Tau Pathology Drives Dementia Risk-Associated Gene Networks toward Chronic Inflammatory States and Immunosuppression

Graphical Abstract

1) Consensus Networks
2) Modules associated with disease progression
3) 7 modules related to p-Tau pathology
4) Microglia neuroimmune state transitions across disease
5) Genetic Drivers
6) Modeling and validating regulatory drivers

Highlights

- Significant changes in microglial-immune signaling mark stages of neurodegeneration
- Pro-inflammatory pathways yield to early interferon-driven immune suppression
- A key hub in the immune suppression module, USP18, modulates this response
- Validation of chemogenetic predictions identifies drugs modulating microglial function

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In Brief

Rexach et al. use transcriptional network analysis to define dynamic microglial transitions across neurodegeneration, discovering that three dementias with tau pathology involve dysregulated microglial viral and antiviral pathways. Bioinformatics coupled with experimental validation identifies regulatory drivers, implicating double-stranded RNA and interferon-response genes as drivers of early immune suppression in disease.
Article

Tau Pathology Drives Dementia Risk-Associated Gene Networks toward Chronic Inflammatory States and Immunosuppression

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https://doi.org/10.1016/j.celrep.2020.108398

SUMMARY

To understand how neural-immune-associated genes and pathways contribute to neurodegenerative disease pathophysiology, we performed a systematic functional genomic analysis in purified microglia and bulk tissue from mouse and human AD, FTD, and PSP. We uncover a complex temporal trajectory of microglial-immune pathways involving the type 1 interferon response associated with tau pathology in the early stages, followed by later signatures of partial immune suppression and, subsequently, the type 2 interferon response. We find that genetic risk for dementias shows disease-specific patterns of pathway enrichment. We identify drivers of two gene co-expression modules conserved from mouse to human, representing competing arms of microglial-immune activation (NAct) and suppression (NSupp) in neurodegeneration. We validate our findings by using chemogenetics, experimental perturbation data, and single-cell sequencing in post-mortem brains. Our results refine the understanding of stage- and disease-specific microglial responses, implicate microglial viral defense pathways in dementia pathophysiology, and highlight therapeutic windows.

INTRODUCTION

Microglia and CNS-resident macrophages are the principal immune cells of the brain, playing critical roles in coordinating the neural-immune response (Hickman et al., 2013). During nervous system injury, microglia can be directly activated by myelin, lipids, or nucleotides released from injured cells to activate pro-inflammatory signaling, such as through the NLRP3 inflammasome complex (Kigerl et al., 2014), and contribute to neuronal dysfunction in multiple ways (Deczkowska et al., 2018; Edwards, 2019; Song and Colonna, 2018). Functional studies in animal models support roles for microglia in the clearance of age-related amyloid beta (Abeta) plaques (Lee et al., 2018) and in the progression of tau pathology and neurodegeneration (Bussian et al., 2018; Kodama et al., 2020; Litvinchuk et al., 2018; Shi et al., 2019). However, microglial responses in neurodegeneration are not monolithic; single-cell genomic studies have begun to delineate substantial heterogeneity among disease-associated microglial states and their trajectories (Grubman et al., 2019; Keren-Shaul et al., 2017; Mathys et al., 2017, 2019). There is a pressing need for biological models to refine our understanding of microglia in human brain disease, including their relationship to specific clinical dementia syndromes and causal genetic factors.

We recently used a systems biology approach to identify a robust, evolutionarily conserved signature of neurodegeneration across humans and multiple mouse models, which is composed of two interconnected neurodegeneration-associated synaptic (NAS) and inflammatory (NAI) modules (Swarup et al., 2019). The NAI module was strongly enriched for markers of astrocytes and microglia. This, coupled with growing evidence that microglial and astrocyte neuroimmune signaling contributes causally to Alzheimer’s disease (AD) (Effthymiou and Goate, 2017), suggested that the NAI module would be a powerful starting framework from which to identify early disease-associated microglial changes relevant to human disease. Since our original study was based on bulk tissue RNA sequencing (RNA-seq), downregulated microglial
Figure 1. Purified Microglia-Brain Tissue Consensus Gene Co-expression Network Analysis
(A) Experimental schema, showing approach for microglia-tissue consensus WGCNA.
(B) Module enrichment heatmap for (left) top 100 genes differentially expressed between progressive microglia single-cell states, as indicated, and (right) genes differentially expressed between microglia single-cell states identified from the prefrontal cortex of patients with Alzheimer’s disease (AD) and controls, as indicated; Mic0, Mic1 (AD-associated microglia subpopulation), Mic2, and Mic3, microglia subclusters as defined in Mathys et al. (2019); n = 7 modules with 4 comparisons per module. *FDR < 0.05, **FDR < 0.005, ***FDR < 0.001. Hom, homeostatic; DAM1, type 1 disease-associated microglia; DAM2, type 2 DAMs, as defined in Keren-Shaul et al. (2017).
(C) Signed Pearson’s correlation of the module eigengene (ME) with transgenic condition calculated in the rTg4510 microglia gene expression dataset at each age; unpaired 2-tailed t test; n = 7 modules, n = 4 mice per genotype (P301L MAPT or wild type [WT]); *p < 0.05, **p < 0.01, ***p < 0.005. Graphed with theoretical zero plotted at time zero.
(D) Module PPI network enrichment p value (STAR Methods).
(E) Module annotation showing representative module hub genes (disease genes in red), enriched Gene Ontology terms (Z score > 2), transcription factors (TFs) with binding site enrichment (p < 0.05; STAR Methods), and genes that function as receptors for pathogen- or damage-associated molecular patterns (“immune sensors”). TF labels are bold and italic if unique, and red if a module hub gene.
(F) Scatterplot of gene-module connectivity (kME) (y axis) versus gene correlation with sample pT231 tau levels (x axis) in TPR50 mouse brain (n = 36; frontal cortex, 6 months of age, n = 18 per group of WT or P301S MAPT; p values from 2-sided test for Pearson correlation).
(G) Module preservation in AD patient and control temporal cortex (control n = 74, AD n = 86), PSP patient and control temporal cortex (control n = 37, PSP n = 37) (Allen et al., 2016), and FTD patient and control frontal cortex (dataset 1 [Chen-Plotkin et al., 2008] control n = 8, FTD n = 10; dataset 2 [Swarup et al., 2019] control n = 14, FTD n = 16). The bottom line is at the lower cutoff for preservation (Zsummary = 2) and the upper line is at the cutoff for high preservation (Zsummary = 10) (Langfelder et al., 2011).
See also Figure S1.

RESULTS
Microglial Neurodegeneration-Associated Modules (MNMs) Capture Neuroimmune Interactions Enriched for Microglial Genes and Pathways
We performed consensus weighted gene co-expression analysis (WGCNA; Langfelder and Horvath, 2008; STAR Methods) to combine gene expression data from multiple independent transgenic models expressing human mutant MAPT, including sorted, purified microglia at different stages of neurodegeneration (from the rTg4510 model expressing the human P301L MAPT mutation; Wang et al., 2018) and whole-brain tissue (TPR50 model expressing the human P301S MAPT mutation; Swarup et al., 2019) to identify conserved modules of co-expressed genes present in both purified microglia and bulk brain tissue sampled from multiple mouse transgenic lines and genetic backgrounds (Figure 1A; STAR Methods). We identified 13 distinct, robust co-expression modules, including 7 modules enriched for microglial genes, but varying in their relationship to disease, cell type, and temporal trajectories; we call these microglial neurodegeneration-associated modules, MNMs (Figures 1B, S1, and S2; Table S1). We compared them with six independent microglia-specific gene expression datasets from transgenic mouse models and humans with AD pathology to identify overlapping patterns of gene expression (Figures 1B, S1C–S1E, and S2F; Table S2), supporting their generalizability. We observed significant overlap between MNMs and disease-associated microglia (DAM), including in their temporal progression (Keren-Shaul et al., 2017), exemplified by a downregulation of homeostatic and upregulation of DAM1 and DAM2 genes (Figure 1B). We note that the microglial modules defined here provide substantially more refinement than the original binary classification. Furthermore, M_UP1 significantly overlaps with a microglial subtype observed in human AD patients (Mathys et al., 2019; Figure 1B).

We capitalized on the sorted, purified microglia, combined with bulk tissue to deconvolute MNM modules into three distinct temporal trajectories with respect to progressive disease stages modeled in the rTg4510 mouse: (1) changing at the earliest
disease stage, before neuronal loss, and persistent through later stages (M_UP1, M_DOWN1, M_DOWN2); (2) changing during early periods of neuronal loss and transient (M_UP2, M_DOWN3); and (3) most significantly changing during the late stage of continued neuronal loss and cumulative pathology (M_UP3) (Figure 1C). We also tested whether these co-expression modules were preserved in protein-protein interaction data. We observed significant conservation of protein-protein interactions (PFIs) for all modules (Figure 1D; STAR Methods), further validation of their biological coherence. We next annotated the MNM in detail to uncover disease-associated microglial changes related to the progressive stages of tau-associated neurodegeneration and to identify regulatory drivers of specific microglia disease states and transitions.

We confirmed that the MNMs include expected traits indicative of neuroinflammatory activity, including: (1) known neuroregulatory pathways, such as complement activation (Schafer et al., 2013), Toll-like receptor (TLR), and interleukin-1β (IL-1β) (Heneka et al., 2013; Figure 1E); (2) strong association with tau pathology, a neuronal driver of neurodegeneration (Figures 1F and S2A); and (3) high overlap with the conserved neuroinflammatory module we identified in our prior study (NAI; Swarup et al., 2019; Figures S2B and S2C). In contrast, when we analyzed WGCNA modules generated using only sorted microglial cell gene expression data from the rTg4510 model, rather than consensus modules based on network edges shared between bulk tissue and the sorted cell data, the correlation with pathological forms of tau was substantially reduced (Figure 1F). Thus, combining bulk tissue and microglial-specific consensus WGCNA identified co-expression patterns that were present in latent forms, but not detected in the analysis of bulk tissue alone. This demonstrates the complementary nature of using both cell-specific and bulk tissue data to resolve neuroimmune-related microglial disease signatures that are closely tied to neuronal pathology.

To explore their preservation across neurodegenerative dementias, we tested module preservation in multiple mouse and human disease datasets (total n = 525 samples from 17 datasets; STAR Methods; Table S2), finding significant preservation in transcriptome data from post-mortem human brain tissue from AD (Allen et al., 2016), FTD (Chen-Plotkin et al., 2008; Swarup et al., 2019), and PSP (Allen et al., 2016) patients (Figure 1G). In addition, all MNMs are preserved in three different transgenic mouse models expressing human MAPT mutations (Swarup et al., 2019; Figure S2D) and in microglial-specific datasets from mouse models expressing PSEN2 (Srinivasan et al., 2016) and APP (Wang et al., 2015) mutations, with the exception of M_UP3, which is only weakly preserved in one of the two datasets from mice expressing early AD mutations (Figure S2E). A few modules display variability in their differential expression in different transgenic models, suggesting that they may be conditional on mutational interactions with disease-stage and disease-specific pathology, or possibly by other technical factors distinguishing these mouse models (Figure S2F).

**Pathway Analysis to Expand Biological Insights into Microglial Transitions across Disease**

The distinct module trajectories suggested that they represented discrete pathways associated with different stages of disease. The time course of the MNMs painted a detailed picture of microglia transitions across disease progression, such that MNMs represented specific biological regulators and pathways, disease genes, immune receptors, and transcription factors (TFs) associated with progressive stages of disease (Figures 1B–1E, 2A, and 2B). M_UP1 represents an early pro-inflammatory response, and the nearly mirror image early M_DOWN1 represents a loss of homeostasis. These are soon followed by the downregulation of cell-cycle and RNA-stabilizing genes represented by M_DOWN2, along with a transient pro-phagocytic response expressed by M_UP2. Finally, we observed an increased expression of genes involved in viral response and type II IFN response represented by M_UP3 (Figures 1E, 2A, 2B, and S2G).

Further examination of the genes within these modules known to regulate immune responses to exogenous signals suggested that gene expression patterns reflected the microglia response to different damage-associated immune activators at different stages of disease progression (Kigerl et al., 2014; Vénéreau et al., 2015; Figure 1E). For example, the earliest upregulated module M_UP1 includes sensors of peptide and lipopeptide immune activators (Tlr1 and Tlr2), whereas the subsequently upregulated module M_UP2 includes sensors of lipid immune activators (Trem2 and Scarb2) and nucleotides (Tlr7, Tlr9, and Ifih1). These nucleotide receptors are known to be activated by viruses or by damaged endogenous DNA or chromatin (Ahmad et al., 2018; Dhir et al., 2018; Dias Junior et al., 2019; Figure 1E). Therefore, this time course analysis suggests that microglia transition from an early response to peptide modulators toward responding to DNA and RNA and expressing genes involved in viral defense over the progression of pathology and neurodegeneration (Figure 2B).

**Genetic Risk Factors for FTD, PSP, and AD Converge on Different Viral Response Pathways**

We integrated genome-wide common genetic risk using MAGMA (de Leeuw et al., 2015) to determine whether any of the identified MNMs enrich for causal genetic factors. We identified the earliest interconnected MNM genes present in presymptomatic disease tissue and called them early MNM sub-modules, reasoning that casual disease pathways should enrich to regulatory drivers of specific microglia disease states and transitions.

To explore their preservation across neurodegenerative dementias, we tested module preservation in multiple mouse and human disease datasets (total n = 525 samples from 17 datasets; STAR Methods; Table S2), finding significant preservation in transcriptome data from post-mortem human brain tissue from AD (Allen et al., 2016), FTD (Chen-Plotkin et al., 2008; Swarup et al., 2019), and PSP (Allen et al., 2016) patients (Figure 1G). In addition, all MNMs are preserved in three different transgenic mouse models expressing human MAPT mutations (Swarup et al., 2019; Figure S2D) and in microglial-specific datasets from mouse models expressing PSEN2 (Srinivasan et al., 2016) and APP (Wang et al., 2015) mutations, with the exception of M_UP3, which is only weakly preserved in one of the two datasets from mice expressing early AD mutations (Figure S2E). A few modules display variability in their differential expression in different transgenic models, suggesting that they may be conditional on mutational interactions with disease-stage and disease-specific pathology, or possibly by other technical factors distinguishing these mouse models (Figure S2F).
Therefore, human genetic variation contributing to tau-associated dementias involves pathways typically associated with host viral defense. However, in AD, the causal genetic association is with positive regulation of viral defense response (upregulated), whereas in FTD and PSP, the causal association is with viral defense response in a downregulated module (Figures 3B and 3C). Overall, these results show that distinct aspects of microglial biology and viral response pathways are influenced by causal risk in AD versus FTD and PSP. Consistent with these causal relationships defined by genetic enrichment, we were able to demonstrate that the expression of genes involved with viral defense, including the type I IFN response
Figure 3. GWAS Variants for AD, FTD, or PSP Implicate Modules Associated with Viral Response in Causal Disease Biology

(A) Module enrichment for disease variants for AD (Lambert et al., 2013), FTD (Ferrari et al., 2014b), or PSP (Höglinger et al., 2011) (horizontal line: $-\log_{10}(FDR) = 1$; FDR corrected, competitive gene-set analysis p value from MAGMA [de Leeuw et al., 2015]).

(B) Gene co-expression network plots of the top 25 genes, ranked by kME, from each module; with enriched TFs (bold, if unique) shown ("TFBS"; enrichment p < 0.05).

(C) GO terms enrichment among corresponding module genes (Z score > 2).

See also Figure S3.
and dsRNA binding, were highly associated with the level of pathological tau phosphorylation in the brains of TPR50 mice (Figures S3H–S3J).

**Opposing Neuroimmune Activation and Suppression Modules**

We observed that early in disease, microglia upregulate a mixture of signaling receptors and immune response pathways, including viral defense and IFN response genes in two upregulated modules—M_UP1 and M_UP2 (Figures 1 and 2). To further explore the early microglial response in disease, we re-clustered only the genes from M_UP1 and M_UP2 to identify additional co-expression relationships among them (Figure 4A, Module A and Module B; Table S1). This resulted in two new modules upregulated early in disease with nearly identical trajectories (Figure 4B), but demonstrating strongly anti-correlated gene–module connectivity (anti-correlated gene-module connectivity [kME]; Figures 4C and 4D), suggestive of opposing or competing pathways (Langfelder and Horvath, 2008). To test this hypothesis, we leveraged the CMAP database to ask whether large-scale gene perturbations have opposing effects on the trajectories of these two modules (Subramanian et al., 2017). We found that gene overexpression has inverse effects on modules A and B (Figure 4D; 2,161 genes; STAR Methods), which we also confirmed at the levels of gene connectivity (Figure S4A) and PPI (Figure S4B). These patterns were confirmed in 12 independent transcriptome datasets (Figures S4D–S4I), indicating that these two modules represent robust, early microglial responses mediating opposing signaling pathways.

Module A includes protein complexes and pathways of microglial innate neuroimmune activation, including the NLRP3 inflammasome (Nlrp3, PyCARD, Il1a, Il1b, and Casp1) (Ahmed et al., 2017; Ising et al., 2019; Luciùnaitė et al., 2019), TLRs (Tlr1, Tlr2, Tlr7, and Tlr9), and nuclear factor κB (NF-κB) targets (Figures 4E, 4G, and S4A). Since this module represents early microglial innate immune activation in response to extracellular disease-associated signaling molecules (Ahmed et al., 2017), we called it the neuroimmune activation module (NAct). Supporting this interpretation, we found that purified pathological Aβ, an extracellular inducer of pro-inflammatory microglial activity (Luciùnaitė et al., 2019), stimulates NAct in microglia (Figures 4F and S4C). In contrast, module B includes viral and stress response genes, drivers of immune suppression (Cd274 [PD-L1], Ifit1, Il10b, Lag3, Usp18, and Nfkbia), and the senescence marker, Cdkn1a (Figures 4H and S4A). Therefore, we hypothesized that module B represents microglial neuroimmune suppression and called it the neuroimmune suppression module (NSupp).

We next used the CMAP database to find candidate drivers of NSupp and NAct (STAR Methods), identifying genes that regulate the viral activation of type I IFN among the top drivers of NSupp and suppressors of NAct (Prdf; Marazzi et al., 2012; Table S1). Consistent with this, the NSupp module contains both type I IFN receptor genes (Ifnar1 and Ifnar2), the principal downstream TF, Stat1, and multiple IFN response activators (Ifit1, Ifit2, and Ifit3) (Figures 4G and 4H). In contrast, the NAct module contains inhibitors of IFN-β production, including Ifitm3 (Jiang et al., 2018) and Prdm1 (Doody et al., 2010).

Therefore, we also hypothesized that type I IFN is a direct upstream driver of NSupp, which we were able to verify using data from mice treated with IFNb (Figure 4I). In contrast, we found that the treatment of mice with IFN-β decreases the microglial expression of NAct (Figure 4I). Because NAct contains no IFN receptor or activators of IFN signaling, our experimental evidence suggested that in microglia, NAct and NSupp appear to represent opposing disease-associated microglial states, likely orchestrated by direct type I IFN activation of NSupp, leading to secondary suppression of NAct.

Type I IFN is a critical driver of immune suppression and tolerance in certain chronic viral infections (Teijaro et al., 2013). Several features of NSupp suggest that in the context of tauopathy it may also represent an immunosuppressive type of IFN-β signaling in disease-associated microglia that are expressing inhibitory immune checkpoint genes (e.g., Cd274 [PD-L1], Ifit1, Ifit2, and Ifit3) (Snell et al., 2017; Wykes and Lewin, 2018) and inhibitors of immune hyperactivity (e.g., Usp18; Basters et al., 2018; Taylor et al., 2018; Figures 4G, 4H, and S4A). Consistent with the model of NSupp activation causing the partial suppression of immune defense, the IFN-induced immune suppressor gene Usp18 is a NSupp module hub (Figure 5A). Moreover,
USP18 modifies protein ISGylation to coordinate regulation of multiple genes and pathways in the NSupp module, including Isg15, proteostasis, translation, viral response, and exosome formation (Honke et al., 2016; Figure 4H).

Consequently, these bioinformatic analyses support Usp18 as a candidate regulator of the integrated biological response represented by NSupp, and therefore we predicted that Usp18 knockout would disrupt NSupp module connectivity. Remarkably, and consistent with Usp18 being a critical driver of NSupp (Figure 5A), we found that gene co-expression relationships in NSupp were severely disrupted by Usp18 knockout. This was despite these animals having a dramatic brain cellular inflammatory response (Goldmann et al., 2015). NAct remains intact in Usp18 knockout brain, illustrating the specificity of the relationship of Usp18 to NSupp integrity (Figure 5B) and supporting Usp18 as a critical driver of the integrated biological response to IFN-β in NSupp. In addition, we find that NAct is highly upregulated in the Usp18 knockout mouse brain in an IFNAR1-dependent fashion and in Usp18 knockout primary microglial cultures (Figures 5C and 5D). These data show that the immune suppressor Usp18 drives the NSupp module, which partially inhibits microglial NAct module gene upregulation in disease, such that when Usp18 is deleted, NSupp is essentially dissolved, and NAct is de-repressed and up-regulated. These results, summarized in Figure 5H, suggest that the microglial response to IFN-β in disease includes both coordinated changes in cellular function related to viral response (represented by NSupp) and partial suppression of TLR signaling (represented by NAct).

To facilitate proof-of-concept hypothesis testing of our findings, we synthesized the following key observations into a cohesive, testable model (Figure 5H). NAct is activated by extracellular stimuli working through TLR and related receptors (Figures 4E, 4F, 5A, and 5C). NAct is independently activated by the detection of aberrant intracellular nucleotides leading to type I IFN signaling (Figures 4G–4I) and this NSupp activation secondarily inhibits NAct (Figures 4C, 4D, 5C, and 5D). Genes that drive NSupp inhibit NAct, and vice versa (Figures 4C, 4D, and 4I). This model also describes how NAct and NSupp are functionally interconnected in disease (Figure 5H). NAct is activated initially, representing pro-inflammatory microglia activated by TLR signaling (Figures 4E, 4F, and 5H). As disease progresses, NSupp follows closely due to aberrant nucleotide detection and type I IFN signaling, which in turn partially suppresses NAct. Therefore, the model predicts that the inhibition of NSupp would cause a relative increase in NAct and a net increase in NAct activity, whereas the inhibition of NAct would cause a net decrease in NAct activity.

We experimentally validated a prediction of this model, that NSupp would suppress and NAct would activate microglial neuroimmune activity, using a chemical genomics approach (STAR Methods), identifying 2 compounds predicted to coordinate NAct and NSupp module-wide expression. Then, we tested the functional consequences of altering NAct activity using a co-culture of neurons with microglia-like BV2 cells, where BV2 expression of pathways that are highly represented in NAct drives the clearance of neuronal processes in cell culture (Höing et al., 2012; Figures 5E–5G). We found that high-dose saracatinib, a Src kinase inhibitor (Wölfli et al., 2013), increases NAct expression and decreases NSupp expression in primary mouse microglial cultures as predicted (Figures 5E and 5F, CMAP score = −90.42 [NSupp] and 36.88 [NAct]). In contrast, we found that fatostatin, a SREBP inhibitor, decreased NAct expression (CMAP score = −90.18 [NAct]), which we confirmed in primary mouse microglial cultures (Figures 5E and 5F). Therefore, we hypothesized that by reducing NSupp, saracatinib would increase the microglial clearance of neuronal process, and by decreasing net NAct activity, fatostatin would decrease the microglial clearance of neuronal processes in cell culture, which is what was observed (Figure 5G). This confirmed predictions that the chemical inhibition of NAct and NSupp has opposing effects on neuroimmune activation and suppression, respectively.

Figure 5. Microglial Immune Suppression Is Prominent in Disease

(A) PPI plot showing that Usp18 is a central NSupp module gene based on high PPI and ME connectivity (node color) in microglia purified from IFN-β and control treated mice (Deczkowska et al., 2017).

(B–D) Module preservation of NAct and NSupp modules (B); ME trajectory of NAct in Usp18 knockout, IFNAR knockout, double knockout, and WT mouse brain (C) (2-tailed unpaired t test; n = 3 per group [Goldmann et al., 2015]; boxplot with center line at median and upper and lower lines at 75th and 25th percentiles) and average module gene expression of NSupp and NAct in Usp18 knockout and WT mouse microglia (D) (2-tailed unpaired t test; n = 2 per group [Goldmann et al., 2015]).

(E) Module preservation of NAct and NSupp in primary mouse microglia cultures treated with drugs or DMSO vehicle as indicated in (F).

(F) ME trajectory of NAct and NSupp in primary mouse microglia cultures treated with high-dose saracatinib (1 μM, n = 10, 24 h) or DMSO vehicle control (n = 10, 24 h), and fatostatin (1 μM, n = 5, 72 h) or DMSO vehicle (n = 6, 72 h) (unpaired 2-sample Wilcoxon rank-sum test).

(G) Image of neuronal-BV2 co-cultures (left) showing neuronal processes (synaptophysin) and BV2 cells (non-neuronal DAPI+ cells), and density of neuronal processes in cell culture, which is what was observed (Figure 5G).

(H) Module enrichment for genes that are differentially expressed in microglia of AD compared to control brain based on published single nuclear sequencing studies (genes upregulated or downregulated in AD with log fold change [LFC] > 0.1 and FDR < 0.05 = “AD_up,” and “AD_down,” respectively; Fisher’s 2-tailed exact test; “FDR < 0.05,” “FDR < 0.001,” “FDR < 0.005” [Grubman et al., 2019; Mathys et al., 2019]).
A Model of Early Immunosuppression in Tau-Associated Neurodegeneration

Cumulatively, these unbiased, genome-wide data support a more refined model of early microglial neuroimmune signaling in neurodegenerative disorders with tauopathy (Figures 2B and 5H). At the onset of cellular dysfunction, a pro-inflammatory microglial neuroimmune response, NAct, is activated by extracellular peptide and lipopeptide damage signals. Then, as tau pathology leads to chromatin and nucleotide dysregulation before neuronal loss, nucleotide damage signals activate dsRNA receptors to trigger a microglial type I IFN response that drives the expression of NSupp pathways involved in stress response, exosome, and viral entry (Figure 4H), while suppressing the specific microglial immune attack pathways represented by NAct, including the NLRP3 inflammasome and TLR1 and -2 signaling (Figures 4E and S4A). This results in reactive microglia that suppress pathways early in disease that are related to pathogen clearance consistent with the known immunosuppressive role of IFN-β elsewhere (Snell et al., 2017). As disease progresses to include frank neurodegeneration, microglia undergo additional dynamic states, including increased type II IFN signaling, exogenous antigen presentation, T cell regulation, and cell death (Figures 1E, 2A, 2B, and S2G). Importantly, this model suggests that tau pathology may contribute to the inhibition of microglial immune clearance activity early in disease, causing a state of relative immunosuppression, which may lead to disease progression.

We tested this model with a proof-of-concept validation experiment in human microglia, asking whether type I IFN can functionally inhibit NAct. We pre-treated human-induced pluripotent stem cell (iPSC)-derived microglia (Haenseler et al., 2017; STAR Methods) with IFN-β and tested the ability of fibrillar Aβ, a NAct activator (Figures 4F and S4C), to induce the secretion of IL-1β, a prominent member of the NACT pathway in these human cells (Figures 4E and 4G). We observed that Aβ increased IL-1β secretion in untreated microglial cultures and that IFN-β treatment significantly suppressed Aβ-induced IL-1β secretion in human iPSC-derived microglial cultures (Figure 5I).

Validation by Single Nuclear Sequencing of Human Disease Tissue

Having annotated and functionally validated the NSupp and NAct modules, we levered them as biomarkers to measure microglial neuroimmune activity in human post-mortem disease samples. We found that NSupp upregulation is a consistent feature seen in the brain tissue of subjects with AD and PSP (Figure S5J), and in single-cell data from AD (Figure 5K), suggesting that microglial NAct suppression may be a shared feature of the chronic phases of these two tau-associated dementia syndromes.

Another prediction based on the mouse microglia module trajectory analysis (Figure 2) is a delayed, biphasic type II IFN response with early suppression and late increase (M_DOWN1, M_UP3; Figures 1C, 1E, 2A, 2B, and S2G). To validate the relevance of these findings to humans, we performed deep single-cell sequencing in human post-mortem brain from dementia patients and controls to determine the microglia changes associated with tau pathology. We performed single nuclear sequencing of 107,620 nuclei from pre-central gyrus from patients having behavioral variant FTD (bvFTD) with tau immunoreactive inclusions (Pick’s disease) and matched controls, identifying 2,891 microglial nuclei for this analysis after quality control (QC), clustering and cluster annotation (Figure 6A). Remarkably, we observed concordance between disease-associated signaling pathways represented in M_UP3 and microglia from patients with FTD-Pick’s; in particular in type II IFN, a specific activator of M_UP3 (Figures 2A, 2B, 6C, and S2G). Significantly upregulated in bvFTD microglia are genes that can stimulate IFN-γ production (IL18, LILRB1, TLR7, and SPP1) (Renkl et al., 2005) and genes that mediate IFN-γ response (IFNGR1, IRF8, B2M, and JAK2; Figures 6C, 6D, and S5E). Gene Ontology (GO) and PPI analyses demonstrate additional pathway-level alignment between M_UP3 and bvFTD microglia, including antigen presentation, IFN-γ signaling, and regulation of cell death (Figure 6C), further confirming that the latent microglia disease states that we identified in mice are relevant to human disease.

DISCUSSION

Through an integrative systems-biology approach, we have identified microglia neuroimmune networks related to early stages of neurodegeneration modeled in mice harboring mutant tau protein. Combining bulk tissue and cell-type-specific data from multiple divergent transgenic mice and human single-cell data, we identified seven conserved microglia modules that were also represented in post-mortem tissue from patients and controls. By integrating data from brain tissue with sorted cell data, we achieved a unique perspective on neuroinflammatory signaling in neurodegeneration that we show neither bulk tissue nor single-cell-type data can achieve on its own. The seven microglial co-expression modules identified represent a refined, more integrative, and complementary view of microglial neuroimmune trajectories representing distinct signaling and neuroimmune states in neurodegeneration.
Our refined analyses of microglia-associated changes at different stages of tauopathy suggest that early immune activation is accompanied by immune suppression, likely driven by the activation of IFN-β, and we provide multiple analyses supporting this model. Recent experimental data show that IFN receptor blockade reduces sustained microgliosis and synaptic clearance (Roy et al., 2020), and IFN treatment inhibits microglial phagocytosis (Mudu et al., 2019) and release of pro-inflammatory cytokines (Moore et al., 2020). Here, we provide complementary evidence that IFN-β may also suppress genes involved in microglial immune clearance (NAct; including Iilb, CD74, IL27ra, B2m, Fcerv1g, Cd14, Ptprc, Tril, Trem2, Cd68, and Cxcr4) and drive genes that function in early immune suppression (NSupp; PD-L1, Isg15, Tgfbr2, Usp18, and Zc3h12a; Mao et al., 2017). At later disease stages, microglia transition to expressing chronic viral response pathways, together with pathways that were relatively quiescent or downregulated at earlier stages, such as the type II IFN response.

Several endogenous stimuli are capable of activating type I IFN in disease (Cuellar et al., 2017; Dhir et al., 2018), but our observations suggest that pathological tau may trigger the IFN pathway through cytosolic dsRNA detection. We find that both dsRNA detection and IFN pathways are highly correlated with pathological tau burden in brain (Figures S3H–S3J) and both the cytosolic dsRNA receptor Ifi1f1 (MDAS) and RIG1 pathway are hubs of the NSupp module (Figure 4H). This is particularly salient based upon the recent observation that pathological tau drives chromatin destabilization (Guo et al., 2018; Sun et al., 2018), a known source of endogenous dsRNA that can activate Ifi1f1 (MDAS) and trigger an IFN response outside of the CNS (Ahmad et al., 2018; Cuellar et al., 2017). These intracellular events contrast with extracellular tau seeds and fibrils, which experimental evidence suggests activate microglia NAct pathways (Pampuscenko et al., 2020; Stancu et al., 2019).

These data suggest a parsimonious model wherein dsRNA, released following chromatin destabilization in injured neurons and/or other cells in response to tau pathology, activates early suppressive pathways driven by type I IFN (NSupp) to suppress acute microglia defense pathways that would clear infection (NAct). These observations predict that the inhibition of NSupp, either through blockade of dsRNA, IFNAR1 pathways, or immune checkpoints, would reduce early immune dysregulation triggered by pathological tau and, at least in part, restore microglia damage response and promote viral clearance mechanisms. Our model also predicts that without this suppression, disease pathology may drive immune hyperactivity and cellular injury.

These data fit with recent observations that IFN-driven microglial immunosuppression in aging may also contribute to age-related susceptibility to neurodegeneration (Deczkowska et al., 2017). In addition, our observation that AD, FTD, and PSP susceptibility genes converge on viral response pathways is consistent with the notion that the microglial type I IFN response may influence early disease progression, including the propagation of tau pathology (Ising et al., 2019; Stancu et al., 2019). These observations suggest an important causal connection between viral defense and pathological tau, with interactions varying over the course of disease.

From this perspective, different stages of dementia are associated with different forms or levels of immune activation. These analyses suggest that early immune suppression and delayed viral response, rather than immune activation alone, may contribute to disease progression and promote chronic inflammation as disease progresses into its clinical phase, particularly in response to tauopathy. Future functional and mechanistic studies will be needed to experimentally test and extend this model, which has significant implications for the development and timing of therapeutic interventions targeted at the neuroimmune response.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at [https://doi.org/10.1016/j.celrep.2020.108398](https://doi.org/10.1016/j.celrep.2020.108398).

ACKNOWLEDGMENTS

The results published here are in part based on data obtained from the AMP-AD Knowledge Portal (AMP-AD Knowledge Portal: [https://doi.org/10.7303/syn2580853](https://doi.org/10.7303/syn2580853)). We thank Eli Lilly and Company scientists for generating the rTg4510 microglia RNA-seq data (Wang et al., 2018) and providing us access to them. For the FTD GWAS summary statistics used for MAGMA, we acknowledge the investigators of the original study (Ferraro et al., 2014a). We thank Dr. Timothy Hammond and Dr. Marta Olah for use of their microglial
single-cell data and discussion and Dr. Chris Hartl for helpful complementary analysis and discussion. Funding for this work was provided by Takeda Pharmaceu-
ticals (D.H.G.), Rainwater Charitable Foundation (D.H.G. and W.W.S.), Roche Pharmaceuticals (D.H.G.), BrightFocus (D.H.G. and J.E.R.), NIH grants to J.E.R and T.S.C. (R01 NS065723) and J.E.R (K08 NS105916), Fineberg Foundation (J.E.R.), and the John Douglas French Alzheimer’s Foundation (J.E.R.). The UCSF Neurodegenerative Disease Brain Bank is supported by NIH grants AG023501 and AG019724, the Rainwater Charitable Foundation, and the Bluefield Project to Cure FTD. The University of Pennsylvania Center for Neurodegenerative Disease Research is supported by NIH grants P30-AG010123 Alzheimer’s Disease Core Center, U19-AG062418 Center on Alpha-synuclein Strains in Alzheimer Disease & Related Dementias, P01-AG017586 Frontotemporal Dementias: Genotypes and Phenotypes, U19-NS110456U54 Center without Walls for Imaging Proteinopathies with PET (CW2IP2), and U54-RFA-NS-19-030 Connect-TBI.

AUTHOR CONTRIBUTIONS

J.E.R. and D.H.G. designed and supervised the experiments, analyzed and inter-
preted the results, and wrote the manuscript. J.E.R. completed the experi-
ments, performed the analyses, and generated figures and tables. D.P. per-
formed single nuclear sequencing analysis and differential gene expression on bvFTD and control brain samples. A.Y. performed the IPSC-microglia ex-
periments. J.H. and A.Y. assisted with single nuclear isolation experiments. D.M. contributed to the snRNA-seq study design and sequencing. L.-C.L., W.S., and J.O.T. curated and provided disease and control brain tissues used for snRNA-seq. V.S. and L.C. contributed to the preparation of raw sequencing data, including QC and normalization, and V.S. provided technical training and supervision of the cWGCNA experiments. T.S.C. contributed exome-based validation of FTD, AD, and PSP module associations. T.N. assisted with image analysis and quantification of the BV2 cell co-culture experi-
ments. A.S. assisted with the BV2 cell co-culture experiments.

DECLARATION OF INTERESTS

D.H.G. has received research funding from Takeda Pharmaceuticals and Hoff-
man-LaRoche. D.M. is a full-time employee of F. Hoffmann-La Roche, Basel, Switzerland.

Received: June 17, 2020
Revised: June 29, 2020
Accepted: October 28, 2020
Published: November 17, 2020

REFERENCES


Ahmed, M.E., Iyer, S., Thangavel, R., Kumpuraj, D., Selvakumar, G.P., Raik-


# STAR METHODS

## KEY RESOURCES TABLE

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Critical Commercial Assays

NucleoSpin RNA Plus | Takara | Cat# 740984.50
QuantSeq 3’ End Labeling Kit | Lexogen | Cat# 015.96
Total Tau Human ELISA Kit | Thermo Fisher | Cat# KH80041
pT231 Tau Human ELISA Kit | Thermo Fisher | Cat# KHBB051
IL-1 beta Mouse ELISA Kit | R&D Systems | Cat# DY401
Chromium Single Cell 3’ Reagent Kits V2 | 10X Genomics | Cat# 1000075

Deposited Data

TPR50 Mouse (male P301S MAPT and WT) microarray or RNaseq transcriptomics dataset | (Swarup et al., 2019) | GEO: GSE90696
rTg4510 microglia dataset | (Wang et al., 2018) | AMP-AD Knowledge Portal: https://www.synapse.org/#!/Synapse:syn884351
JNPL3 mouse datasets | AMP-AD | AMP-AD Knowledge Portal: https://adknowledgeportal.synapse.org/Explore/Studies/DetailsPage?studyId=3157182
PS2APP microglia RNaseq | (Srinivasan et al., 2016) | GEO: GSE75431
USP18 Knockout and IFNAR Knockout | (Goldmann et al., 2015) | GEO: GSE61499; GEO: GSE61500
5xFAD microglia RNaseq | (Wang et al., 2015) | GEO: GSE65067
CK-p25 | (Mathys et al., 2017) | GEO: GSE103334
In vivo Abeta-42 | (Johansson et al., 2015) | GEO: GSE57181
in vitro treatments with Abeta-42 | (Woodling et al., 2014) | GEO: GSE55627
Microglia isolated from IFN-beta AAV infected mouse brain | (Deczkowska et al., 2017) | GEO: GSE98401
IFN-gamma human microglia | (Flock et al., 2005) | GEO: GSE1432
Human postmortem data-FTD RNaseq | (Chen-Plotkin et al., 2008) | GEO: GSE13162
Human postmortem data-FTD frontal cortex | (Swarup et al., 2019) | Synapse: https://www.synapse.org/#!Synapse:syn7818788
Single nuclear sequencing data from Alzheimer’s and control prefrontal cortex | (Mathys et al., 2019) | Synapse: https://www.synapse.org/#!Synapse:syn1845175
Single nuclear sequencing data from Alzheimer’s and control Entorhinal cortex | (Grubman et al., 2019) | GEO: GSE138852
Single cell data from injured mouse brain | (Hammond et al., 2019) | GEO: GSE121654
Alzheimer’s disease, FTD, and PSP exome array data | (Chen et al., 2015) | NIAGADS: https://www.niagads.org/, accession number NG00040
Single nuclear sequencing data from Pick’s disease and control precentral gyrus | This study | dbGAP: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs002197.v1.p1
Mouse microglia treated with fatostatin or saracatinib | This study | GEO: GSE148866
5xFAD microglial single cell data | (Keren-Shaul et al., 2017) | GEO: GSE98969

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RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Professor Daniel H. Geschwind (dhg@mednet.ucla.edu).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
RNaseq data of mice microglia cultures treated with fatostatin and saracatinib are available at the NCBI Gene Expression Omnibus (GEO) database under accession number GEO: GSE146866.

FTD-tau and control snSeq data are available from dbGAP under accession number dbGAP: phs002197.v1.p1

Custom code used for the analysis is available on github: https://github.com/dhglab/Tau-pathology-drives-dementia-risk-associated-gene-networks-towards-chronic-inflammatory-states-and

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Subjects
Frozen brain tissue (precentral gyrus) were obtained from the UCSF Neurodegenerative Disease Brain Bank and University of Pennsylvania Center for Neurodegenerative Disease Research Brain Bank. We obtained samples from 16 total individuals, including 8

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subjects with clinical diagnosis of behavior variant frontotemporal dementia and neuropathological diagnosis of Pick's disease (Tau protein pathology) and controls (n = 8). Samples were balanced age (mean: 68 years old -Pick’s, 66 years old -control), PMI (mean: 15.98 hours – Pick’s, 12.78 hours - control) and tissue RNA integrity number (RIN; mean: 6.1 -Pick’s, 5.6 -controls) (Table S2). Samples were obtained with informed consent and institutional IRB approval for each brain bank. IRB exemption was obtained from the UCLA IRB to authorize use of de-identified human postmortem brain single nuclear sequencing data in this study. Neuropathological diagnosis was verified by Professor William Seeley at UCSF.

**Cell Lines**

The induced pluripotent stem cells (iPSC) line #1205 (a de-identified, human control line; Sloan et al., 2018) was provided by the lab of professor Sergiu Pasca (Stanford University); and obtained with informed consent and institutional IRB approval by the Pasca lab. Use of this line in the Geschwind lab is approved by the UCLA Embryonic Stem Cell Research Oversight (ESCOR) Committee. The iPSC line was checked when thawed and confirmed for expression of markers of pluripotency (OCT4, SSEA4). For line maintenance and quality control, the line was tested to confirm negative mycoplasma contamination, and checked for chromosomal copy number changes using comparative genomic hybridization array. CO2 was tightly controlled. iPSC cell morphology was visually inspected to ensure against spontaneous differentiation. For cell cultures, iPSCs were plated on 5μg/mL Vitronectin (Thermo Fisher, A14700) coated plates in Essential 8 (E8) media (Thermo Fisher, A1517001). Cells were maintained with daily media changes and kept at 37°C and 5% CO2. Cells were passaged approximately every 4 days or at 60%–70% confluency using 0.5mL EDTA/DPBS for 3 minutes at RT. EDTA/DPBS was aspirated and cells were blown off by gently using 1mL of media. iPSCs were passaged as small cellular clusters at a 1:10 dilution to help proliferation.

iPSC derived microglia-like cells were generated and validated as previously described (Haenseler et al., 2017). Embryoid bodies (EBs) were made by adding 3x10^5 iPSCs to each Aggrewell-800 well (24-well plate; StemCell Technologies, 34850) in 2mL of E8 media supplemented with 50ng/mL BMP-4 (Peprotech, 120-05ET), 50ng/mL VEGF (Biologic, 583702), and 20ng/mL SCF (Peprotech, 300-07). ROCK inhibitor Y-27632 was added to the media only on day 0. For days 1-4, media was replaced by performing two-half changes daily. On day 5, EBs were gently collected with wide-boar pipette tips and transferred to T-75 flasks with 12mL of MacPre media. EBs were maintained in macrophage precursor (MacPre) media, consisting of AIM-V media (Thermo Fisher, 12055083) supplemented with 100U/mL penicillin/streptomycin, 0.055mM beta-mercaptoethanol (Life Technologies, 31350-010), 100ng/mL M-CSF (Biologic, 574806), 25ng/mL IL-3 (Biologic, 578006), and 2mM GlutaMAX (Thermo Fisher, 35050071). EBs seeded in T-75 flasks were cultured at 37°C and 5% CO2 for 7-12 days before performing weekly media changes to ensure EBs attached to the flask. Macrophage precursor cells were released from EBs into the media approximately 3-4 weeks post-seeding of EBs into flasks. At that point, the supernatant was collected weekly and passed through a 40μm cell strainer. Supernatant was spun down at 500xg for 5 minutes to pellet cells. Macrophage precursor cells were resuspended in microglia media to differentiate cells toward a microglia-like state and plated on non-treated tissue culture plates. Human 1205 iPSC-derived microglia cells were maintained in RPMI media (Thermo Fisher, 11875093) supplemented with 2mM GlutaMAX, 100U/mL penicillin/streptomycin, 0.055mM beta-mercaptoethanol, 1x N2 supplement (Thermo Fisher, 17502048), 100ng/mL IL-34 (Biolegend, 577906), and 10ng/mL GM-CSF (Biolegend, 766104). Microglia-like cells can be maintained up to 14 days after initial plating with media changes every 3-4 days.

BV2 female C57BL/6 mouse microglia cultures (RRID: CVCL_0182) were passaged at 70%-80% confluency weekly. 0.25% Trypsin/EDTA was used to dissociate cells and suspended down to 1000rpm for 5 minutes to pellet the cells. Supernatant was aspirated and cells were resuspended and passed at 1:10 on 6-well plates for maintenance with complete media. Cells were kept in 37°C with 5% CO2 in DMEM media (Thermo Fisher, 11995065) supplemented with 5% heat-inactivated fetal bovine serum (HI-FBS) and 1% Penicillin/Streptomycin. Media was replaced every 3-4 days.

**Primary Cell Cultures**

Mouse primary embryonic cortical neurons were prepared from E15 C57BL/6J embryos. Briefly, cortical tissue from day 15 (E15) C57BL/6J embryos was collected, dissected and washed in ice-cold Hank’s balanced salt solution (HBSS; Thermo Fisher, 14175095). Tissue was incubated in 0.25% trypsin (Invitrogen) in the presence of Dnase I (Roche, 4536282001) at 37°C for 10 minutes. Tissue was washed with cold HBSS and triturated in plating media (Neurobasal Medium; Thermo Fisher Scientific, 10888-022), 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, 16140071), 25mM sucrose and 0.25% GlutaMAX (Thermo Fisher, 35050061) in the presence of Dnase I. Dissociated cells were centrifuged at 125xg for 5 minutes at 4°C, resuspended in culture medium, counted and plated in poly-D-lysine coated and etched glass coverslips. Neurons were cultured in Neurobasal Medium supplemented with 2% B27 (17504-044), 2mM GlutaMAX and penicillin/streptomycin (100 U/mL). BV2-neuronal cocultures were also maintained in this neuronal culture media.

Mouse primary embryonic microglial cultures were prepared from P1 C57BL/6J pups as previously described (Saura et al., 2003). This protocol has two steps – first the culturing of mixed glia followed by isolation of microglia. Cortical tissues were collected in 0.1% glucose/PBS. Meninges and blood vessels were removed and brains were transferred to 6mL of 0.05% trypsin/PBS (Invitrogen) and pipetted through P1000 tip one time to gently mince. Tissue was incubated for 20 minutes at 37°C and transferred to 50mL conical tube containing 25mL of DMEM-F12 (Thermo Fisher, 1132003) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS; Thermo Fisher). Tissue settled to bottom of tube, and the media was replaced with fresh 30mL of DMEM-F12 (Thermo Fisher) + 10% HI-FBS. Samples were triturated until completed homogenized and aliquoted (at 1/2 brain per plate) into 150mm Petri dish containing
were removed by incubating in 5mL of 0.25% Trypsin/HBSS for 3 minutes at 37°C off the plate. Media containing astrocytes was aspirated and cells washed 3x with 10mL of PBS. Subsequently, adherent microglia were removed by incubating in 5mL of 0.25% Trypsin/HBSS for 3 minutes at 37°C. Microglia were then collected in 5mL of DMEM-F12 + 10% HI-FBS, spun down, and resuspended in 5mL culture media (DMEM-F12 supplemented with 2% HI-FBS, penicillin/streptomycin (100U/mL) and 50μM beta-mercatopentanol) for 24 hours prior to drug treatment. Microglia purity was assessed by immunocytochemistry.

**METHOD DETAILS**

**Dataset Acquisition and Filtering**

Both RNAseq datasets used as input for consensus WGCNA were previously generated. The TPR50 dataset (Swarup et al., 2019) includes gene expression data from frontal cortex dissected from male mice expressing P301S MAPT or WT controls (TPR50 transgenic model (Onishi et al., 2014)) in three different genetic backgrounds (C57BL6/J, F1 C57BL6/J x DAB, F1 C57BL6/J x FVB), and includes samples collected at 3 months of age (n = 6 per group) and 6 months of age (n = 5-6 per group). The Tg4510 microglia dataset includes gene expression data obtained from microglia purified using C11b FACS collected from mice expressing P301L MAPT and WT controls (Tg4510 transgenic model (Santacruz et al., 2005)), pooled to include microglia from 8-10 forebrains per sample, with n = 4 replicate samples per time points (2, 4, 6, and 8 months of age) (AMP-AD Knowledge Portal: https://doi.org/10.7303/syn2580853) (Wang et al., 2018). Data were filtered for low read counts (>80% of the sample with >10 reads with HTSeq quantification) and normalized using log2-transformation and linear regression prior to use for consensus WGCNA and module expression trajectory analysis, as previously described (Swarup et al., 2019). The Tg4510 transgenic mouse is a model of neurodegeneration secondary to human mutant MAPT and additional factors (Gamache et al., 2019).

Additional publicly available datasets were used throughout the study for validation or comparison (Table S2; and Key Resources Table). Mouse datasets consist of microarray or RNAseq transcriptomics data from a variety of transgenic mice models –PS19 (Swarup et al., 2019), rTg4510 and JNPL3 (AMP-AD Knowledge Portal, available on Synapse: https://www.synapse.org), PS2APP (Srinivasan et al., 2016), USP18 knockout (Goldmann et al., 2015), IFNAR knockout (Goldmann et al., 2015), 5xFAD (Wang et al., 2015), CK-p25 (Mathys et al., 2017), and in vitro and in vivo treatments – Abeta42 (Johansson et al., 2015; Woodling et al., 2014), IFN-beta-expressing AAV (Deczkowska et al., 2017), and IFN-gamma (Rock et al., 2005). Human postmortem data consist of AD temporal cortex (Allen et al., 2016), FTD frontal cortex, and PSP temporal cortex (Allen et al., 2016), as well as single nuclear sequencing data from Alzheimer’s and control prefrontal (Mathys et al., 2019) and entorhinal cortex (Grubman et al., 2019). IRB exemption was obtained from the UCLA IRB to authorize use of de-identified human postmortem sequencing data in this study.

Microarray or RNAseq datasets downloaded from the Gene Expression Omnibus (GEO) were read into R and processed as follows. Microarray data were log2-transformed and normalized by quantile normalization. Gene counts were filtered to remove low read counts (>80% of the sample with >10 reads with HTSeq quantification), corrected for guanine-cytosine content, gene length and library size, and log2-transformed using the COIN package in R (Hansen et al., 2012). The resulting data was used as an input to test module preservation, average gene expression and/or eigengene expression.

**mRNA Weighted Co-expression Network Analysis**

In order to identify gene co-expression networks present both in purified microglia and frontal cortical brain tissue, and across multiple transgenic mouse strains and genetic backgrounds, we utilized consensus WGCNA as previously described (Swarup et al., 2019) using the WGCNA R package (Langfelder and Horvath, 2008), applied to the TPR50 dataset of forebrain RNAseq from mice aged 6 months, and the Tg4510 dataset of purified microglia described above. The input data were generated from (1) microglia purified from P301L MAPT and WT mice from the Tg4510 model (Santacruz et al., 2005) at ages 2, 4, 6 and 8 months (n = 4 mice per condition) (AMP-AD Knowledge Portal: https://doi.org/10.7303/syn2580853) (Wang et al., 2018), and (2) frontal cortex from P301S MAPT and WT mice from the TPR50 model with three different genetic backgrounds (C57BL6/J, F1 C57BL6/J x DAB, F1 C57BL6/J x FVB) at 6 months of age (n = 5-6 per group), a period with extensive gliosis and neuronal Tau pathology but prior to frank atrophy (Swarup et al., 2019).

Similarity matrices were created using the Consensus WGCNA method as previously described (Li and Horvath, 2007). In the signed network, the similarity between genes reflects the sign of the correlation of their expression profiles. The signed similarity matrix was then raised to power β to emphasize strong correlations and reduce the emphasis of weak correlations on an exponential scale. A thresholding power of 14 was chosen (as it was the smallest threshold that resulted in a scale-free R2 fit of 0.8) and the consensus network was created using the function blockwiseConsensusModules() to calculate the component-wise minimum values for topologic overlap (TOM), with parameters set as networkType = "signed," deepSplit = 2, detectcutHeight = 0.995, consensusQuantile = 0.0, minModuleSize = 100, mergeCutHeight = 0.2. Using 1 – TOM (dissTOM) as the distance measure, genes were hierarchically clustered. The resulting modules or groups of co-expressed genes were used to calculate module eigengenes (MEs; or the 1st principal component of the module). Gene – module eigengene connectivity score (kME, was calculated using the WGCNA.
function consensusKME() with parameters set to consensusQuantile = 0.20, signed = TRUE. Modules were annotated using the GOElite package (Zambon et al., 2012). We performed module preservation analysis using consensus module definitions (Langfelder et al., 2011). MEs were correlated with transgenic condition to find disease-associated modules. Module hubs were defined by calculating module membership (kME) values which are the Pearson correlations between each gene and each ME. Gene expression was correlated with pT231 Tau levels measured by ELISA to calculate the “gene significance” relationship with pT231 Tau, as defined by the WGCNA method (Langfelder and Horvath, 2008), using gene expression data from the TPR50 model (6 months, n = 36), and this was further correlated (Pearson’s) with kME to assess the relationship between pT231 Tau and gene-module connectivity. All network plots were constructed using the Cytoscape software (Saito et al., 2012). Module definitions from the network analysis were used to create synthetic eigengenes from which to calculate the expression trajectory of various modules in different gene expression datasets.

Clustering of Gene Subsets
To apply gene co-expression methods to understand co-expression relationships among subsets of module genes in either the original consensus dataset, or in the TPR50 dataset of pre-symptomatic mice at 3 month of age, we again used the WGCNA package (Langfelder and Horvath, 2008). Biweighted mid-correlations were calculated for a subset of genes from selected consensus modules to create an adjacency matrix that was further transformed into a topological overlap matrix (with TOMType = “unsigned”). Using 1 – TOM (disTOM) as the distance measure, genes were hierarchically clustered using the following parameters (deepSplit = 2, detectcutHeight = 0.999, minModuleSize = 40, dtresh = 0.1, softPower = 7). The resulting modules, or groups of co-expressed genes, were used to calculate module eigengenes (MEs; or the 1st principal component of the module). The significance of intramodular connectivity was assessed for each module using a permutation test (10,000 permutations), and all modules were confirmed to have permuted p value < 0.001. “Early submodules,” described in Figures 3 and S3, were derived by re-clustering M_UP1 and M_UP2 genes to generate “earlyUP” modules, or M_DOWN1, M_DOWN2 and M_DOWN3 genes to generate “earlyDOWN” modules, using the 3 month of age frontal cortex TPR50 gene expression data (Swarp et al., 2019). “NSupp and NAct,” described in Figures 4 and 5, were derived from re-clustering the M_UP1 and M_UP2 genes, keeping only genes with annotated PPI in either Biogrid (Stark et al., 2006) or Inweb databases (Rossin et al., 2011), using the same gene expression data used for the consensus WGCNA analysis (purified microglia from the Tg4510 model and frontal cortex TPR50 dataset (6 months of age)).

Module Preservation Analysis
We used module preservation analysis (Langfelder et al., 2011) to validate co-expression in independent mouse and human datasets. Module definitions from consensus network analysis were used as reference and the analysis was used to calculate the Zsummary statistic for each module. This measure combines module density and intramodular connectivity metrics to give a composite statistic where Z > 2 suggests moderate preservation and Z > 10 suggests high preservation (Langfelder et al., 2011).

Module Gene Set Enrichment Analysis
Gene set enrichment analysis was performed using a two-sided Fisher exact test with 95% confidence intervals calculated according to the R function fisher.test(). We used p values from this two-sided approach for the one-sided test (which is equivalent to the hypergeometric p-value) as we do not assume a priori enrichment (Rivals et al., 2007). To reduce false positives, we used FDR adjusted p values (Benjamini and Hochberg, 1995) for multiple hypergeometric test comparisons. For cell-type enrichment analysis we used published mouse brain dataset (Zhang et al., 2014). The test background for over-representation analyses was chosen as total genes input into the consensus analysis (overlap of genes expressed in Tg4510 microglia and TPR50 frontal cortex RNaseq datasets).

To test module enrichment for single cell microglial gene expression signatures, we used signatures defined from publicly available single-cell studies pertaining to microglia and/or neurodegenerative disease (Hammond et al., 2019; Keren-Shaul et al., 2017; Mathys et al., 2017; Olah et al., 2018; Scott et al., 2018). Specifically, for disease-associated microglia (Keren-Shaul et al., 2017; Mathys et al., 2017), we set cluster signatures to be the top 100 differentially expressed genes between two microglia clusters, as defined in their corresponding publications. For microglial and macrophage clusters defined from young and aged mouse brain in (Hammond et al., 2019), we defined clusters signatures as published except duplicated genes were removed among the young cluster group (C1, C2a, C2b, C3, C4, C5, C6, C7a, C7b, C7c, C8, C9, mono_macA, mono_macB), and aged cluster group (aging_C1a, aging_C1b, aging_C2, aging_C3, aging_C4) to increase the distinctiveness of each cluster. To define genesets from the single-cell microglial trends from injured mouse brain published in (Hammond et al., 2019), we used the genes with fold change > 1.5 in control versus injured, and injured versus control mice, respectively, to define the injury_C1 and injury_C2 genesets. For human microglial gene clusters defined in (Olah et al., 2018), we defined cluster signatures as genes with expression fold > 1.8 compared to any other clusters. For human Alzheimer’s disease brain single cell signatures (Grubman et al., 2019; Mathys et al., 2019), we defined differentially expressed genes as those with FDR < 0.05 and logFC > 0.1 using the published differential gene expression data.

Gene Set Annotation
Genes in network modules were characterized using GO-Elite (version 1.2.5), using as background the set of input genes used to generated the modules being annotated (Zambon et al., 2012). GO-Elite uses a Z-score approximation of the hypergeometric distribution to assess term enrichment, and removes redundant GO or KEGG terms to give a concise output. We used 10,000
permutations and required at least 3 genes to be enriched in a given pathway at a Z score of at least 2. We report only biological process and molecular function category output.

**Protein-Protein Interaction Analysis**

To assess and visualize protein-protein interactions among module genes, we used STRING (version 10.5; [Szklarczyk et al., 2017] with the following setting (organism: *Mus musculus* for mouse data, and *Homo sapiens* for human data; meaning of network edges: confidence; active interaction sources: experiments and databases; minimal required interaction score: medium confidence (0.400), max number of interactors to show: none). Data was exported and visualized using the Cytoscape software ([Saito et al., 2012]).

**Transcription Factor Binding Site Enrichment Analysis**

Transcription Factor Binding Site (TFBS) enrichment analysis using an in-house package that incorporates TFBS as previously described ([Chandran et al., 2016]). Briefly, this published code uses TFBS position weight matrices (PWMs) from JASPAR and TRANSFAC databases ([Matys et al., 2003; Portales-Casamar et al., 2010]) to examine the enrichment for TFBS within each module using the Clover algorithm ([Frith et al., 2004]). To compute the enrichment analysis, we utilized three different background datasets (1000 bp sequences upstream of all mouse genes, mouse CpG islands, and mouse chromosome 20 sequence).

**Connectivity Map (CMAP) Analysis**

For a given module, the top 150 module genes (ranked by kME) were used as input for the QUERY app in the Broad’s CMAP database, version CLUE ([Subramanian et al., 2017]). This signature was used to query 7,494 gene overexpression or knockdown experiments carried out across 9 cell lines for similar (positive connectivity score) or opposite (negative connectivity score) effects on gene expression signatures, incorporating Kolmogorov-Smirnov statistics (a nonparametric, rank-based pattern-matching strategy) as described ([Lamb et al., 2006; Subramanian et al., 2017]). Per the CMAP website ([https://clue.io](https://clue.io)), for each module-perturbagen pair, the connectivity score (\(\tau\)) is a standardized percentile score that compares the similarity of the query geneset to the perturbagen compared to all other reference genesets in CMAP; such that 95 indicates that 5% of reference genesets show stronger connectivity to the perturbagen than the query dataset. For our analysis, we used the mean “connectivity scores” which is calculated from the combining data generated independently in 9 cell lines (see Table S1).

**MAGMA**

Summary statistics for genome-wide association studies for AD ([Lambert et al., 2013]), PSP ([Höglinger et al., 2011]) and FTD ([Ferrari et al., 2014a]) were used as an input for MAGMA (v1.06) ([de Leeuw et al., 2015]) for gene annotation to map SNPs onto genes (with annotate window = 20,20) and the competitive gene set analysis was performed to test module associations with GWAS variants (permutations = 100,000). All genes assigned to a given module were used as the input for each module. Consensus modules and re-clustered modules were run as separate groups in MAGMA given that they contain overlapping genes. Additional FDR correction was applied across all the competitive p value outputs from MAGMA for all modules used in the study.

**Exome-based Validation of MAGMA**

Summary statistics from Alzheimer’s disease, frontotemporal dementia and progressive supranuclear palsy exome array analyses were downloaded from ([Chen et al., 2015]). To incorporate protein-protein interaction, summary statistics were used as input to the network burden test, NetSig ([Hom et al., 2018]). NetSig determines a gene’s network association with disease. Generalized least-squares regression was used to determine if NetSig results were enriched in gene modules. Regression covariates included gene length and mean protein expression, including the log of these values. To account for linkage disequilibrium, error was correlated for genes within 5 megabase pairs.

**Drug Treatments**

Saracatinib (S1006) and fatostatin (F8932) were purchased from Selleckchem and Sigma. Primary microglia were treated with saracatinib (1 \(\mu\)M, 24h), fatotastatin (1 \(\mu\)M, 72h) or corresponding DMSO vehicle control as indicated. BV2 cells were pretreated with saracatinib (1 \(\mu\)M, 72h), fatotastatin (1 \(\mu\)M, 72h) or DMSO vehicle control (equal volume as drug, 72h) in BV2 cell culture media. Pre-treated BV2 cells were dissociated using trypsin/EDTA, washed and resuspended in neuronal culture media, counted, and plated on top of mouse primary neuronal cultures (aged 14 days *in vitro*) at a ratio of 1 BV2 cell: 3 neurons. BV2 cell-neuronal co-cultures were treated with DMSO or DMSO vehicle control for three days in neuronal culture media (Neurobasal medium (Thermo Fisher) supplemented with 2% B27 (17504-044), 2 mM GlutaMAX Supplement (Thermo Fisher) and penicillin/streptomycin (100 U/mL)).

iPSC-induced microglia cultures were plated on black 96-well clear bottom plates (Corning, 3603) and maintained in RPMI media (Thermo Fisher, cat no 11875085) supplemented with 100ng/mL IL-34 (Biolegend, 577906) and 10ng/mL GM-CSF (Biolegend, 766404) for 10 days prior to experimentation. Human iPSC-induced microglia and were then treated in culture media with 1\(\mu\)M fibrillar amyloid beta for 6 hours at 37°C and 5% CO2 with or without pretreatment of 20ng/mL recombinant human interferon beta (IFN-\(\beta\); R&D Systems, cat no. 8499-IF) for 2 hours.
Cell Staining and Quantification

Following BV2-neuronal coculture, cells were fixed in 4% paraformaldehyde, permeabilized for 20 minutes in 0.25% Tween-20, and immunostained with a microglial marker rabbit anti-Iba1 (1:1000, Wako, 019-19741), and guinea pig anti-synaptophysin (1:1000; Synaptic Systems) for 2 hours at room temperature, followed by secondary antibodies (donkey anti-rabbit 488 (1:500; Invitrogen, A21206); goat anti-guinea pig 555 (1:500; Invitrogen, A21435) for one hour at room temperature. After washing the slides with Tris-buffered saline with Tween-20 (TBST), slides were stained with DAPI (4',6-diamidino-2-phenylindole, a blue-fluorescent DNA stain). Cells were imaged at 20x using Zeiss Axiosplan II microscope with six representative images captured and quantified per coverslip in a blinded fashion. The local density of neuronal processes surrounding BV2 cells was quantified in ImageJ by measuring the synaptophysin staining density within the BV2 cell and surrounding area (defined by BV2 nuclei (DAPI) area and adjacent area extending out from the DAPI image perimeter using the ImageJ function “Mask of Image Points” (version 1.1) with settings “add mask points within distance” = 1 micron. We used the expanded DAPI area to localize BV2 cells rather than the Iba1 signal because Iba1 staining intensity varied from cell to cell.

RNA sequencing

RNA was isolated from primary mouse microglia using NucleoSpin RNA Plus (Takara, 740984.50) and RNA sequencing libraries were prepared using the QuantSeq 3’ end labeling kit (Lexogen, 015.96) according to the manufacturer’s protocol, and sequenced on a HiSeq4000 to 50M single end reads (1 × 65bp). Reads were aligned against GRCm38 using STAR (Dobin et al., 2013). Transcripts were quantified and annotated against GencodeM11 using Rsubread (Liao et al., 2019). Sample QC was performed using network connectivity from the WGCNA package (Langfelder and Horvath, 2008) and z-score relative to the mean gene PCA, excluding samples that were > 3 standard deviations. Samples were normalized with CQN (Hansen et al., 2012). Sequencing batch correction, when needed, was performed using ComBat (Johnson et al., 2007).

ELISA

Total tau and pT231 tau contents were measured by commercial tau ELISA kits according to the manufacturer’s instructions (total tau - KHB0041; pT231 tau - KHB8051, Invitrogen), as previously described (Swarup et al., 2019). Mouse IL-1beta was quantified using cell culture supernatant via ELISA (R&D Systems, DY401) according to the manufacturer’s protocol, with absorbance measured at 450nm.

Single Nuclear Sequencing (snRNaseq)

Nuclei were prepared from 60-70mg of frozen brain tissue per sample, with all procedures carried out on ice or at 4°C with RNase-free reagents. Briefly, postmortem frozen brain tissue was lysed in 3mL homogenization buffer (250mM sucrose, 150mM KCl, 30mM MgCl2, 60mM Tris, 0.1% v/v Triton X-100, 1mM DTT, supplemented with 0.2U/µL RNase Inhibitor (NEB, M0314), Complete protease inhibitor cocktail (Roche, 11697498001)) using a Wheaton Dounce Tissue Grinder (30 strokes with pestle B). The lysate was filtered with buffer of 250mM sucrose, 150mM KCl, 30mM MgCl2, then layered over 600mL of 29% v/v combined with buffer of 250mM sucrose, 150mM KCl, 30mM MgCl2, 60mM Tris) and centrifuged at 13500xg for 20 minutes. The supernatant was discarded, and nuclei gently resuspended and washed in 1mL of 1% BSA/PBS. The nuclei were visually inspected to confirm complete lysis and nuclear integrity. Nuclei were manually counted and diluted to a concentration of 1000 nuclei/µL in 1% BSA/PBS. For single-nucleus RNA sequencing (snRNA-seq), libraries were prepared using the Chromium Single Cell 3’ Reagent Kits v2 according to the manufacturer’s protocol (10X Genomics, 1000075). The snRNA-seq libraries were sequenced on a Novaseq S2 with paired end reads (read 1: 26 bp, read 2: 96 bp) targeting 50,000 paired reads per nucleus.

snRNaseq Data Processing and Analysis

Raw single-nuclei RNA-seq data was processed using the 10X Genomics Cell Ranger (v3.0) pipeline. Reads were aligned to the Ensembl release 93 Homo sapiens genome. Cells were selected for downstream analysis using the cell barcodes associated with the most UMIs. We estimated the number of cells expected to be captured based on input nuclei concentration and retained this many cell barcodes for downstream analysis. Cells with < 200 unique genes detected were removed (gene detection: > 1 count). Cells with > 8% of their counts mapping to MT genes were removed. Genes detected in < 3 cells were removed. Normalization was performed using Seurat (v3.1) (Butler et al., 2018). Briefly, raw counts are read depth normalized by dividing by the total number of UMIs per cell, then multiplying by 10,000, adding a value of 1, and log transforming (ln (transcripts-per-10,000 + 1)). Raw UMI counts data were assessed for the effects from biological covariates (clinical dx, anatomical region, donor, age, sex), and technical covariates (RIN, PMI, library batch, number of UMI, number of genes detected, percentage MT). The effects of number of UMI (sequencing depth) were removed from the read depth normalized expression values using a linear model. One outlier sample was identified based on abnormal frequencies of major cell types and divergent gene expression patterns, and was removed from the analysis (Table S2). To isolate microglia cells for further analysis, clustering was performed using Seurat (v3.1) (Butler et al., 2018). Each cluster
was then annotated as a major cell type using mean expression of groups of cell type marker genes, including the microglial markers CD74, CSF1R, C3 (Mathys et al., 2019), PTPRC, and DOCK8 (Kelley et al., 2018) (Figure S5A). Microglia clusters were selected for further analysis.

To identify genes affected by diagnosis, differentially expressed genes were determined using a linear model implemented in R comparing all disease microglia versus all control microglia (expression ~clinical_dx + number_umi). P values were then Benjamini-Hochberg corrected. We used the top 250 genes associated with bvFTD (at significance thresholds of beta > 0.1 and p-adjusted < 5.0 E-04) for further analysis (Table S3). We report the effect size output from the linear models as beta; which is approximate to the log2-fold change between groups (Table S3).

While RIN, PMI and age were not significantly different between case and control subjects (Mann Whitney U test; Table S2); sex and rank bank were not evenly split across groups (UCSF brain bank –bvFTD = 70%, control = 12%; male sex –bvFTD = 62%, controls = 50% (Table S2). To identify genes potentially affected by sex or brain bank in our analysis, differentially expressed genes were determined using a linear model comparing control microglia from males and females (expression ~sex + number_umi), and control microglia from UCSF versus University of Pennsylvania brain banks (expression ~finalsite + number_umi). We identified all genes significantly associated with either UCSF brain bank or sex using the thresholds applied to diagnosis (beta > 0.1, p value < 1.0 E-05, p-adjusted < 5.0 E-04) (Table S3) and excluded them from further analysis. To further identify genes potentially impacted by low tissue quality in our analysis, we ran independent linear models for post-mortem interval (PMI; expression ~pmi), RNA integrity (RIN; expression ~rin), number of genes detected per cell (expression ~number_genes_detected), and number of UMI detected per cell (expression ~umi) using both case and control samples. No genes were significantly associated with PMI or number of genes or UMI detected; and genes found to be significantly associated with RIN (Table S3) were excluded from further analysis (beta >0.1, p value < 1.0 E-05, p-adjusted < 5.0 E-04).

In addition, to check that our findings were representative of group effects rather than driven by a few individuals, we compared all disease and control samples for average gene expression and total number of microglia contributed to the analysis (Figure S5E). In addition, to account for the random effect of subject in our analysis, we calculated differential expressed genes using a mixed effects model with subject as a random effect, and confirmed a very high Pearson’s correlation between the diagnosis effects (beta) calculated from the linear model and mixed effects models (Figure 6B; Pearson’s correlation = 0.89, p < 2.2e-16). These results confirm that the disease trends we report are representative of group differences (control versus disease) rather than based on a few non-representative individuals.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

For each result, the figure legend describes the type of plot, statistical analysis, and sample n and definition. For all boxplots, the center line is at the median and the upper and lower lines are at the 75th and 25th percentiles, respectively (Figures 4I, 5C, 5F, 5K, S3B, S3C, S4G–S4I, and S5C). Results of tissue culture experiments show each sample data point as a separate dot, the center line at the group mean, and error bars showing s.e.m. (Figures 5D, 5G, and 5I) The barplot in Figure S2G has the center line at the group mean and error bars as s.e.m. In Figure S5D, the center line of the boxplot is the sample mean, the upper and lower lines are the 75th and 25th percentiles, respectively, and each dot is the average gene expression among the microglia from one subject, with the size of the dot proportional to the number of microglia contributing to the final analysis from that subject.

For each computational analysis performed from published code or resources, we used the statistical tests specified by the code and associated publications, as described and referenced under the corresponding experimental section under Methods Details. For single nuclear sequencing studies, the statistical approaches and methods, including for outlier removal and covariate analysis, are described in detail under the “single-nuclei RNA-seq data processing and analysis” subheading of Methods Details. Prior to single nuclear and RNA sequencing, samples were randomized across sample prep and library synthesis batches, and then randomized again across sequencing lanes to minimize sequencing batch effects. For cell culture experiments that measured one readout and compared one condition (Figure 5), a normal distribution was assumed and a two-tailed Student’s t test was performed; with samples paired if they were run together in an experimental batch. P values were corrected for multiple testing using FDR throughout the study, with significance values reported in the figures and figure legends.