Review

Pathways linking Alzheimer's disease risk genes expressed highly in microglia

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Abstract

Microglia in the brain are exquisitely vigilant to their surroundings. They are dispersed throughout the brain parenchyma where they continually receive and integrate large numbers of incoming signals. They become activated once a tightly controlled signalling threshold is reached. This can lead to a cascade of cellular and molecular changes culminating in the recognition and engulfment of self and non-self structures ranging from macromolecules to whole cells depending on the initiating signal. Once internalised, they digest and where appropriate, present antigens to aid future recognition of pathogens. Their response to pathogenic signals in diseases such as Alzheimer's disease (AD) has long been recognised, but recent genetic findings have cemented their direct causal contribution to AD and thus the potential to target them or their effector pathways as a possible treatment strategy. Around 25% of the ~84 AD risk genes have enriched or exclusive expression in microglia and/or are linked to immune function*. Ongoing work suggests many of these genes connect within important microglial molecular networks as ligand activators (*IL34*), immune receptors (***TREM2, MS4A4A, HLA-DQA1 & CD33***), signalling intermediates (***PLCG2***, *PTK2B* & *INPP5D*) or effector mechanisms (*ABI3* & *EPHA1*). In some cases, evidence links them to specific core pathogenic immune responses and cell mechanisms such as complement (*CR1* & *CLU*) or cytoskeletal machinery (***ABI3***, *EPHA1* and *FERMT2*). However, more work is needed to establish whether these risk variants lead to gain or loss of protein function and to connect them to other genes within effector pathways and downstream cell processes which themselves could be tractable targets for treatment development. Brain tissue analysis and cell models of genetic risk carriers will help enormously to

expand the knowledge of pathways and cell processes important to AD. It will also help direct efforts to develop suitable agonist or antagonist tool compounds for testing target tractability for therapeutic development and for establishing appropriate biomarkers to evaluate efficacy in AD. Ultimately, it should pave the way for developing treatments for AD and potentially other diseases where boosting or dampening key microglia pathways could significantly impact disease outcomes irrespective of genetic status.

Keywords: Alzheimer's disease, microglia, immune, risk genes, genetics, inflammatory

INTRODUCTION

Microglia have a finely tuned array of receptors that enable them to discriminate incoming homeostatic, damage and pathogenic signals and respond appropriately. In Alzheimer's disease (AD), microglia are exposed to a complex array of damage and/or pathogenic signals which challenge their capacity to respond adequately or appropriately. AD is a slow progressive neurodegenerative disease characterised not only by a characteristic pattern of neuronal cell loss in the brain, but also by extracellular protein aggregates composed of Aβ (processed from APP) depositing as plaques and intracellular protein aggregates composed of hyperphosphorylated Tau (MAPT) depositing as neurofibrillary tangles. This pathological sequelae is the same in people with sporadic disease where the cause is unknown and in patients developing AD who also have a risk variant in one or more AD risk genes. We have collated a list of 84 AD risk genes of which 20 have highly enriched or exclusive expression in microglia. Many of these genes can be linked to common signalling pathways, particularly converging on the cytoskeletal system, and thus along with other pathways are expected to have important consequences for the coordinated phagocytic response of microglia in the AD brain. We discuss the various roles for microglia in the brain, including their normal function and behaviour and when presented with different pathogenic and damage signals in AD. We review the current knowledge of AD risk genes and the variants impacting their function. We establish which of these genes are likely to have causal effects in microglia and connect AD risk genes to signalling pathways in microglia. This provides a framework by which further work can test and evaluate these pathways and identify tractable targets to guide therapeutic development.

THE BRAIN IMMUNE SYSTEM

Unlike other tissues in the body, the blood brain barrier is tightly regulated, leading to a largely localised and autonomous immune system. Resident microglia are the predominant immune cell present in the brain. They provide innate immune defence against infection and trauma, and maintain healthy tissue and homeostasis by clearing apoptotic cells and performing synaptic pruning. Although the brain is a highly vascularised organ, the blood brain barrier prevents the adaptive immune system from having a significant role in the brain (discussed below). There is evidence however that microglia have the capacity for immune memory from prior exposure to the same activating signals, leading to an enhanced (training) or diminished (tolerance) response to the same signal during subsequent exposures[1].

The extent to which peripheral immune cells can enter or leave the brain parenchyma is controversial. The three main barriers controlling entry (or exit) of cells and solutes are (1) the blood-brain barrier (BBB, consisting of a neurovascular unit containing endothelial cells with connecting tight junctions, pericytes and astrocytes); (2) the blood-CSF barrier (BCSFB, consisting of either epithelial cells and their joining tight junctions within the choroid plexuses or the arachnoid and dura mater surrounding microvessels, arteries, veins and lymph vessels at the brain surface); and (3) the CSF-brain barrier (consisting of ependymal cells at the ventricular surface and the pial-glial barrier of the outer surface of the brain parenchyma)[2]. Despite these barriers, there are still many examples of their breach in autoimmune diseases such as Multiple Sclerosis[3], Systemic Lupus Erythematosus[4,5], primary and metastatic brain tumours[6,7],

infections[8] and in advanced stages of diseases such as AD[9-11]. Under normal homeostatic conditions, there is negligible cell movement between these compartments, although the selective influx/efflux of proteins and other molecules can occur particularly in early life[12]. However, the view that the brain is immune privileged is no longer considered true. Evidence shows antigens and antigen presenting cells originating in the brain parenchyma can reach the dural and cervical lymphatics[2,13,14], and thereby communicate with the deep cervical lymph nodes[15]. Via the blood they can then illicit adaptive and humoral responses which can return full circle to have effector function in the brain[16]. Whilst the adaptive arm of the immune system has less evidence for direct activity in the brain parenchyma, it has long been recognised that the innate immune system plays a central role in brain health and disease. The main cell type mediating this is the microglial cell. Microglia emerge very early in development in the yolk sac[17,18] where they expand to populate ~5%-12% of the total brain cell population[19].

Long-lived homeostatic microglia[20-22] reside alone in distinct territories as ramified cells under normal circumstances. The cells survey their immediate surroundings for damage or pathogenic signals in the brain parenchyma by extending and retracting their cell membranes to form specialised highly motile filopodia protrusions. Once receptors on the filopodia (such as purinergic receptors) engage an external or internal signal (such as ATP), microglia typically respond by retracting and repurposing their membrane protrusions. Either through direct contact or migration following detection of a diffusible signal, they surround, engulf and digest the pathogen or damaged tissue, cells or cell fragments thus potentially remodelling tissue or cell activity in the immediate vicinity. Depending on the signal, they may also generate toxic cytokine or chemokine signals which attract other microglia and in so doing can form nodules of microglia clusters. Activation of so-called inflammatory or anti-inflammatory cascades in microglia can involve engagement of Fcγ receptors, complement receptors, toll-like receptors (TLR), RIG-like receptors (RLRs), Aim-2-like receptors (ALRs), C-type lectins, advanced glycosylation end product receptor (RAGE), scavenger receptors, N-formyl peptide receptors (FPRs) or NOD-like receptors (NLRs, particularly NALP3) or **TREM2** either working alone or together with other neurotransmitter receptors expressed on microglia[23-26].

In addition to degradation through the lysosomal pathway, phagocytic/endosomal engulfment can lead to the presentation of antigens through the MHC Class II system which can initiate the adaptive and humoral systems either locally within the brain or through the systemic immune system. Other minor populations of immune cells can be detected in the brain under homeostatic conditions. These lymphocytes, macrophages and dendritic cells usually reside in the margins of brain parenchyma or in specific niches such as the meninges, but nevertheless under the right conditions can play important roles in health and disease[27-29].

Microglia impact normal behaviour and development

Beyond their role in combatting pathogens and responding to damage, microglia are increasingly recognised for their important role in shaping behaviour. They can have direct effects on neuronal cell function during normal development and respond to external experience. There is much evidence to demonstrate microglia make activity-dependent contact with neuronal cells[30-35], impacting the output of local neural circuits[36,37]. But more than this, they are able to engulf synaptic components[38-41] and they can "nibble" the membranes of presynaptic neurons at the peak period of brain remodelling in early development, altering the structure and composition of these membranes and impacting postsynaptic spine head filopodia[42]. Others have shown that microglia respond to IL-33 produced by stimulated neuronal cells by remodelling the extracellular perineuronal matrix which in turn affects dendritic spine and synaptic density and behaviours associated with learning and memory[43]. Not only do these activities occur during developmental remodelling, but also throughout life in response to experience[44,45]. Synaptic remodelling involving microglia is commonly found to be dependent on the activation of the complement cascade[39,41,46-48], although not all phagocytic activities of microglia have been shown to rely

on a complement-mediated mechanism. Microglia can also associate with axon tracts in white matter undergoing myelination during early postnatal development and phagocytose oligodendrocytes or their precursors, even in the absence of overt apoptotic signals[49,50]. Recently, microglia were found to directly phagocytose excessive and ectopic myelin during this period, presumably an additional function for shaping neural circuits and behaviour[51]. The molecular signals facilitating this have still to be elucidated. Many of the activities described above are particularly prominent in adolescence where they appear to coincide with the peak of neuronal plasticity, which incidentally is when **TREM2** is most active (discussed below).

Microglia respond to damage in Alzheimer's disease

Microglia can efficiently detect and clear cellular and acellular material following their detection. This includes acute damage caused by trauma or ongoing damage which emerges in chronic diseases such as AD. In the case of AD, there are a myriad of possible "competing" damage related signals simultaneously arising from amyloid plaques, fibrils and insoluble Aβ aggregates, damaged myelin, extracellular matrix and stressed/degenerating neurons. Important challenges for AD therapeutics are reconciling which microglia responses are protective and which are detrimental, identifying the most impactful pathways to target for drug development, identifying the best treatment window and targeting pathways unique to microglia to minimise off-target effects. This is a big challenge. Many receptors in microglia are promiscuous, activated by a myriad of damage and pathogen associated signals[52]. Further functional complexity arises through receptor clustering and dimerisation. TLR4, widely found on myeloid cells, can be activated by the bacterial wall component lipopolysaccharide (LPS), hyaluronan, biglycan, heparan sulphate and CD36 associated Aβ[53]. **TREM2** is microglia-specific, but appears able to bind a number of ligands including Aβ[54,55], apolipoproteins such as **APOE**[56,57], anionic and zwitterionic lipids[58] and anionic carbohydrates[59]. RAGE (AGER) which binds Aβ, S100A12 and S100B is expressed by a number of brain cell types including microglia, neurons and endothelial cells[60] where it is considered an important receptor for regulating amyloid angiopathy[61].

As diseases such as AD develop, microglia can lose their solitary behaviour and form clusters around amyloid plaques either through local proliferation or migration[62-70]. The morphology of microglia become more amoeboid as they pivot towards a so-called reactive profile, altering the repertoire of proteins they express in favour of those needed to mount an immune response. Central to this is phagocytosis[71-74]. Some suggest that the purpose of the clustering behaviour of microglia is a form of amyloid containment or "corralling" to neutralise its toxic effects, rather than for the purpose of amyloid removal[75-77]. Certainly, the capacity for aged microglia to phagocytose amyloid is considered by some to be very poor in AD[78-82] and microglia may even propagate rather than reduce amyloid deposition via NLRP3-ASC speck seeding[83]. This may explain why amyloid accumulates in AD[84]. However, many others have shown that microglia under the right conditions can very efficiently engulf and remove amyloid. For example, following amyloid immunotherapy[68,85-92]. Microglia propagation has also been suggested for Tau aggregates either through microglia engulfment of neuronal cells containing Tau aggregates or via Tau aggregates released at synapses which are subsequently repackaged and released by microglia within exosomes[93-96]. Because of the unique combination of genes expressed by amyloid-associated microglia, the term "DAM" microglia (Damage-Associated Microglia) has been coined to describe a relatively consistent expression profile that marks the activation journey of these cells in mouse models of AD[71-74,97-99]. This phenotype is broadly characterised by high levels of expression of genes involved in complement, antigen presentation, actin regulation and ribosomes consistent with immune activation and phagocytic function. **TREM2** (discussed below) occupies a central position in the DAM cell expression profile and when absent, causes a rippling impact on other brain cell types associated with AD, notably oligodendrocytes and endothelial cells[73,99,100]. A broadly similar expression profile has been observed in "PAM" (Proliferative-region-Associated Microglia) in white matter during normal early development[49], suggesting there is some conservation of function in reactive

microglia across different brain contexts. However, there may be important species differences. Recently, the mouse DAM profile was reported to not have strong overlap with human "HAM" (Human Alzheimer's Microglia) profiles from superior frontal gyrus generated from bulk single-sorted ITGAM (CD11b) expressing cells from late-stage pathology cases[101].

Fibrillar Aβ appears to interact with CD36, AGER and **TREM2** receptors expressed by microglia. In the case of CD36, binding Aβ and additional interactions with TLR4:TLR6 triggers an inflammatory response. This leads to NFKB1 (NF-κβ)-dependent production of CXCL1, CXCL2 and CCL9 cytokines, via the MYD88 signalling pathway, CCL5 cytokine production via the TICAM1 signalling pathway and IL1β secretion, through the priming and activation of the NLRP3 inflammasome. Activation of the NLRP3 inflammasome leads to the release of ASC specks[102-104]. As mentioned earlier, ASC specks can bind extracellular Aβ and facilitate further aggregate seeding[83], thus perpetuating a continuous cycle of pathology. NLRP3-ASC mediated activity also exacerbates Tau hyperphosphorylation and aggregation[105]. Interaction of fibrillar Aβ with AGER leads to activation of NF-κb, phosphoinositide 3-kinase (PI3K), Janus kinase, signal transducer and activator of transcription and mitogen-activated protein kinase (MAPK) signalling pathways, which leads to induction of pro-inflammatory cytokines such as TNF[53,106]. In contrast, activation of **TREM2**-TYROBP (DAP12) is considered to largely occur without the production of pro-inflammatory molecules and is thus considered beneficial for AD. **TREM2**-TYROBP signalling triggers many protein and lipid phosphorylation cascades causing calcium mobilization, integrin activation, mTOR and MAPK signalling, activation of energetic metabolism[107,108] and cytoskeleton activation via SYK[109]. Many of these pathways culminate in cytoskeletal changes that help drive phagocytosis[110]. Conversely, loss of **TREM2** leads to deficits in phagocytosis, at least in the uptake of Aβ, myelin debris, apoptotic neurons and/or *E.coli*[111-115] and an inability to switch to a glycolytic state[108].

The impact of **TREM2** in AD appears to be determined by the pathogenic and temporal context. Over-expression, activation or restoration of normal **TREM2** function has been shown to have largely beneficial outcomes in amyloid-driven mouse models[116-119] while knock-out or haploinsufficiency exacerbates amyloid-mediated pathologies[76,120-123]; although some studies are at variance with this[124]. In Tau-driven models, the picture is more complex with some studies showing loss of **TREM2** to be protective in the presence of Tau pathology[124,125] while others show it leads to worse outcomes[126,127]. These differences may lie in the timing and/or severity of Tau pathology. **TREM2** knock-out or bi-allelic hypomorphic variant mouse models (modelling Nasu-Hakola disease) by themselves do not lead to amyloid or Tau pathology, but in keeping with a protective role for **TREM2**, these mice exhibit greater pro-inflammatory markers, synaptic dysfunction, neuronal cell loss and worse cognition with age[128,129] and mirror the symptoms described for Nasu-Hakola disease[130]. Thus knock-out models of **TREM2** recapitulate the more extreme variants rather than the subtle rs75932628, R47H heterozygous variant linked to AD[108,112].

Complement-mediated remodelling of synapses has long been recognised as an important driver of normal brain plasticity and can significantly impact memory performance[39,41]. The complement cascade has also been shown to be activated in AD mouse model brains mirroring areas of pathology[48]. Activation of C1q (C1QA, B and C) within the C1 complement complex leads to opsonisation or tagging of material which can then activate Fcγ receptor-dependent or -independent pathways in microglia. The Fcγ receptor-independent C1q pathway signals through complement receptor 3 (CR3; ITGAM/ITGB2) while C1q-IgG mediated signalling is through complement receptor 1 (CR1) and Fcγ receptor pathways. Loss of protective sialic acid residues from glycoproteins on cell surfaces such as neuronal cells by neuraminidase activity can also lead to their tagging by C1q and subsequent CR3 activation resulting in neuronal phagocytosis[39,131,132]. Alternatively, desialylated glycoprotein-C1q-calreticulin binding on neurons activates the microglia LRP1 receptor, also leading to phagocytosis[133]. It is noteworthy that LRP1 is also a receptor for **APOE**, Aβ and is responsible for Tau spreading, although this appears to be through neuronal-mediated endocytosis[134-136].

Engagement of specific Fcγ receptor subtypes is dependent on the IgG subtype, with the degree of activation and subsequent activation further impacted by IgG avidity. Both subunits of the CR3 receptor (Mac-1; ITGAM/ITGB2; CD11b/CD18) have previously been found to have highly correlated and elevated expression in AD[137,138]. Following receptor engagement, Rho-dependent (CR3) or Rac/cdc42-dependent (Fcγ/CR1) signalling in microglia are initiated, leading to clearance of tagged cells/proteins by phagocytosis (ADCP, antibody-dependent cell phagocytosis or CDCP, complement-dependent cell phagocytosis) and/or toxin producing pathways in microglia leading to cell death and phagocytic clearance (ADCC, antibody-dependent cell cytotoxicity or CDCC, complement-dependent cell cytotoxicity). ADCC and CDCC are considered pro-inflammatory and therefore usually only activated to kill pathogens or cells infected by pathogens. Thus, there are many checks and balances to control complement ADCC or CDCC activation because of the particular risk they pose in causing significant bystander damage to tissue and cells[139].

Lipid signals in AD exposed by apoptotic cells (dying neurons), demyelination or metabolic changes are a further source of signalling for microglia during AD pathogenesis. Lipids are a considerably diverse and complex class of molecules having significant structural, metabolic and signalling properties. Phosphatidylserine, phosphatidylethanolamine and sphingomyelin are major components of cell membranes and myelin, a specialist membranous extension of oligodendrocytes[140,141]. Stressed or dying cells, including neurons in AD, can cause "eat me" lipid signals such as phosphatidylserine or phosphatidylethanolamine to become exposed on the cell surface, which mark them out for clearance[142,143]. This can occur following interaction with **TREM2**[58,144,145] and/or TAM receptors (Tyro3, Axl, and Mertk)[146] and/or brain-specific angiogenesis inhibitor 1 (BAI1) or vitronectin receptor (VNR)[147] on microglia. **TREM2** can additionally bind phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, sulfatides[58] and cardiolipin, a component of the outer mitochondrial membrane found in pathogen cell membranes[144]. Furthermore, once phagocytosed, **TREM2** knock-out or ageing can cause a failure of lipids such as free cholesterol, cholesteryl esters, and myelin-derived ceramides to be adequately degraded through the lysosomal pathway in microglia. This leads to their accumulation and inflammatory activation[130,148,149].

Response of microglia to pathogens in Alzheimer's disease
Microglia have a multitude of receptors to detect the wide array of molecules found uniquely on pathogens such as fungi, bacteria, viruses and protozoa. Plasma membrane receptors able to detect pathogens include toll-like receptors (TLRs 1-6), C-type lectin receptors, CLRs (dectin 1, dectin 2), DC-specific ICAM3 grabbing non-integrin (DC-SIGN), mincle and the mannose receptor galectin family proteins (such as galectin 3), CR3, chitin receptors and the class B scavenger receptor CD36. Internal membrane receptors to detect viruses gaining entry include toll-like receptors (TLRs 7-9), RIG-I and MDA5[150,151]. Once engaged by pathogen-specific ligands such as lipopolysaccharide in bacterial cells walls, microglia generate an immediate innate immune response to protect the brain. Furthermore, APP processing leading to amyloid deposition is considered by some to be at the heart of an antimicrobial immune defence mechanism in the brain which can sequester certain microbes in an attempt to neutralise their pathogenicity[152,153]. This has led to the "Antimicrobial Protection Hypothesis of AD" which proposes that viral infectivity lies at the heart of AD aetiology with the deposition of amyloid heralding that infectivity has occurred in an individual[152-155]. In this model, amyloid is a protective mechanism for pathogen entrapment, and together with microglia encasement prevents further infectivity as well as Aβ toxicity, thereby avoiding the need for a much larger and potentially damaging pro-inflammatory response[75-77]. Interestingly, the failure to mount a successful Fc-mediated response in the presence of a viral pathogen such as Herpes Simplex Virus type 1, has recently been suggested as a possible mechanism leading to AD[156]. There is also evidence for AD-associated infectivity by certain viruses. Herpes virus re-activation has greater prevalence in people who develop AD[157-159], infectivity by different strains of herpesvirus causes pathologies reminiscent of AD[153,160,161] and viruses such as West Nile virus have an impact on cognition through complement- and microglia-mediated changes to synapses[47].

Microglia Heterogeneity

Technical advances enabling sequencing of the full repertoire of expression in individual cells has paved the way for defining the molecular identity of immune cells in the brain in health and disease with unprecedented depth and breadth. These data provide important knowledge about cell identity and function, as well as a fantastic resource for the research community. In many cases, authors have publicly provided data for individual gene interrogation via highly accessible Shiny apps, e.g., http://celltypes.org/brain/[162] and http://research-pub.gene.com/BrainMyeloidLandscape[101]. These data provide: (1) a set of exclusive or highly enriched genes for each cell type and subtype; (2) knowledge of genes expressed under different conditions; and (3) the full extent of cell heterogeneity across different microniches, microenvironments and temporal patterns throughout the brain. Knowledge of cell type subsets can be exploited to offer much greater precision to target particular cells whilst sparing others. Whole-genome expression profiling of microglia from normal and disease brain samples has been extensively investigated[163]. This includes non-neurologically affected human brains[71,73,74,164-172], normal mouse brain[71-73,173-182], human brain affected by AD[71,73,74,138,171] or Multiple Sclerosis[164] and AD or other neurodegenerative disease mouse models[71-73,98,100,183], EAE/injury mouse models of Multiple Sclerosis[175,180] or acute injury[182]. Despite using different approaches such as whole sample sequencing with post hoc cell type inference, or prior whole cell or nuclei separation by immunopanning or droplet single cell sorting, many of these studies showed remarkable concordance in the gene expression profiles obtained for different cell types and cell subsets. What emerges from the above human brain studies is stratification of between one and nine major microglia subgroups ranging from homeostatic to activated cell profiles. There is a good deal of conservation in the DAM profile across different disease contexts including developmentally active microglia and in the presence of different pathologies such as in AD (as discussed above). The DAM profile will be further explored below, with respect to AD risk genes.

Genetic Risk of Alzheimer's Disease

Although largely sporadic, AD has a significant heritable component[184-187]. In rare AD cases, usually with disease onset before 65 years, there are fully penetrant missense or splicing mutations in APP or genes forming part of the γ-secretase complex which cleaves APP (PSEN1 or PSEN2) to a more amyloidogenic fragment (Aβ1-42). For late onset disease (> 65 years), variants in a much larger number of genes has been found, but their contribution to disease risk is generally significantly lower. Over the years, the list of true positive AD-associated risk genes has been extended, consolidated and refined for sporadic AD. In a large part, the risk gene list has evolved following independent validation, improved analytical approaches and a vast expansion in the number of study participants, which minimises false positives and identifies increasingly smaller effect size variants. **APOE** has been convincingly associated with AD for nearly 30 years[188], reflecting its very large contribution to AD risk. The double SNP haplotype which gives rise to **APOE**ε2, 3 and 4 genotypes confers a 12-fold higher AD risk in those with two **APOE**ε4 alleles, while **APOE**ε2 carriers are protected. Two large studies published around the same time in 2013 identified many gene associations which have continued to be found in subsequent studies[189,190]. Prior to this, ~700 genes had been variously published as having AD association[191]. While some associations were missed by the methods available at the time, many others could not be replicated in these earlier studies. Although substantial for their time, many studies suffered from being statistically underpowered, with small sample sizes leading to stochastic associations. Since then, well-powered and more technically sophisticated gene wide association studies (GWAS) have further consolidated and refined the gene list. They are frequently the product of significantly larger meta-analyses of former cohorts and/or use imputation to improve genome coverage[192-194]. The largest of the recent studies has involved ~500,000 samples from UK Biobank (using parental AD status as a proxy for diagnosis)[195,196]. Whilst GWAS is good at assessing genes with frequency > 5%, more recent AD association studies have used sequencing strategies to identify much rarer high impact variants in genes such as **TREM2**, **PLCG2** (rare protective allele), **ABI3** and *IL34*[193,197-200]. Further work has continued to use family-based approaches which in addition to the well-established mutations in APP, PSEN1 and PSEN2, has begun to expand this list in familial late onset AD[201].

Review of genes associated with Alzheimer's Disease
We have reviewed GWAS, exome and whole genome sequencing studies and present a list of 84 genes which have been reliably associated with AD in independent and well-powered studies [Table 1]. Studies are from literature searches conducted up to 1st August 2020 in Pubmed, Biorxiv and Medrxiv using the terms "Alzheimer*", "genetic", "exome", "sequencing", "genome wide association" and "gene". Only large-scale, familial or genome-wide studies were included. Candidate gene studies were excluded. Appendices and supplementary data from each paper were accessed and included in the analysis. Where associations are SNP-based, SNP-to-gene mapping was performed using the L2G (Locus-to-Gene) score in Open Targets Genetics (OTG) from an index GWAS study if the locus was included[195] or from other papers where the same locus was identified[190,192,194,196]. Genes within each locus were prioritised using the V2G (Variant-to-Gene) score where the L2G (Locus-to-Gene) score was not available from OTG. Prioritisation also considered the SNP-to-gene information from the relevant studies[192,202-204] and the MAGMA (Multi-marker Analysis of GenoMic Annotation) and FUMA (Functional Mapping and Annotation) scores[195].

An over-representation of risk genes with lipid and/or immune function was first recognised in 2010[205]. Since then, further consolidation of the list of risk genes and the addition of genes with rare risk variants has further reinforced this finding. In fact, there is even stronger evidence for the immune system and microglia having a role in AD vulnerability in the list we have assembled (details below and in Table 1). Whilst knowledge of these risk variants in these genes may never be useful by themselves for establishing individual clinical diagnosis, they nevertheless have considerable value in refining pathways of vulnerability which can be targeted for therapeutic development, for identifying and enriching high risk participants for clinical trials related to these pathways and supporting diagnosis as part of a panel of factors including clinical and biomarker assessments as part of a composite risk score.

The degree of knowledge about the function of each gene is highly variable. Nevertheless, by combining evidence from the literature, and integrating this with transcriptomic and biochemical data, it may be possible to achieve a cell- and systems-level understanding of how these genes are linked in pathways that are responsible for the brain immune response in AD which could be exploited therapeutically.

Expression of Alzheimer's Disease Risk Genes in Microglia
With advances in single-cell isolation and sequencing, it is now possible to establish which of the AD risk genes have unique or highly enriched expression in microglia in the brain. To achieve this, we used published data from FACS-sorted cell populations isolated from human donor parietal cortex[172,206]. Genes were given a score reflecting whether they met the criteria used by the authors for microglial expression[172], our own internal criteria of RPKM (Reads Per Kilobase of transcript, per Million mapped reads) (either RPKM > 10 or > 20 RPKM) or a 4- 8- 10- or 20-fold-increase in differential expression (DE) over background. Genes meeting any of these criteria are shown in bold, along with their microglia score using these criteria (0-6) [Table 1]. Twenty (shown in bold throughout this review) out of the eighty-four genes linked to AD risk variants had a microglia enrichment score ≥ 1 [Table 1]. In separate data generated by us, we confirm the most highly expressed of these genes with a score of 6 (*INPP5D*, *TREM2*, *PILRA*, *RIN3*, *MAF*, *PLCG2*, *ABI3*) had highly correlated co-expression with other microglia genes in whole human brain hippocampal tissue, either from neurologically normal or AD donors (unpublished data). Other studies also confirmed these genes have high or enriched expression in microglia from normal brain tissue[166,167]. Some of them also appear to have highly correlated and enriched expression with genes more likely to be found in particular microglia subsets in normal brain - either in highly abundant homeostatic microglia (Cluster 3: **BIN1**, **INPP5D**, **MEF2C**, **SORL1**, **MAF**, **PLCG2**), age-associated homeostatic cells with high levels of MHC Class II expression (Cluster 6: **RAB10**, **TREM2**, **RIN3**, **APOE**, **CD33**) or in a small population of pro-inflammatory microglia (Cluster 8: **CASS4**, **CASP8**, **PILRA**)[165] suggesting there may be multiple routes in microglia contributing to greater AD vulnerability.

Table 1. Review of genes associated with Alzheimer's Disease

Locus	Study type	Diagnosis	Lead variant	Closest gene	Variant type	Functional variant suppport?	Most plausible gene	Microglial score	Figure 1 localisation/ pathway in microglia	Alternative genes	Microglial score2	Ref.
1q23.2	GWAS	AD proxy	rs4575098	ADAMTS4	3 Prime UTR Variant		ADAMTS4	0	Not included	DEDD	0	[2]
1q32.2	GWAS	AD	rs2093760	CR1	Intron variant	CR1; PMID: 31104630 PMID: 29675612	CR1	0	Microglia receptor	CR1L, CD55, YOD1	0	[1,2,3]
1q42.12	GWGAS	AD	[gene-wise]	ENAH			ENAH	0	Not included		0	[2]
2p22.2	GWAS	AD + AD proxy	rs876461	PRKD3	Intron variant		CEBPZOS	0	Not included	PRKD3, NDUFAF7	0	[10]
2p23.3	WGS	AD	rs142787485	RAB10	Intron variant		RAB10	2	Phagosome	DRC1	0	[46]
2q14.3	GWAS	AD	rs4663105	AC110926.2	Intergenic	BIN1; PMID: 26101835	BIN1	3	Phagosome?			[1-5]
2q33.1	WES (familial)	AD	[gene-wise]	CASP8	Missense variant	CASP8; PMID: 28985224	CASP8	5	Not included			[38]
2q33.2	GWGAS	AD	[gene-wise]	WDR12			WDR12	0	Not included			[2]
2q37.1	GWAS	AD	rs10933431	INPP5D	Intron variant		INPP5D	6	Signalling intermediate			[2-5,13]
3p14.3	GWAS	AD proxy	rs184384746	HESX1	Intergenic		IL17RD	0	Not included			[2]
3q25.31	GWGAS	AD	[gene-wise]	PLCH1			PLCH1	0	Not included			[2]
4p15.1	GWGAS	AD	[gene-wise]	PPARGC1A			PPARGC1A	0	Not included			[11]
4p16.1	GWAS	AD + AD proxy	rs6448453	CLNK	Intergenic		CLNK	0	Not included	HS3ST1	0	[2,10,45]
4q22.3	Family-based + GWAS	AD	rs137875858	UNC5C	Missense variant	UNC5C; PMID: 25419706	UNC5C	0	Not included		0	[41]
4q24	WES (familial)	AD	[gene wise]	TACR3	Missense variant	TACR3; 10.1101/2020.01.28.923789	TACR3	0	Not included		0	[30]
4q24	WES (familial)	AD+ NDother	[gene wise]	TET2	Missense variant	TET2; PMID: 32330418	TET2	0	Not included		0	[33]
5q13.1	GWAS	AD	rs4704171	ANKRD31	Intron variant		ANKRD31	0	Not included	GCNT4	0	[1]
5q14.3	GWAS	AD	rs190982	MEF2C-AS1	Intron variant		MEF2C	2	Transcription factor			[5,13]
5q31.3	GWAS	AD	rs11168036	HBEGF	Intergenic		HBEGF	0	Not included	PFDN1	0	[6,44]
6q14.1	GWGAS	AD	[gene-wise]	TPBG			TPBG	0	Not included			[6]
6p22.3	GWGAS	AD	[gene-wise]	PRL			PRL	0	Not included			[2]
6p21.32	GWAS	AD	rs6931277	HLA-DQA1	Intergenic		HLA-DQA1	5	Microglia receptor	HLA-DRB5, HLA-DRA, HLA-DRB1	5	[2-5]
6p21.1	WES	AD	[gene-wise]	TREML2	Missense variant	PMID: 24439484	TREML2	5	Microglia receptor		5	[20]

Cytoband	Study	Disease	SNP/ID	Gene	Variant	Evidence (PMID)	Gene	Count	Category	Additional genes	Count	Ref
6p21.1	GWAS	AD+AD proxy	rs187370608	LOC101929555	intron Variant	TREM2; PMID: 23150908 PMID: 23150934	TREM2	6	Microglia receptor	APOBEC2, TREML2	5	[2,3]
6p21.1	GWAS	AD	rs75932628	TREM2	missense Variant	TREM2; PMID: 23150908 PMID: 23150934	TREM2	6	Microglia receptor			[7,9,21]
6p12.3	GWAS	AD	rs9381563	CD2AP	intergenic	CD2AP: PMID: 26101835	CD2AP	0	Not included			[1-3,31]
7p22.1	GWGAS	AD	[gene-wise]	WIPI2			WIPI2	0	Not included			[2]
7p22.3	GWGAS	AD	[gene-wise]	TMEM184A			TMEM184A	0	Not included			[2]
7p14.1	GWAS	AD	rs2718058	NME8	Intergenic		GPR141	5	Microglia receptor?			[5]
7p21.3	GWAS+	LOAD subtype	rs1990620	TMEM106B	Intron variant		TMEM106B	0	Not included			[41]
7q11.23	GWGAS	AD	[gene-wise]	RHBDD2	Intron variant		RHBDD2	0	Not included			[36]
7q22.1	GWGAS	AD	[gene-wise]	ZNF655	Intron variant		ZNF652	0	Not included			[9]
7q22.1	GWAS	AD	rs1859788	PILRA	Missense variant	PILRA; PMID: 29181857 PMID: 30388101	PILRA	6	Microglia receptor			[2-5,13]
7q35	GWAS	AD	rs7810606	EPHA-AS1	Intron variant	EPHA1: PMID: 26101835	EPHA1	0	Not included	FAM131B, ZYX	0	[1-5,8]
7q35	GWAS	AD+AD proxy	rs114360492	CNTNAP2	Intron variant		CNTNAP2	0	Not included			[1,2]
7q36.3	WES	AD	7:154988675:G:A	AC099552.4			AC099552.4	0	Not included			[9]
8p21.1	GWAS	AD	rs28834970	PTK2B	Intron variant		PTK2B	0	Signalling intermediate	TRIM35	0	[1,5,6]
8p21.2	GWAS	AD	rs4236673	CLU	Intron variant		CLU	0	Not included			[1-5,7]
8q22.1	GWAS	AD	rs10098778	NDUFAF6	Intron variant		NDUFAF6	0	Not included	NDUFAF6	0	[1]
8q24.3	GWAS	AD+AD proxy	rs34173062	SHARPIN	Missense variant	SHARPIN; PMID: 31216982 https://doi.org/10.1101/196410	SHARPIN	0	Not included			[10,35,42]
10p14	GWAS	AD	rs11257238	USP6NL	Intergenic		USP6NL	0	Not included	ECHDC3	0	[6,3,44]
10q21.2	GWGAS	AD	[gene-wise]	CCDC6			CCDC6	0	Not included			[2]
10q22.1	GWGAS	AD	[gene-wise]	CDH23			CDH23	0	Not included			[36]
10q25.3	WES (familial)	AD	[gene wise]	CASP7	Missense variant	CASP7; PMID: 30503768	CASP7	0	Not included			[16]
11p11.2	GWAS	AD	rs12292911	PSMC3	Intergenic	SPI1: doi: 10.1038/nn.4587	SLC39A13	0	Not included	SPI1	6	[3-5]
11q12.2	GWAS	AD	rs2081545	MS4A6A	Intergenic		MS4A4A	5	Microglia receptor			[1-6,8]
11q14.2	GWAS	AD	rs867611	PICALM	Intron variant		PICALM	2	Not included	EED	0	[1-6,8,31]
11q24.1	GWAS	AD	rs11218343	SORL1	Intron variant	SORL1; PMID: 22472873 PMID: 28595629	SORL1	3	Phagosome Receptor			[1-5]
14q11.2	GWGAS	AD	[gene wise]	C14orf93	Intron variant		C14orf93	0	Not included			2

Locus	Study	Phenotype	SNP	Gene	Variant	PMID/Reference	Gene	#	Category	Nearby genes	#	Ref
14q22.1	GWAS	AD	rs17125924	FERMT2	intron variant		FERMT2	0	Not included	STYX	0	[3-5,13]
14q24.1	GWGAS	AD	[gene-wise]	PSEN1		PSEN1; PMID: 32020597	PSEN1	0	Not included		0	[34]
14q32.12	GWAS	AD	rs12590654	SLC24A4	intron variant	RIN3; PMID: 28738127	RIN3	6	Phagosome?	SLC24A4	0	[2-5]
14q32.33	GWGAS	AD	[gene-wise]	IGHV1-67			IGHV1-67	0	Not included		0	[12]
14q32.33	WES	AD	rs12890621	IGHG3	missense variant	IGHG3; PMID: 30108311	IGHG3	0	Not included		0	[9]
15q21.2	GWAS	AD proxy	rs10467994	SPPL2A	intron variant		SPPL2A	0	Not included		0	[2,3,44]
15q21.3	GWAS	AD	rs442495	ADAM10	intron variant		ADAM10	0	Microglia receptor Cleavage		0	[2-4]
15q22.2	GWAS	AD proxy	rs117618017	APH1B	missense variant	APH1B; PMID: 30617256	APH1B	0	Not included	RAB8B	0	[2]
15q22.2	GWGAS	AD	[gene-wise]	RORA			RORA	0	Not included		0	[11]
15q22.31	GWAS	AD	rs74615166	TRIP4	intron variant		CSNK1G1	0	Not included	TRIP4	0	[14]
16p12.3	GWAS	AD	rs7185636	IQCK	intron variant		IQCK	0	Not included		0	[4]
16p11.2	GWAS	AD proxy	rs59735493	KAT8	intron variant		KAT8	0	Not included	FBXL19, BCL7C, TAOK2, ZNF646	0	[2,3]
16q12.1	GWGAS	AD	[gene-wise]	ZNF423	intron variant		ZNF423	0	Not included		0	[11]
16q22.1	GWAS	AD+AD proxy	rs4985556	IL34	missense variant	IL34; PMID: 29777097 https://doi.org/10.1101/19012021	IL34	0	Microglia receptor Ligand		0	[2,3,10]
16q23.1	GWAS	AD	rs62039712	WWOX	intergenic		MAF	6	Transcription factor	WWOX	6	[4]
16q23.3	GWAS	AD	rs72824905	PLCG2	missense variant	PLCG2; PMID: 28714976	PLCG2	6	Cytoskeletal regulator		6	[7]
17p13.2	GWAS	AD+AD proxy	rs72835061	CHRNE	intron variant		PLD2	0	Not included	CHRNE, ZFP3, ZNF653, USP6, ZNF652, ZNF232	0	[2,3]
17q13.2	GWAS	AD+AD proxy	rs113260531	LOC100130950	intron variant		RABEP1	0	Not included	SCIMP	5	[1,10]
17q21.31	GWGAS	AD	[gene-wise]	SPPL2C			SPPL2C	0	Not included		0	[30]
17q21.31	WES-Non-APOE*4	AD	rs199533	NSF	missense variant	NSF	NSF	0	Not included		0	[37]
17q21.32-33	GWAS	AD	rs616338	ABI3	missense variant	ABI3; PMID: 28714976	ABI3	6	Cytoskeletal regulator		6	[7]
17q21.32-33	GWAS	AD	rs28394864	AC091180.5	intron variant		ZNF652	0	Not included		0	[2]
17q22	GWAS	AD	rs2632516	TSPOAP-AS1	intron variant		BZRAP1	0	Not included	MPO, SUPT4H1, TRIM37, TSPOAP1	0	[2,6]

Locus	Method	Disease	Variant	Gene	Variant type	Gene / PMID		Function	Other gene		Ref
17q23.2	WES	AD	[gene-wise]	ERN1	Intergenic	ERN1	0	Not included			[30]
17q23.3	GWAS	AD	rs6504163	ACE	intron Variant		0	Not included	PSMC5, CYB561	0	[3,4]
17q25.1	WGS	AD	[gene-wise]	SLC9A3R1	intron Variant		0	Not included			[36]
18q12.1	GWAS	AD	rs8093731	DSG2	intron Variant		0	Not included			[2,4]
18q21.31-21.32	GWAS	AD proxy	rs76726049	ALPK2	intergenic		0	Not included			[2]
19p11.3	GWAS	AD	rs111278892	CNN2	intergenic	ABCA7: PMID: 26101835 PMID: 25807283 PMID: 27289440	0	Not included	CNN2	0	[1,2]
19p13.3	GWAS - APOE4 int.	AD	rs9749589	NFIC	Intron variant		0	Not included			[6]
19p13.3	GWAS	AD	rs5117	APOC1	Intron variant	APOE	2	Microglia receptor ligand			[1]
19q13.2	WES (familial)	AD		PLD3		PLD3; PMID: 24336208	0	Not included			[40]
19q13.41	GWAS	AD	rs3865444	CD33	Intergenic	CD33; PMID: 28747436 PMID: 24381305	5	Microglia receptor			[1-5,8]
20q11.2-q13.31	GWAS	AD	rs6014724	CASS4	Intron variant		5	Cytoskeletal Regulator			[2-5,31]
21q21.3	GWAS	AD	rs2830500	ADAMTS1	Intergenic		0	Not included	ADAMTS5	0	[4]
21q21.3	GWAS	AD	rs2154481	APP	Intron variant	APP	0	Not included			[10]

Functional Impact of Risk Variants in Microglia Genes

For some genes and risk variants, the functional consequence of the risk variant(s) and knowledge about the role of the protein in normal and AD brain is quite well established (**TREM2** and **CD33**, discussed below). While for other genes where the associated "tag" SNP is intergenic (**BIN1, HLA-DQA1**, GPR141, **MS4A4A, MAF**), the causal driver of the association is far more opaque. In many cases, considerable experimental work is still needed to link the tag SNP to the causal association. Furthermore, it is important to emphasise that as more information comes to light, the gene responsible for the association may also change as the causal variant becomes known. For coding SNPs, particularly missense variants (**CASP8, TREML2, TREM2, PILRA, PLCG2, ABI3**) or where a stop codon leads to a truncated protein (IL34), the tag and causal SNP are likely to be one and the same. *In silico* evaluation of the functional consequences of amino acid substitution (using software such as SIFT, PolyPhen, REVEL, MetaLR, MutationAssessor and PROVEAN linked with output integrated within NCBI Ensembl)[207-212] can be a good first step to providing evidence for pathogenicity of any SNP identified by association analysis.

In **TREM2**, a number of rare hypomorphic AD missense risk variants have been shown to impact **TREM2** production (rs104894002, Q33X), expression or turnover at the cell surface (rs75932628, R47H)[115,213], α-secretase cleavage of the extracellular soluble ectodomain (rs2234255, H157Y)[115,214,215] and/or ligand binding (rs75932628, R47H; rs143332484, R62H; rs2234253/rs2234258/rs2234256, T96K/W191X/L211P; rs2234255, H157Y)[216-218]. Additional rare missense

variants affecting both alleles primarily alter protein structure, cell surface expression and shedding, and cause Nasu-Hakola disease[115,216,219]. In **CD33**, the intergenic tag SNP (rs3865444) is in perfect linkage disequilibrium with an intronic SNP (rs12459419) which affects splicing of exon 2[220]. The risk allele leads to inclusion of exon 2 which codes for an extracellular Ig V-set domain and a longer isoform (designated **CD33**M) which has higher cell surface expression. The protective allele missing from this domain due to the exclusion of exon 2 leads to a shorter isoform (designated **CD33**m). The Ig V-set domain is important for the sialic acid-binding properties of **CD33,** but may also be important for changing the conformation of ligand binding to **CD33** and thereby a shift between inhibitory and activation signalling in microglia which has important consequences for AD risk[221].

Pathways Connecting Alzheimer's Disease Risk Genes in Microglia
It is possible to link a number of the microglia specific/enriched genes within key microglia signalling pathway(s), either from direct evidence or through their homology with proteins where greater functional data exists (e.g., **ABI3** cellular localisation and function can be inferred from family members ABI1 and ABI2) [Figure 1 and Table 1]. This is a starting point for gaining a holistic understanding of how microglia behaviour may impact brain function and the disease process. It will also support the development of testable hypotheses including possible treatment targets. The pathways will be refined further in the coming years as greater functional knowledge of each gene and pathways emerges. Many of the microglia enriched/specific AD risk genes are linked to PAMP/DAMP responsive cell membrane receptors linked to phagocytosis and the cell processes necessary for phagocytosis - cytoskeletal machinery, endocytosis and lysosomal degradation within specialist phagosomes as well as changes in transcription and secretory pathways. In this way, we are beginning to consolidate knowledge about microglia that present opportunities to manipulate them towards a more protective response in AD. For example, **TREM2** activating antibodies[222,223] including the "first in man" Phase I clinical study recently published for agonistic monoclonal antibody, AL002c[119].

The majority of the microglia risk genes code for receptors or proteins which associate with receptors at the inner cell membrane, having transited through internal membranes such as ER, Golgi or endosomes before becoming active on the cell surface (**TREM2, TREML2, CD33, MS4A4A, INPP5D, HLA-DQA1, GPR141, PILRA, PICALM, SORL1, PLCG2**) [Figure 1A]. Frequently, the ectodomains of these receptors are shed by proteolytic processing by secretases at the cell surface (e.g. ADAM10 cleaves **TREM2** while **SORL1** is cleaved by presenilin activity)[115,214,215,224-226]. The functional significance of this is not yet fully understood. Some ligands for these receptors including PAMPs and DAMPs are known. For example, **TREM2** is activated by damage-associated anionic and zwitterionic lipids, including sphingomyelin, phosphatidylserine and phosphatidylethanolamine, which are likely to be displayed by damaged myelin or apoptotic cells such as dying neurons[58,59,144,145]. Interestingly, in addition to being a receptor for sialic-acid containing proteins such COLEC12, NPDC1, CLEC4G, CD99, complement component 4A and PIANP, **PILRA** also appears to be a co-receptor for the cell entry of Herpes virus[227] and thus could be a potential initiator of AD in the viral-induced model of pathogenesis (discussed above). The AD missense risk variant (rs1859788; G78R) results in reduced ligand binding to **PILRA**, although it isn't yet clear how this would impact AD risk[228].

Many of these receptors share the same adaptor proteins and/or downstream signalling cascades and they appear to propagate complex inhibitory or activating signals depending on the avidity of ligand binding, as reported for **TREM2** and **CD33**[221]. TYROBP (DAP12) is in fact a common adaptor for many immune cell receptors which do not have their own ITAM domain and therefore potentially adds further biological complexity as it presumably must respond and coordinate the competing needs of different receptors simultaneously. It is also common for immune receptors (including those coded for by AD microglia risk genes) to signal via the phosphatidylinositol signalling pathway. The phosphatidylinositols are important
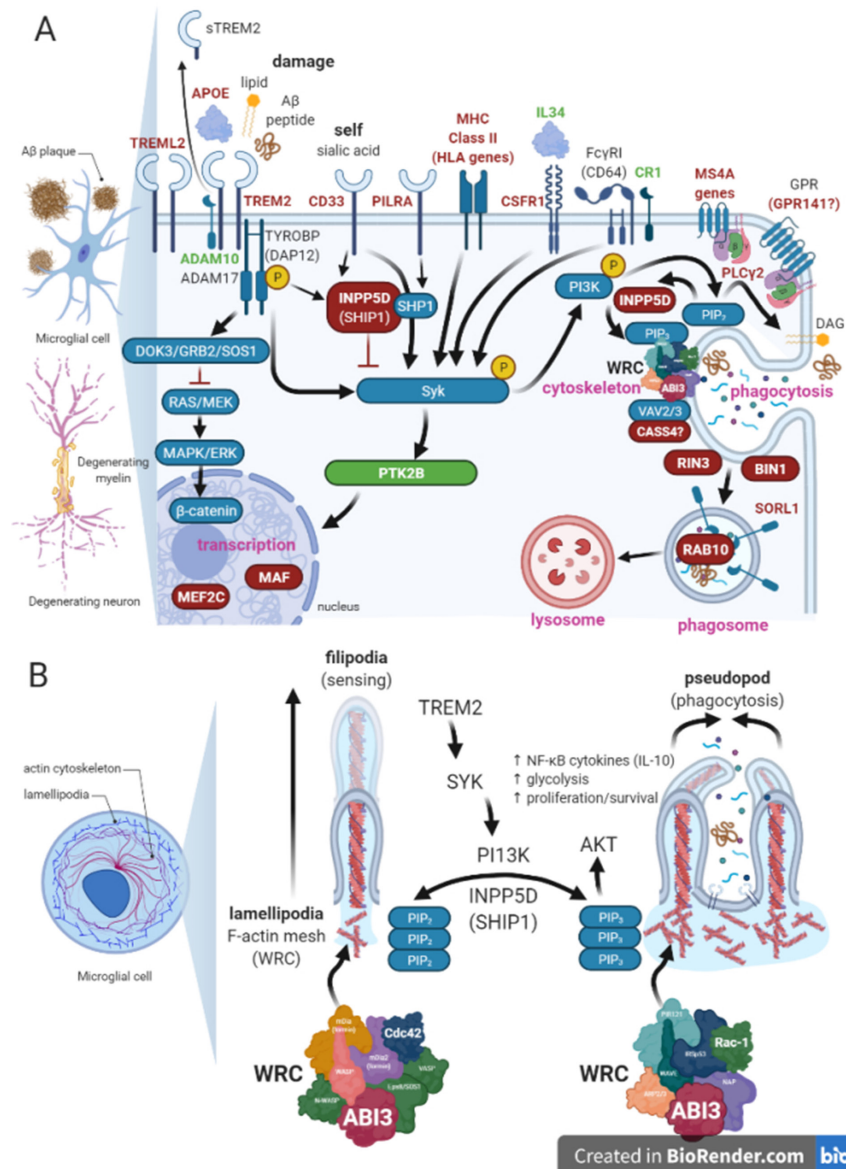
Figure 1. Microglia signalling pathways involving AD risk genes. (A) Key signalling pathways in microglia involving AD risk genes (microglia enriched highlighted in blue, other AD risk genes, purple); (B) signalling converging on the Wave Regulatory Complex (WRC) can impact cytoskeletal polymerisation/depolymerisation to inhibit lamellipodia capping and promote formation of filopodia for cell sensing (homeostatic microglia) or promote lamellipodia capping which promotes phagocytosis in response to damage/pathogen signals (activated microglia).

lipid sensing messengers, regulating many cell processes reliant on cytoskeletal dynamics - sensing, phagocytosis, cell division, vesicle trafficking, adhesion and motility[229,230]. The key elements of this pathway are the conversion of PIP2 (phosphatidylinositol 4,5-bisphosphate) to second messengers IP3 (inositol 1,4,5-trisphosphate) and DAG (diacylglycerol) by PLC enzymes, of which the AD risk gene **PLCG2** is a member, or, conversion of PIP2 to PIP3 [Phosphatidylinositol (3,4,5)-trisphosphate] by the enzyme PI3K [Figure 1A].

TREM2-DAP12 signalling triggers many protein and lipid phosphorylation cascades causing calcium mobilization, integrin activation, mTOR and MAPK signalling, activation of energetic metabolism[107] and cytoskeleton rearrangement via Syk[109] and is thus considered necessary for protection against damage and

pathology in AD. PI3K activation via Syk is considered an important downstream target of **TREM2**[231,232], expected to convert PIP2 to PIP3 and subsequently the type of membrane extensions from lamellipodia.

Lamellipodia are structures at the inner membrane leaflet. They consist of a mesh of F-actin and interacting proteins, which give membranes their irregular appearance at high magnification. It is from here that processes/filopodia can develop when F-actin capping is blocked, allowing actin polymerisation to continue. The balance between lamellipodia and process/filopodia formation is regulated by the large multiprotein Wave Regulatory Complex (WRC) [Figure 1A]. Microglia processes/filopodia are dynamic membrane protrusions extending (actin polymerisation) and retracting (actin depolymerisation) 100mm/3mm from the cell soma or process end, respectively[233]. Processes/filopodia are characteristic of homeostatic/ramified microglia, allowing microglia to efficiently survey their territories for signals. Their formation relies on high local levels of PIP2 at the inner cell membrane leaflet. PIP2 to PIP3 conversion generates shorter extensions or podosomes to develop, which allow the membrane to collapse to form phagocytic cups able to engulf and internalise damaged material or pathogens [Figure 1B]. The composition and activity of the WRC can change according to the signals received, either favouring the clustering of PIP2 or PIP3. Signalling through cdc42 via WRC: cdc42-mDia2 (formin)-WASP (WAS)-N-WASP (Eps8/SOS1, VASP, **ABI3**) clusters PIP2 and favours filopodia formation, while signalling through Rac via WRC: Rac-IRSp53-WAVE-Arp2/3-**ABI3**-NAP-PIR121) clusters PIP3 and favours lamellipodia[234-236]. Loss of **TREM2** reduces the degree of membrane ruffling, leads to increased filopodia number with reduced length and impairs phagocytic capability when BV2 microglia are stimulated with ATP or M-CSF (CSF1)[237]. Likewise, an absence of **TREM2** impairs formation of actin rings and podosomes in osteoclasts, essential for bone resorption[238], consistent with a block in PIP2 to PIP3 which appears to be essential for "sealing off" the membrane edges.

PI3K activity can be inhibited by the AD risk gene **INPP5D** (SHIP-1), which can cooperate with the protein SHP-1 to inhibit immune receptor signalling. In fact, SHP-1 is a key downstream effector of **CD33**, at least when activated through the Ig V-set domain (discussed above). PI3K activity is complex, but as indicated above, downstream signalling is expected to regulate PIP2 to PIP3, as well as regulating other complexes which promote membrane flexibility. SHIP-1 is also a phosphatase involved in PIP2 to PIP3 production. Loss of SHIP-1 activity might therefore be expected to lead to a failure in PIP3 production leading to a block in phagocytosis.

ABI3, another microglia specific AD risk gene, appears to physically interact with VASP within the WRC which controls cytoskeletal activity[239-241] [Figure 1]. **ABI3** activity is expected to favour Cdc42-dependent filopodia formation since high levels of **ABI3** decrease phosphorylation of Src, Lyn, Lyk, Yes, Hck, Fyn, Pyk2, FAK and FGR and members of P13K/AKT, AMPK, p38 MAPK, SAPK/JNK, mTOR, consistent with a role in maintaining high levels of PIP2 permissive for filopodia formation in homeostatic microglia[242]. When **ABI3** is phosphorylated at residue p.S342 by AKT, it has reduced association with the WRC[242]. This would be expected to be permissive for PIP3 and impact not only phagocytosis and cell morphology, but also potentially cell motility and cytokinesis[239]. Although it still needs to be tested for **ABI3**, it is possible that the phosphorylation of **ABI3** may also be modulated by **TREM2**-dependent activity, but this and the functional impact of the missense AD risk variant rs616338; c.626C>T; p.S209F which may abolish such signalling needs further testing.

Human Microglia Cell Models
In many ways, we have entered a new golden age for microglia research. Not only have they become centre stage for their clear contributions to disease and the remarkable new insights in to their roles in modifying human behaviours[41], but new knowledge about their developmental origin and expression profiles have paved the way for authentic human microglia to be generated from blood or other accessible tissue

samples[243-249]. Microglia are myeloid cells formed in the yolk sac during the first wave of haematopoiesis[17]. They share close molecular identity with other haematopoietic cells and specialist macrophages in different tissue niches, such as osteoclasts in bone and Kupffer cells in liver. However, different macrophages can exhibit a unique response , such as found with albumin exposure[250]. Human iPSC-microglia are becoming a well-established tool to understand the molecular events linking microglia activation with signalling pathways and functional outcomes. They appear to display many of the expected morphological and functional behaviours and express genes expected of authentic human microglia, where comparisons have been made with primary *ex vivo* foetal and/or adult microglia or isolated and profiled donor post mortem cells[243,245-247,249]. They can also be grown in co-culture which allows more complex investigations in to their relationship with other brain cell types[251]. Importantly, they can be generated from patient samples, including those with different genetic risk variants So overall, they offer a step change in the breadth of research questions which can be investigated and increased confidence in the results which accurately capture human microglia activities.

CONCLUSION

Genetic findings provide very clear evidence that fundamental processes controlling how microglia behave can impact vulnerability to AD. How microglia recognise and respond to age-associated signals determines whether they are ultimately protective or damaging in the brain and provide hope that microglia behaviour is modifiable. Identifying the functional links between AD risk genes, their causal variants and the pathways they operate in, particularly those highly expressed by microglia, will pave the way for identifying tractable targets and new approaches for therapeutic development. Methodological advances in generating authentic human microglia and the ability to selectively survey every gene they express under different conditions supports these efforts.

DECLARATIONS

*Genes highlighted in bold throughout the review are AD risk genes with highly enriched or exclusive expression in microglia. Additional AD risk genes may also have significant expression in microglia, but are also expressed by other brain cell types.

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Authors' contributions
Made substantial contributions to conception and design of the study and performed data analysis and interpretation and drafted the manuscript: Hodges AK
Made substantial contributions to conception and design of the study and performed data analysis and interpretation and revised the manuscript for intellectual content: Collier D, Pocock JM
Revised the manuscript for intellectual content: Piers TM, Cousins O

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