novel radiotracer [^{11}C]CURB. *Biol Psychiatry*. 2016. 80(9). pp691-701
16. Chen Y, Fu AKY, Ip NY. Synaptic dysfunction in Alzheimer's disease. *Pharmacology and Therapeutics*. 2019. 195. pp186-198
17. Cristino L, Bisogno T, Di Marzo V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nat Rev Neurosci*. 2020. 16. pp9-29
18. Csukly G, Siraly E, Fodor Z, Horváth A, Salacz P, Hidasi Z, Csibri É, Rudas G, Szabó. The differentiation of amnestic type MCI from non-amnestic types by structural MRI. Front Agin Neurosci. 2016.
19. D'Addario C, Di Francesco A, Arosio B, Gussago C, Dell'Osso B, Bari M *et al*. Epigenetic regulation of fatty acid amide hydrolase in Alzheimer disease. *PLoS One*. 2012. 7(6). e39186
20. Declercq T, Petrovic M, Azermai M, Vander Stichele R, De Sutter AI, van Driel ML, Christiaens T. Withdrawal versus continuation of chronic antipsychotic drugs for behavioural and psychological symptoms in older people with dementia. *Cochrane Database Syst Rev*. 2013.(3). CD007726

21. De Petrocellis L, Nabissi M, Santoni G, Ligresti A. Actions and regulation of ionotropic cannabinoid receptors. *Adv Pharmacol*. 2017. 80. pp249-289

22. Di Marzo V. New approaches and challenges to targeting the endocannabinoid system. *Nature Rev Drug Disc*. 2018. 17(9). pp623-639

23. Donvito G, Nass SR, Wilkersen JL, Curry ZA, Schurman LD, Kinsey SG et al. The endogenous cannabinoid system: a budding source of targets for treating inflammatory and neuropathic pain. *Neuropsychopharmacology*. 2017. 43. pp52-79

24. Doot RK, Kurland BF, Kinahan PE, Mankoff DA. Design considerations for using PET as a response measure in single- and multi-centre clinical trials. *Acad Radiol.* 2012. 19(2). pp184-190

25. Ehrhart J, Obegron D, Mori T, Hou H, Sun N, Bai Y et al. Stimulation of cannabinoid receptor 2 (CB2) supresses microglial activity. *J Neuroinflammation*. 2005. 2. 29

26. Farkas S, Nagy K, Palkovits M, Kovacs GG, Jia Z, Donohue S, Pike V et al. [^{125}I]SD-7015 reveals fine modalities of CB1 cannabinoid receptor density in the prefrontal cortex during progression of Alzheimer's disease. *Neurochemistry International*. 2012. 60. Pp286-291

27. Farooqui AA, Liss L, Horrocks LA. Stimulation of lipolytic enzymes in Alzheimer's disease. *Ann Neurol.* 23(3). 1988. pp306-308

28. Fonteh AN, Chiang J, Cipolla M, Hale J, Diallo F, Chirino A, Arakaki X, Harrington MG. Alterations in cerebrospinal fluid glycerophospholipids and phospholipase A2 activity in Alzheimer's disease. *J Lipid Res*. 2013. 54(10). pp2884-97

29. Fowler CJ. NSAIDs: eNdocannabinoid stimulating anti-inflammatory drugs. *Trends Pharmacol Sci*. 2012. 33(9). pp468-473

30. Galiegue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *European Journal of Biochemistry*. 1995. 232(1). pp54-61

31. Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J et al. Cerebrospinal fluid anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*. 2004. 29. pp2108-2114

32. González-Domínguez R, Javier-Rupérez F, García-Barrera T, Barbas C, Gómez-Ariza. Metabolomic-driven elucidation of serum disturbances associated with Alzheimer's disease and mild cognitive impairment. *Curr Alzheimer Research*. 2016. 13(6). pp641-653

33. Goonawardena AV, Sesay J, Sexton CA, Riedel G, Hampson RE. Pharmacological elevation of anandamide impairs short term memory by altering the neurophysiology of the hippocampus. *Neuropharmacology.* 2011.61(5-6). pp1016-25

34. Grünblatt E, Zander N, Bartl J, Jie L, Monoranu C-M, Arzberger T, Ravid R et al. Comparison analysis of gene expression between sporadic Alzheimer's and Parkinson's disease. *J Alzheimers Dis*. 2007. 12. pp291-311

35. Grünblatt E, Bartl J, Zehetmeyer S, Ringel TM, Bauer P, Riederer P, Jacob CP. Gene expression as peripheral biomarkers for sporadic Alzheimer's disease. *J Alzheimers Dis*. 2009. 16. pp627-634

36. Halleskog C, Mulder J, Dahlström J, Mackie K, Hortobágyi T, Tanila H et al. WNT signalling in activated microglia is proinflammtory. *Glia*. 2011. 59(1). Pp119-131

37. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J Clin Biochem.* 2018. 217(2).pp459-472

38. Henry RJ, Kerr DM, Flannery LE et al. Pharmacological inhibition of FAAH modulates TLR-induced neuroinflammation, but not sickness behaviour: an effect partially mediated by central TRPV1. *Brain Behav Immun*. 2017. 62. pp318-331

39. Henstridge CM, Hyman BT, Spires-Jones TL. Beyond the neuron – cellular interactions early in Alzheimer disease pathogenesis. *Nat Rev Neurosci*. 2019. 20. pp94-108

40. Jung K-M, Astarita G, Yasar S, Vasilevko V, Cribbs DH, Head E, Cotman CW, Piomelli D. An amyloid β42-dependent deficit in anandamide mobilization is associated with cognitive dysfunction in Alzheimer's disease. *Neurobiol Aging.* 2012. 33. pp1522-1532

41. Jung YH, Park S, Jang H, Cho SH, Kim SJ, Kim JP et al. Frontal-executive dysfunction affects dementia conversion in patients with amnestic mild cognitive impairment. *Sci Rep.* 2020. 10. 77. https://doi.org/10.1038/s41598-020-57525-6

42. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev*. 2009. 89. pp309-380

43. Kantae V, Nahon KJ, Straat ME, Bakker LEH, Harms AC, van der Stelt M et al. Endocannabinoid tone is higher in healthy lean South Asian than white Caucasian men. *Sci Rep*. 2017. 7(1). 7558

44. Koppel J, Bradshaw H, Goldberg TE, Khalili K, Marambaud P, Walker MJ et al. Endocannabinoids in Alzheimer's disease and their impact on normative cognitive performance: a case-control and cohort study. *Lipids Health Dis*. 2009. 8.2. doi:10.1186/1476-511X-8-2

45. Laurikainen H, Tuominen L, Tikka M, Merisaari H, Armio R, Sormunen E et al. Sex difference in brain CB1 receptor availability in man. *Neuroimage*. 2019. 184. pp834-842

46. Lee JH, Agacinksi G, Williams JH, Wilcock GK, Esiri MM, Francis PT, Wong PTH, Chen CP, Lai MKP. Intact cannabinoid receptors in the Alzheimer's disease cortex. *Neurochemistry International.* 2010. 57. pp985-989

47. Legge SE, Jones HJ, Kendall KM, Pardiñas AF, Menzies G, Bracher-Smith M et al. Association of genetic liability to psychotic experiences with neuropsychiatric disorders and traits. *JAMA Psychiatry*. 2019. doi:10.1001/jamapsychiatry.2019.2508

48. López A, Aparicio N, Pazos MR, Grande MT, Barreda-Manso MA, Benito-Cuesta et al. Cannabinoid CB2 receptors in mouse brain: relevance for Alzheimer's disease. J Neuroinflammation. 2018. 15 (158) https://doi.org/10.1186/s12974-018-1174-9

49. Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, Cedarbaum J, Brashear R, Miller DS. Neuropsychiatric symptoms in Alzheimer's disease. *Alzheimers Dement*. 2011.7(5). pp532-539

50. Malek N, Starowicz K. Dual-acting compounds targeting endocannabinoid and endovanniloid systems – a novel treatment option for chronic pain management. *Front Pharmacol*. 2016. 7. 257

51. Manuel I, Gonzalez de san Roman E, Giralt MT, Ferrer I, Rodriguez-Puertas R. Type-1 Cannabinoid receptor during Alzheimer's disease progression. *J Alzheimers Dis*. 2014. 42. pp761-766

52. Mayo LM, Asratian, Lindé J, Morena M, Haataja R, Hammar V. Elevated anandamide, enhanced recall of fear extinction, and attenuated stress responses following inhibition of fatty acid amide hydrolase: a randomized, controlled experimental medicine trial. *Biol Psychiatry*. 2020. 87 (6). pp538-547

53. Meurs J. The experimental design of post-mortem studies: the effect size and statistical power. *Forensic Sci Med Pathol*. 2016. 12. pp343-349

54. Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, Drago F et al. Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels. *Neuropsychopharmacology.* 2009. 34. 593-606

55. Minichino A, Senior M, Brondino N, Zhang SH, Godwlewska BR, Burnet PWJ, Cipriani A, Lennox B. Measuring disturbance of the endocannabinoid system in psychosis A systematic review and meta-analysis. *JAMA Psychiatry*. 2019. 76(9). pp914-923

56. Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., Group, P., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med. 6*. e1000097. doi: 10.1371/journal.pmed.1000097.

57. Montanari S, Scalvini L, Bartolini M, Belluti F, Gobbi S, Andrisano V et al. Fatty acid amide hydrolase (FAAH), Acetylchoinesterase (AChE), and butyrylcholinesterase (BuChE): networked targets for development of carbamates as potential anti-Alzheimer's disease agents. *J Med Chem*. 2016. 59 (13). pp6387-6406

58. Morena M, Campolongo P. The endocannabinoid system: an emotional buffer in the modulation of memory function. *Neurobiol Learn Memory*. 2014. 112. pp30-43

59. Mulder J, Zilberter M, Pasquire SJ, Alpar A, Schulte G, Ferreira SG et al. Molecular reorganization of endocannabinoid signalling in Alzheimer's disease. *Brain*. 2011. 134. Pp1041-1060

60. Nelson PT, Dickson DW, Trojanowski JQ, Jack CR, Boyle PA, Afanakis K et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. *Brain*. 2019. 142 (6). 1503-1527

61. Nichols E, Szoeke CEI, Vollset SE, Abbasi N, Abd-Allah F, Abdela J et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1996-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019. 18(1). pp88-106

62. Okello A, Koivunen J, Edison P, Archer HA, Turkheimer FE, Någren K et al. Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C -PIB PET study. *Neurology*. 2009. 73(10). pp754-60

63. Oropeza VC, Page ME, Van Bockstaele EJ. Systemic administration of WIN 55,212-2 increases norepinephrine release in rat frontal cortex. *Brain Res*. 2005. 1046(1-2).45-54

64. Pascual AC, Martin-Moreno AM, Giusto NM, de Ceballos ML, Pasquaré. Normal aging in rats and pathological aging in human Alzheimer's disease decrease FAAH activity: modulation by cannabinoid agonists. *Exp Gerontol*. 60. 2014. pp92-99

65. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR et al. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacol Rev.* 2010. 17(14). pp1360-81

66. Ramirez B, Blazquez C, Gomez del Pulgar T, Guzman M, de Ceballos ML. Prevention of Alzheimer's Disease Pathology by Cannabinoids: Neuroprotection Mediated by Blockade of Microglial Activation. *J Neurosci*. 2005. 25(8). pp1904-1913

67. Reeves SJ, Gould RL, Powell JF, Howard RJ. Origins of delusions in Alzheimer's disease. *Neurosci Biobehav Rev*. 2012. 36. pp2274-2287

68. Rossi S, Motta C, Musella A, Centonze D. The interplay between inflammatory cytokines and the endocannabinoid system in the regulation of synaptic transmission. *Neuropharmacology*. 2015. 96 (Pt A). pp105-12

69. Santabárbara J, Lipnicki DM, Bueno-Notivol J, Olaya-Guzmán B, Villagrasa B, López-Antón R. Updating the evidence for an association between anxiety and risk of Alzheimer's disease: a meta-analysis of prospective cohort studies. *J Affect Disord*. 2020. 262. pp397-404

70. Soethoudt M, Grether U, Fingerle J, Grim TW, Fezza F, de Petrocellis L et al. Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. *Nat Communications.* 2017. 13958. https://doi.org/10.1038/ncomms13958

71. Solas M, Francis PT, Franco R, Ramirez MJ. CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients. *Neurobiol Aging*. 2013. 34. pp805-808

72. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016. 8(6). pp595-608

73. Stempel VA, Stumpf A, Zhang HY, Özdoğan T, Pannash U, Theis AK et al. Cannabinoid type 2 receptors mediate a cell-type specific plasticity in the hippocampus. *Neuron*. 2016. 90(4). pp795-809

74. Takkinen JS, Lopez-Picon FR, Kirjavainen AK, Pihlaja R, Snellman A et al. [18F]FMPEP-d2 PET imaging shows age- and genotype-dependent impairments in the availability of cannabinoid receptor 1 in a mouse model of Alzheimer's disease. *Neurobiol Aging*. 2018. 69. pp199-208

75. Tzavara ET, Li DL, Moutsimilli L, Bisogno T, Di Marzo V, Phebus LA et al. Endocannabinoids activate transient receptor vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. *Biol Psychiatry*. 2006. 59(6). pp508-15

76. Tolon RM, Nunez E, Pazos MR, Benito C, Castillo AI, Martinez-Ogado JA *et al*. The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Res*. 2009. 1283.pp148-154

77. van der Stelt M, Mazzola C, Esposito G, Matias I, Petrosino S, De Filippis D et al. Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol Life Sci*. 2006. pp1410-1424

78. Van Sickle MD, Duncan M, Kingsley PJ et al. Identification and functional characterisation of brainstem CB2 receptors. *Science*. 2005. 310 (5746). pp329-332

79. Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ. Effects of dronabinol on anorexia and disturbed behaviour in patients with Alzheimer's disease. *Int J Geriatr Psychiatry*. 1997. 12. pp913-919

80. Vos, SJB, van Rossum IA, Verhey F, Knol DL, Soininen H, Wahlund L-O et al. Prediction of Alzheimer disease in subjects with amnestic and non-amnestic MCI. *Neurology.* 2013. 80(12). pp1124-1132

81. Walther S, Schüpbach B, Seifritz E, Homan P, Strik W. Randomised, controlled crossover trial of dronabinol, 2.5mg, for agitation in 2 patients with dementia. *J Clin Psychopharmacol*. 2011. 31. pp256-258

82. Woodward MR, Harper DG, Stolyar A, Forester BP, Ellison JM. Dronabinol for the treatment of agitation and aggressive behaviour in acutely hospitalised severely demented patients with noncognitive behavioural symptoms. *Am J Geriatr Psychiatry.* 2014. 22. pp415-419

83. Westlake TM, Howlett AC, Bonner TI, Matsuda A, Herkenham M. Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience*. 1994. 63(3). pp637-652

84. Wood PL, Medicherla S, Sheikh N, Terry B, Phillipps A, Kaye JA, Quinn JF, Woltjer RL. Targeted lipidomics of frontal cortex and plasma diacylglycerols (DAG) in mild cognitive impairment and Alzheimer's disease: validation of DAG accumulation early in the pathophysiology of Alzheimer's disease. *J Alzheimers Dis*. 2015. 48(2). Pp537-546

85. Yamasaki T, Mori W, Zhang Y, Hatori A, Fujinaga M, Wakizaka H, Kurihara Y et al. First demonstration of in vivo mapping for brain monoacylglycerol lipase using PET with [^{11}C]SAR127303. *Neuroimage*. 2018. 176. pp313-320

86. Zetterberg H, Skillbäck T, Mattson N, Trojanowski JQ, Portelius E, Shaw LM et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol*. 2016. 73(1). 60-67

6. Figures:

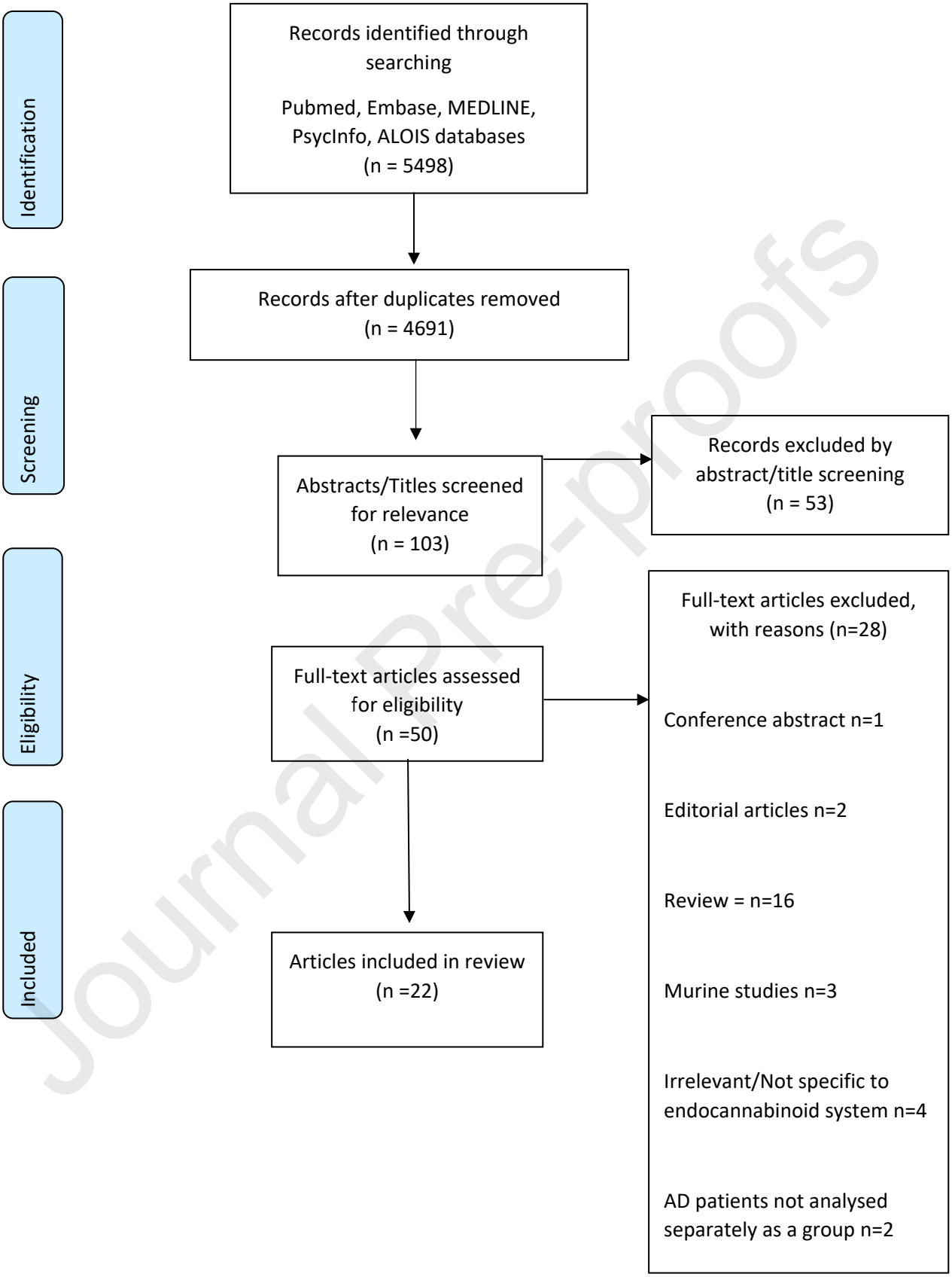

Figure 1: PRISMA Flowchart

7. Tables:

Authors (year)	Number of AD cases	Number of controls	Number of MCI cases	Study Type	AD and MCI diagnostic criteria used	Confirmed AD diagnoses?
Farooqui et al (1988)	6	2	-	Post-mortem	Not stated	100% "moderately advanced to advanced" AD pathology
Westlake et al (1994)	5	3	-	Post-mortem	DSM-III R criteria, Khachaturian criteria	100% had neuropathologic determined AD
Benito et al (2003)	7	4	-	Post-mortem	CERAD clinical and neuropathological criteria	100% Probable or definite diagnoses
Ramirez et al (2005)	6	5	-	Post-mortem	Not stated	Not stated
Grünblatt et al (2007)	13	9	-	Post-mortem	Clinical criteria not stated, Braak staging	100% probable/definite cases
Grünblatt et al (2009)	18	34	-	In vivo, whole blood samples	NINCDS-ADRDA	Not stated.
Koppel et al (2009)	19	12	-	In vivo, plasma and cerebrospinal fluid samples	NINCDS-ADRDA, DSM-IV	Not stated.
Tolon et al (2009)	4	-	-	Post-mortem	CERAD clinical and neuropathological criteria	Not stated.
Lee et al (2010)	17	16	-	Post-mortem	CERAD, Braak Staging	100% neuropathologically definite AD
Mulder et al (2011)	18	10	-	Post-mortem	National Institute on Aging-Reagan Institute, CERAD, Braak staging	100% of cases
Halleskog et al (2011)	19	4	-	Post-mortem	NIA-Reagan criteria, CERAD clinical criteria, Braak staging	31.6% possible (68.4% probable/definite)
Jung et al (2012)	38	17	-	Post-mortem brain tissue	NIA-Reagan criteria for intermediate or high likelihood AD.	100% Cases intermediate-high likelihood for AD

Farkas et al (2012)	11	5	-	Post-mortem	Braak staging	Not stated - 3x AD patients had Braak 1-2 disease
D'Addario et al (2012)	32 (13 for gene expression analysis)	33 (12 for gene expression analysis)	-	In vivo, gene expression in PBMCs	NINCDS-ADRDA	Not stated.
Fonteh et al (2013)	29	70	40	In vivo - cerebrospinal fluid	NINCDS-ADRDA, Petersen (2004) criteria (MCI)	100% (clinically probable LOAD)
Solas et al (2013)	15	16	-	Post-mortem	CERAD	100% definite AD diagnoses
Ahmad et al (2013)	11	7	-	In vivo	NINCDS-ADRDA, [11C]PIB positive binding on PET imaging	100% Had clinically probable AD
Pascual et al (2014)	9	9	-	Post-mortem	Not stated.	Not stated
Manuel et al (2014)	34	17	-	Post-mortem	Braak Staging, Khachaturian criteria	Not stated
Altamura et al (2015)	41	30	-	In vivo - plasma	NINCDS-ADRDA	100% probable AD
Ahmad et al (2016)	9	8	-	In vivo	NINCDS-ADRDA, [11C]PIB positive PET	100% clinically probable AD
González-Domínguez et al (2016)	75	45	17	In vivo - serum samples	NINCDS-ADRDA, Petersen (2004) criteria (MCI)	Not stated

Table 1: Study Characteristics

Legend: Aβ = beta-amyloid, AD= Alzheimer's disease, CERAD = Consortium to establish a registry for Alzheimer's disease, MCI = mild cognitive impairment, NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and related disorders association, PET = positron emission tomography, PiB = Pittsburgh B

Year	Authors	A. Selection Bias	B. Study Design	C. Endocannabinoid Biomarker	D. Correlates	E. Analysis & Confounders	Global Rating
1988	Farooqui et al.	Weak	Weak	Moderate	N/A	Weak	Weak
1994	Westlake et al.	Strong	Weak	Strong	Strong	Moderate	Moderate
2003	Benito et al.	Strong	Weak	Strong	Strong	Weak	Weak
2005	Ramirez et al.	Strong	Weak	Strong	N/A	Strong	Moderate
2007	Grünblatt et al.	Strong	Weak	Strong	N/A	Moderate	Moderate
2009	Koppel et al.	Strong	Weak	Strong	Strong	Moderate	Moderate
2009	Tolon et al.	Strong	Weak	Strong	N/A	Moderate	Weak
2009	Grünblatt et al.	Weak	Weak	Strong	Strong	Moderate	Weak
2010	Lee et al.	Strong	Weak	Strong	Strong	Weak	Weak
2011	Halleskog et al.	Weak	Weak	Strong	N/A	Moderate	Weak
2011	Mulder et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2012	Jung et al.	Strong	Weak	Strong	Strong	Moderate	Moderate
2012	D'Addario et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2012	Farkas et al.	Strong	Weak	Strong	N/A	Weak	Weak
2013	Fonteh et al.	Strong	Weak	Strong	N/A	Moderate	Moderate
2013	Solas et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2014	Manuel et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2014	Pascual et al.	Strong	Weak	Strong	N/A	Strong	Moderate
2014	Ahmad et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2015	Altamura at al.	Strong	Weak	Strong	Strong	Strong	Moderate
2016	Ahmad et al.	Strong	Weak	Strong	Strong	Strong	Moderate
2016	González-Domínguez et al.	Strong	Moderate	Strong	N/A	Strong	Strong

Table 2: Study Quality Ratings

Author (year)	Number of AD or MCI cases and controls	Main Investigative method(s)	Findings
Genetic or epigenetic studies			
D'Addario et al (2012)	Gene methylation analysis arm - 32 AD cases, 33 controls Gene expression analysis arm - 13 AD cases, 12 controls	Quantitative RT-PCR, and methylation-specific primer of real-time PCR, from PBMC	↑ FAAH mRNA in AD (p<0.05). ↓DNA methylation at FAAH gene site AD (p=0.003) ↑ FAAH protein expression (Western Blotting, n=5, p<0.05) and degradative activity (p ↓DNA methylation at FAAH gene site in severe dementia (0-10 on MMSE) compared w
Grünblatt et al (2009)	18 AD cases, 34 controls	Quantitative real time RT-PCR (whole blood samples)	*CNR2* gene expression did not correlate with MMSE scores
Imaging studies:			
Ahmad et al (2016)	9 AD cases, 8 controls	PET study, CB2R radioligand [^{11}C]NE40	↓ Binding potential (non-displaceable) [^{11}C]NE40 in all cortical areas of interest in AD (
Ahmad et al (2014)	11 AD cases, 7 controls	PET study, CB1R radioligand [^{18}F]MK-9740	No differences identified between standard uptake value ratio (SUVR) of [^{18}F]MK-9740
Blood or cerebrospinal fluid studies:			
González-Domínguez et al (2016)	75 AD cases, 17 MCI cases, 45 controls	Ultrahigh-performance LC MS (quadrupole time-of-flight MS) - serum	Serum oleamide ↓ in AD and MCI (p=0.025) compared to controls. Serum monopalmiti in AD compared to controls.
Altamura et al (2015)	41 AD cases, 30 control cases	High-performance LC MS – serum	↑ 2-AG concentration in in AD cases (p=0.02) compared to controls. 2-AG serum levels group (r=0.415, p=0.015). Positive correlation of serum 2-AG with memory function on p=0.018), Lower constructional apraxia scores correlated with higher PEA concentration PEA and OEA positively correlated with AEA concentrations (r=0.381, p<0.05)r=0.647, p
Koppel et al (2009)	19 AD cases (35 AD cases in CSF arm), 12 controls	LC MS (triple quadrupole tandem MS) - plasma and CSF	Inverse correlation identified between plasma 2-AG and TNFalpha concentration (Pears
Fonteh et al (2013)	29 AD cases, 40 MCI cases, 70 controls	LC MS (tandem MS) – CSF PLA2 activity assay	↓PC (1-radyl-2-acyl-sn-glycerophosphocholine) in AD and MCI in supernatant CSF (p<0 AD (p<0.05). Total content of GPC lipids (PC+LPC+PAF) decreased in AD (p<0.05). ↓ PE (p<0.05), but not significantly increased in MCI.
Postmortem studies:			
Farooqui et al (1988)	10 AD cases, 2 controls	Radiolabelled hydrolysis assay of membrane and synaptosomal fractions	↑concentration of MAGL in synaptosomal plasma membrane isolates (p-value not calc not calculated).
Westlake et al (1994)	5 AD cases, 3 controls	Autoradiography, in situ hybridisation	↓CB1R binding density noted relative to controls in entorhinal cortex (40%), subiculum (p<0.01). CB1R binding density ↓ in the substantia nigra pars compacta and GPi (p<0.0 observed between AD and control brains. Numbers of individual neurons intensely exp dentate hilus regions) were reduced in AD compared to controls (p<0.05).
Benito et al (2003)	7 AD cases, 4 controls	Western blotting, immunohistochemistry, FAAH activity assay	FAAH activity could be detected in plaques from AD cases, but never in tissuefrom cont associated microglia only. FAAH immunoreactivity mainly detected in cell bodies and hy neuritic tangles in the entorhinal cortex and parahippocampal areas.
Ramirez et al (2005)	Immunocytochemistry arm, 6 AD cases, 5 controls Pharmacological study arm -18 AD cases, 18 controls	Immunocytochemistry, [35S]GTPγS binding assay, Western blotting	CB1R and CB2R colocalized with senile plaques in frontal cortex, and markers of microg protein nitration). CB1R positive plaques with CB1R positive neurons co-localised in the minority of sampl CB2R expression observed in AD brains in tangle-like neurons and dystrophic neurites. CB1R and CB2R nitration was markedly increased in AD (n=6, p<0.01) Immunolabelling revealed a reduction in CB1R positive neuron density in AD compared Pharmacological study arm – binding density and binding affinity unaltered in AD brains GTPgammaS G protein binding assay demonstrated decreased cannabinoid receptor ac ↓CB1R expression in AD (p<0.05) compared to controls. No differences in CB2R protein
Grünblatt et al (2007)	13 AD cases, 9 controls	Gene chip microarray, quantitative real-time RT-PCR	↑ *CNR2* gene expression in hippocampal samples of AD compared with controls (n=10)
Tolon et al (2009)	4 AD cases	Aβ removal assay, incubation with SR 144528 (CB2R inverse agonist) and JWH-015 (CB2R agonist)	Incubation of AD tissue sections with a CB2R agonist and THP-1-derived macrophage ce plaque (p<0.05). Plaque clearance was inhibited by SR 14452B. Similar plaque clearance

Lee et al (2010)	17 AD cases, 16 controls	Immunoblotting, radioligand binding density studies	Frontal cortical CB1R immunoreactivity correlated positively with pre-death MMSE and
Halleskog et al (2011)	19 AD cases, 4 controls	Immunohistochemistry, immunoblotting	↑ CB2R expression in Braak VI stage AD (n=7) compared to stage II-IV cases, and age-m In Braak VI cases (n=7), Iba-1+ cells co-expressed CB2R, and were closely associated wit Positive association observed between beta-catenin and CB2R protein expression in Bra
Mulder et al (2011)	2x arms - CB1R investigation arm - 18 AD cases, 10 controls, MAGL/FAAH investigation arm - 18 AD cases, 18 controls	Immunofluorescence histochemistry, Western blotting, AEA and 2-AG degradation assays	↓membrane-associated 2-AG degradation in frontal cortex in AD (p<0.05). ↑ 2AG degr controls(p<0.05). ↑MAGL expression in Braak VI stage (though not stage III/IV disease) CB1R expression on presynaptic terminals remains unaltered in AD. CB1R +ve neurons s MAGL+ve and IBA1+ve microglia accumulate around senile plaques. Increased density o stage VI. AT8+ve (marker of hyperphosphorylated tau) pyramidal cells retain MAGL expression b
Farkas et al (2012)	11 AD cases, 5 controls	Autoradiography using [^{125}I]SD-7015	CB1R density↑ in Braak Stage 1-2 disease AD (n=3, p<0.05), and ↑ overall in AD group
Jung et al (2012)	38 AD cases, 17 controls	LC MS (tandem MS)	↓ AEA availability in midfrontal and temporal cortices (p<0.05) in AD. 2AG in midfronta Midfrontal cortical AEA concentration negatively correlated with insoluble beta amyloid Midfrontal cortex AEA did not correlate with amyloid plaque load, Aβ40 isoform concen Midfrontal AEA concentration positively correlated with KDCT performance (p=0.004) a cortex AEA concentration was positively correlated with BNT performance (n=18, r=0.5
Solas et al (2013)	15 AD cases, 16 controls	Western blot, optical denistometry	↓ CB1R expression on Western blot of BA10 homogenates in AD (p<0.001 and <0.05 re Strong correlation between CB2R expression and Aβ42 levels and senile plaque score (r Hypophagia scores on the Present Behavioural Examination correlated with CB1R expre
Pascual et al (2014)	9 AD cases, 9 controls	Western blotting, AEA hydrolysis assay	Frontal cortex neuronal membrane-associated AEA hydrolysis in AD decreased significa Incubation with URB597 (FAAH inhibitor) decreased AEA hydrolysis to a lesser extent in stimulated hydrolysis of AEA by 11% in control sample tissue, but no changes in hydroly WIN55,212-2 (Mixed CB1/CB2 agonist) inhibited hydrolysis by 34% in control human tis with AD (p<0.001).
Manuel et al (2014)	34 AD cases, 17 controls	[35S]GTPγS binding assay, WIN55,212-2 autoradiography	No correlation between WIN55,212-2 and [35S]GTPγS binding densities. ↑CB1R-media stage compared to controls (p<0.05). ↓CB1R-mediated Gi/o protein activity within the lateral nucleus of the amygdala in sta ↑ CB1R density in caudate/putamen in stage I-II AD compared to controls (p<0.05). ↑CB1R binding density in Braak stages III-IV relative to controls (in layer VI of frontal co (p<0.05). ↓ CB1R binding density in stage V-VI disease compared to III-IV disease in the entorhin subfield (p<0.05). ↓CB1R density in V-VI stage AD in the caudate/putamen compared with stage I-II AD (p ↓CB1R binding density in dentate gyrus granular cells in V-VI stage compared to I-II sta ↓CB1-mediated activation of Gi/o proteins in the CA1 subfield in stage V-VI AD compar ↓CB1R-mediated G-protein coupling observed in stage V-VI disease compared with I-II gyrus (p<0.05). ↓CB1R-mediated Gi/o activity in the pyramidal layer of the subiculum in stage V-VI sta

Table 3: Results

Legend: ↓ = Decreased (p<0.05), ↑ = Increased (p<0.05), +ve = positive, -ve = negative, 2-AG = 2-arachidonoylglycerol, AEA = arachidonoylethanolamine, AD = Alzheimer's disease, APOE e4 = apoliporotein gene e4 allele, AT8+ = positive staining with monoclonal antibody for phosphorylated tau, AVLT = Auditory verbal learning test, BA10 = Brodmann Area 10, BNT = Boston Naming Test, CAMCOG = Cambridge Cognition Examination, CA = Cornu Ammonis (hippocampal subfields), CB1R = cannabinoid 1 receptor, CB2R = cannabinoid 2 receptor, *CNR2* = cannabinoid 2 receptor gene, DAG = diacylglycerol, DAGL = diacylglycerol lipase, DNA = deoxyribonucleic acid, FAAH = fatty acid amide hydrolase, GPC = glycerophosphocholine, GPi = globus pallidus interna, GTPγS = guanosine 5'-O-[gamma-thio]triphosphate, Iba1+ = positive monoclonal antibody staining with Iba-1 protein (a marker of activated microglia), KDCT = Kendrick Digit Copy Test, LOAD = late-onset Alzheimer's disease, LCMS = liquid chromatography mass spectrometry, LPC = lysophosphatidylcholine, MAGL = monoacylglycerol lipase, MCI = mild cognitive impairment, MMSE = Folstein's mini mental state examination, mRNA = messenger ribonucleic acid, NFT = neurofibrillary tangles, OEA = oleoylethanolamide, PAF = platelet activating factor, PBMC = peripheral blood mononuclear cells, PC = phosphatidylcholine, PCR = polymerase chain reaction, PE = 1-radyl-2-acyl-sn-glycerophosphoethanolamine, PEA = palmitoylethanolamine, PET = positron emission tomography, PiB = Pittsburgh compond B, PLA2 = phospholipase A2, RT-PCR = reverse transcriptase polymerase chain reaction, TNFalpha = tumour necrosis factor alpha TRPV1 = transient receptor potential cation channel family V member 1, VOI = volume of interest

Highlights:

- **Endocannabinoid system functioning is altered in Alzheimer's disease**
- **Expression and activity of CB2R, MAGL and FAAH may be increased in Alzheimer's disease**
- **Very few studies included patients with mild cognitive impairment (MCI)**
- **TRPV1 expression appears unaltered in Alzheimer's disease**
- **Few studies have investigated correlations between neuropsychiatric symptomatology and endocannabinoid alterations**

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Endocannabinoid system alterations in Alzheimer's disease: a systematic review of human studies

CRediT author statement:

Alex Berry: Conceptualization, methodology, investigation, writing – original draft, writing – review and editing **Olga Zubko:** Conceptualization, methodology, investigation, validation **Suzanne Reeves:** Conceptualization, methodology, writing – review and editing, supervision **Rob Howard:** Conceptualization, methodology, writing – review and editing, supervision