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REVIEW

Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review

KE Hopperton[1], D Mohammad[1,2], MO Trépanier[1], V Giuliano[1] and RP Bazinet[1]

Neuroinflammation is proposed as one of the mechanisms by which Alzheimer's disease pathology, including amyloid-β plaques, leads to neuronal death and dysfunction. Increases in the expression of markers of microglia, the main neuroinmmune cell, are widely reported in brains from patients with Alzheimer's disease, but the literature has not yet been systematically reviewed to determine whether this is a consistent pathological feature. A systematic search was conducted in Medline, Embase and PsychINFO for articles published up to 23 February 2017. Papers were included if they quantitatively compared microglia markers in post-mortem brain samples from patients with Alzheimer's disease and aged controls without neurological disease. A total of 113 relevant articles were identified. Consistent increases in markers related to activation, such as major histocompatibility complex II (36/43 studies) and cluster of differentiation 68 (17/21 studies), were identified relative to nonneurological aged controls, whereas other common markers that stain both resting and activated microglia, such as ionized calcium-binding adaptor molecule 1 (10/20 studies) and cluster of differentiation 11b (2/5 studies), were not consistently elevated. Studies of ionized calcium-binding adaptor molecule 1 that used cell counts almost uniformly identified no difference relative to control, indicating that increases in activation occurred without an expansion of the total number of microglia. White matter and cerebellum appeared to be more resistant to these increases than other brain regions. Nine studies were identified that included high pathology controls, patients who remained free of dementia despite Alzheimer's disease pathology. The majority (5/9) of these studies reported higher levels of microglial markers in Alzheimer's disease relative to controls, suggesting that these increases are not solely a consequence of Alzheimer's disease pathology. These results show that increased markers of microglia are a consistent feature of Alzheimer's disease, though this seems to be driven primarily by increases in activation-associated markers, as opposed to markers of all microglia.

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INTRODUCTION

Elevations in neuroinflammatory markers are widely reported in Alzheimer's disease (AD), both in animal models[1–3] and human subjects.[4–7] This has contributed to the development of the neuroinflammatory hypothesis of AD that suggests that aberrant activation of immune cells may drive neuronal death and dysfunction in AD.[8] This is supported by genome-wide association studies that have identified polymorphisms in inflammation-associated genes as risk factors for the development of AD.[9–11]

Microglia are the resident immune cells of the brain, and are thought to be the main cells responsible for initiating the immune response to AD pathology. Several of the inflammation-associated genetic risk factors for AD, including human leukocyte antigen (HLA)-DRB1/B5,[9] cluster of differentiation (CD) 33,[12] triggering receptor expressed on myeloid cell-2 (TREM2)[10] and phospholipase C γ2,[10] are highly expressed in microglia where they are involved in cell function and activation. This suggests that aberrant microglial activation is a causal factor in the development of AD, as opposed to a consequence of AD pathology. Although it is commonly accepted that there are increased microglia markers in the brains of patients with AD relative to controls, no one has yet systematically synthesized the literature to see whether this is supported by the totality of the evidence. Here, we describe the results of a systematic review examining microglia in post-mortem human brain samples from patients with AD and healthy controls. We find that some markers associated with cell activation, such as major histocompatibility complex- II (MHCII) and CD68, are consistently increased in the AD brain, but that studies using other common microglial markers that stain both resting and activated cells, such as ionized calcium-binding adaptor molecule-1 (Iba1) and CD11b, are heterogeneous and do not demonstrate a consistent elevation. We further identify brain regions, such as the white matter and the cerebellum, that appear to be more resistant to inflammation in AD.

METHODS

The systematic search was conducted in Medline, Embase and PsychINFO covering articles published up to 23 February 2017. The search protocol was developed based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and World Health Organization (WHO) Review Protocol Template Guidelines where applicable for a systematic review of descriptive (non-interventional) data, and is provided in Appendix 1 of the Supplementary Materials section. The search queried the

[1]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, ON, Canada and [2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada. Correspondence: Dr RP Bazinet, Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, FitzGerald Building, 150 College Street, Room 306, Toronto, ON M5S 3E2, Canada.
E-mail: richard.bazinet@utoronto.ca
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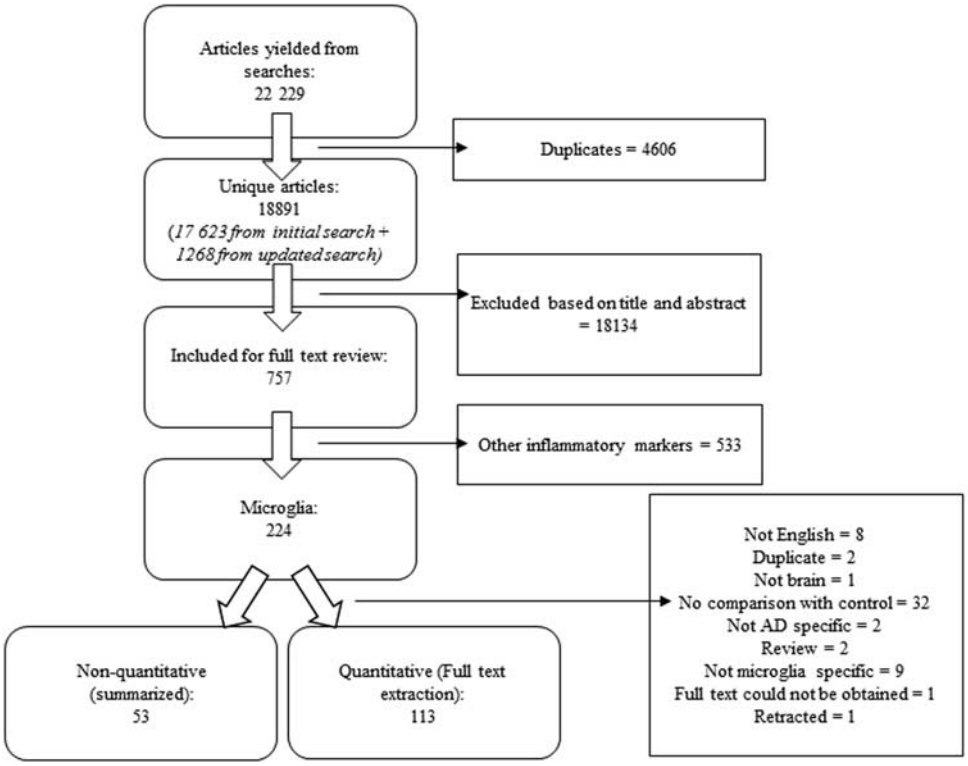

Figure 1. Flow diagram of systematic search.

following terms with numerous synonyms and related words as both MeSH/Emtree terms (where applicable) and as keywords (for title, abstract and keyword searches): Alzheimer's Disease AND brain AND inflammation. The additional term 'AND post-mortem' with its synonyms and related words was included for the Embase and PsychINFO searches. A full list of terms used for each search can be found in Appendix 2.

Studies were included if they used human brain samples from patients with AD, included a measure of inflammation, were conducted post-mortem and included a comparison to a control group without AD or a confounding neurological disease. This review initially set out to include all inflammatory markers, including for astrocytes, complement, cytokines, lipid mediators and other immune cells, but the title and abstract screening returned 757 eligible papers, making it unfeasible to summarize all the evidence in a single paper. It was decided to proceed with a review of microglia, as these are the main immune effector cells in the brain, and are the source of many of the other inflammatory mediators measured in other studies. Microglia terms in the initial search include the MeSH term: neuroglia and neurogenic inflammation, the Emtree terms: neurogenic inflammation, glia and leukocyte antigen, and keywords: microglia, HLA, MHC, CD11b, CD68, Iba1, OX-42 and CD45 along with their synonyms and alternate spellings (see Appendix 2 of the Supplementary Materials for the full list of terms). In addition, any other papers returned in the full search that used other markers identified by the study authors as being specific to microglia or their activation were included. Studies were excluded if they were not in English or not published in full in a peer-reviewed journal. Papers that measured markers associated with an M1 or M2 phenotype, such as interleukin (IL)-1β, tumour necrosis factor-α, IL-4 or IL-10 but that did not localize these markers specifically to microglia, were not included.

Data from included studies were extracted by at least two independent reviewers (KEH and DM, VG or MT), with a third reviewer employed in the case of a conflict. Extracted data included origin of brain samples, number of subjects, sex, age, apolipoprotein E (APOE) genotype, histological confirmation of AD status, Braak stage, control history of neurological or psychiatric disease, post-mortem interval (length of time between death and retrieval of brain), brain regions examined, medications at the time of death, anti-inflammatory drug use at death, technique for measuring microglia, marker of microglia used and the results of the comparison between the AD subjects and healthy controls. Where available, information on relationship between labelled microglia and amyloid-β plaques or neurofibrillary tangles was also extracted. The terms 'higher' or ↑ and 'lower' or ↓ are used in the text and tables referring to significantly higher or lower levels of the microglial marker used in the study relative to the nonneurological aged control group. As the type of outcome reporting was extremely heterogeneous, results were reported as higher, lower or unchanged for AD relative to control as identified by the study authors. Meta-analysis or other summary statistics were not used because of the large variability in assessment techniques and brain regions examined between studies. Data were listed as 'Not Reported' if the relevant information could not be found in the article, or in a previous article specifically referred to by the authors in the Methods section. In a few instances where information in the article was unclear, the corresponding authors were contacted to provide clarification or additional detail.

RESULTS

A total of 22 229 articles were screened, of which 757 met inclusion for full text review for inflammatory markers, including 224 that examined microglia (Figure 1). A total of 113 papers that quantified microglia and compared the results statistically between control and AD groups were fully extracted and are presented in the tables below (see Figure 2 for a visual summary of the results). Fifty-three papers that made a nonquantitative comparison between AD and control, often as a qualitative assessment of the intensity of immunohistochemistry staining, were not fully extracted but are summarized under the 'Non-quantitative comparisons' heading.

The microglial markers analysed in this review all serve distinct functions within the cell, and as such, the interpretation of an up-

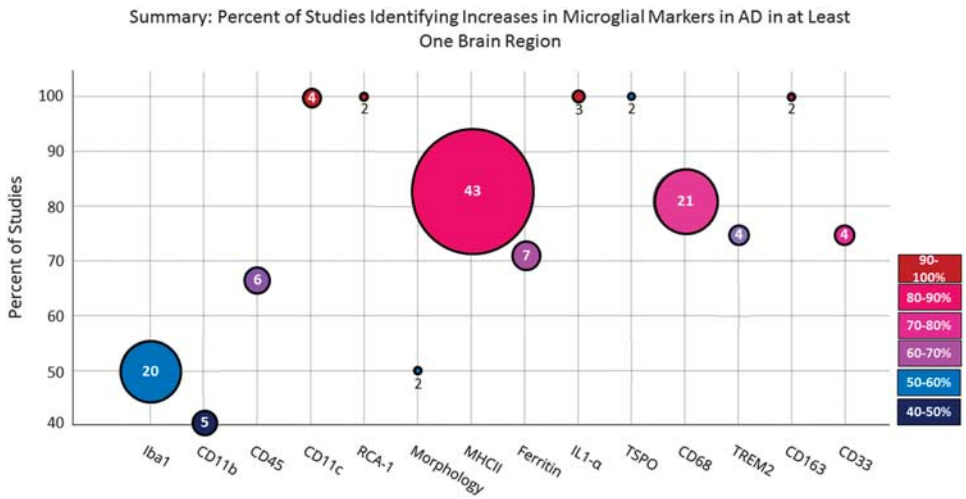

Figure 2. Summary results of systematic search. Size of circle is proportional to the number of identified studies, whereas the colour and position on the graph illustrates the percentage of studies that identified an increase in AD in at least one brain region. AD, Alzheimer's disease; CD, cluster of differentiation; Iba1, ionized calcium-binding adapter molecule 1; IL, interleukin; MHC, major histocompatibility complex; RCA-1, Ricinus communis agglutin-1; TSPO, translocator protein; TREM2, triggering receptor expressed on myeloid cells 2.

or down-regulation of expression will vary depending on the marker used. The functions of all major markers included in this review, their association with polarization and their expression on other cell types besides microglia is shown in Supplementary Table 1. M1 and M2 polarization is increasingly regarded as an oversimplification of many diverse functions of activated microglia; however, it is used here to provide a reference for whether a marker is associated with the pro-inflammatory or phagocytic microglial phenotype. As shown in the table, it is important to note that all the major markers in this review are also expressed on other cell types, particularly on macrophages, and hence it is possible that other infiltrating or perivascular immune cells may have contributed to the results.

Major histocompatibility complex II

MHCII is expressed on the surface of antigen-presenting cells and is responsible for antigen recognition and the activation of the adaptive immune system. Within the brain, MHCII is primarily expressed on microglia, where it is generally considered a marker of activated cells, though it may have weaker expression in resting cells.[14] Forty-three papers were identified that quantitatively compared markers of MHCII between AD and control in post-mortem human brains (Table 1). The majority (41/43) used immunohistochemistry for HLA-DR or for multiple isoforms of HLA (HLA-DR-DQ-DP) quantified by cell counts, scoring or staining area, whereas other techniques included gene expression by quantitative PCR (qPCR) or protein quantification by western blot.

Thirty-six papers reported higher MHCII in AD relative to control brains in at least one of the measured brain regions,[7,15–49] whereas 7 identified no difference between AD and control,[50–56] and 5 identified no difference in at least one brain region.[21,32,34,41,44] Increased MHCII staining, counts or expression was noted in: the entorhinal cortex,[7,18,20,35,43,47] frontal and temporal gyri,[7,19,24,32,33,39,45,56] hippocampus[20,25,27,30,32–38,40,42–44,47] and frontal,[18,21,25,40,41,44,46,48,49,52] temporal[16–18,28,30,35,43,44,48,52] and occipital cortices.[25,35,41,43,44] HLA-DR was found to increase with AD plaque stage and clinical dementia rating in the entorhinal cortex, hippocampus and occipital and temporal cortices.[43,47] HLA-DR-stained microglia were reported to take on an activated morphology in AD,[19,23,45,48,49] indicating that AD pathology may stimulate the activation of microglia and upregulation of MHCII. In contrast, seven papers identified no difference in MHCII between AD and control in several regions including the hippocampus,[21,55] frontal, temporal and parietal grey matter,[52] the temporal polar association cortex[54] and the subventricular zone of the lateral ventricle.[53]

Of the five studies examining HLA-DR immunoreactivity in the cerebellum, four found no differences between AD and control,[25,34,44,51] suggesting that regional differences may explain some of the null findings. Overmyer *et al.*[52] noted that women had higher HLA-DR reactivity than men in AD, whereas the reverse was true in the controls, suggesting that sex differences in the AD and control groups could also contribute to between-study variability. HLA-DR reactivity increased in both AD and control patients >75 of age in one study,[52] but decreased in AD patients >80 of age in another,[28] making the effect of age on HLA-DR expression unclear. APOE genotype can also impact the results, with the ε4 risk allele increasing HLA-DR-positive cells or area in the frontal and temporal cortices[48] and middle frontal gyrus.[56]

Ionized calcium-binding adaptor molecule 1

Iba1 is a cytoplasmic protein expressed in monocyte lineage cells and in the brain and is primarily restricted to microglia.[57] Although its expression is thought to increase with microglial activation,[58] where it may be involved in membrane ruffling and phagocytosis,[59] it is considered a marker of all microglia, rather than an activated subset.[60] Twenty papers quantitatively compared Iba1 between AD and control post-mortem human brains (Table 2). Ten studies identified increases in Iba1 cell counts, staining intensity or expression in AD compared with control samples in at least one brain region,[20,60–68] whereas 10 studies identified no difference from controls[32,54,69–76] and 5 reported lower Iba1 in AD than controls[32,54,56,75,76] in at least one of the regions measured.

The results of studies measuring Iba1 are relatively heterogeneous. Higher Iba1 was noted in the AD frontal cortex in four studies,[20,63,64,68] but was the same as control in three others[32,70,74] and lower in the white matter of the frontal lobe in one study.[32] Iba1-positive cell density[72] and gene expression[74] was the same in AD and control in the temporal cortex in two studies, but greater by western blot or immunocytochemistry in three other studies.[20,61,66] Similarly, although Iba1 was higher in AD than control in the hippocampus in three studies,[20,62,67] it was unchanged in five studies[32,69,71,73,75] and reduced in one study.[76] Iba1 was higher in the entorhinal cortex[20,62] and inferior parietal cortex[65] in AD compared with control, but this is based on a limited number of studies. All but 1 of the 10 studies that identified increases in Iba1 in AD used expression-based assays.

Table 1. Major histocompatibility complex II (MHCII)

First Author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Marker	Results (AD vs C unless otherwise specified)
Akiyama[15]	NR	AD: 9 C: 6	AD: 77 C: 69	NR	NR	NR	NR	No neurological disease	All within 2–12 h	Temporal lobe	IHC	HLA-DR	↑
Carpenter[19]	NR	AD: 5 C: 5	AD 4/1 C: 5/0	AD: 75.4 C: 73.6	NR	Khachaturian	NR	No history of neurological or systemic diseases affecting the brain	AD: 3.6 C: 2.4	Grey matter of the middle temporal gyrus	IHC	HLA-DR (LN3)	↑ Density of cells per mm, staining area and percent of area. Most cells of resting morphology in Cs vs activated in AD (not compared statistically)
Dal Bianco[20]	NR	AD: 9 C: 15	AD: 0/9 C: 13/2	AD: 81 C: 70	NR	Braak, CERAD	AD: IV: 2, V: 4, VI: 3	No neurological disease or brain lesions	NR	Cortical areas of the temporal lobe, including entorhinal cortex, hippocampus and temporal cortex	Immunocytochemistry	MHCII	↑ MHCII
Desai[21]	Religious Orders Study	AD:8 C: 7	AD: 3/5 C: 4/3	AD: 85.2 C: 79.5	NR	NIA-Reagan criteria	AD: III–VI	NR	AD: 5.4 C: 15.5	Hippocampus, midfrontal cortex, locus ceruleus, substantia nigra pars compacta	IHC	HLA-DR-DQ- DP (Cr3/43)	↑ In the midfrontal cortex, and locus ceruleus ↔ In density of HLA-DR microglia in hippocampus
Dhawan[22]	University of Washington ADRC	NR	NR	NR	NR	NR	NR	NR	NR	Temporal lobe	IHC	HLA-DR	↑ HLA-DR+ microglia
Mattiace[49]	NR	AD: 15 C: 14	AD: 2/13 C: 3/11	AD: 76.6 C: 67.8 (as young as 6 months)	NR	Khachaturian	NR	Depressive psychosis, manic depressive psychosis ($n = 2$), Parkinson's disease ($n = 1$)	AD: 6.4 C: 6.3	Midfrontal cortex (BA9)	IHC	HLA-DR, double staining with Leu-M5 (CD11c)	↑ Activated microglia (morphology) ↑ Proportion of HLA-1DR+ microglia in grey matter ↔ In white matter Results not compared statistically
Egensperger[48]	Institute of Neuropathology of the University of Munich	AD: 20 C: 5	AD: 5/15 C: NR	AD: 75.9 C: 71.6	APOE: AD: 3/3: 7 3/4: 10 4/4: 3 C: NR	Braak, CERAD	NR	No neurological or neuropathological disorder	NR	Frontal and temporal cortex	IHC	HLA-DR-DQ-DP (CR3/43)	↑ Counts and area —Plaque-associated microglia demonstrate activated morphology— Microglia number correlates with neuritic plaques and NFT
Flanary[24]	BSHRI	AD: 4 HPC: 3 C: 4	AD: 1/3 HPC: 2/1 C: 3/1	AD: 82.0 HPC: 87.7 C: 81.5	NR	Yes	NR	NR	AD: 2.4 HPC: 3.1 C: 2.6	Superior frontal and temporal gyri	IHC	HLA-DR	↑ Dystrophic microglia in AD vs C, HPC vs C and AD+HPC vs C ↑ HPC vs AD (not clear if statistically significant)
Giulian[25]	NR	AD: 6 C: 5	NR	NR	NR	CERAD	NR	No neuropathological disorder	NR	Cerebellum, hippocampus, frontal, occipital, parietal cortices and neocortical white matter	IHC, confocal microscopy	HLA-DR	↑ Hippocampus, frontal, occipital and parietal cortices ↔ Cerebellum, white matter
Gouw[26]	NBB and Vrije University Medical Centre	AD: 11 C: 7	AD: 3/8 C: 3/4	AD: 82.6 C: 78.3	NR	Braak, CERAD	AD: V C: I	All had white matter hyperintensities (small vessel disease), other neurological diseases excluded	AD: 6.1 C: < 24 h	Normal white matter and white matter hyperintensities in frontal, parietal and prefrontal lobes	IHC	HLA-DR	↑ Overall than C ↑ Higher in white matter hyperintensities than normal white matter

Table 1. (Continued)

First Author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Marker	Results (AD vs C unless otherwise specified)
Halliday[16]	Dementia clinics at the Repatriation General Hospital Concord and Lidcombe Hospital in Sydney, Australia	AD: 12 C: 10	NR	AD: 79 C: 77	APOE: AD: 3/4: 1 C: 3/4: 1	CERAD, NIA-Reagan Criteria	NR	NR	All <45 h, mean 19	Anterior cingulate, hippocampal, inferior temporal, parahippocampal and superior frontal regions	IHC	HLA-DR	↑ In AD vs C ↔ AD patients taking NSAIDs and AD patients not taking NSAIDs
Hensley[27]	NR	AD: 3 C: 3	Whole sample: AD: 9/13 C: 4/3	Whole sample: AD: 78.1 C: 79.7	NR	Khachaturian, Mirra	NR	No history of neurological and/or psychiatric disorders	Whole sample: AD: 4.6 C: 4.4	Cerebellum, hippocampus, inferior parietal lobule	IHC	HLA-DR	↑ In all regions
Hoozemans[17]	NBB	Braak stage 0: 5, I–II: 16, III–IV: 10, V–VI: 9	Braak stage 0: 3/2, I–II: 6/10, III–IV: 0/10, V–VI: 3/6	Braak stage 0: 62, I–II: 83, III–IV: 89, V–VI: 76	NR	Braak	0–VI (not divided into C and AD)	NR	Braak stage 0: 8, I–II: 7.5, III–IV: 6.5, V–VI: 5	Temporal cortex	IHC	HLA-DR-DQ-DP (CR3/43)	↑ With increasing Braak NFT or plaque stage ($P<0.05$ for trend), significant for NFT group V–VI vs O
Hoozemans[28]	Netherlands Brain Bank	AD: 19 C:19	AD: 3/16 C: 8/11	AD: 83.5 C: 76.8	APOE4: AD: 12 C: 8	Braak	AD: avg IV C: avg I	NR	AD: 5.1 C: 8.6	Midtemporal cortex	IHC	HLA	↑, ↑ In AD patients ‘80 years compared with those >80 years
Imamura[29]	NR	AD: 6 C: 6	AD: 2/4 C: 2/4	AD: 65.4 C: 62.8	NR	Yes	NR	NR	NR	Temporal lobe	IHC	HLA-DR	↑
Itagaki[30]	NR	AD: 10 C: 5	NR	NR	NR	Yes	NR	No neurological complications	All within 2–12	Mixed: hippocampus and temporal cortex	IHC: semiquantitative scoring of staining	HLA-DR, LCA	↑
Jantaratnotai[31]	Kinsmen Laboratory Brain Bank at the University of British Columbia	AD severe: 9 AD mild: 6 C: 9	NR	AD severe: 74.2 AD mild: 77.7 C: 83	NR	Braak, NIA-Reagan criteria	NR	No neurological disorders	NR	Medial temporal cortex	IHC	HLA-DR	↑
Kellner[18]	NR	AD: 48 C: 48	AD: 19/29 C: 24/24	AD: 80.3 C: 77.5	NR	Braak, CERAD	AD: II–VI ($38>4$) C: I–III ($45=0$)	NR	NR	Entorhinal, frontal cortex, temporal cortex	IHC	HLA-DR	↑
Korvatska[32]	University of Washington Neuropathology Core Brain Bank	AD (normal TREM2): 6 AD (with TREM2 R47H variant): 7 C: 3	NR	Whole sample: 84.9	NR	CERAD	AD: III: 1, V: 4, VI: 1, AD R47H: V: 6, VI: 1, C: 0: 1, I: 1, III: 1	NR, One C CERAD score A and one B	Whole sample: 4.5	Frontal lobe: grey and white matter Hippocampus: CA1, hilus, parahippocampal gyrus and white matter	IHC	MHCII	↑ Staining in hippocampus CA1, hilus, parahippocampal gyrus and white matter ↑ Activated counts in hippocampus white matter
Lopes[33]	BSHRI	AD: 7 young C: 3 Aged C: 7 HPC: 7	AD: 4/3 young C: 2/3 Aged C: 6/1 HPC: 5/2	AD: 80.3 young C: 36.3 aged C: 80.0 HPC: 83.4	NR	Yes	NR	NR	AD: 2.3 young C: 2.8 aged C: 2.5 HPC: 2.90	Amygdala, hippocampus, superior frontal gyrus, superior, middle, and inferior temporal gyri	IHC and morphometric analyses	HLA-DR	Microglia counts HLA-DR: ↑ vs all other groups Dystrophic microglia HLA-DR: ↑ vs C, ↔ vs HPC
Lue[7]	NR	AD: 6 HPC: 6 C: 6	AD: 3/3 HPC: 5/1 C: 2/4	AD: 81 HPC: 78 C: 77	NR	Markesbery	NR	NR, Cs had minimal AD pathology or sufficient plaques or tangles to qualify for AD diagnosis	AD: 3.2 HPC: 3.2 C: 1.9	Entorhinal cortex, superior frontal gyrus	IHC	HLA-DR (LN3)	↑ AD>HPC>C
Lue[34]	BSHRI	AD:11 C:10	AD: 5/6 C: 4/6	AD: 80.8C: 80.5	APOE: AD: 3/4: 4 4/4: 4 C: 3/4: 3 4/4: 1	Braak, CERAD	AD: IV–VI C: I–III	NR	AD: 2.6 C: 2.3	Hippocampus, cerebellum, superior frontal gyrun	IHC	HLA-DR (LN3)	↑ In the hippocampus, parahippocampal gyrus and superior frontal gyrus ↔Cerebellum
Matsuo[35]	NR	AD: 8 C: 5	NR	NR	NR	Yes	NR	Neurologically normal	All 2–24	Angular, entorhinal, hippocampus, occipitotemporal cortices	IHC	HLA-DR	↑ HLA-DR (more intense staining in more severe AD cases)

Table 1. (Continued)

First Author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Marker	Results (AD vs C unless otherwise specified)
McGeer[38]	Pathology Department of the University of British Columbia	NR	NR	NR	NR	NR	NR	NR	NR	Hippocampus	PCR	HLA-DR	↑
McGeer[36]	Autopsy Service of the University of British Columbia	AD: 9 C: 7	AD: 5/4 C: NR	AD: 77.2 C: 73.4	NR	NR	NR	No neurological disorders	All >3 days, most >10 h	Hippocampus, substantia nigra	IHC	HLA-DR	↑
McGeer[37]	NR	AD: 6 C: 5	NR	AD: 78, C: 73	NR	NR	NR	No neurological disease	All within 2-12	Hippocampus	IHC	HLA-DR	↑
Minett[56]	Medical Research Council Cognitive Function and Ageing Study—six centres in UK	AD: 83 C: 130	AD: 30/53 C: 64/66	AD: 89 C: 84	NR	CERAD	NR	NR	NR	Middle frontal gyrus	IHC	HLA-DR	↔
Narayan[39]	Neurological Foundation of New Zealand Human Brain Bank (Centre for Brain Research, University of Auckland)	AD: 14 C: 17	AD: 6/8 C: 10/7	AD: 74.1 C: 58.9	NR	Braak or CERAD	NR	Neurologically normal, four have high plaque load	AD: 16.7 C: 16.7	Inferior temporal gyrus	IHC	HLA-DP-DQ-DR	↑ HLA-DP, DQ, DR-positive cells correlate with Iba1-positive cells in C but not AD
Overmyer[52]	Kuopio University Hospital	AD: 73 C: 22	AD: 12/61, C: 12/10	AD: 84 C: 78	APOE4 carriers: AD: 31 C: 7	CERAD Patients with possible AD and vascular dementia included	NR	NR—55% demonstrated plaque and tangles, 32% enough for diagnosis of possible AD	All within 48	Grey and white matter of frontal, temporal and parietal cortices	IHC	HLA-DR	↔ With dementia diagnosis (trend) ↑ With CERAD in grey matter ↔ White matter (counts and area) ↔ With plaque burden but ↑ correlation with NFT
Parachikova[40]	Institute for Brain Aging and Dementia Tissue Repository, and the BSHRI	AD: 10 HPC: 10 C: 4	AD: 6/4 HPC: 4/6 C: 3/1	AD: 85.3 HPC: 86.6 C: 76.3	NR	Braak	AD: IV–V HPC: 1–V	NR	AD: 2.6 HPC: 2.8 C: 3.0	Hippocampus and prefrontal cortex (gene chip only)	Gene chip, western, IHC	GeneChip: MHCII western: HLA-DR-DQ-DP IHC: CD4/43	GeneChip and western: ↑ vs HPC +C (pooled) IHC: ↑ MHCII (not quantified)
Pugliese[53]	Neurological Tissue Bank (Serveis Cientifico-Tècnics), Universitat de Barcelona	AD: 7 C: 3	AD: 2/5 C: 1/2	AD: 84.0 C: 63.3	NR	Braak, Newell Criteria	AD: II: 3, V: 1, VI: 3	NR	AD: 8.8 C: 5.1	Subventricular zone of the lateral ventricle	IHC	HLA-DR	↔ Number of microglia ↑ Activated microglia
Rezaie[41]	MRC London Neurodegenerative Diseases Brain Bank	AD: 10 C: 10	AD: 4/6 C:7/3	AD: 79.3 C: 70.2	Not reported	CERAD	NR	No history of neurological disease or neuropathology	AD: 20.9, C: 43.2	Frontal blocks included agranular-intermediate frontal cortex (BA 6/8), cingulate cortex (BA 24/32) Occipital blocks included the calcarine sulcus (BA 17) and striate cortex)	IHC	HLA-DR-DP-DQ (CR3/43)	↑ In frontal white matter, occipital white matter, plaque associated frontal grey matter, plaque associated occipital grey matter ↔ In MHCII in frontal grey matter, or occipital grey matter
Serrano-Pozo[54]	Massachusetts ADRC Brain Bank	AD: 40 C: 32	AD: 14/26 C: 13/19	AD: 77.3 C: 81.3	APOE4: AD: 21/40 C: 5/27	NIA—Reagan criteria	NR	No clinical history of neurological disorders and did not meet the pathological criteria for any neurodegenerative disease	AD: 14.1 C: 17.9	Temporal polar association cortex (BA38)	IHC, stereology	HLA-DR-DQ-DP	↔ Total microglia ↓Iba1+/MHC2- microglia ↑Iba1-/MHC2+ microglia ↔Iba1+/MHC2+ microglia ↑ Iba1+/MHC2+ microglia in APOE4 + ↔with microglia by genotype

Table 1. (Continued)

First Author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Marker	Results (AD vs C unless otherwise specified)
Shepherd[42]	Collected brains from a regional brain donor program for neurodegenerative diseases in 1993	AD:10 C: 11	NR	AD:76 C: 71	NR	CERAD	AD: V or VI	No history of neurological disease or neuropathology	AD: 16 C: 21	Cortex and hippocampus (grey and white matter)	IHC	HLA-DR	↑ White and grey matter
Szpak[23]	NR	AD: 18 (7 had Lewy body variant of AD) C: 6	NR	Whole sample: 63–86 years old	NR	CERAD	NR	No neuropathological abnormality	NR	Cortical layers of limbic, cingulate cortex and temporal association cortex	IHC	CR 3/43 clone HLA-DP-DQ-DR	↑
Thal[43]	Pathological Institute of the University of Leipzig and University of Frankfurt	159 participants (68 non-demented, 24 and 19 in GDS scores 6 and 7)	NR	Ages 46–93 (most between 71 and 90)	NR	Braak	Whole sample: 0: 23, I: 23, II: 42, III: 36, IV: 16, V: 13, VI: 6	No confounding neurological diagnosis	All within 12–72	Entorhinal cortex, hippocampus (CA1, CA4), occipital region (BA 17), temporal cortex	IHC	HLA-DR	↑
Valente[55]	Hospital Clinic-University of Barcelona	AD: 7 AD with diabetes: 7 C: 6	AD: 2/5 AD with diabetes: 5/2 C:3/3	AD: 83.9 AD with Diabetes: 73.0 C: 70.0	NR	Braak	AD: VI AD with diabetes: VI	NR	AD: 8.9 AD with diabetes: 11.5 C: 9.6	Hippocampus	IHC	HLA	↔ AD vs C ↑ AD +diabetes vs C
Van Everbroeck[44]	NR	AD: 10 C: 10	NR	NR	NR	Braak	AD: at least III–IV C: 0, Av or A 1	Some had protein deposition and some had core containing plaques (numbers not given)	NR	Cerebellum, hippocampus (CA1, CA4, subiculum), frontal, temporal and occipital neocortices	IHC	HLA-DR	↑ In grey matter and hippocampus ↔ In white matter and cerebellum
Vehmas[45]	Johns Hopkins University ADRC and Baltimore Longitudinal Study of Aging	AD: 9 HPC: 15 C: 11	AD: 3/6 HPC: 10/5 C: 11/0	AD: 83.2 HPC: 86.3C: 81.7	NR	Braak, CERAD	AD: II–V HPC: I–IV C: 0–III	NR, free of plaque	NR	Mixed: middle frontal gyrus, middle and superior temporal gyrus	IHC	HLA-DR	↑ Than C ↔ high pathology C
Verwer[50]	NBB	AD: 14 C: 7	AD: 4/10 C: 2/5	AD: 83.9 C: 79.0	NR	Braak	AD: IV: 3 V: 8 VI: 2 NA: 1 C: 0: 3 I: 1 II: 1 III: 1 NA: 1	No neurological causes of death	AD: 4.6 C: 4.6	Neocortex	IHC	HLA-DR-DQ-DP	↔ $P = 0.08$
Wilcock[46]	Irvine ADRC, the Maryland Developmental Disorders Brain Bank and the University of Kentucky Alzheimer's Disease Center.	AD: 9 C: 9	IHC: AD: 6/2 C: 3/6 qPCR +western: AD: 6/4 C: 12/4	IHC: AD: 81.3, C: 81.6 qPCR +western: AD: 80 C: 81.6	NR	NR	NR	NR	IHC: AD: 5.5, C: 3.3 qPCR +western: AD: 6.8 C: 3.3	Frontal cortex	IHC for HLA-DR staining, RT-qPCR and western for expression of M2 and M1 markers	HLA-DR M1 markers: IL-1B, IL-6, IL-12, TNF-α M2a markers: CH13L1, IL1Ra, IL-10, MRc1, M2b markers: CD86, FCGR1B M2c markers: TGFB	↑ HLA-DR in AD vs C ↑ HLA-DR in AD vs. AD+DS (in grey and white matter) —Pattern of increases in both M1 and M2 markers: IL6, IL-12, IL-10, CHI3L1, TGFB1 in AD vs C
Wojtera[51]	NR	AD: 4 C: 2	NR	NR	NR	NIA-Reagan criteria	NR	NR	NR	Mixed: cerebellum, cerebral cortex	IHC	HLA-DR	↔, ↔ in HLA-DR/CD68 ratio between AD and C (activation)
Xiang[47]	ADRC of the Mount Sinai School of Medicine	AD: 26 with clinical dementia rating 0.5–5, 6 rated 5	AD: 7/19 C: 0/5	AD: 88.7 C: 83.2	NR	CERAD	NR	NR	AD: 4.2 C: 4.2	Entorhinal cortex and dorsal hippocampus (CA1 pyramidal cell layer, DG granule cell layer and	IHC	HLA-DR	Entorhinal cortex: ↑ In grey and white matter at CDR 5, in grey matter only at CDR 2 ↔ For CDR

Table 1. (Continued)

First Author	*Brain bank*	*N*	*Sex*	*Age*	*AD genetic risk factors*	*AD histologically confirmed*	*Braak stage*	*C history of neurological or psychiatric disease*	*PMI (h)*	*Brain region*	*Technique*	*Marker*	*Results (AD vs C unless otherwise specified)*
		(very severe) C: 5								upper molecular layer)			scores of 0.5 to 1 vs 0 Hippocampus: ↑ In all regions for CDR > 2 vs 0, for CA1 pyramidal layer and upper molecular layer for CDR 1 and for the upper molecular layer only for CDR 0.5—HLA-DR score correlates with plaque and tangle scores in various regions

Abbreviations: AD, Alzheimer's disease; ADRC, Alzheimer's Disease Research Center; APOE, apolipoprotein E; Avg, average; BSHRI, Banner Sun Health Research Institute; BA, Brodmann area; C, Control; CA, Cornu ammonis; CD, cluster of differentiation; CDR, Clinical Dementia Rating score; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CHI3L, chitinase 3-like; DG, dentate gyrus; GDS, Global Deterioration Scores; HLA, human leukocyte antigen; HPC, high pathology control; Iba1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemistry; IL, interleukin; MHC, major histocompatibility complex; MRc, mannose receptor; MRC, Medical Research Council; NA, not available; NBB, Netherlands Brain Bank; NFT, neurofibrillary tangle; NIA, National Institute on Aging; NR, not reported; NSAID, nonsteroidal anti-inflammatory drug; PMI, post-mortem interval; TNF-α, tumour necrosis factor-α; TREM2, triggering receptor expressed on myeloid cells 2. Results are expressed relative to control unless specified otherwise. Where there are both young and older controls, values are reported for the older (age-matched controls).

The remaining positive study quantified plaque-associated microglia.[20] In contrast, 6 of the 10 null studies used cell counting, 2 of which used stereology, the gold standard for quantifying cells without bias. Expression-based assays like qPCR, western blot, or intensity of immunohistochemistry staining indicate the amount of Iba1 in a sample. An increase could reflect a change in cell numbers, cell size or function, as Iba1 expression is thought to increase with microglial activation. In contrast, the studies using cell counting for Iba1, which is expressed by all microglia,[58,77] assess the absolute number of microglia in the samples. The discrepancy between the studies using expression-based assays and those that used cell counting suggests that Iba1 expression, and thus microglial activation, increases in AD without affecting the absolute number of microglia.

Cluster of differentiation 68

CD68 is a common marker for macrophage lineage cells, primarily localized to microglia within the brain parenchyma, and perivascular macrophages in the cerebral blood vessels and, occasionally, parenchyma.[78] Although there is some CD68 expression on resting microglia,[14] it labels the lysosome and is therefore commonly considered a marker of activated phagocytic microglia.[60] Twenty-one studies were identified that compared CD68 between AD and control post-mortem brain samples (Table 3). Twenty of these studies used immunohistochemistry to visualize CD68-positive cells and measured cell counts or staining area, whereas one study measured CD68 gene expression by qPCR. Seventeen identified an increase in CD68 expression, staining or positive cell counts in AD relative to control samples in at least one region,[17,18,20,28,41,56,69,76,78–86] whereas four found no difference between AD and control,[51,87–89] and four reported no difference in at least one of the brain regions measured.[20,41,69,80]

CD68-positive cell counts, staining area or gene expression were measured in the hippocampus in eight studies: six identified higher levels in AD[20,69,76,78,79,86] and two identified no differences.[69,87] CD68 was higher in AD than control in the frontal cortex in three studies,[18,41,82] though for white matter only in one of the studies.[41] Elevations in CD68 were also reported in the temporal cortex,[20,28] the olfactory bulb,[80] the calcarine cortex,[69,86] the superior temporal sulcus,[83] the orbitofrontal cortex,[86] parahippocampal gyrus[76] and temporal association isocortex.[84] No difference between AD and control was reported in the caudate nucleus,[88] combined cerebellum and cerebral cortex,[51] mediodorsal nucleus of the thalamus[88] and the middle frontal gyrus.[56] CD68 immunoreactivity appears to increase with age in control subjects, but decreases with age in patients with AD.[28] It also increases with APOE ε4 genotype.[56] Characteristics of AD and control groups could therefore contribute to between-study variability. On balance, CD68 appears to be increased in the brains of patients with AD, though there is some variation between studies and brain regions.

Cluster of differentiation 11b

CD11b forms part of complement receptor 3 that aids in the recognition and phagocytosis of antigens, including amyloid-β.[90] Like Iba1, CD11b is expressed by both resting and activated microglia, though it too is inducible with activation.[14] Five studies were identified that compared CD11b between AD and control post-mortem human brain samples (Supplementary Materials Table 2). Two papers reported an increase in CD11b,[15,38] whereas three reported no differences between AD and control.[74,76,91]

Like Iba1, the results of studies measuring CD11b in AD and control post-mortem human brain samples are heterogeneous. Of two studies in the hippocampus, one identified an increase in CD11b gene expression,[38] whereas the other identified no difference in expression between AD and controls.[76] Similarly, Akiyama *et al.* reported an increase in CD11b-positive cells in the

Table 2. Ionized calcium-binding adapter molecule 1 (Iba1)

First author	*Brain bank*	N	*Sex*	*Age*	*AD genetic risk factors*	*AD histologically confirmed and criteria*	*Braak stage*	*C history of neurological or psychiatric disease*	*PMI (h)*	*Brain region*	*Technique*	*Marker*	*Direction of results*
Bachstetter[69]	University of Kentucky Alzheimer's Disease Center	AD: 7 C: 9	AD: 4/3 C: 6/3	AD: 77 C: 86	APOE: AD: NA: 2, 4/4: 1, 3/4: 2, C: 3/4: 1	CERAD	AD: ~VI C: ~II	NR	AD: 4.2 C: 2.4	Hippocampus: CA1, CA2/3, CA4, DG, subiculum and adjacent white matter Morphology assessed in the CA1 only	IHC	Iba1	↔Iba1 staining or cell counts in any hippocampal area ↔ in CA1 Iba1+ microglial morphology
Griciuc[70]	Massachusetts ADRC	AD: 25 C: 15	AD: 7/18 C: 6/9	AD: 79.2 C: 79.9	APOE carrier: AD: 18 (8 homozygous) C: 5 (0 homozygous)	NIA-Reagan Institute Criteria	NR	NR	AD: 17 C: 29	Frontal cortex	IHC (stereology), western	Iba1	↔ Iba1+ microglia (data not shown) ↔ Iba1 protein (nonsignificant increase)
Magistri[71]	BSHRI	AD: 4 C: 4	AD: 1/4 C: 2/2	AD: 83.75 (not exact, one age just listed >90) C: 83.5	AD: all APOE3/3 C: APOE2/3: 2 APOE3/3: 1 NR: 1	NIA-Reagan criteria	AD: V: 1 VI: 3 C: I: 1 II: 3	NR	AD: 2.5 C: 2.5	Hippocampus	RNA seq (gene expression)	Iba1	↔
Nielsen[62]	NBB	AD: 4 C: 5	AD: 1/3 C: 1/4	AD: 76.3 C: 77.6	NR	Yes	AD: III: 1, IV: 2, V: 1 C: 0: 1, I: 3 II: 1,	NR	AD: 6.3 C: 6.1	Entorhinal cortex, hippocampus	IHC	Iba1	↑
Dal Bianco[20]	NR	AD: 9 C: 15	AD: 0/9 C: 13/2	AD: 81 C: 70	NR	Braak, CERAD	AD: IV: 2, V: 4, VI: 3	No neurological disease or brain lesions	NR	Cortical areas of the temporal lobe, including entorhinal cortex, hippocampus and temporal cortex	Immunocytochemistry	AIF-1	↑ AIF-1 near plaque only
Davies[72]	New South Wales Brain Bank	AD: 7 C: 5	AD: 3/4 C: 2/3	AD: 83.6 C: 83.0	NR	Braak, CERAD, National Institute on Aging-Alzheimer's Association Guidelines for Neuropathological Assessment of AD	AD: V: 3 VI: 4 C: 0: 4 I: 1	No co-existing pathology	AD: 12.3 C: 15.4	Cingulate cortex, inferior temporal cortex	IHC	Iba1	↔ Cell density ↑ Microglia with dystrophic morphology, activated morphology (lower ramification)
Satoh[68]	NR	: AD: 7 C: 14	AD: 5/5 C: 6/5	AD: 70 C: 75	NR	Braak, CERAD	AD: VI: 10	4 Died of nonneurological causes, 3 with Parkinson's, 4 ALS	NR	Frontal cortex	IHC, qPCR	Iba1	PCR: ↑ IHC: ↔
Tang[65]	University of Kentucky Alzheimer's Disease Center Autopsy Program	AD: 10 C: 10	AD: 5/5 C: 6/4	AD: 82.1 C: 82.7	NR	NIA-Reagan criteria	AD: VI: 6 C: I–III, (avg 1.6)	No neuropathology	AD: 26.2 C: 6.2	Inferior parietal cortex	Western	Iba1	↑
Ekonomou[73]	United Kingdom MRC Cognitive Function and Ageing Study	AD: 13 C: 15	14/28 (both AD and C)	Whole sample: 84.8	NR	Braak	Whole sample: 0–II: 12, III–IV: 11, V–VI: 5	No neurological disease	All within 17.5–25.0	Hippocampus DG	IHC	Iba1	↔ Between AD and C ↑ in Braak stage 3–4 than 0–2 or 5–6
Korvatska[32]	University of Washington Neuropathology Core Brain Bank	AD (normal TREM2): 6 AD (with TREM2 R47H variant): 7 C: 3	NR	Whole sample: 84.9	NR	CERAD	AD: III: 1, V: 4, VI: 1 AD R47H: V: 6, VI: 1 C: 0: 1, I: 1, III: 1	NR, one C CERAD score A and one B	All avg 4.5	Frontal lobe: grey and white matter Hippocampus: CA1, hilus, parahippocampal gyrus and white matter	IHC	Iba1	↓ Staining in hippocampus and frontal lobe white matter ↔ Staining in hilus, CA1, parahippocampal gyrus or frontal lobe grey matter ↔ Counts in frontal lobe grey matter or activated counts in hippocampus white matter in AD, though ↓ in R47H

Table 2. (Continued)

First author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed and criteria	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Marker	Direction of results
Lastres-Becker[67]	Banco de Tejidos de la Fundacion CIEN	AD: 4 C: 4	NR	AD: 73–90 C: 78–90	NR	Braak	AD: II–IV	No neuropsychiatric disease or neuropathology	All within 5 h	Hippocampus	IHC, immunoblot	Iba1	↑
Lee[74]	OPTIMA and Newcastle Brain Tissue Resource (NBTR)	AD: 12 C: 11	AD: 7/5 C: 6/5	AD: 73.1 C: 81.1	NR	Braak, CERAD	AD: V–VI C: I–II	NR	AD: 61.2 C: 41.5	Prefrontal (BA9) and temporal (BA22) cortices	PCR	Iba1	↔
Lue[61]	BSHRI	AD: 11 C: 11 HPC: 11	AD: 6/5 C: 7/4 HPC: 3/8	AD: 82.4 C: 85.4 HPC: 86.5	APOE 4 Carriers: AD: 5/6 C: 1/10 HPC: 2/9	Braak, CERAD	AD: Avg 5.2 C: Avg 2.8 HPC: Avg 2.9	NR	NR	Middle temporal cortices	Western	Iba1	↑ Than C and HPC
Marlatt[75]	Netherlands Brain Bank	AD: 8 C: 8	AD:4/4 C: 4/4	AD: 81 C: 80	NR	Braak	AD: Avg 4.8 C: Avg 1.4	NR	All within 5–7	Hippocampus (CA1/2, CA3, DG/SCZ, Hilus)	IHC	Iba1	↔ In cell number or in morphology
Minett[56]	Medical Research Council Cognitive Function and Ageing Study—six centres in UK	AD: 83 C: 130	AD: 64/53 C: 51/66	AD: 89 C: 84	NR	CERAD	NR	NR	NR	Middle frontal gyrus (BA9)	IHC	Iba1	↓ Iba1 no association with cognition (MMSE), positive association with AD pathology (plaques, tangles)
Rangaraju[63]	Emory ADRC Neuropathology Core, Atlanta	AD: 10 C: 10	AD: 6/4 C: 6/4	AD:71.5 C: 71.5	APOE: AD: 8 with APOE4 (3 homozygous) C: 1 APOE4 (0 homozygous	Yes	AD: All VI C: 0	NR	NR	Frontal cortex	IHC	Iba1	↑ Iba1 staining density, $P = 0.06$ for staining intensity
Rivera[64]	KPBBB, University Medical Center	Braak stage 0–1: 12, 2–3: 12 4–5: 12 6: 9	Braak stage: 0–1: 6/6 2–3: 5/7 4–5: 3/9 6: 0/9	Braak stage: 0–1: 74.4 2–3: 81.1 4–5: 82.1 6: 71.8	APOE4/4: Braak stage 0–1: 1 2–3: 4 4–5: 5 6: 0	Braak, NIA-Reagan Criteria	AD: II–VI C: 0–I	NR	All < 16 h	Anterior frontal cortex	PCR	AIF1 IBA1	↑
Sanchez-Mejias[76]	Tissue bank at Fundación CIEN	Braak stage 0: 8 II: 13, III–IV: 9, V–VI: 17	Braak stage 0: 5/3, II: 7/13, III–IV: 4/5, V–VI: 7/11	Braak stage 0: 19, II: 78, III–IV: 80, V–VI: 79	NR	Braak Braak V–VI clinically classified as AD, Braak II age-matched and used as C	Braak stage 0: 8, II: 13, III–IV: 9, V–VI: 17	NR	Braak stage 0: 8 II: 7, III–IV: 6, V–VI: 8	Hippocampus CA1, CA3, parahippocampal gyrus	IHC, PCR	Iba1	PCR: ↔ Iba1 IHC: ↓ in DG and CA3 ↔CA1 and parahippocampal gyrus More activated morphology
Serrano-Pozo[54]	Massachusetts ADRC Brain Bank	AD: 40 C: 32	AD: 14/26 C: 13/19	AD: 81.3 C: 77.6	APOE4: AD: 21/40 C: 5/27	NIA-Reagan criteria	NR	No clinical history of neurological disorders and did not meet the pathological criteria for any neurodegenerative disease	AD: 18.0 C: 14.1	Temporal polar association cortex (BA 38)	IHC, stereology	Iba1	↔ Total microglia ↓IBA1+/MHC2- microglia ↑IBA1-/MHC2+ microglia ↔IBA1+/MHC2+ microglia ↑ IBA1+/MHC2+ microglia in APOE4+ ↔with microglia by genotype
Walker[60]	BSHRI	AD: 30 C: 41	Whole sample: AD: 49/48 C: 50/46	Whole sample: AD: 82.2 C: 84.9	APOE4/4 genotypes excluded	NIA-Reagan criteria	NR	NR	Whole sample: AD: 3.6 C: 4	Temporal cortex	Western	Iba1	↑ For CD33 rs3865444 allele A/C genotype ↔ For C/C and A/A genotypes

Abbreviations: AD, Alzheimer's disease; ADRC, Alzheimer's Disease Research Center; ALS, amyotrophic lateral sclerosis; APOE, apolipoprotein E; Avg, average; BSHRI, Banner Sun Health Research Institute; BA, Brodmann area; C, Control; CA, Cornu ammonis; CD, cluster of differentiation; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CIEN, Centro Investigación Enfermedades Neurológicas; DG, dentate gyrus; HPC, high pathology control; Iba1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemistry; KPBBB, Kathleen Price Bryan Brain Bank; MRC, Medical Research Council; MMSE, mini-mental state examination; NA, not available; NBTR, Newcastle Brain Tissue Resource; NBB, Netherlands Brain Bank; NIA, National Institute on Aging; NR, not reported; OPTIMA, Oxford Project to Investigate Memory and Aging; PMI, post-mortem interval; Seq, sequencing; TREM2, triggering receptor expressed on myeloid cells 2. Results are expressed relative to control unless specified otherwise. Where there are both young and older controls, values are reported for the older (age-matched controls).

Table 3. Cluster of differentiation 68 (CD68)

First author	*Brain bank*	N	*Sex*	*Age*	*AD genetic risk factors*	*AD histologically confirmed and criteria*	*Braak stage*	*C history of neurological or psychiatric disease*	*PMI (h)*	*Brain region*	*Technique*	*Direction of results*
Alvarez[87]	NR	AD: 24 (for cortex and CA1) ADaβ−: 5 Control: 24 (16 for cortex and CA1)	NR	AD: 70–86 ADaβ−: 70-76 Control: 70-86	NR	Braak, CERAD Adaβ− group had no aβ pathology	AD: V–VI	No mental disorder	NR	Cerebellar cortex and hippocampus white matter molecular layer, Purkinje cell layer, granule cell layer, white matter core of the folium, central white matter, layer V of the cortex and CA1	IHC	↔ Between AD, Adaβ− and control
Arnold[86]	University of Pennsylvania Alzheimer Disease Center Core	AD: 10 C: 14	AD: 5/5 C: 6/8	AD: 81.8 C: 75.3	NR	Khachaturian	NR	No major psychiatric illness, no neuropathologic abnormality except 1 patient with lacunar infarct, one with small temporal contusions	AD: 9.8 C: 11.4	Calcarine cortex (BA17), enthorinal cortex (BA 28) hippocampus CA1, midfrontal cortex (BA9 and 46) orbitofrontal cortex (BA11), subiculum	IHC	↑ In all regions
Arnold[79]	AD and FTD from: University of Pennsylvania's Alzheimer Disease Center Core	AD: 10 C: 10	AD:5/5 C: 4/6	AD: 81.8 C: 76.1	NR	Yes	NR	No history of major neurological or psychiatric disorder, no neuropathologic abnormalities relevant to mental status	AD: 9.8 C: 11.6	Calcarine cortex, frontal lobe, hippocampus	IHC	↑ (Not compared statistically)
Bachstetter[69]	University of Kentucky Alzheimer's Disease Center	AD:7 C: 9	AD: 4/3 C: 6/3	AD: 77 C: 86	APOE: AD: NA: 2 4/4: 1 3/4: 2 C: 3/4: 1	CERAD	AD: Median VI C: Median II	NR	AD: 4.2 C: 2.4	Hippocampus: CA1, CA2/3, CA4, DG, subiculum and adjacent white matter. Morphology assessed in the CA1 only	IHC	↑ CD68 staining in subiculum, CA1, DG, and mean of hippocampal regions ↔ in CA2/3, CA4 or white matter ↔ in CD68+ amoeboid in any region except ↑ DG
Dal Bianco[20]	NR	AD: 9 C: 15	AD: 0/9 C: 13/2	AD: 81 C: 70	NR	Braak, CERAD	AD: IV: 2, V: 4, VI: 3	No neurological disease or brain lesions	NR	Cortical areas of the temporal lobe, including entorhinal cortex, hippocampus and temporal cortex	Immunocytochemistry	↑ CD68 near plaque only
DeLuca[80]	Oxford Brain Bank	AD: 4 C: 8	AD: 3/1 C: 5/3	AD: 76.3 C: 63.0	NR	NR	AD: V or VI	No neurological disease	NR	Olfactory bulb/ tract	IHC	↑ In parenchyma and meninges ↔ Perivascular
Doorn[81]	NBB or Department of Pathology, Vrije Universiteit, University Medical Center in Amsterdam, The Netherlands	AD: 8 C: 11	AD: 3/5 C: 5/6	AD: 74.5 C: 84	NR	Braak	NFT AD: IV–VI C: 0–III Amyloid: AD: C: 7 B: 1 C: 0:4 A: 3 B: 3 C: 1	Without neurological or psychiatric diseases	AD: 6.2 C: 5.9	Olfactory bulb	IHC	↑ Amoeboid microglia ↔ Ramified

Table 3. (Continued)

First author	*Brain bank*	N	*Sex*	*Age*	*AD genetic risk factors*	*AD histologically confirmed and criteria*	*Braak stage*	*C history of neurological or psychiatric disease*	*PMI (h)*	*Brain region*	*Technique*	*Direction of results*
Falke[88]	University of Pennsylvania ARDC	AD: 12 C: 11	AD: 2/10 C: 7/4	AD: 79.4 C: 77.6	NR	NR	NR	No neuropsychiatric disease—3 control subjects had abnormality at autopsy (haemorrhagic microinfarct, bilateral contusion, adenocarcinoma metastasis). 1 AD subject had microinfarct, all had aβ plaques and NFT	AD: 10.9 C: 12.4	Caudate nucleus (6 AD, 7 Control), mediodorsal nucleus of the thalamus (12 AD, 10 Control)	IHC	↔ In caudate nucleus ↔ In MEDIODORSAL nucleus of the thalamus ($P = 0.06$)
Fiala[78]	UCLA ADRC Brain Bank	AD: 8 C: 5	NR	AD: 77.6 C: 74.6	NR	Yes, one patient with vascular dementia not excluded	NR	No neuropathological findings	All 5–6 h	Mix of areas (different for different cases): hippocampus, frontal lobe (mix of left and right), superior temporal lobe	IHC	↑ CD68 staining in AD than C
Hoozemans[17]	NBB	Braak stage 0: 5, I–II: 16, III–IV: 10, V–VI: 9	Braak stage 0: 3/2, I–II: 6/10, III–IV: 0/10,V–VI: 3/6	Braak stage 0: 62, I–II: 83, III–IV: 89, V–VI: 76	NR	Braak	Subjects vary from 0–VI (not divided into C and AD)	NR	Braak stage 0: 8, I–II: 7.5, III–IV: 6.5, V–VI: 5	Temporal cortex	IHC	↑ With increasing Braak NFT or plaque stage ($P < 0.05$ for trend), significant for NFT group V–VI vs 0
Hoozemans[28]	Netherlands Brain Bank	AD: 19 C:19	AD: 3/16 C: 8/11	AD: 83.5, C: 76.8	APOE4: AD: 12 C: 8	Braak	AD: avg IV C: avg I	NR	AD: 5.1 C: 8.6	Midtemporal cortex	IHC	↑ ↑ In AD patients ˂80 years compared with those ˃80 years
Hultman[82]	KPBBB, Duke University, North Carolina	AD: 36 C: 22	AD: 13/23 C: 10/12	AD: 76.9 C: 79.1	APOE4 Carriers: AD: 14 C: 0	CERAD, NIA-Reagan criteria	AD: III: 11, IV: 3, V: 13, VI: 9 C: I: 18, II: 3, III: 1	NR, some cases and Cs had mild to severe atherosclerosis	AD: 9.2 C: 7.7	Frontal cortex—perivascular	IHC	↑
Kellner[18]	NR	AD: 48 C: 48	AD: 19/29 C: 24/24	AD: 80.3 C: 77.5	NR	Braak, CERAD	AD: II–VI (38 > 4) C: I–III (45 = 0)	NR	NR	Entorhinal, frontal cortex, temporal cortex	IHC	↑
Lue[89]	BSHRI	AD: 16 C: 21	NR	NR	NR	NR	NR	NR	All avg 3.1	Mixed: corpus callosum, superior and middle frontal gyri of the right hemisphere	IHC	↔
Minett[56]	Medical Research Council Cognitive Function and Ageing Study—six centres in UK	AD: 83 C: 130	AD: 64/53 C: 51/66	AD: 89 C: 84	NR	CERAD	NR	NR	NR	Middle frontal gyrus (BA9)	IHC	↑ Negative correlation with cognition (MMSE), positively with AD pathology (plaques, tangles)

Table 3. (Continued)

First author	Brain bank	N	Sex	Age	AD genetic risk factors	AD histologically confirmed and criteria	Braak stage	C history of neurological or psychiatric disease	PMI (h)	Brain region	Technique	Direction of results
Perez-Nievas[83]	Massachusetts General Hospital, Mayo Clinic and University of Pittsburgh ADRC Brain Banks	AD:15 LPC: 15 IPC: 12 HPC: 8	NR	AD: 87.2 LPC: 84.4 IPC: 89.8 HPC: 88.4	NR	Braak, CERAD	NR	NR	NR	Superior temporal sulcus	IHC, stereology	↑ In AD vs LPC, IPC and HPC ↔ in IPC or HPC vs C
Rezaie[41]	MRC London Neurodegenerative Diseases Brain Bank	AD: 10 C: 10	AD: 4/6 C: 7/3	AD: 79.3 C: 70.2	NR	CERAD	NR	No history of neurological disease or neuropathology	AD: 20.9, C: 43.2	Frontal blocks included agranular-intermediate frontal cortex (BA 6/8), cingulate cortex (BA 24/32) Occipital blocks included the calcarine sulcus (BA 17) and striate cortex)	IHC	↑ In frontal white matter, occipital white matter, plaque associated frontal grey matter, plaque associated occipital grey matter ↔ Frontal grey matter, or occipital grey matter
Sanchez-Mejias[76]	Tissue bank at Fundación CIEN	Braak stage 0: 8, II: 13, III–IV: 9, V–VI: 17	Braak stage 0: 5/3, II: 7/13, III–IV: 4/5, V–VI: 7/11	Braak stage 0: 19, II: 78, III–IV: 80, V–VI: 79	NR	Braak Braak V–VI clinically classified as AD, Braak II age-matched and used as C	Braak stage 0: 8, II: 13, III–IV: 9, V–VI: 17	NR	Braak stage 0: 8, II: 7, III–IV: 6, V–VI: 8	Hippocampus CA1, CA3, parahippocampal gyrus	PCR	↑ With increasing Braak stage Braak stage V–VI had clinical AD and were compared with stage II Cs
Serrano-Pozo[84]	Massachusetts ADRC Brain Bank	AD: 91 C: 15	AD: 33/58 C: 5/10	AD: 79.0 C: 79.9	APOE E4 carriers AD: 59/32 C: 4/11	NIA-Reagan Criteria	NR	NR, 10/15 had some plaque burden	AD: 13.9 C: 22.3	Temporal association isocortex (BA 38)	IHC, stereology	↑ With increasing disease stage and NFT, no correlation with amyloid burden
Van Everbroeck[85]	NR	AD: 21 C: 40	NR	NR	NR	Yes	NR	NR, 14 cases/Cs suffered from inflammatory conditions	NR	Mixed: Cerebellum (when available), frontal, occipital and temporal cortices	IHC	↑
Wojtera[51]	NR	AD: 4 C: 2	AD: 4 C: 2	NR	NR	NIA-Reagan Criteria	NR	NR	NR	Mixed: cerebellum, cerebral cortex	IHC	↔ Microglia number ↔ cortex and cerebellum ↔ in HLA-DR/CD68 ratio between AD and control (activation)

Abbreviations: AD, Alzheimer's disease; ADRC, Alzheimer's Disease Research Center; APOE, apolipoprotein E; Avg, average; BSHRI, Banner Sun Health Research Institute; BA, Brodmann area; C, Control; CA, Cornu ammonis; CD, cluster of differentiation; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CIEN, Centro Investigación Enfermedades Neurológicas; DG, dentate gyrus; FTD, frontotemporal dementia; HLA, human leukocyte antigen; HPC, high pathology control; IHC, immunohistochemistry; IPC, intermediate pathology control; KPBBB, Kathleen Price Bryan Brain Bank; LPC, low pathology control; MRC, Medical Research Council; NA, not available; NBB, Netherlands Brain Bank; NFT, neurofibrillary tangle; NIA, National Institute on Aging; NR, not reported; PMI, post-mortem interval; UCLA, University of California Los Angeles. Results are expressed relative to control unless specified otherwise. Where there are both young and older controls, values are reported for the older (age-matched controls).

temporal lobe of AD cases,[15] whereas Lee *et al.*[74] found no difference in gene expression relative to controls in the same region. Two studies that compared CD11b gene expression in the prefrontal cortex identified no difference between AD and control.[91] Interestingly, two of the studies that identified increases in CD11b also measured MHCII, and both reported greater increases in MHCII than CD11b in the AD brain.[15,38] Based on these studies, CD11b does not appear to be consistently increased in the AD brain.

Cluster of differentiation 45

CD45 is a cell surface antigen that is expressed on most hematopoietic lineage cells, where it is involved in the regulation of numerous processes, including cell division and differentiation. CD45 is expressed by both resting and activated microglia, but it does not appear to be inducible with activation in humans.[92] Six papers were identified that compared CD45 in AD and control human brains (Supplementary Materials Table 3). Two used immunohistochemistry with cell counting, three measured gene expression and one used a combination of western analysis and immunohistochemistry with cell counting. Four studies reported higher CD45 in AD in at least one brain region,[15,76,93,94] whereas two reported no difference relative to control[71,95] and one reported no difference in at least one region.[94]

All three of the studies that used immunohistochemistry with cell counting reported an increase in CD45-positive cells in AD patients in the temporal lobe,[15] midfrontal cortex,[93] frontal cortex[94] and hippocampus molecular and pyramidal layers,[94] respectively. In contrast, the polymorphous layer of the hippocampus did not demonstrate increased CD45-positive cells.[94] Studies using gene expression were more heterogeneous: Sanchez-Mejias *et al.*[76] detected higher CD45 gene expression in the hippocampus and parahippocampal gyrus in AD, whereas Magistri *et al.*[71] identified no such increase in the hippocampus relative to controls. No difference in expression was also reported in the frontal cortex.[95] The evidence suggests that there are more CD45-positive cells in the brains of patients with AD, but that this is not necessarily accompanied by increased CD45 gene expression.

Ferritin

Iron is stored in the brain as heavy (H) or light (L) subunits. Neurons and other cells primarily contain H ferritin, which is the less reactive form, whereas glial cells contain the L subunit, which can be used to generate free radicals as part of the inflammatory response.[96,97] Although not specific to microglial cells, ferritin immunohistochemistry combined with morphological identification can be used to visualize these cells in the central nervous system, and increases in ferritin levels with inflammation are thought to be caused by increases in microglial number and activation. Seven papers measured ferritin in post-mortem human brain samples from patients with AD and controls (Supplementary Materials Table 4). Five of these papers identified greater levels of ferritin, ferritin-positive microglia or microglia-associated plaques in AD in at least one of the brain regions measured,[96,98–101] whereas two found no difference in cell counts relative to control,[33,51] and one identified no change in ferritin-associated plaques in one region and a decrease in another.[100]

Three papers that measured ferritin-positive cell counts or protein levels in the hippocampus identified higher levels in AD,[96,99,101] whereas one that combined counts from the hippocampus with the amygdala, superior frontal gyrus and the superior, middle and inferior temporal gyri found no significant difference relative to control, though a nonsignificant increase was noted.[33] Higher ferritin-positive microglia was also reported in AD relative to control in the amygdala, entorhinal cortex, and frontal, occipital, parietal and temporal neocortices.[99] No difference between AD and control was reported in one study that counted microglia over 12 slices of combined cerebellum and cerebral cortex.[51]

Cluster of differentiation 33

CD33 is a myeloid cell surface marker that is involved in the regulation of the innate immune system. In microglia, CD33 seems to regulate phagocytosis of amyloid-β, with a reduction in CD33 associated with increased phagocytosis.[70] Two polymorphisms in the CD33 gene have recently been identified that modulate AD risk,[102,103] with the protective allele associated with reduced CD33[12,66,70] and increased phagocytosis.[12] Four papers were identified that used qPCR, western blot or immunohistochemistry to compare levels of CD33 in the brains of patients with AD to controls, three of which reported elevations in AD, whereas one reported no change (Supplementary Materials Table 5).

Higher expression of CD33 genes or of CD33 immunolabelled cells was noted in three studies in the superior and middle temporal gyri, frontal cortex and temporal cortex of patients with AD relative to controls.[66,70,104] In contrast, Sanchez-Mejias *et al.*[76] reported no difference in CD33 gene expression between patients with AD and controls in the hippocampus cornu ammonis 1 (CA1), CA3 and parahippocampal gyrus.[76] CD33 expression was shown to correlate with pan-microglial markers Iba1 or CD11b, supporting its microglial localization.[66,70,104] As CD33 increases when adjusting for Iba1 in most of studies, it is likely a marker of microglial function or activity, and not of the number of microglia. Though few studies are currently available, the evidence indicates that CD33 is likely increased in the brains of patients with AD.

Triggering receptor expressed on myeloid cells 2

TREM2 is a regulatory protein under the control of γ-secretase that controls toll-like receptor 4 signalling. In microglia, TREM2 seems to be important for microglial activation and phagocytosis of apoptotic neurons.[105] Polymorphisms in the R47H allele of the *TREM2* gene have recently been identified as a strong genetic risk factor for the development of AD,[13] though the actual impact of these polymorphisms on microglial function remain unclear. Four papers were identified that compared levels of TREM2 in the brains of patients with AD and controls (Supplementary Materials Table 6). Two identified an increase of TREM2 in AD,[61,106] whereas one reported no difference relative to control[76] and one reported opposing results for qPCR and western analysis.[107]

Both papers that examined the temporal cortex identified an increase in TREM2, either for the protein,[61,106] staining intensity by immunohistochemistry[61] or gene expression.[106] Roussos *et al.*[107] examined the impact of R47H genotype on TREM2 mRNA and protein in the superior temporal gyrus. They also reported higher TREM2 gene expression in AD R47H carriers relative to controls and no difference in TREM2 between AD noncarriers and control. Their protein measurements, however, indicated decreased TREM2 in AD R47H carriers relative to controls with no difference between AD noncarriers and control. The authors speculate that the discrepancy between gene expression and protein quantity may be explained by increased immature TREM2 and increased degradation. In contrast, Sanchez-Mejias *et al.*[76] identified no difference in TREM2 gene expression relative to control in the hippocampus CA1, CA3 and the parahippocampal gyrus. As with CD33, TREM2 expression is increased even when adjusting for Iba1,[106] and hence it can be considered a marker of microglial function as opposed to number.

Cluster of differentiation 11c

CD11c is a transmembrane protein expressed on the surface of various immune cells, including microglia, macrophages and neutrophils. Four studies were identified that compared CD11c in

AD and control post-mortem brains, all of which used immunohistochemistry and reported higher staining scores or cell counts in AD in at least one brain region[15,108–110] (Supplementary Materials Table 7).

Three studies examined CD11c in the hippocampus. Two reported higher levels of staining quantified by a semiquantitative scoring system in AD in the CA1 and subiculum;[108,110] however, Paulus *et al.*[109] quantified CD11c by unbiased cell counts and identified no difference in CA1 or the granular layer of the dentate gyrus relative to control. Elevated CD11c was also reported in the temporal lobe,[15] frontal cortex,[109] entorhinal cortex[110] and superior temporal gyrus.[108] No difference between AD and control was detected in the frontal white matter.[109] Based on the limited number of studies, it appears that CD11c is increased in brain of patients with AD relative to controls, but this may be influenced by differences in methodology between the studies.

IL-1α-expressing microglia

IL-1α is a proinflammatory cytokine that plays a central role in the induction of an immune response. Although IL-1α is not a microglia marker, three papers measuring it were included in this review because they used immunohistochemistry and cell counting to examine IL-1α-positive microglia as an indication of activation (Supplementary Materials Table 8). All three papers are from the same research group and reported higher IL-1α-positive microglia in AD in at least one of the measured brain areas, but two reported no difference relative to control in one region.

Elevated IL-1α-positive microglia were reported in the parahippocampal cortex,[111] the hippocampus[112] and the frontal,[112] occipital[112] and temporal lobes.[112,113] Within the temporal cortex, layers III–VI were found to be enriched in IL-1α-expressing microglia in AD, whereas layers I–II were no different from controls.[113] No difference between AD and control brains was detected in the cerebellum.[112] Based on the available evidence, IL-1α-expressing microglia seem to be increased in the brains of patients with AD.

Ricinus communis agglutinin-1

Microglia appear to be the only resident brain cells that express the lectin Ricinus communis agglutinin-1 (RCA-1).[114] Two papers were identified that used RCA-1 immunohistochemistry to compare microglia counts between AD and control post-mortem human brain samples (Supplementary Materials Table 9). Both reported significantly more RCA-1-labelled microglia in AD in the inferomedial temporal lobe[115] and the association cortex, periallocortex/allocortex and primary cortex, respectively.[116]

Translocator protein

Translocator protein (TSPO), also known as the peripheral benzodiazepine receptor, is a mitochondrial protein expressed on activated microglia in the brain. It is the ligand for (11)C-PK11195 that is commonly used in positron emission tomography imaging of activated microglia *in vivo*. Two papers returned in the systematic search compared *in situ* PK11195 binding or expression of TSPO in control and AD post-mortem human brain samples (Supplementary Materials Table 10). Kravitz *et al.*[117] identified higher [3H]-PK11195 binding in the entorhinal cortex, the subiculum, the striatum and various areas of the hippocampus. In contrast, Marutle *et al.*[118] identified no difference in PK11195 binding in the hippocampus between control and AD brains, though they reported higher levels in the frontal cortex. Kravitz *et al.*[117] also measured TSPO mRNA and identified higher expression in the hippocampus in AD relative to controls, but no difference in the striatum. Because these two studies have somewhat opposing results for the brain region they have in common, the hippocampus, it is unclear whether TSPO is increased in post-mortem brain samples from patients with AD. PK11195 has a lower affinity for TSPO and a lower signal-to-noise ratio than newer ligands which may explain some of the discrepancy. A recent review of positron emission tomography imaging of microglia in AD *in vivo* found that of the five papers using the PK11195 ligand, three identified increases in AD relative to controls and two identified no differences, whereas for the second-generation radioligands, 5/6 reported increases in AD in various brain regions.[119]

Cluster of differentiation 163

CD163 is a scavenger receptor expressed on monocyte/macrophage lineage cells. Two papers identified higher CD163 in AD than control post-mortem human brains (Supplementary Materials Table 11). Dal Bianco *et al.*[20] identified a greater number of CD163-positive cells in the cortical areas of the temporal lobe, including the hippocampus and entorhinal and temporal cortices. Like Dal Bianco *et al.*[20] Pey *et al.*[120] also found higher levels of CD163 staining in the hippocampus, as well as the frontal and occipital cortices. Based on this small number of studies, CD163 seems to be upregulated in AD relative to control post-mortem brains.

Microglia identified by morphology

Two studies used nonspecific cell stains to visualize microglia that were identified based on morphology (Supplementary Materials Table 12). Shefer[121] identified both a greater absolute number of glia and a greater number of microglia per volume in the subiculum of the archicortex in the hippocampal fissure. This combination of relative and absolute counts provides evidence that the apparent increase in microglia was not just a function of tissue shrinkage. In contrast, Pelvig *et al.*[122] also identified microglia based on morphology and quantified them using stereology, but found no difference in the total number of cells in the neocortex.

Other

Seventeen papers returned in the systematic search used markers other than those discussed in previous sections (Supplementary Materials Table 13). Some of these, such as Ox-42 and GLUT-5, are known microglial markers, whereas others were identified by the study authors as being generalizable either to microglia or their activation. Most (16/17) reported changes in markers consistent with increased numbers or activation of microglia in AD.

High-throughput techniques: microarray and proteomics

Microarrays and proteomic studies that specifically discussed microglia or their markers were included in the interest of comprehensiveness, but they are presented separately (Supplementary Materials Table 14) as there is significant risk that those presented here do not represent the balance of the literature. Any microarray would include some microglial markers, such as HLA-DR, but studies that did not identify significant differences between AD and control for these markers may be less likely to have mentioned them by name in the title, abstract or article keywords, and would therefore have been missed by the systematic search. Six studies used high-throughput techniques, either microarray[11,123–126] or proteomics,[127] to examine genes, proteins or patterns of gene expression associated with microglia in AD and control in various brain regions, including the entorhinal cortex, hippocampus, postcentral gyrus, superior frontal gyrus, prefrontal cortex, cerebellum, dorsolateral prefrontal cortex, visual cortex and precuneus. All high-throughput studies reported increases in AD relative to control in some of the microglial markers measured.

Nonquantitative comparisons

Fifty-three papers that would otherwise have met the inclusion and exclusion criteria for this review were not included in the full extraction because they were nonquantitative, though results and key methodological details are presented in Supplementary Materials Table 15. Papers were considered nonquantitative if quantitative data for the comparison between AD and control were not presented and/or the methods did not indicate that quantification had occurred. These papers used immunohistochemistry or immunohistochemistry with western blot to measure microglia using various markers, and commented qualitatively on the number of cells, amount of staining/expression or morphology. The most common brain region investigated by the nonquantitative papers was the hippocampus (24/53 studies); however, like in the quantitative papers in the review, many other regions were also investigated, including the entorhinal cortex, temporal cortex, occipital cortex, frontal cortex and anterior cingulate gyrus. Nonquantitative studies used many of the same markers discussed previously, such as HLA,[39,128–144] Iba1,[77,145] CD68,[142,146] ferritin,[140,147–150] CD45,[142,151–156] RCA-1,[138,157] and various other markers or combinations of markers, considered to be related to microglial activation.[158–175] All but two of these papers,[97,176] measuring CD68 and Iba1, respectively, reported elevations in microglial markers in AD relative to control postmortem brains. Thus, the null finding rate in the nonquantitative papers is less than a third of the rate of quantitatively assessed markers like MHCII.

DISCUSSION

Most (76/113) of the studies included in this review measured microglia using one of three common markers: MHCII, CD68 and Iba1. Although studies measuring MHCII or CD68 consistently identified increases in AD relative to control in most brain regions studied, 10 of the 20 studies that compared Iba1 identified no difference or a decrease relative to controls. Importantly, 9/10 studies noting an increase in Iba1 in AD relative to controls used expression-based quantification methods (qPCR, western, fluorescence intensity) that indicate only the amount of Iba1 in the sample, whereas most that identified no difference used cell counting, including two studies that used stereological quantification. Iba1 is a pan-microglial marker whose expression increases with microglial activation,[58,77] and hence these results indicate that there are increases in the expression of Iba1, but not the absolute number of microglia in AD. This, along with the increases in MHCII and CD68, which are both markers of activated microglia, suggest that the apparent increases in microglial markers in AD are attributable to increases in activation rather than the absolute number of microglia. This is supported by findings with CD11b that, like Iba1, labels resting and activated microglia, that also had mixed results, and by studies using other activation markers, such as ferritin, IL-1α and CD33, that were consistently increased in AD. However, there is still controversy surrounding what markers are indicative of activation, as well as the type of activation they are associated with,[60] and hence more research on the physiological significance of increases in these markers is needed.

The nine studies identified by this review that compared microglial markers between AD and a high pathology control group, the cognitively intact subjects with AD neuropathology, may shed light on whether increased microglial activation is a cause or consequence of the disease. Five of these studies reported higher levels of microglia markers in AD, three using HLA-DR,[7,33,40] one using CD68,[83] and one using both TREM2 and Iba1.[61] In contrast, three studies, using CD36, HLA-DR or proteomics, reported no difference between AD and the high-pathology control group despite increases relative to the regular control group,[45,127,177] whereas one reported more HLA-DR-positive microglia in the high pathology control group than in AD.[24] Though there appears to be increased microglia markers in AD relative to high pathology control subjects, more studies using this reference group are needed to help elucidate the role of microglia in AD pathogenesis. Polymorphisms in genes encoding microglial markers HLA-DR, CD33 and TREM2 have been implicated as risk factors for late-onset AD,[9,10,103] supporting the notion that increased microglial activation is a contributor to AD development, and not merely a response to established AD pathology.

Some of the heterogeneity identified between studies may be attributed to differences in the brain regions examined. More than half the studies that used tissue from the cerebellum identified no difference between control and AD across a range of microglial markers.[25,34,44,112,178] This lack of reactivity in the cerebellum has been remarked upon previously, leading the cerebellum to be proposed as a reference region for positron emission tomography imaging of TSPO.[179] Similarly, half the studies examining microglia in the white matter showed either no difference between AD and control[25,44,52,69,94,109,139] or higher levels in control.[32] Several studies report higher levels of HLA-DR-expressing microglia in the white matter than the gray matter of nondemented cases, suggesting that microglia in this tissue may be constitutively activated.[49,52,143] The lack of consistent differences in the white matter between patients with AD and controls in the studies in our review could therefore indicate that AD pathology does not stimulate further increases in microglial markers on top of their already activated state, whereas elevations in AD can be observed in the grey matter that does not demonstrate this constitutive elevation.

Heterogeneity can also exist within a brain region. For example, Bachstetter *et al.*[69] counted CD68-positive microglia in the hippocampus CA1, CA2/3, CA4 and the dentate gyrus, and noted increases in the CA1 and dentate gyrus relative to controls, but no difference in CA2/3 or CA4. Similarly, Masliah *et al.*[94] quantified microglia in different layers of the hippocampus and reported higher levels relative to control in the molecular and pyramidal layer, but lower levels in the stratus polymorphous. Thus, studies that examined staining in the whole hippocampus could dilute potential differences between AD and control by examining multiple regions simultaneously, and this may help to explain why nearly a third of studies using hippocampal tissue reported no difference between AD and control. The potential for heterogeneity of results between tissues is particularly important for studies that performed their analyses in a mix of different tissues, rather than examining each region separately.[30,89,98,122,180]

Only one of the identified studies reported on the use of anti-inflammatory drugs in their subjects.[16] Though this study did not identify significant differences in microglia counts in users and nonusers, nonsteroidal anti-inflammatory drug treatment reduces activated glial cells in a mouse model of AD,[181] and the use of nonsteroidal anti-inflammatory drugs is associated with less microglial activation in the brains of elderly patients postmortem,[182] suggesting that unreported nonsteroidal anti-inflammatory drug use could be a potential source of confounding. It is therefore feasible that unreported differences in medication use between AD and controls within and between studies contributed to the variability in the results.

The genotypes of the subjects could also offer a source of heterogeneity. Microglial activation appears to be affected by APOE genotype, with carriers of the ε4 risk allele exhibiting greater activation in some[48,56] but not all studies.[82] Only 29 of the studies included in this review reported the APOE genotype of their subjects. Among those that reported genotype, there was variability, with some studies excluding those with the ε4 allele,[68,172] and others including a majority of AD subjects carrying the risk allele.[30,62,79]

Differences in control group characteristics could also influence the results. Over half the included studies did not report screening controls for neurological or neuropathological abnormalities. In addition, only 11 studies reported excluding subjects with a history of psychiatric disorders. As various neurological and psychiatric diseases have been associated with neuroinflammation in some post-mortem studies,[183–185] this lack of screening could contribute heterogeneity to the results.

The severity of AD pathology in AD cases and controls also varies between studies. Braak stage was the most widely reported pathological score, and is presented in the tables where available. Most studies used AD cases with a Braak score of V–VI, but some used AD cases with scores as low as I and II.[18,45,53,64,67,123] Similarly for controls, Braak stages less than III were most commonly used, but some studies included controls with Braak stages IV or higher.[123,127] This variation may be important, as some studies identified associations between Braak stage or neurofibrillary tangles and microglia markers, including HLA-DR, Iba1 and CD68.[17,48,52,64,84,186] Few studies reported on the amount of plaque in the brains included in their cohorts, but differences in plaque load between studies could also influence the results, as microglial markers were found to correlate with plaque loads in several studies.[47,48,52,56,186] These correlations could be functionally important to the progression of AD, as post-mortem analyses from brains of AD patients administered amyloid-β vaccines demonstrate positive correlations between Iba1 and CD68 and markers of neuronal loss and degeneration that are attenuated following immunization and amyloid-β clearance,[187] when the number of activated CD68-positive but not total Iba1-positive microglia are reduced.[186]

Very few studies reported using stereology to complete their cell counts,[54,70,83,84,122] the gold standard for quantifying cells in a tissue without bias. In our review, Serrano-Pozo *et al.*[54] reported that the absolute number of microglia was the same in AD and control samples when stereology was used, but higher in AD when nonstereology-based counting was applied because of an apparent concentration of cells caused by cortical atrophy. The use of nonstereological counting could therefore have introduced some bias into the results. Interestingly, the three stereology studies that counted all microglia, identified by either Iba1 or morphology, reported no differences in total microglial counts in AD relative to control samples,[54,122,70] whereas increases were noted in the number of cells positive for activation-associated markers MHCII, CD68 and CD33.[54,70,83,84] This supports the suggestion that microglial activation is increased in AD without an absolute increase in the number of microglia.

Technical differences could also contribute to variation between studies. For immunohistochemistry, the most commonly used technique in this review, the method and duration of tissue fixation,[188,189] the thickness of the brain sections,[189] the use of antigen retrieval,[190] the antibody selected[77,188] and variation in many steps in the immunohistochemistry protocol itself can all influence the intensity of staining that could affect the ability of studies to pick up differences between AD and control subjects. Differences in the methods used for measuring gene expression or for quantifying protein by western blot or enzyme-linked immunosorbent assay could similarly contribute to variability in the results. This technical information, with the exception of technique used and post-mortem interval, was not extracted in the tables because the wide variability in methods and reporting made it unfeasible, but it is an important potential contributor to study heterogeneity.

Limitations

Though efforts were made to identify every published paper that compared microglia markers between AD and control post-mortem human brain samples, it is possible that some papers have been missed. The large number of papers returned in the initial search ($>20\,000$) may have increased the risk of misidentifying a paper. In addition, studies that measured a microglial marker, but did not mention it in the title, abstract or keywords would have been missed by the systematic search. Thus, studies that did not examine microglia as a primary objective are more likely to have been omitted which may introduce some bias into the results of this review.

Microglia interact with astrocytes to initiate and drive inflammation within the brain. Like with microglia, markers of astrocytes are reported to be elevated in patients with AD relative to controls,[191,192] where they seem to colocalize with amyloid-β plaques.[193,194] Although our review focused only on microglial markers, it had initially set out to include other inflammatory markers as well, including, astrocytes, cytokines, complement, lipid mediators and other immune cells. Our title and abstract screening uncovered over 700 papers, nearly 297 for astrocytes alone, an unfeasibly large number of papers that led us to focus on microglia for this review. Not including astrocytes and other inflammatory mediators is a limitation of our review because it results in an incomplete picture of neuroinflammation in AD.

Another important limitation of this review is that although it describes the results of the included studies in the context of microglia, it should be noted that nearly all microglia markers are also expressed by other myeloid cells such as infiltrating or perivascular macrophages, neutrophils or T cells (see Supplementary Table 1 for a summary of marker expression in other cell types). Although there are generally few of these cells in the brain parenchyma under normal conditions, there may be increased accumulation and infiltration in AD that could lead to an overestimation of the quantity of microglial markers in diseased relative to control brains.

CONCLUSION

Microglia markers are increased in various brain regions in patients with AD relative to controls. The balance of the evidence suggests that this increase is more attributable to increased activation than to an increase in absolute cell number, but more research measuring microglial proliferation in post-mortem human brain samples would be useful for clarifying this point.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

KEH designed the search with the assistance of MT, reviewed the articles returned by the search for eligibility with the assistance of DM, reviewed all data extraction and wrote the paper. DM, MT and VG assisted with the full-text assessments, data extraction and provided feedback on the paper. RPB oversaw the project, provided feedback on all steps of the search and data extraction and reviewed the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)