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Permalink
https://escholarship.org/uc/item/57p7w0hp

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Publication Date
2019

DOI
10.3389/fimmu.2019.01170

Peer reviewed
The Transcriptional Landscape of Microglial Genes in Aging and Neurodegenerative Disease

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Microglia, the brain-resident myeloid cells, are strongly implicated in Alzheimer's disease (AD) pathogenesis by human genetics. However, the mechanisms by which microglial gene expression is regulated in a region-specific manner over the course of normal aging and in neurodegenerative disease are only beginning to be deciphered. Herein, we used a specific marker of microglia (TMEM119) and a cell-type expression profiling tool (CellMapper) to identify a human microglial gene expression module. Surprisingly, we found that microglial module genes are robustly expressed in several healthy human brain regions known to be vulnerable in AD, in addition to other regions affected only later in disease or spared in AD. Surveying the microglial gene set for differential expression over the lifespan in mouse models of AD and a related tauopathy revealed that the majority of microglial module genes were significantly upregulated in cortex and hippocampus as a function of age and transgene status. Extending these results, we also observed significant upregulation of microglial module genes in several AD-affected brain regions in addition to other regions using postmortem brain tissue from human AD samples. In pathologically confirmed AD cases, we found preliminary evidence that microglial genes may be dysregulated in a sex-specific manner. Finally, we identified specific and significant overlap between the described microglial gene set—identified by unbiased co-expression analysis—and genes known to impart risk for AD. Our findings suggest that microglial genes show enriched expression in AD-vulnerable brain regions, are upregulated during aging and neurodegeneration in mice, and are upregulated in pathologically affected brain regions in AD. Taken together, our data-driven findings from multiple publicly accessible datasets reemphasize the importance of microglial gene expression alterations in AD and, more importantly, suggest that regional and sex-specific variation in microglial gene expression may be implicated in risk for and progression of neurodegenerative disease.

Keywords: microglia, Alzheimer's disease, genetics, TMEM119, cell-type profiling, frontotemporal dementia, autoimmune disease, RNAseq

INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disorder involving the progressive loss of memory and cognitive abilities. Discoveries over the last decade suggest that many, perhaps even a majority, of the genes contributing risk to AD are expressed primarily by microglia (1, 2), the resident myeloid cells of the brain parenchyma. Furthermore, work from the past few years
demonstrates that microglial gene expression changes during aging and may contribute to risk for AD (3) as well as autoimmune disorders of the central nervous system (CNS) such as multiple sclerosis [MS; reviewed in (4)] and lupus (5), but precisely how these cells contribute to risk for autoimmune disorders or AD remains unclear. Although AD is not considered a classical autoimmune disease, genetic pleiotropy studies have found striking genetic overlap between AD and several autoimmune disorders, including psoriasis and Crohn disease (6). Beyond these findings, a specific class II human leukocyte antigen (HLA)-DR haplotype, DR15, has been suggested to impart risk for both AD and MS (7, 8). These findings suggest that microglia may represent a common cellular link between canonical autoimmune disorders and AD, but it remains unclear whether shared or distinct microglial activities underlie the etiologies of these disorders.

To better understand the role of microglia in normal aging as well as in AD, robust and selective markers enabling unambiguous identification of microglia are required. Prior to the development of single-cell sequencing technologies, it was difficult to systematically identify and characterize *bona fide* microglia—as opposed to other myeloid cells, including perivascular macrophages and infiltrating monocyte-derived macrophages—at the molecular level and across multiple brain regions throughout the lifespan. However, advances within the last 5 years have enabled the identification of highly specific microglial markers not shared by other myeloid cell populations. In particular, the identification of the transmembrane protein TMEM119 as a specific marker of microglia has enabled global gene expression profiling of highly pure preparations of microglia (9). Beyond the identification of specific microglial markers, advances in cell-type expression profiling have enabled the identification of cell-type-specific gene expression profiles (10). These techniques do not require microdissection of the target cell type, but rather rely on a single cell-restricted marker (e.g., *TMEM119*) to reveal additional genes expressed by a given cell type.

In this study, we used multiple publicly available datasets to explore microglial gene expression in both healthy aging and disease. We leveraged the specificity of *TMEM119* expression and the CellMapper tool (10) to identify robustly co-expressed genes in human microglia. We utilized this microglial gene expression profile to explore how microglial genes are expressed in both healthy human aging and neurodegenerative disease. Our data-driven, expression-based microglial gene set demonstrated significant upregulation during normal aging and dysregulation in regions known to show atrophy in AD along with other brain regions. Further, it overlapped with genes implicated in risk for AD. Taken together, our results demonstrate that co-expressed microglial genes display regional heterogeneity in terms of expression level, are found at high levels in brain regions vulnerable in AD, and are significantly dysregulated in neurodegenerative disease. These results strengthen the known association of microglia with neurodegenerative disease and suggest that brain regions selectively vulnerable in AD may show greater numbers of microglia even in the healthy brain.

**METHODS**

Identification of Microglia-Specific Genes

We identified microglia-specific genes using previously described techniques to identify and characterize cell-specific expression profiles for rare or difficult-to-isolate cell types (10). Briefly, these methods take advantage of inherent cell type variability within a given bulk tissue sample set (e.g., undissociated brain tissue) by identifying genes with similar expression profiles. Although many techniques are available to identify a cell-specific expression profile, they often require multiple cell type markers and large training datasets. The technique we chose, CellMapper, requires only a single cell marker and a smaller training dataset than other techniques, and enables analysis of expression data derived from bulk brain tissue samples (10). These characteristics are especially important for native microglia in brain tissue—publicly available brain tissue samples are limited and many native microglial markers are shared with other ostensibly similar cell populations (e.g., infiltrating peripheral macrophages).

Validated cell markers specific for brain-resident microglia remained, until the last 5 years, elusive and difficult to confirm. There are multiple proposed markers, including *P2ry12, Fcrls, Siglec-H, Olfm13*, and *Tmem119* (9, 11, 12), but several of these markers are not yet validated or derived from models of neurodegeneration. We analyzed *TMEM119* in bulk human brain tissue data from the Allen Brain Institute (13) because it is a highly specific and well-validated marker of brain-derived microglia in both mice and humans.

**Gene Set Expression Enrichment Analyses in Normal Human Tissue**

We next determined the spatial patterns in microglial gene expression using tissue samples from the Allen Brain Institute. These analyses relied on one dataset available to the public (13) through the Allen Brain Institute (human.brain-map.org). The dataset included 6 adult control brain samples (H0351.2001, H0351.2002, H0351.1009, H0351.1012, H0351.1015, and H0351.106) finely dissected as described in the documentation available at http://help.brain-map.org/display/humanbrain/Documentation.

We tested whether microglial genes were regionally enriched by using novel methods to compare the number of query genes expressed above baseline in each region (14). Briefly, enrichment was calculated using the number of query genes expressed above baseline for each tissue type compared to the background gene expression for the aforementioned region. Statistical significance was calculated using a bootstrapping procedure comparing the provided gene list against the overlap occurring in randomly generated gene sets. Multiple testing correction was conducted using the FDR technique (15).

**Differential Expression Analyses in Mouse Models of Neurodegenerative Disease**

To better understand the dynamics of the microglial gene module in both healthy aging and disease, we examined its expression in two mouse models of neurodegenerative disease alongside wild type mice. We utilized data from the Mouseac project,

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which includes brain tissue samples from mouse models of neurodegenerative disease and wild type (WT) mice of the same background strain at varying ages (i.e., 8, 16, 32, and 72 weeks). The Mouseac project has been described in detail elsewhere (16). Briefly, samples were collected from three brain regions (cortex, hippocampus, and cerebellum) from wild-type, TASTPM (TAS10 × TPM AD mouse models; APPswe × PS1M1466V), and P301L-tau transgenic mice, which model a related neurodegenerative disorder, frontotemporal lobar degeneration (FTLD). Amongst the mouse models of AD available from the Mouseac project, we chose to analyze the TASTPM model because it involves two mutant, disease-causing transgenes (APP and PS1) identified in human familial AD cases and demonstrates a more severe pathological burden than either mutation alone. In addition, we analyzed the P301L mouse model of FTLD. Both mouse models are known to demonstrate a proinflammatory phenotype (17, 18). Of note, both heterozygous and homozygous carriers of the TASTPM transgenes were available for analysis, while only heterozygous carriers of the P301L-tau transgene were available. For the TASTPM mouse data, we analyzed heterozygous and homozygous mice together, accounting for gene dosage.

Gene expression was measured using microarrays (Illumina Ref8 v2) and processed by the Mouseac project staff. Briefly, raw expression levels were normalized using a log2 transformation and quantile normalization was performed for all samples together. An individual probe was excluded if the p-value for detection was >0.05 in >50% in a given group’s samples at any age. Additionally, samples were excluded if <95% of the probes for a given gene were detected.

We tested whether microglial gene expression in AD mouse models varied from control tissue with respect to both age and brain region using ANOVA.

### Differential Expression in Pathologically Diagnosed Human AD Tissue

RNA sequencing (RNAseq) data from pathologically confirmed AD cases was used to explore whether areas that display enriched microglial gene expression in the healthy human brain also show enhanced expression in AD cases relative to pathologically normal controls. This data was obtained through the Accelerating Medicines Partnership—Alzheimer’s Disease (AMP-AD) portal. Samples used for this portion of the study included those from the Mount Sinai School of Medicine (MSSM) Brain Bank (19), Mayo Clinic Brain Bank (20), and Religious Orders Study and Memory and Aging Project (ROSMAP) Study (21, 22). In aggregate, over 500 individuals were included in these analyses; cohort characteristics and sample distribution by region are available in Table 1. Data from the three brain banks’ collective samples was reprocessed and harmonized using a consensus toolset at the Mount Sinai Icahn School of Medicine Minerva HPC system. The results are accessible online through Synapse (ID # syn14237651). Technical details describing the reprocesing and analysis are explained in detail online through Synapse (https://www.synapse.org/#! Synapse:syn14237651). The resulting dataset includes tissue samples from many relevant brain regions, including some impacted early in AD (e.g., superior temporal cortex, parahippocampal gyrus, inferior frontal gyrus) as well as regions not impacted until much later in AD (e.g., cerebellum and frontal pole). In all analyses, the effect of diagnosis on gene expression was tested using linear regression controlling for biological factors such as sex, age, postmortem interval, and technical confounders accounting for more than 1% of variance of the principal components. To illustrate the results of our analyses in multiple brain regions in the context of atrophy patterns typically seen in AD, we created an atrophy map using voxel-based morphometry. The map included data from 120 individuals (60 clinically diagnosed AD cases compared to 60 normal controls). All individuals were seen at the UCSF Memory and Aging Center and scanned on a 3 Tesla scanner as previously described (23). The images were processed using SPM12 (24, 25) and analyzed as previously described (26).

Given that AD shows a sex-specific incidence and findings indicating that microglia show sex-specific differentiation and gene expression profiles in adult mice (27–29), we also examined whether there was a statistical interaction between AD diagnosis and sex (i.e., Gene expression × Sex + covariates), using the covariate selections described above.

### Microglial Gene Enrichment in Alzheimer’s Disease Genome-Wide Association Study (GWAS) Data

We tested whether our microglial gene set showed specific enrichment for AD risk genes using FUMA GWAS (Functional Mapping and Annotation of Genome-Wide Association Studies), a platform developed to characterize and interpret the results of genetic analyses (30). Briefly, FUMA compares a user’s list of submitted genes to publicly reported disease-associated genes (e.g., AD risk genes) and computes an associated p-value using

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**Table 1 | AD cohorts used for differential expression analysis.**

| Study | Total cohort size | Tissue type | Sample count by diagnosis |
|-------|-------------------|-------------|--------------------------|
|       |                   |             | AD | Control |
| Mayo  | 179               | CBE         | 47 | 32 | 35 | 37 |
| MSSM  | 164               | FP          | 63 | 27 | 23 | 22 |
| ROSMAP| 241               | DLPFC       | 109| 46 | 47 | 39 |

For each cohort, the total number of participants is provided along with sample counts for each tissue type grouped by diagnosis and sex. Note, not all participants provided samples for all brain regions. RNA expression data from the cohorts shown above corresponds to differential expression analyses presented in Figure 4.
hypergeometric testing. The background GWAS datasets used for this analysis come from the NHGRI-EBI catalog of published genome-wide association studies (https://www.ebi.ac.uk/gwas/), which contains over 2,500 publications and over 24,000 single nucleotide polymorphism (SNP) trait associations (31). We tested the 30 microglial genes identified using CellMapper against all available genes and associations in the catalog (a total of over 3,000 unique diseases and traits).

Statistical Analyses
All statistical analyses were performed using R (version 3.3.3) unless otherwise specified.

Ethics Statement
This study was carried out in accordance with the recommendations of University of California, San Francisco Committee on Human Research. The protocol was approved by the University of California, San Francisco Committee on Human Research. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

RESULTS
TMEM119 Identifies 30 Additional Microglial Genes
Using CellMapper and TMEM119 as a marker of native microglia, we identified 30 additional genes associated with a microglial gene expression profile (Table 2; \( p_{\text{FDR}} < 0.05 \)). Some of these genes, like TREM2, are known to be expressed by microglia and are associated with neurodegenerative diseases (32–34). Others, like SUCNR1, have established functions in inflammatory pathways, but are not known to be associated with neurodegenerative diseases (35). In Table 3, we provide a summary describing the protein product encoded by each gene, its cellular localization, regions of expression in the brain, and potential roles in normal aging as well as neurodegenerative disease. Using a stricter significance threshold of \( p_{\text{FDR}} < 0.01 \), 11 genes remained significant (IGSF6, ADORA3, ALOX5AP, CSF2RA, HPGDS, P2RY13, ACY3, SUSD3, SASH3, TXBAS1, and RASAL3). In the analyses that follow, we evaluated the entire 30 gene set when possible, omitting specific microglial genes only when expression data was not available.

Microglial Genes Are Enriched in Healthy Human Temporal and Parietal Cortex, Basal Ganglia, and Brainstem Nuclei
Utilizing the microglial gene set identified above, we tested which brain regions demonstrate enriched microglial gene expression in finely-dissected healthy human tissue samples. Our analyses revealed significant enrichment in 48 out of 194 testable brain regions at a \( p_{\text{FDR}} < 0.05 \) (Table 4, Figure 1). Of note, enrichment was particularly notable in the parietal cortex, areas of the temporal cortex (e.g., temporal pole, parahippocampal gyrus, amygdalolhippocampal transition), basal ganglia (e.g., putamen and globus pallidus), and brainstem nuclei (e.g., vestibular nuclei, pontine nuclei, and paraventricular nuclei) (Table 4, Figure 1). Although the frontal lobes showed comparably less enrichment relative to other brain regions, there was significant enrichment in the inferior frontal gyrus and frontal pole. Notably, both the cerebellum and occipital cortex showed minimal microglial gene enrichment.

Mouse Models of Neurodegenerative Disease Differentially Express Microglial Genes in Cortex and Hippocampus
Given that our analysis of healthy human brain revealed enriched expression of the microglial gene set in several regions affected early in the course of AD, we next explored the expression profile of microglial genes in several mouse models of AD and FTLD [(16); Mouseac project; www.mouseac.org]. Of the 30 human microglial genes identified in our prior analyses, 20 had data mapping to an orthologous mouse gene and passing quality control (as described above).

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**TABLE 2** | Microglial genes identified by TMEM119 expression profile.

| Gene symbol | Name |
|-------------|------|
| ACY3        | Aminoacylase 3 |
| ADAM28      | ADAM metallopeptidase domain 28 |
| ADORA3      | Adenosine A3 receptor |
| ALOX5AP     | Arachidonate 5-lipoxygenase activating protein |
| C1QB        | Complement C1q B chain |
| C3          | Complement C3 |
| CD33        | CD33 molecule |
| CD84        | CD84 molecule |
| C11T4A      | Class II major histocompatibility complex transactivator |
| CPE31       | Cadherin like and PC-esterase domain containing 1 |
| CSF2RA      | Colony stimulating factor 2 receptor alpha subunit |
| DHR59       | Dehydrogenase/reductase 9 |
| FCER1G      | Fc fragment of IgE receptor Ig |
| FYB         | FYN binding protein |
| GPR34       | G protein-coupled receptor 34 |
| HPGDS       | Hematopoietic prostaglandin D synthase |
| IGSF6       | Immunoglobulin superfamily member 6 |
| LAPT56      | Lysosomal protein transmembrane 5 |
| LY86        | Lymphocyte antigen 86 |
| P2RY13      | Purinergic receptor P2Y13 |
| RASAL3      | RAS protein activator like 3 |
| SASH3       | SAM and SH3 domain containing 3 |
| SELPLG      | Selectin P ligand |
| SPN         | Sialophorin |
| SUCEIR1     | Succinate receptor 1 |
| SUSD3       | Sushi domain containing 3 |
| SYK         | Spleen associated tyrosine kinase |
| TXBAS1      | Thromboxane A synthase 1 |
| TLR7        | Toll like receptor 7 |
| TREM2       | Triggering receptor expressed on myeloid cells 2 |

Using TMEM119 as a marker of native microglia, we identified a set of 30 additional genes whose expression profile suggested relevance to microglial function. The names of these genes along with their associated gene symbol are provided.
### TABLE 3 | Protein function, cellular location, and disease associations for microglial genes.

| Gene symbol | Protein function | Cellular locations of protein | Brain location of protein | Notes | References |
|-------------|------------------|------------------------------|--------------------------|-------|------------|
| ADAM28      | Modulate cell-cell and cell-matrix interactions; implicated in neurogenesis | Mitochondria; plasma membrane | Cerebral cortex | Expression lower in AD CSF | (36–39) |
| CD33        | Cell-cell interactions; maintenance of resting state in immune cells | Nucleus; plasma membrane | Cerebral cortex | Known AD risk gene | (37–40) |
| CD84        | Cell-cell interactions; modulate activation and differentiation of innate and adaptive immune system | Plasma membrane | Not yet determined | Upregulated during plaque development in mouse models of AD | (37, 41, 42) |
| FYB         | Adapter protein of FYN and LCP2 signaling cascades; modulate expression of IL2 | Cytosol | Cerebral cortex | | (37–39) |
| FCER1G      | Tyrosine kinase-based activation motif for transduction of immune activation signals | Plasma membrane | Cerebral cortex; hippocampus; caudate; cerebellum | RNA and protein expression noted to be discrepant. Upregulated in AD cases | (37–39, 43, 44) |
| GPR34       | Orphan Gi protein-coupled receptor implicated in immune response, receptor for short chain fatty acids | Nucleus; cytosol | Cerebral cortex; hippocampus; caudate; cerebellum | Appears to have multi-pass membrane component | (37–39, 45, 46) |
| RASAL3      | Negative regulation of RAS signaling | Cytoplasm near the plasma membrane | Cerebral cortex | | (37, 39, 47) |
| SASH3       | Signaling adapter protein in lymphocytes | Plasma membrane | Cerebral cortex | | (37–39) |
| ADORA3      | Adenosine receptor | Plasma membrane | None | Primarily expressed in lung, liver, kidney, and heart. Downregulated in aging | (37, 48, 49) |
| ACY3        | Deacetylation of mercaptyric acids, classically associated with kidney proximal tubule and gastrointestinal tract function | Plasma membrane; cytosol | Unspecified, but present at low levels in mouse | See Pushkin et al. (50) for brain expression data | (37, 50, 51) |
| ALOX5AP     | Leukotriene synthesis and promotion of inflammatory responses | Nuclear envelope; endoplasmic reticulum | Cerebral cortex | Associated with stroke and AD | (37, 39, 52–54) |
| CPED1       | Not well understood. Multiple likely protein products. | Nucleus; endoplasmic reticulum | Hippocampus; caudate | | (37–39, 55) |
| CIIA        | Required for transcriptional activity of Class II MHC receptor and Class I MHC receptor to a lesser extent | Nucleus | Cerebral cortex; hippocampus; caudate; cerebellum | | (37–39) |
| CSF2RA      | Controls production, differentiation, and function of granulocytes and macrophages | Extracellular; plasma membrane | Detected throughout the CNS | Reduced protein expression in hippocampus of human AD cases | (37, 56–58) |
| C1QB        | Initiation of the complement cascade | Extracellular; blood microparticle | Cerebral cortex; cerebellum | | (37, 39, 59, 60) |
| C3          | Activation of the classical and alternative complement pathways | Plasma membrane; extracellular; endoplasmic reticulum; lysosome | Hippocampus | Primarily extracellular; broadly implicated in AD | (37, 39, 61–63) |
| DHR59       | Steroid and retinoid synthesis | Endoplasmic reticulum | Caudate; cerebellum | | (37, 39, 64) |
| HPGDS       | Prostaglandin synthesis | Cytosol | Frontal cortex; hippocampus | Expression localizes to microglia and astrocytes in human AD cases | (37, 65, 66) |
| IGSF6       | Not well understood. Associated with transmembrane signaling receptor activity. | Plasma membrane | Not yet determined | Associated with inflammatory bowel disease | (37, 67, 68) |
| LY86        | Innate immune response to lipopolysaccharide and cytokine production | Plasma membrane | Cerebral cortex | | (37, 39, 69) |

(Continued)
We started by comparing the main effects of brain region, age, and transgene status. ANOVA analyses revealed markedly different microglial gene expression relative to wild type mice when compared by brain region and with increasing age. Using a strict Bonferroni correction for multiple testing \((p = 0.000357)\), 16/20 microglial genes (80%) showed significant differences by brain region in the TASTPM mouse model data (including both homozygote and heterozygote mutation carriers, and accounting for gene dosage) combined with WT data (Table 5, Figure 2A) and 14/20 of microglial genes (70%) demonstrated significant differences in the P301L mouse model data combined with WT data (Table 6, Figure 2B). All 14 genes identified in the P301L mouse model overlapped with genes upregulated in the TASTPM model.

We next explored statistical interactions between age, brain region, and transgene status. In the TASTPM model, we observed significant interactions for transgene status by age (15/20), transgene status by region (13/20), and transgene status by age and region (12/20) (Table 5). Similar results were observed in the P301L model, with significant interactions for transgene status by age (16/20) and transgene status by age and region (14/20), but not transgene status by region (0/20) (Table 6). With respect to the transgene status by age analyses, the genes identified in each mouse model were almost identical, with the exceptions being Fvb in the TASTPM model and Acy3 as well as Sucnr1 in the P301L model. For the transgene status by age and region analyses, the two additional genes identified in the P301L tau model that were not identified in the TASTPM mouse were Alox5ap and Cd33—both of which were close to the significance cutoff \((p = 0.000357)\).

Given the large number of genes implicated by our analyses, we chose three genes (Trem2, Laptm5, and Alox5ap) as exemplars of the expression patterns observed in the transgenic mice.
TABLE 4 | Regional gene set enrichment analyses in healthy human brain tissue.

| Structure                                      | Fold change | Raw p-value | Adjusted p-value |
|------------------------------------------------|-------------|-------------|-------------------|
| Corpus callosum                                | 1.667       | 2.52E-13    | 4.86E-11          |
| Temporal pole, right, medial aspect            | 1.404       | 3.10E-09    | 5.94E-07          |
| Globus pallidus, internal segment, right        | 1.376       | 4.85E-09    | 9.27E-07          |
| Principal sensory nucleus of trigeminal nerve, right | 1.333       | 6.66E-08    | 1.27E-05          |
| Parahippocampal gyrus, left, lateral bank of gyrus | 1.136       | 1.75E-07    | 3.32E-05          |
| Parolfactory gyrus, left                        | 1.259       | 1.94E-07    | 3.65E-05          |
| Globus pallidus, external segment, right        | 1.280       | 2.04E-07    | 3.81E-05          |
| Posterior orbital gyrus, right                  | 1.200       | 2.05E-07    | 3.82E-05          |
| Frontal pole, left, medial aspect              | 1.250       | 2.32E-07    | 4.29E-05          |
| Subcallosal cingulate gyrus, left               | 1.173       | 2.58E-07    | 4.74E-05          |
| Lateral group of nuclei, right, dorsal division | 1.070       | 2.71E-07    | 4.97E-05          |
| Paratemporal gyrus, right                       | 1.161       | 3.39E-07    | 6.18E-05          |
| Cochlear nuclei, left                           | 1.232       | 6.52E-07    | 1.18E-04          |
| Putamen, right                                  | 1.037       | 1.28E-06    | 2.31E-04          |
| Short insular gyr, left                         | 1.102       | 1.42E-06    | 2.54E-04          |
| Temporal pole, left, inferior aspect            | 1.180       | 1.44E-06    | 2.56E-04          |
| Parahippocampal gyrus, left, bank of the cos    | 1.223       | 1.49E-06    | 2.65E-04          |
| Gyrus rectus, right                             | 0.950       | 1.57E-06    | 2.77E-04          |
| Lateral parabrachial nucleus, left              | 1.118       | 2.21E-06    | 3.87E-04          |
| Superior frontal gyrus, right, medial bank of gyrus | 0.903       | 6.95E-06    | 0.001             |
| Lateral orbital gyrus, left                     | 0.913       | 8.32E-06    | 0.001             |
| Precentral gyrus, left, bank of the precentral sulcus | 0.941       | 9.36E-06    | 0.002             |
| Locus ceruleus, right                           | 1.055       | 9.79E-06    | 0.002             |
| Vestibular nuclei, left                         | 1.013       | 1.31E-05    | 0.002             |
| Pontine raphe nucleus                           | 1.000       | 2.98E-05    | 0.005             |
| Paraventricular nuclei, right of thalamus, right | 0.987       | 3.92E-05    | 0.007             |
| Middle frontal gyrus, left, inferior bank of gyrus | 1.004       | 4.19E-05    | 0.007             |
| Temporal pole, right, superior aspect           | 0.904       | 4.59E-05    | 0.008             |
| Frontal pole, right, superior aspect            | 0.984       | 4.63E-05    | 0.008             |
| Medial orbital gyrus, left                      | 0.945       | 4.68E-05    | 0.008             |
| Pontine nuclei, right                           | 0.989       | 4.73E-05    | 0.008             |
| Inferior rostral gyrus, right                   | 0.948       | 4.91E-05    | 0.008             |
| Frontal pole, left, inferior aspect             | 0.987       | 5.14E-05    | 0.008             |
| Planum polare, right                            | 0.968       | 5.22E-05    | 0.008             |
| Frontal operculum, left                         | 1.036       | 5.50E-05    | 0.009             |
| Gigantocellular group, left                     | 1.027       | 6.73E-05    | 0.011             |
| Medial parabrachial nucleus, right              | 0.905       | 7.22E-05    | 0.011             |
| Amygdalohippocampal transition zone, right      | 0.962       | 1.94E-04    | 0.030             |
| Inferior olivary complex, left                  | 0.860       | 1.97E-04    | 0.030             |
| Superior rostral gyrus, left                    | 0.925       | 2.20E-04    | 0.034             |
| Lateral group of nuclei, left, ventral division | 0.871       | 2.21E-04    | 0.034             |
| Inferior frontal gyrus, opercular part, right   | 0.943       | 2.21E-04    | 0.034             |

(Continued)
FIGURE 1 | Microglial gene enrichment analysis in normal human brain. We utilized gene set enrichment analyses to test whether the 30 microglial genes we identified using cell-type profiling showed a tropism for specific brain regions. Our analyses suggest that there is diffuse involvement of multiple brain regions, but also that there is focal involvement of specific regions such as the frontal pole, temporal cortex, basal ganglia, parietal cortex, and brain stem nuclei. For additional details, please refer to the manuscript text and methods described in Linker et al. (14).

TABLE 5 | Microglial gene expression changes in the TASTPM mouse model.

| Gene   | TASTPM | Age  | Region | TASTPM:age | TASTPM:region | Age:region | TASTPM:age:region |
|--------|--------|------|--------|------------|---------------|------------|-------------------|
| Cd33   | 3.75E-23 | 1.43E-14 | 3.77E-36 | 5.62E-08   | 2.51E-05   | 0.003      | 6.62E-04   |
| Cd84   | 5.34E-31 | 4.23E-25 | 9.24E-16 | 3.55E-14   | 1.97E-10   | 5.22E-06   | 5.80E-07   |
| Fyb    | 1.73E-18 | 6.02E-16 | 1.21E-10 | 6.24E-06   | 3.11E-04   | 1.93E-05   | 9.05E-06   |
| FceR1g | 6.39E-34 | 1.04E-25 | 3.47E-49 | 7.08E-11   | 1.47E-06   | 0.106      | 3.35E-05   |
| Gpr34  | 2.26E-21 | 6.37E-18 | 3.42E-67 | 9.21E-09   | 1.43E-05   | 0.016      | 4.16E-05   |
| Adora3 | 3.52E-13 | 4.56E-18 | 3.95E-24 | 9.84E-11   | 6.22E-06   | 1.37E-05   | 1.89E-07   |
| Acy3   | 0.071   | 0.494  | 0.010   | 0.772      | 8.30E-04   | 0.027      | 0.019      |
| Alox5ap| 1.17E-15 | 5.69E-28 | 5.86E-32 | 6.58E-09   | 2.02E-04   | 0.153      | 5.10E-04   |
| Csf2ra | 0.608   | 0.013  | 1.31E-12 | 0.090      | 0.004      | 0.984      | 0.639      |
| C1qb   | 1.29E-38 | 2.49E-29 | 6.95E-51 | 1.37E-10   | 1.87E-04   | 0.198      | 8.80E-06   |
| C3     | 3.24E-16 | 9.05E-34 | 6.32E-06 | 2.53E-10   | 0.290      | 0.003      | 3.31E-04   |
| Igf6   | 0.260   | 0.510  | 0.845   | 0.712      | 0.910      | 0.160      | 0.969      |
| Ly86   | 2.91E-29 | 3.04E-26 | 8.80E-33 | 2.74E-10   | 2.27E-07   | 0.037      | 2.93E-06   |
| Laptm5 | 9.51E-21 | 9.32E-23 | 1.70E-10 | 8.07E-09   | 3.16E-05   | 0.417      | 2.19E-04   |
| P2ry13 | 2.00E-15 | 1.82E-18 | 2.30E-74 | 2.99E-05   | 0.005      | 0.177      | 0.001      |
| Sprn   | 0.137   | 0.518  | 0.648   | 0.593      | 0.723      | 0.746      | 0.698      |
| Sucnr1 | 0.186   | 0.011  | 0.481   | 0.003      | 0.044      | 0.026      | 0.044      |
| Tbxas1 | 8.07E-29 | 1.77E-15 | 2.19E-21 | 2.35E-09   | 4.10E-10   | 1.35E-04   | 1.23E-04   |
| Tlr7   | 6.24E-29 | 3.88E-17 | 8.02E-38 | 9.58E-12   | 2.89E-08   | 1.03E-04   | 1.22E-06   |
| Trem2  | 1.20E-35 | 1.73E-30 | 3.46E-42 | 5.36E-14   | 5.45E-7    | 3.54E-04   | 2.11E-08   |

Analysis of variance (ANOVA) results from the TASTPM mouse model of Alzheimer’s disease are shown when transgenic mice and wild type mice were compared by age and brain region (e.g., cerebellum, hippocampus, or cortex). Significant findings (Bonferroni p-value threshold of 0.000357) are shown in bold. Data are courtesy of the Mouseac project (15).
FIGURE 2 | Microglial gene expression demonstrates age- and region-specific effects in neurodegenerative disease. To explore the temporal and spatial patterns of microglial gene expression in neurodegenerative disease, we used data from transgenic mouse models of Alzheimer’s disease (AD; TASTPM mouse model) and tauopathy (Tau; P301L mouse model) from the Mouseac project (16). Hierarchical clustering analyses revealed that microglial gene expression is broadly divided into four groups, the first three of which are regionally-specific and attributable to whether the tissue sample was cerebellum, hippocampus, or cortex [shown toward the left in (A, B)]. The fourth group [shown farthest to the right in (A, B)] was driven by transgene status. In TASTPM mice, the fourth group exclusively included transgenic model tissue either heterozygous or homozygous for the TASTPM transgenes from either the hippocampus or cortex (A). Further, the samples in the disease specific group tended to come from older mouse groupings, with all entries aged at least 8 months (A). Data from the tau P301L mouse demonstrated congruent patterns when compared to the TASTPM mouse model (B). CRB, Cerebellum; HIP, Hippocampus; CTX, Cortex; Mo, Month; AD, TASTPM mouse model; Tau, P301L tau mouse model; Het., Heterozygous; Ho., Homozygous.
TABLE 6 | Microglial gene expression changes in the P301L tau mouse model.

| Gene   | P301L Tau | Age | Region | Tau:age | Tau:region | Age:region | Tau:age:region |
|--------|-----------|-----|--------|---------|------------|------------|---------------|
| Cx33   | 1.47E-04  |     | 1.89E-08 | 2.40E-24 | 4.56E-08   | 0.178      | 0.029         | 2.14E-07      |
| Cdo4   | 0.002     |     | 2.08E-11 | 1.54E-04 | 3.81E-09   | 0.167      | 0.019         | 1.28E-06      |
| Fyb    | 0.004     |     | 2.96E-06 | 1.07E-04 | 0.002      | 0.476      | 0.035         | 3.72E-05      |
| Fcer1g  | 2.92E-04 |     | 3.62E-18 | 5.55E-35 | 1.45E-12   | 0.591      | 0.615         | 2.38E-05      |
| Gpr34   | 0.483     |     | 9.15E-08 | 1.89E-50 | 2.63E-04   | 0.828      | 0.599         | 2.21E-04      |
| Adora3  | 0.017     |     | 2.17E-09 | 7.11E-14 | 6.78E-09   | 0.307      | 0.016         | 4.90E-07      |
| Acy3    | 3.39E-04  |     | 0.002   | 0.023    | 1.73E-05   | 0.011      | 0.660         | 0.382         |
| Alox5ap | 0.017     |     | 2.39E-17 | 1.80E-18 | 8.91E-07   | 0.793      | 0.291         | 4.30E-06      |
| Cst2la  | 0.018     |     | 0.048   | 0.003    | 0.015      | 0.408      | 0.851         | 0.13          |
| C1qB   | 1.81E-05  |     | 7.78E-19 | 6.21E-35 | 9.04E-11   | 0.596      | 0.552         | 2.38E-04      |
| C3     | 5.94E-05  |     | 8.44E-15 | 0.185    | 2.28E-08   | 0.086      | 0.005         | 5.97E-05      |
| Igsf6   | 0.625     |     | 0.286   | 0.412    | 0.144      | 0.411      | 0.286         | 0.241         |
| Lyb6    | 0.007     |     | 3.88E-15 | 2.87E-18 | 9.18E-08   | 0.424      | 0.710         | 2.35E-05      |
| Laptm5  | 0.207     |     | 1.69E-10 | 5.48E-38 | 4.46E-05   | 0.984      | 0.207         | 0.120         |
| P2ry13  | 0.278     |     | 1.16E-11 | 2.93E-55 | 5.36E-05   | 0.566      | 0.358         | 0.060         |
| Snn     | 0.750     |     | 0.278   | 0.466    | 0.277      | 0.996      | 0.564         | 0.101         |
| Sucnr1  | 7.77E-04  |     | 0.005   | 0.697    | 1.23E-05   | 0.364      | 0.018         | 4.95E-07      |
| Tbxas1  | 7.99E-04  |     | 7.48E-09 | 4.48E-09 | 1.24E-08   | 0.679      | 0.303         | 2.88E-04      |
| Tr7     | 1.50E-04  |     | 2.50E-06 | 1.25E-19 | 2.38E-06   | 0.810      | 0.151         | 1.62E-04      |
| Trem2   | 3.84E-04  |     | 2.15E-19 | 2.76E-30 | 2.81E-11   | 0.973      | 0.378         | 1.97E-05      |

Analysis of variance (ANOVA) results from the P301L mouse model of tauopathy are shown when transgenic mice and wild-type mice were compared by age and brain region (e.g., cerebellum, hippocampus, or cortex). Significant findings (Bonferroni p-value threshold of 0.000357) are shown in bold. Data are courtesy of the Mousesc project (16).
microglial marker, TMEM119. This microglial gene set was generated using CellMapper, a novel tool that enables the identification of networks of co-expressed genes. Analyzing this microglial gene expression module with finely dissected samples of healthy human brain, we identified 48 regions (25% of tested regions) showing significant enrichment for the microglial gene set, including particularly robust enrichment in a number of regions affected early in the course of AD (e.g., parahippocampal gyrus and inferior frontal gyrus). However, we also detected enrichment in regions that are not particularly affected in AD (e.g., frontal pole and DLPFC) which indicates that enrichment of microglial genes is not necessarily sufficient to predict region-specific vulnerability in AD.

Analysis of orthologous members of this microglial gene set in mouse models of AD and tauopathy revealed striking variation in expression level that was dictated by brain region, age, and transgene status, with highest expression observed in the aged cortex and hippocampus of mouse models of neurodegenerative disease. We then surveyed the microglial gene expression module in pathologically confirmed human AD cases and found striking upregulation of the microglial module in regions impacted early in the course of AD (e.g., superior temporal cortex, parahippocampal gyrus, and inferior frontal gyrus), and somewhat less robust upregulation in the cerebellum, which is largely unaffected in AD. Finally, we observed significant overlap between our microglial gene expression module and genes independently found to impart risk for AD.

Collectively, our findings demonstrate that microglial gene expression varies greatly by brain region and as a function of age, and responds dynamically to neurodegenerative processes in pathologically affected brain regions. Our findings for human AD are consistent with prior studies using independent datasets and modes of analysis which similarly implicated microglial...
and myeloid expression modules in AD risk and pathobiology (89, 90). Taken together, our findings and those cited above help explain why pathogenic mutations and rare variants in microglial genes may exert a disproportionately strong impact on risk for neurodegeneration.

**Study Limitations—Interpretation of Dynamic Gene Expression Networks**

As with any study focusing on dynamic changes in gene expression networks, it is difficult to determine with certainty whether the measured changes in gene expression are a reflection of increases in microglial density (i.e., microglial “cellularity”), a reduction in the proportion of other cell types (e.g., loss of neurons due to ongoing neurodegeneration), or altered transcription of particular members of the microglial gene set [e.g., due to activation of a specific microglial gene expression program; for an in-depth discussion of these issues, see (91)]. These limitations are especially difficult to address when analyzing bulk tissue as was done in our study. A potential solution to the problems associated with microglial density is to analyze individual microglia using microdissection or single-cell RNA sequencing (scRNAseq; see below). Unfortunately, scRNAseq data have not, to our knowledge, been generated from finely dissected human brain regions demonstrating significant...
upregulation in this study. Such a dataset would enable important follow-up analyses that could provide additional insight into our current findings.

Microgliosis is a known feature of AD [reviewed in (92, 93)]; therefore, a relative increase in microglial density in pathologically affected regions is likely at least partially responsible for the microglial gene set upregulation we observed in human AD. This finding is further supported by evidence from AD cases demonstrating that microglial density is proportional to neurofibrillary tangle frequency and distribution (94). In addition, TMEM119 has been shown to be stably and selectively expressed in adult microglia (9, 95), and appears to exhibit stable expression even in response to a variety of inflammatory conditions (9, 96). Taking all of the above into consideration, it would be tempting to speculate that the expression changes we observed in this study are primarily a reflection of microglial cellularity. Intriguingly, P2ry13 and Gpr34, mouse orthologs of genes we identified in our human microglial gene set, are known to show down-regulation in so-called DAM (86–88) that are found in AD and other neurological disorders. P2ry13 is an ADP receptor and part of the G-protein coupled receptor family—it is thought to play a role in hematopoiesis and immune function (97). The function and importance of Gpr34 and its protein product remain largely undetermined, though its suspected role in detecting short chain fatty acids may implicate it in both metabolic and immune pathways (45, 98). That we observed increased expression of these genes (in both mouse and human) in bulk tissue from regions affected in neurodegeneration further indicates that the results we observe are driven, at least in part, by increased microglial cell density. Other potential explanations of the increased expression we observed include age differences at tissue collection and use of different AD mouse models.

On the other hand, TREM2 is also a member of our gene expression module and is known to be upregulated in DAM (87, 99). Dynamic changes in our microglial gene expression module therefore likely reflect a combination of increases in microglial cellularity and activation of specific gene expression programs (e.g., microgliosis and concomitant activation of the DAM program). It should be mentioned here that our knowledge of the DAM program is currently limited primarily to what has been gleaned from mouse models. Thus, future studies are needed to elucidate human-specific aspects of DAM dynamics in neurodegeneration.

Our study relies heavily upon publicly available data from human and mouse studies of neurodegenerative disease and healthy aging. Strengths of this approach are that it enabled us to build cohorts of large sample size, detect subtle disease effects, and ensure replicability of our findings; using large public datasets is also a limitation because our analyses required pooling of samples from multiple cohorts (especially in the postmortem human tissue analyses). Combining cohorts from multiple studies requires detailed and careful correction for batch effects and covariate selection but was necessary to achieve adequate statistical power. Despite pooling data from multiple sources, we cannot fully disentangle whether our findings are specific to AD, due to microglial density patterns, or both. For instance, we do not have AD samples from globus pallidus or corpus callosum, which would enable us to test whether microglial genes showed increased expression as a function of AD in regions that show highly enriched microglial expression in healthy brain, but which are not impacted until very late in AD.

The mouse models we utilized harbor genetic mutations that cause familial AD and FTLD, and thus present a unique set of limitations. Familial neurodegenerative disease is rare and the pathological processes driving it may be substantially different than those driving sporadic disease (100). Further, mouse models often mimic only limited aspects of the corresponding human neuropathology. For instance, the TASTPM mouse model of AD has amyloid plaques and demonstrates memory deficits, but does not show neurofibrillar tangles (101).

The primary limitations of CellMapper include that it was designed to use microarray data (not yet validated using RNAseq data) and that it requires validated as well as highly specific cell markers to accurately generate a cellular expression profile. Although many of our secondary analyses utilized RNAseq data, we generated the microglial expression profile using microarray data from the Allen Brain Institute. We thus were not subject to the former limitation. Secondly, we utilized TMEM119 as a marker of microglial expression, which was shown in 2016 to be a highly specific marker of brain-derived microglia (9). Prior to TMEM119’s discovery as a marker of brain-derived microglia, we would have been hindered by the latter limitation.

**Microglia-Mediated Synapse Loss in AD and Autoimmune Disease**

A major question in the field remains whether microglial activity in AD is largely beneficial or detrimental. Data from human genetics, particularly with respect to the hypomorphic R47H allele of TREM2 that strongly increases risk for AD (32, 33, 102), provide strong evidence for a protective role for microglia. However, it is currently unknown whether the protective effect of “normally” functioning microglia (via sufficient TREM2 function) is a manifestation of normal microglial activity specifically during aging, over the entire lifespan, or perhaps even during development.

In contrast to developments in human genetics, many studies in mouse models of neurodegenerative disease have identified a harmful role for microglia, particularly as drivers of synapse loss [for example, (103)]. In particular, tauopathy has been suggested to be a driver of complement- and microglia-mediated synapse engulfment (104, 105). Strikingly, mechanistic dissections of the CNS manifestations of the autoimmune disorder, lupus, have demonstrated a role for type I interferon signaling and autoantibodies in promoting inappropriate complement- and microglia-mediated engulfment of synapses (5, 106). Thus, while microglia are clearly capable of protecting against neurodegeneration when functioning in a homeostatic manner, inappropriate activation may be sufficient to induce synapse loss and neurodegeneration. A critical area of future research will be to determine at which point(s) in
aging and/or disease microglia are protective, and at which point (or in which specific contexts) their activities become harmful. A clear understanding of these issues will be critical in determining which microglial molecules and pathways to target for therapeutic intervention, and at which point in the course of disease.

**Future Directions: Single-Cell RNA Sequencing and Microglial Sexual Dimorphism**

Several publications within the last 2 years have begun to employ scRNAseq and mass cytometry of microglia and other CNS immune cells as ways to understand (i) global alterations in immunological gene expression and (ii) dynamic changes in immune cell-type abundance as a function of brain anatomy, aging, and neurodegeneration (87, 95, 107, 108). The major advantage of these techniques is that they enable unbiased, global profiling of gene expression uninfluenced by changes in cellularity, as well as characterization of cell-type fluctuations as a function of anatomy, age, and disease. Given this, replication of our findings using scRNAseq technology will be critical to determine whether our findings were influenced primarily by microglial cellularity or by changes in microglial gene expression programs. Another critical issue for future work will be to determine the mechanisms responsible for sexually dimorphic microglial gene expression profiles reported in mice (28, 88) and to what extent they apply to human microglia. Our work comparing microglial gene expression in human brain tissue from AD cases suggests that the relationships observed in mice may apply to the human condition and is consistent with reports from humans showing that there is a sex dimorphism in non-diseased human tissue (109) and that microglial gene expression changes with aging (110–112). Our sex-specific findings, despite modest effect size, are a promising addition to this emerging literature, given that AD is one of the most common diseases of aging and shows sex-specific incidence and progression (113). As described above, extending our study in the future with the use of scRNAseq would likely provide deeper insight into sex- and age-specific effects on microglial gene expression changes in AD.

**ETHICS STATEMENT**

This study was carried out in accordance with the recommendations of University of California, San Francisco Committee on Human Research. The protocol was approved by the University of California, San Francisco Committee on Human Research. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

**REFERENCES**

1. Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, et al. An environment-dependent transcriptional network specifies human microglia identity. Science. (2017) 356:eaal3222. doi: 10.1126/science.aal3222
2. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer’s disease. J Cell Biol. (2018) 217:459–72. doi: 10.1083/jcb.201709069
3. Olah M, Patrick E, Villani A-C, Xu J, White CC, Ryan KJ, et al. A transcriptomic atlas of aged human microglia. Nat Commun. (2018) 9:539. doi: 10.1038/s41467-018-02926-5
4. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Neurol. (2015) 11:545–58. doi: 10.1038/nrneurol.2015.87
5. Bialas AR, Presumey J, Das A, van der Poel CE, Lapchak PH, Mesin L, et al. Microglia-dependent synapse loss in type I interferon-mediated lupus. Nature. (2017) 546:539–43. doi: 10.1038/nature22821

**AUTHOR CONTRIBUTIONS**

LB conceived the study, performed statistical analyses, produced the figures, and drafted the manuscript. DS provided feedback on analyses, produced the figures, and drafted the manuscript. JY conceived the study, supervised all study activities, and drafted the manuscript.

**FUNDING**

Primary support for data analyses was provided by the Larry L. Hillblom Foundation 2016-A-005-SUP (JY), NIA K01 AG049152 (JY), and the Tau Consortium (JY). Study data were provided by the following sources: The Mayo Clinic Alzheimer’s Disease Genetic Studies, led by Dr. Nilüfer Taner and Dr. Steven G. Younkin, Mayo Clinic, Jacksonville, FL using samples from the Mayo Clinic Study of Aging, the Mayo Clinic Alzheimer’s Disease Research Center, and the Mayo Clinic Brain Bank. Data collection was supported through funding by NIA grants P50 AG016574, R01 AG032990, U01 AG046139, R01 AG018023, U01 AG006576, U01 AG006786, R01 AG025711, R01 AG017216, R01 AG003949, NINDS grant R01 NS080820, CurePSP Foundation, and support from Mayo Foundation. Study data includes samples collected through the Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson’s Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer’s Disease Core Center), the Arizona Department of Health Services, the Arizona Alzheimer’s Research Center, the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Alzheimer’s Disease Core Center), and Parkinson’s Disease (contract 211002, Arizona Alzheimer’s Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson’s Disease Consortium) and the Michael J. Fox Foundation for Parkinson’s Research. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

**ACKNOWLEDGMENTS**

We thank Dr. Brad Nelms for a helpful discussion regarding this project.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01170/full#supplementary-material
46. Pugazhenthi S. Metabolic syndrome and the cellular phase of Alzheimer's disease. *Prog Mol Biol Transl Sci.* (2017) 146:243–58. doi: 10.1016/bs.pmbts.2016.12.016

47. Saito S, Kawamura T, Higuchi M, Kobayashi T, Yoshita-Takahashi M, Yamazaki M, et al. RASAL3, a novel hematopoietic RasGAP protein, regulates the number and functions of NKT cells. *Eur J Immunol.* (2015) 45:1512–23. doi: 10.1002/eji.201444977

48. Crotti A, Ransohoff RM. Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. *Immunity.* (2016) 44:505–15. doi: 10.1016/j.immuni.2016.02.013

49. Sajjadi FG, Firestein GS. cDNA cloning and sequence analysis of the human A3 adenosine receptor. *Biochim Biophys Acta.* (1993) 1179:105–7. doi: 10.1016/0167-4889(93)90077-3

50. Pushkin A, Carpenito G, Abuladze N, Newman D, Tsuprun V, Ryazantsev M, et al. Structural characterization, tissue distribution, and functional expression of murine aminocycloisole. *Am J Physiol.* (2004) 286:C848–56. doi: 10.1152/ajpcell.00192.2003

51. Chen Y, Chen T, Zhang S, Lin S, Zhao Y, Ye F, et al. Identification of a novel protein binding to hepatitis C virus core protein. *J Gastroenterol Hepatol.* (2009) 24:1300–4. doi: 10.1111/j.1440-1746.2009.05846.x

52. Kaushal R, Pal P, Alwell K, Haverbusch M, Flaherty M, Moomaw KR, et al. Association of ALOX5AP with ischemic stroke: a population-based case-control study. *J Gastroenterol Hepatol.* (2012) 27:2227–86. doi: 10.1111/j.1440-1746.2011.05915.x

53. Strid T, Svartz J, Franck N, Hallin E, Ingelsson B, Söderström M, et al. Characterization of the extracellular matrix of normal and Alzheimer's disease. *Acta Neuropathol Commun.* (2014) 2:354. doi: 10.1186/s40478-014-0358-9

54. Taniike M, Mohri I, Kagitani-Shimono K, Kanekiyo T, Kudo T, Kadoyama K, et al. Hematopoietic prostaglandin D synthase and DP1 receptor are selectively upregulated in glioblastoma and astrocytomas within senile plaques from human patients and in a mouse model of Alzheimer disease. *J Neurophysiol Exp Neurol.* (2007) 66:469–80. doi: 10.1097/00002402-200703280-00027

55. Bayram D, Cinar D, Schuff N, Hulihan JM, et al. CD40L activation of dendritic cells down-regulates DORA, a novel member of the immunoglobulin superfam. *Mol Immunol.* (1998) 35:513–24. doi: 10.1016/S0168-8278(98)00045-5

56. Bonham et al. Microglial Genes in Normal Aging and AD. Frontiers in Immunology | www.frontiersin.org 16 June 2019 | Volume 10 | Article 1170

57. Bates EEM, Dieu M-C, Ravel O, Zuurawski SM, Patel S, Bridon J-M, et al. CD40L activation of dendritic cells down-regulates DORA, a novel member of the immunoglobulin superfam. *Mol Immunol.* (1998) 35:513–24. doi: 10.1016/S0168-8278(98)00045-5

58. Bates EEM, Kissenpfennig A, Pérone C, Mattei M-G, Fossiez F, Malissen B, et al. The mouse and human IGSF6 (DORA) genes map to the inflammatory bowel disease locus 1 and are embedded in an intron of a gene of unknown function. *Immunogenetics.* (2000) 52:112–20. doi: 10.1007/s002550000259

59. Miura Y, Shimazu R, Miyake K, Akashi S, Ogata H, Yamashita Y, et al. RP105 is associated with MD-1 and transmits an activation signal in human B cells. *Blood.* (1998) 92:2815–22.

60. Sako D, Chang X-J, Barone KM, Vachino G, White HM, Shaw G, et al. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell.* (1993) 75:1179–86. doi: 10.1016/0028-0846(93)90327-M

61. Veldman GM, Beam KM, Cumming DA, Eddy RL, Sait SNJ, Shows TB. Genomic organization and chromosomal localization of the gene encoding human P-selectin glycoprotein ligand. *J Biol Chem.* (1995) 270:16470–5. doi: 10.1074/jbc.270.27.16470

62. Matsuoka A, Walker DG, Terai K, McGregor PL. Expression of CD43 in microglia and its downregulation in Alzheimer's disease. *J Neuroimmunol.* (1996) 71:81–6. doi: 10.1016/0165-5728(96)00134-8

63. Park JK, Rosenstein YJ, Remold-O'Donnell E, Bierer BE, Rosen FS, Burakoff SJ. Enhancement of T-cell activation by the CD43 molecule whose expression is defective in Wiskott–Aldrich syndrome. *Nature.* (1991) 350:706–9. doi: 10.1038/350706a0

64. Schweig JE, Yao H, Beaulieu-Abdelahad D, Ait-Ghazala G, Mouzon B, Crawford F, et al. Alzheimer's disease pathological lesions activate the spleen tyrosine kinase. *Acta Neuropathol Commun.* (2017) 5:69. doi: 10.1186/s40478-017-0472-2

65. Uckun FM, Ma H, Zhang J, Ozer Z, Dovat S, Mao C, et al. Serine phosphorylation by SYK is critical for nuclear localization and transcription factor activation of Ikaros. *Proc Natl Acad Sci USA.* (2012) 109:18072–7. doi: 10.1073/pnas.1209828109

66. Peruzzi-Lametti L, Bernstock JD, Vicario N, Costa ASH, Kowk CK, Leonardi T, et al. Macrophage-derived extracellular succinate licenses neural stem cells to suppress chronic neuroinflammation. *Cell Stem Cell.* (2018) 22:355–68.e13. doi: 10.1016/j.stem.2018.01.020

67. Ryan VH, Primiani CT, Rao JS, Ahn K, Rapoport SI, Blanchard H. Coordination of gene expression of arachidonic and docosahexaenoic acid cascade enzymes during human brain development and aging. *PLoS ONE.* (2014) 9:e100858. doi: 10.1371/journal.pone.0100858

68. Chaturvedi A, Pierce SK. How location governs toll-like receptor signaling. *Traffic.* (2009) 10:621–8. doi: 10.1111/j.1600-0854.2009.00899.x

69. Gambuzza ME, Sofo V, Salmeri FM, Soraci L, Marino S, Bramanti P. Toll-like receptors in Alzheimer's disease: a therapeutic perspective. *CNS Neuro Disord Drug Targets.* (2014) 13:1542–58. doi: 10.1080/14795958.2013.819969

70. Ryan VH, Primiani CT, Rao JS, Ahn K, Rapoport SI, Blanchard H. Coordination of gene expression of arachidonic and docosahexaenoic acid cascade enzymes during human brain development and aging. *PLoS ONE.* (2014) 9:e100858. doi: 10.1371/journal.pone.0100858

71. Bonham LW, Desikan RS, Yokoyama JS. The relationship between complement factor C3, APOE ε4, amyloid and tau in Alzheimer's disease. *Acta Neurologica Commun.* (2016) 4:655. doi: 10.1186/s40478-016-0339-y

72. Hernandez-Encinas E, Aguilar-Morante D, Morales-Garcia JA, Gine E, Sanz-Sanz-Cristobal M, Santos A, et al. Complement component 3 (C3) expression in the hippocampus after excitotoxic injury: role of C/EBPβ. *J Neuroinflammation.* (2016) 13:276. doi: 10.1186/s12974-016-0742-0

73. Stamer WD, Hoffinan EA, Luther JM, Hachez DL, Schey KL. Protein profile of exosomes from trabecular meshwork cells. *J Proteomics.* (2011) 74:796–804. doi: 10.1016/j jäpro.2011.02.024

74. Chetirykin SV, Belyaeva OV, Gough WJ, Kedishivilv NY. Characterization of a novel type of human microsomal 3α-hydroxysteroid dehydrogenase unique tissue distribution and catalytic properties. *J Biol Chem.* (2001) 276:22278–86. doi: 10.1074/jbc.M102076200
84. Borroni B, Ferrari F, Galimberti D, Nacmias B, Barone C, Bagnoli S, et al. Heterozygous TREM2 mutations in frontotemporal dementia. *Neuropath Biol Aging*. (2014) 35:7–10. doi: 10.1016/j.neurobiolaging.2013.09.017

85. Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Bioi Psychiatry*. (2015) 77:43–51. doi: 10.1016/j.biopsych.2014.05.006

86. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci*. (2018) 19:622–35. doi: 10.1038/s41583-018-0057-5

87. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Divr-Sitzenfeld R, Ulland TK, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. (2017) 169:1276–90.e17. doi: 10.1016/j.cell.2017.05.018

88. Krassmann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity*. (2016) 47:566–81.e9. doi: 10.1016/j.immuni.2017.08.008

89. Huang K, Marcera E, Pimenova AA, Di Narzo AF, Kapoor M, Jin SC, et al. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer's disease. *Nat Neurosci*. (2017) 20:1052–61. doi: 10.1038/nm.4587

90. Zhang B, Gaiter C, Bodea L-G, Wang Z, McElwee J, Podtelezhnikov AA, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell*. (2013) 153:707–20. doi: 10.1016/j.cell.2013.03.030

91. Friedman BA, Srivinasa K, Ayaloon G, Meilandt WJ, Lin H, Huntley MA, et al. Diverse brain myeloid expression profiles reveal distinct microglial activation states and aspects of Alzheimer's disease not evident in mouse models. *Cell Rep*. (2018) 22:832–47. doi: 10.1016/j.celrep.2017.12.066

92. Song WM, Colonna M. The identity and function of microglia parallels that of neurofibrillary tangles in Alzheimer's disease. *Neuron*. (2018) 100:1337–53.e5. doi: 10.1016/j.neuron.2018.10.031

93. Yeh FL, Hansen DV, Sheng M. TREM2, microglia, and mechanisms of disease pathogenesis. *Biol Psychiatry*. (2018) 80:255–66. doi: 10.1016/j.biopsych.2018.05.006

94. Dejanovic B, Huntley MA, De Mazière A, Meilandt WJ, Wu T, Srivinasa K, et al. Changes in the synaptic proteome in tauopathy and rescue of tau-induced synaptic loss by C1q antibodies. *Neuron*. (2018) 100:1322–36.e7. doi: 10.1016/j.neuron.2018.10.014

95. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Werler A, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*. (2016) 352:712–6. doi: 10.1126/science.aad8837

96. Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, et al. Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. *Nat Neurosci*. (2018) 21:541–51. doi: 10.1038/s41593-018-0100-x

97. Litvinchuk A, Wan Y-W, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Complement C3aR inactivation attenuates tau pathology and reverses an immune network deregulated in tauopathy models and Alzheimer's disease. *Neuron*. (2018) 100:1337–53.e5. doi: 10.1016/j.neuron.2018.10.031

98. Nestor J, Arinuma Y, Huerta TS, Kowal C, Nasiri E, Kello N, et al. Lupus antibodies induce behavioral changes mediated by microglia and blocked by ACE inhibitors. *J Exp Med*. (2018) 215:2535–46. doi: 10.1084/jem.20180776

99. Zrzavy T, Hametner S, Hametner S, Wimmer I, Butovsky O, Weiner HL, Lassmann H, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci*. (2017) 20:1162–71. doi: 10.1038/nn.4222

100. Litvinchuk A, Wan Y-W, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell*. (2018) 172:500–16.e16. doi: 10.1016/j.cell.2017.11.042

101. Howlett DR, Richardson JC, Austin A, Parsons AA, Bate ST, Davies DC, et al. Cognitive correlates of Aβ deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Brain Res*. (2004) 1017:130–6. doi: 10.1016/j.brainres.2004.05.029

102. Song WM, Joshua S, Zhou Y, Ulland TK, Gilfillan S, Colonna M. Humanized TREM2 mice reveal microglia-intrinsic and -extrinsic effects of R47H polymorphism. *J Exp Med*. (2018) 215:745–60. doi: 10.1088/jem.20171529

103. Hong S, Beja-Glasser VF, Nfoniyem BM, Frouin A, Li S, Ramakrishnan S, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*. (2016) 352:712–6. doi: 10.1126/science.aad8837

104. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci*. (2014) 15:511–20. doi: 10.1038/nrn3807

105. Litvinchuk A, Wan Y-W, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Complement C3aR inactivation attenuates tau pathology and reverses an immune network deregulated in tauopathy models and Alzheimer's disease. *Neuron*. (2018) 100:1337–53.e5. doi: 10.1016/j.neuron.2018.10.031

106. Nestor J, Arinuma Y, Huerta TS, Kowal C, Nasiri E, Kello N, et al. Lupus antibodies induce behavioral changes mediated by microglia and blocked by ACE inhibitors. *J Exp Med*. (2018) 215:2535–46. doi: 10.1084/jem.20180776

107. Litvinchuk A, Wan Y-W, Swartzlander DB, Chen F, Cole A, Propson NE, et al. Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell*. (2018) 172:500–16.e16. doi: 10.1016/j.cell.2017.11.042

108. Galatro TF, Holtman IR, Larario AM, Vainchtein ID, Brouwer N, Sola PR, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci*. (2017) 20:1162–71. doi: 10.1038/nn.4597

109. Grabert K, Michoel T, Karavolos MH, Clohisy S, Baillie JK, Stevens MP, et al. Microglial brain region–dependent diversity and selective regional sensitivities to aging. *Nat Neurosci*. (2016) 19:504–16. doi: 10.1038/nn.4222

110. Soreq L, Rose J, Soreq E, Hardy J, Trabzuni D, Cookson MR, et al. Major shifts in glial regional identity are a transcriptional hallmark of human brain aging. *Cell Rep*. (2017) 18:537–70. doi: 10.1016/j.celrep.2016.12.011

111. Snyder HM, Asthana S, Bain L, Brinton R, Craft S, Dubal DB, et al. Sex biology contributions to vulnerability to Alzheimer's disease: a think tank convened by the Women's Alzheimer's Research Initiative. *Alzheimers Dement*. (2016) 12:1186–96. doi: 10.1016/j.jalz.2016.08.004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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