Genetic variability in response to amyloid beta deposition influences Alzheimer’s disease risk

Dervis A. Salih,1,2 Sevinc Bayram,3 Sebastian Guelfi,4 Regina H. Reynolds,4 Maryam Shoai,2,4 Mina Ryten,4 Jonathan W. Brenton,1 David Zhang,4 Mar Matarin,4 Juan A. Botia,4,5 Runil Shah,4 Keeley J. Brookes,6 Tamar Guetta-Baranes,6 Kevin Morgan,6 Eftychia Bellou,7 Damian M. Cummings,1 Valentina Escott-Price7 and John Hardy2,4

Genome-wide association studies of late-onset Alzheimer’s disease risk have previously identified genes primarily expressed in microglia that form a transcriptional network. Using transgenic mouse models of amyloid deposition, we previously showed that many of the mouse orthologues of these risk genes are co-expressed and associated with amyloid pathology. In this new study, we generate an improved RNA-seq-derived network that is expressed in amyloid-responsive mouse microglia and we statistically compare this with gene-level variation in previous human Alzheimer’s disease genome-wide association studies to predict at least four new risk genes for the disease (OAS1, LAPTMS, ITGAM/CD11b and LILRB4). Of the mouse orthologues of these genes Oas1a is likely to respond directly to amyloid at the transcriptional level, similarly to established risk gene Trem2, because the increase in Oas1a and Trem2 transcripts in response to amyloid deposition in transgenic mice is significantly higher than both the increase of the average microglial transcript and the increase in microglial number. In contrast, the mouse orthologues of LAPTMS, ITGAM/CD11b and LILRB4 (Laptm5, Itgam/CD11b and Lilra5) show increased transcripts in the presence of amyloid plaques similar in magnitude to the increase of the average microglial transcript and the increase in microglia number, except that Laptm5 and Lilra5 transcripts increase significantly quicker than the average microglial transcript as the plaque load becomes dense. This work suggests that genetic variability in the microglial response to amyloid deposition is a major determinant for Alzheimer’s disease risk, and identification of these genes may help to predict the risk of developing Alzheimer’s disease. These findings also provide further insights into the mechanisms underlying Alzheimer’s disease for potential drug discovery.

1 Department of Neuroscience, Physiology and Pharmacology, UCL, Gower Street, London WC1E 6BT, UK
2 UK Dementia Research Institute at UCL, Gower Street, London WC1E 6BT, UK
3 Hitachi Rail Europe Ltd, Holborn, London, UK
4 Department of Neurodegenerative Diseases, Institute of Neurology, UCL, 1 Wakefield Street, London WC1N 1PJ, UK
5 Department of Information and Communications Engineering, Universidad de Murcia, Spain
6 Human Genetics, School of Life Sciences, Life Sciences Building, University Park, University of Nottingham, Nottingham NG7 2RD, UK
7 Dementia Research Institute, MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, UK

Correspondence to: Dervis A. Salih
UK Dementia Research Institute at UCL, Gower Street, London WC1E 6BT, UK
E-mail: dervis.salih@ucl.ac.uk
Correspondence may also be addressed to: John Hardy. E-mail: j.hardy@ucl.ac.uk

Keywords: Alzheimer’s; microglia; amyloid; genome-wide association studies; expression quantitative trait loci

Abbreviations: Aβ = amyloid beta; APP = amyloid precursor protein; eQTL = expression quantitative trait loci; GWAS = genome-wide association studies IFN-γ, interferon-γ; IGAP, International Genomics of Alzheimer’s Projects; SNP, single nucleotide polymorphism

Received July 22, 2019. Revised September 10, 2019. Accepted September 11, 2019. Advance Access publication October 10, 2019
© The Author(s) (2019). Published by Oxford University Press on behalf of the Guarantors of Brain.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

All the known mutations in genes causing early-onset Alzheimer’s disease alter amyloid precursor protein (APP) processing such that amyloid deposition becomes more likely (Hardy and Selkoe, 2002). In contrast, despite some rare variants in APP processing enzymes (Kim et al., 2009; Marioni et al., 2018; Jansen et al., 2019; Kunkle et al., 2019), the majority of the risk in late-onset disease has been shown to be due to sequence variability in genes expressed in the innate immune system (largely microglial genes; Jones et al., 2010). We and others identified the microglial gene TREM2 as a potent risk gene for late-onset disease (Guerreiro et al., 2013; Jonsson et al., 2013), and identified that its expression was strongly increased by amyloid deposition in APP transgenic mice (Guerreiro et al., 2013; Matarin et al., 2015; Cheng-Hathaway et al., 2018; Song et al., 2018). We previously reported a microarray analysis of genome-wide expression of a range of transgenic mice expressing mutant human APP and/or PSEN1 (Matarin et al., 2015). The different lines of mice analysed in this study developed amyloid plaques at different rates and so, by analysis of plaque deposition and gene expression in the same animals, plaque deposition could be correlated with gene expression across the life of a mouse, independent of age. We noted that Trem2 was one of the genes whose expression was up-regulated the most in relation to amyloid deposition. Trem2 expression also showed a strong correlation with the expression of a network of genes in the innate immune system suggesting Trem2 is a ‘hub’ gene, and may regulate the expression of the entire network. This immune module of genes showed a remarkable positive correlation to amyloid deposition (Matarin et al., 2015), and contained orthologues of other already established Alzheimer’s disease risk genes such as Abca7 and Ms4a6d (Lambert et al., 2013). Notably, two genes, ABI3 and PLCG2, that were identified subsequently as being associated with Alzheimer’s disease risk loci (Sims et al., 2017), were also present in this network. Hence, mouse microglia clearly respond to plaques in a manner where the genes co-expressed within these microglia relate closely to the genes that are relevant in human disease. These observations also suggest that this innate immune network that is expressed by these amyloid-responsive microglia may be used to predict future risk genes for Alzheimer’s disease.

An important outstanding question is whether progression of late-onset Alzheimer’s disease to the point of neurodegeneration and diagnosis is largely due to an inadequate innate immune response to rising amyloid beta (Aβ) deposition, resulting in accelerated amyloid-induced damage (Edwards, 2019). This hypothesis is difficult to study in human post-mortem tissue because during pathogenesis the proportion of cell types in the brain changes and the remaining cells show extensive compensatory
changes in gene expression. With this in mind, for this new work we developed the approach outlined below to use the gene expression network that is present within amyloid-responsive microglia in mouse models during pathology progression, and tested for significant overlap with human gene variation associated with Alzheimer’s disease. We then surveyed the gene expression network in mouse amyloid-responsive microglia to investigate if we could identify further Alzheimer’s disease risk loci. Initially, we took advantage of the increased resolution provided by performing RNA-seq to improve the gene expression analysis we had previously undertaken with microarray technology in the same mice. The new higher-resolution transcriptional network containing the co-expressed mRNA that most strongly correlated to amyloid deposition again featured primarily microglial genes. This confirmed the previous analysis in the same mice (Matarin et al., 2015), but the mouse RNA-seq analysis revealed many additional genes not detectable with microarray, and included yet more genes previously identified as human risk genes for Alzheimer’s disease from genome-wide association studies (GWAS). We then investigated whether the genes included in the novel co-expression network present in amyloid-responsive mouse microglia are also significantly associated with Alzheimer’s disease in human GWAS data. We used the data from the International Genomics of Alzheimer’s Projects (IGAP; Lambert et al., 2013; Kunkle et al., 2019) to identify the genes which are present in the mouse network and also significantly associated with Alzheimer’s disease risk. The significance of each human gene was assessed using a gene-based approach, applied to the summary statistics of the IGAP datasets (Brown, 1975; Moskina et al., 2011; Escott-Price et al., 2014; de Leeuw et al., 2015). The gene-based analyses employed here account for the strength of the association of multiple adjacent single nucleotide polymorphisms (SNPs), restricted to the exon boundaries of genes. This approach has important implications for predicting disease risk in people at the gene level (rather than SNP-level), with the potential of providing mechanistic insights into the cellular and molecular processes underlying disease progression.

**Materials and methods**

**Mouse models of Alzheimer’s disease**

The RNA samples used for this study were from the same mice we used previously, described in detail in Matarin et al. (2015), therefore no further mice were bred for this study. The mouse lines used are stated in the Supplementary material. The mice procedures used for Matarin et al. (2015), were performed in agreement with the UK Animals (Scientific Procedures) Act, 1986, with local ethical agreement.

**Human genome-wide association studies data**

The original IGAP (Lambert et al., 2013) summary statistics calculated for each SNP with 17008 Alzheimer’s disease cases and 37154 controls (Stage 1) were used to derive the gene-based P-values, described further below and in Escott-Price et al. (2014). The updated IGAP (Kunkle et al., 2019) summary statistics, derived from 21982 clinically confirmed Alzheimer’s disease cases and 41944 controls (Stage 1) were used to repeat the procedure and generate gene-based P-values to determine if the associations identified from the original IGAP data remained.

**Mouse transcriptome work**

For this study, RNA-seq library preparation and sequencing was performed by Eurofins Genomics (Ebersberg, Germany), details given in Supplementary material together with processing of FASTQ files. Supplementary Fig. 1 shows how the new RNA-seq data and new comparison to IGAP GWAS data for Alzheimer’s disease, relates to total RNA samples collected previously in Matarin et al. (2015).

Weighted gene co-expression network analyses were performed as described in Matarin et al. (2015), using the recommended parameters from the original analysis developers (Zhang and Horvath, 2005; Horvath et al., 2006; Oldham et al., 2006; Langfelder and Horvath, 2008). Further details are in Supplementary material.

**Gene-based human genome-wide association studies data analysis**

The significance of the association to Alzheimer’s disease of human genes was assessed using a gene-based approach as introduced in Brown (1975), Escott-Price et al. (2014), and implemented in de Leeuw et al. (2015; MAGMA software ctg.cnrc.nl/software/magma). Briefly, the updated IGAP (Kunkle et al., 2019) summary statistics calculated for each SNP in a sample of 21982 Alzheimer’s disease cases and 41944 controls were used to derive gene-based P-values. SNPs were assigned to genes if they were located within the genomic sequence corresponding to the start of the first and the end of the last exon of each transcript. Previous analyses including the 10 kb sequence flanking the first and last exons of genes, which may contain potential regulatory SNPs, did not cause substantial differences to the gene-based P-values (Escott-Price et al., 2014). Gene locations were as Build 37, Assembly Hg19 of the National Center for Biotechnology Information (NCBI) database as provided as part of the MAGMA software package. Phase 3 of 1000 Genomes data were used as a reference panel for calculation of linkage disequilibrium between markers (Genomes Project Consortium et al., 2015). The gene-wide analysis was performed based on the summary
P-values while controlling for linkage disequilibrium and different numbers of SNPs per gene using a statistical approach by Brown (1975), and adopted for set-based analysis of genetic data by Moskvina et al. (2011) and de Leeuw et al. (2015). Prior to the gene-based analyses all individual SNP P-values were corrected for the genomic inflation factor ($\lambda$; to normalise for unaccounted variation, due to factors such as population stratification; Devlin and Roeder, 1999).

**Statistical analysis comparing human genes with co-expression network of amyloid-responsive mouse microglia**

The lists of mouse genes in the co-expression networks were converted to lists of human gene names using convertMouseGeneList() function, library biomaRt in R downloaded from https://bioconductor.org/biocLite.R. We tested whether the number of Alzheimer’s disease associated genes (at significance thresholds alpha = 0.05, 0.01 and 0.001) in the mouse co-expression network was greater than that expected by chance given the number of human orthologues present in the mouse network. For that, we counted the observed number of independent significant human genes in the mouse network and compared this with the expected (by chance) number of genes calculated as $N \times \alpha$, whilst accounting for the variance (var=$N^2 \alpha^2(1-\alpha)$), where $N$ was the total number of independent human genes in the mouse network. To account for linkage disequilibrium, the genes within 0.5 Mb of each other were conservatively counted as one. The P-value of the excess of significant genes in the mouse network, between observed and expected, was calculated using a Z-test comparing the number of observed significant genes with the expected number. The observed number of significant genes was significantly higher than

---

**Figure 1** An innate immune network of genes expressed by amyloid-responsive microglia, featuring several orthologues of established GWAS genes associated with Alzheimer’s disease, predicts the importance of four new risk genes that may influence the risk of developing Alzheimer’s disease. Network plot using VisANT reveals key drivers of an innate immune module from RNA-seq derived gene expression from the hippocampus of wild-type and amyloid mice. Red circles show orthologues of established GWAS genes associated with Alzheimer’s disease including Trem2, Abi3, Cd33 and Spil/IPU.1. Blue underline shows gene orthologues predicted to confer altered risk of Alzheimer’s disease by overlapping a gene co-expression network present in mouse microglia that show a strong response to amyloid in transgenic mice with individual human genes significantly associated with Alzheimer’s disease by analysing combinations of adjacent SNPs (see Materials and methods section; Escott-Price et al., 2014). Genes shown in this network are transcribed and co-expressed in amyloid-responsive microglia. Larger red spheres represent ‘hub’ genes, those showing the greatest number of connections to other genes in the network, and include Trem2, Tryobp, Lirb4a, P2ry13, Cts, Ctsz, Mpeg1 and Plek, which are likely to play important roles in driving microglial function.
the expected at all gene $P$-value thresholds (0.05, 0.01, 0.001) for the amyloid-associated network. We report the genes at the gene-based $P$-values at threshold alpha = 0.01.

**Data availability statement**

RNA-seq expression data have been deposited in NCBI’s Gene Expression Omnibus (GEO; Series accession number GSE137313; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137313), and are available at: www.mouseac.org.

**Results**

**High-resolution co-expression network using RNA-seq in amyloid-responsive microglia**

Although mouse models for dementia have clear limitations in that they do not show tau tangles or neuronal loss solely in response to rising Aβ, they allow us to study the time-course response of a healthy innate immune system reacting to Aβ, leading to the possibility that the innate immune cells of the mouse may ultimately be preventing Aβ killing neurons. We previously constructed a transcriptional network using expression arrays that was present in microglia that respond to plaques (Matarin et al., 2015). As microarrays are limited by their probe content and their dynamic range, for this new study we have now sequenced the transcriptome of the same mice, expressing one or two copies of the APP (Swedish) and/or PSEN1 (M146V) transgenes alongside wild-type controls, using RNA-seq to construct a new higher resolution expression network. Similar to our findings with the initial microarray analysis, the RNA-seq analysis revealed a microglial module of genes that showed a strong correlation with Aβ deposition (correlation = 0.94; $P<3e^{-41}$), and contained the mouse orthologues of the identified GWAS loci TREM2, Aβ3, CD33, INPP5D, MS4A6D, SP11/PU.1, PLCG2, GAL3ST4, RIN3, HLA and APOE (Supplementary Table 1), verifying the relevance of this gene network to the human condition. Our hypothesis is that this network contains most of the genes that the microglia need to respond to amyloid plaques, including genes necessary for increases in cell number and activation (thus many cellular responses including proliferation, survival, metabolism, activation into a variety of states and phagocytosis). The genes showing the tightest expression correlation within the module associated with microglia reacting to plaques form the network shown in Fig. 1 and Supplementary Table 2 (top 147 genes from a total of 1584 genes expressed as part of the innate immune module based on the topological overlap measure, connectivity values). This network is broadly similar to the network derived from the analysis of the same RNA by microarray methods (Matarin et al., 2015) and shows common features with microglial networks published by other groups using other amyloid mouse models (Wang et al., 2015; Castillo et al., 2017; Keren-Shaul et al., 2017; Lee et al., 2018; Nam et al., 2018), suggesting this is a conserved core network of genes that can be reliably identified using different methodologies. Trem2 forms a hub gene in our network, using either technique, indicating that Trem2 expression is highly correlated to many other genes in the network, and may drive the response of this network. In line with this idea, Trem2 has been shown to regulate at least part of this immune module (Wang et al., 2015; Keren-Shaul et al., 2017; Lee et al., 2018). The network we identified is also broadly similar to a human network of innate immune genes containing TYROBP, TREM2, MS4A family genes, CIQ members and CD33, identified from human post-mortem tissue bearing in mind the caveats discussed above for human tissue (Forabosco et al., 2013; Zhang et al., 2013). Again this suggests that this gene network expressed by Aβ-responsive mouse microglia behaves similarly in humans.

**Enrichment of human Alzheimer’s disease genes in the mouse gene network expressed by amyloid-responsive microglia**

Traditionally, GWAS projects have focused on single SNPs because single locus tests are the easiest to test and interpret, but these have limitations. For example, if disease risk is conferred by several (semi) independent SNPs within a locus with moderate effect sizes, this locus (gene) will be overlooked by the genome-wide analyses, as the statistical significance of each individual SNP will not pass the Bonferroni correction. Therefore, if only single SNPs are considered, useful disease associations may be lost, despite apparently high sample sizes (Escott-Price et al., 2014). To identify genes associated with Alzheimer’s disease at the gene-based level we initially used the summary statistics from the original IGAP (Lambert et al., 2013), and then re-ran the gene-based analyses using the larger updated IGAP data (Kunkle et al., 2019). We considered multiple SNPs within individual human genes to generate gene-level $P$-values in order to assess whether multiple SNPs together constitute a significant risk factor, using a gene-based approach applied to the Alzheimer’s disease GWAS summary statistics (Brown, 1975; Escott-Price et al., 2014; de Leeuw et al., 2015). Within our mouse innate immune network, we first confirmed the significance of several members of the network that were orthologues of established Alzheimer’s disease loci variants using the gene-level $P$-values, including genes such as Trem2 and Abi3 (Table 1). We then asked whether the other members of the mouse network expressed by amyloid-responsive microglia might predict additional risk for Alzheimer’s...
The SNP data were from the updated IGAP study, using Build 37, Assembly Hg19 (Supplementary Table 1). The top 137 genes from a total of 147 genes (Fig. 1) with individual human genes significantly associated with Alzheimer's disease by analysing combinations of adjacent SNPs (see Materials and methods section; Escott-Price et al., 2014). The SNP data were given for comparison. The genes predicted to contain variants associated with Alzheimer's disease together with established loci of genes for the gene enrichment analysis. To ensure that correlated genes due to linkage disequilibrium (i.e. in close proximity to one another), the genes within 0.5 Mb of each other were counted as one. We found a significant enrichment of orthologues of human genes associated with Alzheimer's disease at the $P = 0.01$ significance threshold within this mouse network expressed by amyloid-responsive microglia over and above that expected by chance ($P = 8.86 \times 10^{-6}$). The enrichment remained significant even after the established GWAS loci were excluded [$P = 1.66 \times 10^{-4}$ for highly connected network of 147 genes (Fig. 1) and similarly $P = 3.68 \times 10^{-4}$ for the entire module of 1584 genes (Supplementary Table 1)]. GWAS loci boundaries were defined as 0.5 Mb from either side of the most significant SNPs of previously identified GWAS genes with exclusion of APOE and HLA which we defined as chromosome 19: 44 500 000–46 500 000 and chromosome 6: 32 200 000 – 32 800 000, respectively.

In contrast to the mouse gene network expressed by amyloid-responsive microglia, the innate immune network expressed by microglia responding to tau pathology in mice transgenic for tau (P301L), was not significantly enriched for human genes associated with Alzheimer's disease using the same methods ($P = 0.78$), although $Apoe$ is part of this module and this module also contained genes largely expressed by microglia (Supplementary Fig. 2, top 137 genes from a total of 2299 genes in the module based on the topological overlap measure). When the entire module of innate immune genes expressed by tauopathy-responsive microglia (2299 genes) was considered there was a modest significant enrichment, $P = 1.74 \times 10^{-2}$, suggesting that a proportion of genes associated with Alzheimer’s disease through multiple SNPs are microglial genes that have mouse orthologues, but are expressed by microglia that are less responsive to tau pathology compared to Ab deposition.

The analysis of the genetic network expressed by amyloid-responsive microglia identified five genes within the central portion of mouse microglial network whose human orthologues were associated with Alzheimer’s disease from the original IGAP data [described in Salih et al. (2018), using the IGAP data from Lambert et al. (2013)]. When we repeated the analysis using the updated IGAP data (Kunkle et al., 2019) containing 29.2% more cases and 12.9% more controls, and 62.7% more SNPs as compared to Lambert et al. (2013), four of the five identified genes from the centre of the co-expression network in mice were still strongly associated with the orthologues containing variants in human Alzheimer’s disease. These four genes, OAS1, LAPTMS1, ITGAM/CD11b and LILRB4, have not been previously reported as having variants significantly associated with Alzheimer’s disease using traditional GWAS approaches.

### Table 1 The genes predicted to contain variants associated with Alzheimer’s disease together with established loci from GWAS

| Mouse symbol (MGI) | Human symbol (HGNC) | NCBI ID | Human chromosome | Start location | End location | Number of SNPs (adj for GC) | Gene | Best SNP | Best SNP p-value | Effect size | Risk Allele | Frequency |
|-------------------|---------------------|--------|------------------|---------------|-------------|---------------------------|------|----------|-----------------|------------|-------------|-----------|
| Laptm5            | LAPTM5              | 7805   | T                | 1             | 31205316    | 71 6.62E–05               | rs7549164 | 31224193 | 4.15E–04       | 0.0655     | T           | 0.1935    |
| Os1a              | OSI1                | 4938   | 12               | 113344582     | 113371027   | 126 1.58E–03               | rs4766676 | 11336581 | 6.16E–04       | 0.0518     | T           | 0.6209    |
| Itgam             | ITGAM               | 3684   | 16               | 31271288      | 31344213    | 168 4.92E–03               | rs79113991 | 31273662 | 4.48E–03       | 0.0656     | A           | 0.1308    |
| Lin5A             | LLRB4               | 11006  | 19               | 55153340      | 55181810    | 148 8.96E–03               | rs731170 | 55176262 | 1.72E–03       | 0.0513     | A           | 0.3023    |

Genes predicted to confer altered risk of Alzheimer's disease by overlapping gene expression data transcribed by microglia that show a strong response to plaques in amyloid mice (Fig. 1) with individual human genes significantly associated with Alzheimer’s disease by analysing combinations of adjacent SNPs (see Materials and methods section; Escott-Price et al., 2014). The SNP data were given for comparison.

To this end, we identified orthologues of human genes in the mouse network and tested whether this set of genes is enriched for the genes which contain variants significantly associated with Alzheimer’s disease. As this set of genes was defined by our biological experiment in contrast to genome-wide analyses, which by their nature are exploratory rather than hypothesis driven, we considered a nominal statistical significance threshold of $P = 0.05$ for human Alzheimer’s disease gene-based associations. We also explored more stringent significance thresholds ($P = 0.01$ and $P = 0.001$), for selection of the genes for the gene enrichment analysis. To ensure that our enrichment analysis results were not inflated by the correlated genes due to linkage disequilibrium (i.e. in close proximity to one another), the genes within 0.5 Mb of each other were counted as one. We found a significant enrichment of orthologues of human genes associated with Alzheimer’s disease at the $P = 0.01$ significance threshold within this mouse network expressed by amyloid-responsive microglia over and above that expected by chance ($P = 8.86 \times 10^{-6}$). The enrichment remained significant even after the established GWAS loci were excluded [$P = 1.66 \times 10^{-4}$ for highly connected network of 147 genes (Fig. 1) and similarly $P = 3.68 \times 10^{-4}$ for the entire module of 1584 genes (Supplementary Table 1)]. GWAS loci boundaries were defined as 0.5 Mb from either side of the most significant SNPs of previously identified GWAS genes with exclusion of APOE and HLA which we defined as chromosome 19: 44 500 000–46 500 000 and chromosome 6: 32 200 000 – 32 800 000, respectively.

In contrast to the mouse gene network expressed by amyloid-responsive microglia, the innate immune network expressed by microglia responding to tau pathology in mice transgenic for tau (P301L), was not significantly enriched for human genes associated with Alzheimer’s disease using the same methods ($P = 0.78$), although $Apoe$ is part of this module and this module also contained genes largely expressed by microglia (Supplementary Fig. 2, top 137 genes from a total of 2299 genes in the module based on the topological overlap measure). When the entire module of innate immune genes expressed by tauopathy-responsive microglia (2299 genes) was considered there was a modest significant enrichment, $P = 1.74 \times 10^{-2}$, suggesting that a proportion of genes associated with Alzheimer’s disease through multiple SNPs are microglial genes that have mouse orthologues, but are expressed by microglia that are less responsive to tau pathology compared to Ab deposition.

The analysis of the genetic network expressed by amyloid-responsive microglia identified five genes within the central portion of mouse microglial network whose human orthologues were associated with Alzheimer’s disease from the original IGAP data [described in Salih et al. (2018), using the IGAP data from Lambert et al. (2013)]. When we repeated the analysis using the updated IGAP data (Kunkle et al., 2019) containing 29.2% more cases and 12.9% more controls, and 62.7% more SNPs as compared to Lambert et al. (2013), four of the five identified genes from the centre of the co-expression network in mice were still strongly associated with the orthologues containing variants in human Alzheimer’s disease. These four genes, OAS1, LAPTMS1, ITGAM/CD11b and LILRB4, have not been previously reported as having variants significantly associated with Alzheimer’s disease using traditional GWAS approaches.

| Predicted genes | Location | Start location | End location | Number of SNPs (adj for GC) | Gene | Best SNP | Best SNP p-value | Effect size | Risk Allele | Frequency |
|------------------|----------|---------------|-------------|-----------------------------|------|----------|-----------------|------------|-------------|-----------|
| Laptm5           | LAPTMS1  | 7805          | T           | 1                           | 31205316    | 71 6.62E–05               | rs7549164 | 31224193 | 4.15E–04       | 0.0655     | T           | 0.1935    |
| Os1a             | OSI1     | 4938          | 12          | 113344582                   | 113371027   | 126 1.58E–03               | rs4766676 | 11336581 | 6.16E–04       | 0.0518     | T           | 0.6209    |
| Itgam            | ITGAM    | 3684          | 16          | 31271288                    | 31344213    | 168 4.92E–03               | rs79113991 | 31273662 | 4.48E–03       | 0.0656     | A           | 0.1308    |
| Lin5A            | LLRB4    | 11006         | 19          | 55153340                    | 55181810    | 148 8.96E–03               | rs731170 | 55176262 | 1.72E–03       | 0.0513     | A           | 0.3023    |
(Table 1, Supplementary Figs 3 and 4). In addition, amongst the entire genetic network expressed by amyloid-responsive microglia (Supplementary Table 1; 1584 genes), a further 12 mouse genes have orthologues associated with human Alzheimer’s disease (P < 0.01) from the updated IGAP study (Supplementary Table 3). We emphasise that the goal of this comparison between the genetic network in mouse amyloid-responsive microglia versus human genes associated with Alzheimer’s disease combining multiple SNPs in a given gene was not to identify new single SNPs with genome-wide significant P-values ≤ 5 × 10⁻⁸. Instead, the alternative approaches we describe here were used to survey for more complex relationships between DNA variation and coding genes associated with Alzheimer’s disease by: (1) selecting a network of biologically relevant genes to Alzheimer’s disease genes (which reduces dramatically the number of genes being surveyed, to 1584 genes in our amyloid-associated network); (2) considering all SNPs together bounded by the coding region of a given gene (the gene-based analysis); and (3) looking at the network as a whole rather than individual genes (the enrichment analysis). Hence the individual gene significance is modest as compared to the genome-wide levels, but the genes are statistically significant and, together with previously identified Alzheimer’s disease genes, form the core of a transcriptional gene network (Fig. 1 and Table 1).

If we consider a sub-network of genes expressed by amyloid-responsive microglia that contains these four novel putative risk genes with the established GWAS loci TREM2, ABI3, CD33, INPP5D, SPI1/PU.1, MS4A6D and GAL3ST4 present in Fig. 1, this sub-network is not highly connected in an innate immune gene network associated with tauopathy (Supplementary Fig. 2), suggesting this sub-network is expressed by microglia that are more responsive to amyloid deposition than other pathological features. Furthermore, in common with the existing seven GWAS-associated genes in Fig. 1, the four novel risk genes we identify that are expressed by microglia that respond to Aβ deposition show transcript levels rising from 4 months of age in the homozygous APP/PSEN1 mice and after 4 months of age in the homozygous APP/PSEN1 mice relative to wild-type at 18 months of age; Laptm5, 4.1-fold increase; Lilra5, 3.8-fold increase; Itgam/CD11b, 2.3-fold increase; compared to Trem2, 9.2-fold increase, and Aif1, 3.3-fold increase; Supplementary Fig. 6). Genes showing higher relative transcript levels such as Oas1a and Trem2 compared to the average transcript level relative to wild-type mice for the entire innate immune network throughout disease progression, thus are likely to be directly up-regulated in response to amyloid by the reacting microglia, considering the number of microglia (3.7-fold increase in microglia at 18 months of age in homozygous APP/PSEN1 mice compared to wild-type; Medawar et al., 2019). In contrast, Laptm5 and Lilra5 relative expression are only significantly increased relative to average transcript level of the entire network when the plaque load starts to become heavy (8 months of age), but returns to the average relative transcript level of the network as disease progresses, suggesting a role in the initial response to Aβ (Supplementary Fig. 6). Itgam/CD11b shows a similar change in relative expression to the average relative transcript level of the entire immune network, and to the increase in microglia numbers, comparable to relative Spi1/PU.1 expression, suggesting that Itgam/CD11b and Spi1/PU.1 transcription is unlikely to be directly regulated by Aβ, but may play a role in regulating the change in microglia number in response to amyloid plaques because of the strong correlation between pathology and Itgam/CD11b expression. The expression patterns for Oas1a, Lilra5 and Itgam/CD11b are similar in both the homozygous and hemizygous APP/PSEN1 mice (Supplementary Fig. 6), whereas Laptm5 shows an expression pattern in the hemizygous APP/PSEN1 mice that is more similar to Itgam/CD11b. The similarity of the expression profiles of Laptm5, Itgam/CD11b and Spi1/PU.1 in the hemizygous APP/PSEN1 mice suggests that these three genes may play a role in regulating microglial number in response to amyloid deposition.

**Transcriptional network expressed by amyloid-responsive microglia containing risk genes is conserved in humans**

Aspects of the transcriptional network associated with amyloid that we identified in our analysis, containing the four predicted risk genes with the existing seven GWAS loci, are broadly similar to microglial networks we and others have previously identified in human brain analyses. Zhang et al. identified an Alzheimer’s disease-relevant network centred on TYROBP and TREM2, which contained ITGAM/CD11b and LAPTM5 (Zhang et al., 2013), and we described a human microglial network containing LAPTM5, ITGAM/CD11b and LILRB4 (Forabosco et al., 2013). We then determined whether these novel Alzheimer’s disease risk genes, derived from a
mouse transcriptional network expressed by amyloid-responsive microglia were present in independent datasets of human brain co-expression networks. Cross referencing our network with the data from the ROS/MAP project (Bennett et al., 2012a, b; De Jager et al., 2018), revealed that LAPTMS, ITGAM/CD11b and LILRB4 clustered together with many of the GWAS risk genes for Alzheimer’s disease (Supplementary Fig. 7; Fisher’s Exact test Bonferroni corrected P = 1.34 × 10⁻¹³ showing a significant overlap between the genes in the mouse amyloid-associated module and human genes in the ROS/MAP module associated with Alzheimer’s disease). Interestingly, SPI1/PU.1, the myeloid cell transcription factor and a newly discovered GWAS risk gene (Huang et al., 2017) was also in the same ROS/MAP module as LAPTMS, ITGAM/CD11b and LILRB4. We confirmed these module memberships in the BRAINEAC data for non-Alzheimer’s disease control human brains generated in our own lab (Ramasamy et al., 2014). Interestingly, we found that SPI1/PU.1 binds to the regulatory regions of Laptms and Itgam/CD11b, as well as established Alzheimer’s disease risk gene orthologues Trem2, Abi3, Inpp5d, Ms4a6d and Spi1/PU.1 itself, by searching data from a chromatin immunoprecipitation experiment against SPI1/PU.1 in mouse microglial-like BV-2 cells (Satoh et al., 2014). This finding was supported by mining for regulatory features and cis-regulatory modules in the gene network expressed by microglia that respond to plaques using i-cisTarget that uses a library of regulatory data (Imrichova et al., 2015). Together, these findings suggest that several of the predicted and established Alzheimer’s disease risk genes may be regulated by SPI1/PU.1, which itself alters Alzheimer’s disease risk by coordinating a program of microglial-expressed genes (Huang et al., 2017).

Discussion

A decade of GWAS projects for Alzheimer’s disease has provided key and initially surprising insights into the progression of late-onset Alzheimer’s disease, particularly the dependence on the innate immune system, with the identification of genes such as TREM2 and SPI1/PU.1 (Guerreiro et al., 2013; Jonsson et al., 2013; Huang et al., 2017; Sims et al., 2017). The latest GWAS studies published during 2019 mark the largest of their kind for Alzheimer’s disease featuring 71880 Alzheimer’s disease cases to identify 9 novel risk loci (Janssen et al., 2019), and 35274 clinically assessed Alzheimer’s disease cases to identify 5 novel risk loci from the updated IGAP study (Kunkle et al., 2019). Despite all the risk genes that have been discovered by GWAS, they still do not account for all of the heritability of late-onset Alzheimer’s disease. Finding further risk genes will become increasingly difficult due to the sheer number of patients required and associated costs, as the remaining risk genes are likely to be of rare mutation frequency or lower effect size. Here, we describe a new approach to identify further risk genes by intersecting transcriptome data from a functional cellular response to rising amyloid with a gene-based statistical approach to identify genes significantly associated with Alzheimer’s disease from the updated IGAP project. We identify four further potential risk genes, OAS1, LAPTMS, ITGAM/CD11b and LILRB4, alongside confirming the importance of seven established GWAS hits TREM2, AB13, CD33, INP5D, SPI1/PU.1, MS4A6D and GAL3ST4. Together these new and established genes form a transcriptional network that is conserved in mice and humans, and so suggests that this sub-network of genes are regulated together, in part by the SPI1/PU.1 transcription factor, and may function together.

Surveying the literature on our genes of interest revealed that OAS1 (2-prime, 5-prime oligoadenylate synthetase 1) is involved in the regulation of cytokine expression (Lee et al., 2019). OAS1 is induced by interferons (Donovan et al., 2013), which supports our eQTL analysis showing that one of the best SNPs we identified for OAS1 appears in a locus which acts as an eQTL in response to interferon-γ (IFN; Fig. 2 and Supplementary Figs 8 and 9). OAS1 can additionally activate ribonuclease L, which degrades viral RNA and inhibits viral replication (Donovan et al., 2013). Interferons are cytokines that are thought to trigger a key

Colocalization between Alzheimer’s disease-related loci and expression quantitative trait loci for gene OAS1

Since most GWAS loci are thought to operate by regulating the expression of neighbouring genes (Bradshaw et al., 2013; Griciuc et al., 2013; Huang et al., 2017), for each of the four potential Alzheimer’s disease-associated genes we performed a colocalization analysis to test the association between Alzheimer’s disease-related loci located within these genes and loci regulating the expression of these genes (eQTLs) (Giambartolomei et al., 2014). eQTLs were obtained from two previously published datasets using baseline and stimulated human-derived monocytes and iPSC-derived macrophages (Kim-Hellmuth et al., 2017; Alasoo et al., 2018). In these studies, macrophages and monocytes were stimulated with various immunostimulants to activate distinct, well-characterised immune signalling pathways, including those broadly associated with bacterial and viral responses. Interestingly, we identified three colocalizations between Alzheimer’s disease loci and eQTLs regulating OAS1 gene expression, all of which were identified in stimulated states, suggesting that this association is only active in certain environmental conditions (Fig. 2 and Supplementary Figs 8 and 9), in particular those designed to model monocyte/macrophage priming or more chronic inflammation.
response to viral and other pathogens. In addition to the mouse orthologue of OAS1 (Oas1a), a number of other genes involved in interferon signalling are also present in our co-expression network from amyloid-responsive microglia, including other Oas family members, Ifit members, and transcription factors such as Irf7, Trp53 and the Stat family (Supplementary Tables 1 and 2). Recent studies have also shown that interferon-related genes are expressed in ageing control mice, and that the expression of interferon-related genes is further elevated in mouse models with amyloid pathology (Friedman et al., 2018; Sala Frigerio et al., 2019), leading to the identification of a population of ‘interferon response microglia’ (Sala Frigerio et al., 2019). The role of OAS1 and the other interferon-related genes in ageing animals and Alzheimer’s disease is not clear, they may be involved in limiting viral infections, recruiting immune cells to sites of damage and/or regulating cytokine production.

LAPTM5 (lysosome-associated protein, transmembrane 5) is associated with amyloid deposition in transgenic mice (Nam et al., 2018). LILRB4 (leukocyte immunoglobulin-like receptor, subfamily B, member 4), orthologues have also been shown to be increased with amyloid deposition and specifically associated with amyloid plaques (Wirz et al., 2013; Kamphuis et al., 2016; Castillo et al., 2017). A paralogue of LILRB4, named LILRB2, and its mouse orthologue Pirb have been shown to bind Aβ, and this interaction with Aβ in mice mediates synapse elimination, and deficits in synaptic plasticity and memory (Kim et al., 2013). The functions of LAPTM5 and LILRB4 have not been well characterised, but are thought to suppress the activation of a variety of immune cells. ITGAM/CD11b (or CR3A), is a cell surface receptor involved in activation, migration and phagocytosis of immune cells, so much so that ITGAM/CD11b is used as a marker of activated microglia (Matsuoka et al., 2001; Heneka et al., 2013; Kamphuis et al., 2016). ITGAM/CD11b was highlighted in recent genetic and functional analyses as likely being important for the progression of Alzheimer’s disease, whose expression was driven by SPI1/PU.1, and related to amyloid deposition in mice and humans (Zhang et al., 2013; Hong et al., 2016; Kamphuis et al., 2016; Olmos-Alonso et al., 2016; Huang et al., 2017; Nam et al., 2018). Most recently, inhibiting the interaction between the blood protein fibrinogen and ITGAM/CD11b reduced synaptic elimination and cognitive decline in a mouse model of Alzheimer’s disease (Merlini et al., 2019), providing strong evidence that ITGAM/CD11b function contributes to disease development. Given the previous studies for ITGAM/CD11b, LAPTM5 and LILRB4, it is tempting to speculate that they are involved in phagocytic processes involving synapses which are known to be reactivated during Alzheimer’s disease progression. More work is...
necessary to understand the molecular mechanisms of all four of these putative risk genes in the progression of Alzheimer’s disease.

It is also useful to consider how microglial proliferation in response to amyloid plaques relates to expression of the four putative risk genes. We have previously shown that microglial number is increased in these homozygous APP/PSEN1 mice, by around 3.7-fold in the CA1 region of the hippocampus (Medawar et al., 2019), and in an elegant study by Srinivasan et al. (2016), delineates the difference between expression changes in bulk tissue versus the influence of increased microglial numbers in response to amyloid by cell sorting to analyse expression changes in purified microglia alone. The expression levels of our putative risk genes relative to expression in age-matched wild-type mice shows a range, with Oas1a showing the greatest relative expression (10.0-fold increase relative to wild-type), and Itgam/CD11b showing the lowest relative expression (2.3-fold increase), suggesting that these genes may fulfil different purposes in microglia in the presence of amyloid plaques. Genes showing higher relative transcript levels such as Oas1a and Trem2 are likely to be directly up-regulated by microglia in response to amyloid, and may be promoting a protective response to amyloid e.g. as described by Lee et al. (2018). Oas1a shows increased expression in purified microglia from a number of different mouse models of Alzheimer’s disease, using the Myeloid Landscape datasets suggesting Oas1a is directly up-regulated by amyloid (http://research-pub.gene.com/BrainMyeloidLandscape/#; Friedman et al., 2018). Laptm5 and Llrα relative expression are only significantly increased in homozygous APP/PSEN1 mice when the plaque load starts to become heavy (8 months of age), suggesting direct regulation by amyloid only as the plaque load increases, implying a specific role for these genes in microglia at this stage. Instead, Itgam/CD11b shows a similar change in relative expression to the average relative transcript level of the entire immune network, and to the increase in microglia number, comparable to relative Spi1/PU.1 expression. This suggests that Itgam/CD11b and Spi1/PU.1 genes may play a role in regulating the change in microglia number in response to amyloid plaques, given the strong correlation between the expression of these genes and amyloid pathology.

The study by Huang et al. (2017) shows that a common SNP in the population delays onset of Alzheimer’s disease, purportedly via reduced expression of SPI1/PU.1. However, in our study we see a positive correlation between Spi1/PU.1 and candidate genes Laptm5 and Itgam/CD11b, as well as established risk genes Trem2 and Abi3, which all have binding sites in their promoters for SPI1/PU.1, suggesting that SPI1/PU.1 is a positive regulator of these genes in this mouse model where heavy amyloid load does not lead to tangles and neurodegeneration. This discrepancy may be due to differences in the increase in microglial number between mice and humans; our data suggest that Spi1/PU.1 may be regulating microglial number, and it is possible that the level of microglial proliferation that can be tolerated by mice and humans is different (particularly given the long course of Alzheimer’s disease in humans). Not all Alzheimer’s disease risk genes have SPI1/PU.1 binding sites; thus, while this core transcription factor plays a substantial role in the progression of disease, there are likely to be auxiliary, environment-dependent transcription factors that modify disease development. In future work, it would be good to complement the bulk RNA-seq analysis here with isolated microglia and single-cell work for microglia to determine how Spi1/PU.1 expression and the transcriptome is different for microglia proximal to plaques versus those away from plaques, and in different regions of the brain. In studies where microglia are isolated, the limitations associated with purifying microglia should be borne in mind, in that the procedure may alter some transcripts, and it is also important to consider the heterogeneity of microglia seen from single-cell work (Sala Frigerio et al., 2019). Further work is required to understand how the putative risk genes respond to amyloid within microglia, both at the transcriptional level, and at the post-translational level. Notably, while there is evidence that these putative risk genes have been coincidentally linked with amyloid plaques, there is no published evidence to date that DNA variation in these genes in the human population is linked to risk for Alzheimer’s disease.

Our data also show that microglia respond differently to amyloid deposition versus tauopathy, with around 29% of transcripts in amyloid-responsive microglia showing a stronger correlation to amyloid pathology. A recent study also presents related data, identifying a co-expression module within microglia that respond more robustly to amyloid pathology compared to tauopathy (Sierksma et al., 2019). In both studies, established and putative Alzheimer’s disease risk genes are more strongly enriched in the amyloid-responsive microglia compared to tauopathy-responsive microglia. These data collectively provide compelling evidence that the microglial response to amyloid pathology determines whether the disease progresses to neurodegeneration and cognitive problems. Further work is required to understand how the microglial response to tauopathy is different, and why mouse models with heavy amyloid plaque loads do not lead to tau tangles and neurodegeneration. It may be that other triggers, in addition to amyloid deposition, are required to push microglia to a state that permits amyloid-dependent tau pathology, such as blood-brain barrier breakdown or priming of the immune system by exposure to environmental pathogens. Alternatively, it may be due to microglial genes expressed more abundantly in human microglia compared to mouse.

This work focuses on the commonality between mice and humans, specifically how expression of mouse microglial genes overlap with human genes showing DNA
variation associated with Alzheimer’s disease. It is worthwhile to bear in mind that a number of important studies have compared gene expression in microglia from mice and humans, and while they have shown a significant overlap between the transcriptomes of the two species, they have also seen a number of genes are expressed selectively more abundantly in human microglia (Miller et al., 2010; Galatro et al., 2017; Gosselin et al., 2017). Our four putative risk genes, OAS1, LAPTMS, IL1RB4 and ITGAM/CD11b are expressed abundantly in the human microglia (Galatro et al., 2017; Gosselin et al., 2017), and more generally there is a substantial overlap in human orthologues expressed by the mouse amyloid-responsive microglia and the transcripts expressed abundantly by human microglia from Galatro et al. (2017) and Gosselin et al. (2017). Genes expressed more abundantly in human microglia and not present in our mouse microglial network are given in Supplementary Table 5. Thus, in future studies it is important to select the appropriate model for the study of specific microglia genes.

The importance of this work is 2-fold. First, by identifying more genetic loci involved in amyloid deposition, we derive a more complete insight into the cellular processes and molecular mechanisms underlying the disease. In this regard, this work is complementary to that of Huang et al. (2017), showing that microglial SPI1/PU.1-driven transcription is a common feature of many Alzheimer’s disease loci. These findings are also consistent with previous work on Trem2 (Wang et al., 2015; Keren-Shaul et al., 2017; Mazaheri et al., 2017; Cheng-Hathaway et al., 2018; Lee et al., 2018), and CD33 (Bradshaw et al., 2013; Griciuc et al., 2013), suggesting these risk genes are crucial in controlling the microglial response to amyloid-induced damage. Understanding the mechanisms of function of TREM2 and the sub-network of genes expressed by amyloid-responsive microglia identified here may be useful to leverage therapeutic opportunities. Second, and perhaps of greater importance, this work implies that, overall, how well an individual responds to amyloid deposition at the cellular and gene expression level plays a part in determining one’s risk of disease, and understanding the genes that control this may be used to predict the chances of developing Alzheimer’s disease and to develop preventative or disease-delaying treatments before irreversible neurodegeneration sets in.

Supplementary material

Supplementary material is available at Brain Communications online.

Acknowledgements

We would like to thank Frances Edwards for very helpful discussions.

Funding

J.H., V.E-P. and D.A.S. are members of the UK Dementia Research Institute (DRI), which receives its funding from the DRI Ltd, funded by the UK Medical Research Council, Alzheimer’s Society and Alzheimer’s Research UK (ARUK). J.H. is supported by the Cure Alzheimer’s Fund, and also by the Dolly Foundation, and by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. R.H.R. is supported through the award of a Leonard Wolfson Doctoral Training Fellowship in Neurodegeneration. D.Z. and M.R. are supported by the UK Medical Research Council, through the award of a Tenure-track Clinician Scientist Fellowship to M.R. (MR/N008324/1). The University of Nottingham Group is funded by ARUK and hosts the ARUK Consortium DNA Bank, with the members given in the Appendix I below. D.M.C. is funded by the UCL DRI.

Competing interests

The authors report no competing interests.

Appendix I

The University of Nottingham Group is funded by ARUK and hosts the ARUK Consortium DNA Bank, with the members: Tulsi Patel,1 David M. Mann,2 Peter Passmore,3 David Craig,3 Janet Johnston,3 Bernadette McGuinness,3 Stephen Todd,3 Reinhard Heun,4 Heike Kölsch,5 Patrick G. Kehoe,6 Emma R.L.C. Vardy,7 Nigel M. Hooper,8 Stuart Pickering-Brown,2 Julie Snowden,8 Anna Richardson,8 Matt Jones,8 David Neary,8 Jenny Harris,8 A. David Smith,9 Gordon Wilcock,9 Donald Warden,9 and Clive Holmes10

1Schools of Life Sciences and Medicine, University of Nottingham, Nottingham NG7 2UH, UK, 2Institute of Brain, Behaviour and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester M13 9PT, UK, 3Centre for Public Health, School of Medicine, Queen’s University Belfast, BT9 7BL, UK, 4Royal Derby Hospital, Derby DE22 3WQ, UK 5Department of Psychiatry, University of Bonn, Bonn 53105, Germany, 6School of Clinical Sciences, John James Laboratories, University of Bristol, Bristol BS16 1LE, UK, 7Salford Royal NHS Foundation Trust, 8Cerebral Function Unit, Greater Manchester Neurosciences Centre, Salford Royal Hospital, Stott Lane, Salford M6 8HD, UK, 9University of Oxford (OPTIMA), Oxford OX3 9DU, UK 10Clinical and Experimental Science, University of Southampton, Southampton SO17 1BJ, UK.

References

Alasoo K, Rodrigues J, Mukhopadhyay S, Knights AJ, Mann AL, Kundu K, et al. Shared genetic effects on chromatin and gene
expression indicate a role for enhancer priming in immune response. Nat Genet 2018; 50: 424–31.
Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the religious orders study. Curr Alzheimer Res 2012a; 9: 628–45.
Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the rush Memory and Aging Project. Curr Alzheimer Res 2012b; 9: 646–63.
Bradshaw EM, Chibnik LB, Keenan BT, Ottoboni L, Raj T, Tang A, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. Nat Neurosci 2013; 16: 848–50.
Brown MB. A method for combining non-independent, one-sided tests of significance. Biometrics 1975; 31: 987.
Castillo E, Leon J, Mazzei G, Abolhassani N, Haruyama N, Saito T, et al. Comparative profiling of cortical gene expression in Alzheimer's disease patients and mouse models demonstrates a link among amyloidosis and neuroinflammation. Sci Rep 2017; 7: 17762.
Cheng-Hathaway PJ, Reed-Geaghan EG, Jay TR, Casali BT, Bemiller SM, Puntambekar SS, et al. The Trem2 R47H variant confers loss-of-function-like phenotypes in Alzheimer's disease. Mol Neurodegener 2018; 13: 29.
De Jager PL, Ma Y, McCabe C, Xu J, Vardarajan BN, Felsky D, et al. A multi-omic atlas of the human frontal cortex for aging and Alzheimer's disease research. Sci Data 2018; 5: 180142.
der Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput Biol 2015; 11: e1004219.
Devlin B, Roeder K. Genomic control for association studies. Biometrics 1999; 55: 997–1004.
Donovan J, Dufner M, Korennykh A. Structural basis for cytosolic double-stranded RNA surveillance by human oligoadenylate synthetase 1. Proc Natl Acad Sci USA 2013; 110: 1652–7.
Edwards FA. A unifying hypothesis for Alzheimer’s disease: from plaques to neurodegeneration. Trends Neurosci 2019; 42: 310–22.
Escott-Price V, Bellenguez C, Wang LS, Choi SH, Harold D, Jones L, et al. Gene-wide analysis detects two new susceptibility genes for Alzheimer's disease. PLoS One 2014; 9: e84661.
Forabosco P, Ramasamy A, Trabuini D, Walker R, Smith C, Bras J, et al. Insights into TREM2 biology by network analysis of human brain gene expression data. Neurobiol Aging 2013; 34: 2699–714.
Friedman BA, Srinivasan K, Ayalong K, Meilandt WJ, Lin H, Huntley PR, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. Nat Neurosci 2017; 20: 1162–71.
Genomes Project Consortium; Auton A, Brooks LD, Durbin RM, Galatro TF, Holtman IR, Lerario AM, Vainchtein ID, Brouwer N, Sola MA, et al. A multi-omic atlas of the human frontal cortex for aging and Alzheimer's disease research. Nat Neurosci 2013; 16: 848–50.
Giangreco N, et al. Genetic regulatory effects modified by immune activation contribute to autoimmune disease associations. Nat Commun 2017; 8: 266.
Kim T, Vidal GS, Djurisic M, William CM, Garcia KC, et al. Human LilRb2 is a beta-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. Science 2013; 341: 1399–404.
Kim-Ha J, Kim YJ. OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages. BMB Rep 2019; 52: 133–8.
Kim T, Vidal GS, Djurisic M, William CM, Garcia KC, et al. Human LilRb2 is a beta-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. Science 2013; 341: 1399–404.
Kimmelman AC, Younes L, Bieche I, Maltzahn D, et al. Genetic meta-analysis identifies new loci and functionally implicated pathways influencing Alzheimer’s disease risk. Nat Genet 2015; 47: 1004–12.
Lee WB, Choi WY, Lee DH, Shim H, Kim-Ha J, Kim YJ. OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages. BMB Rep 2019; 52: 133–8.
Lee CYD, Daggett A, Gu X, Jiang LL, Langfelder P, Li X, et al. Elevated TREM2 gene dosage reprograms microglia responsiveness and ameliorates pathological phenotypes in Alzheimer’s disease models. Neuron 2018; 97: 1032–48.
Lee WB, Choi WY, Lee DH, Shim H, Kim-Ha J, Kim YJ. OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages. BMB Rep 2019; 52: 133–8.
Lee CYD, Daggett A, Gu X, Jiang LL, Langfelder P, Li X, et al. Elevated TREM2 gene dosage reprograms microglia responsiveness and ameliorates pathological phenotypes in Alzheimer’s disease models. Neuron 2018; 97: 1032–48.
Matarin M, Salih DA, Yasvoina M, Cummings DM, Guelfi S, Liu W, et al. A genome-wide gene-expression analysis and database in transgenic mice during development of amyloid or tau pathology. Cell Rep 2015; 10: 633–44.

Matsuoka Y, Picciano M, Malester B, Lafrancois J, Zehr C, Daeschner JM, et al. Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer’s disease. Am J Pathol 2001; 158: 1345–54.

Mazaheri F, Snaidero N, Kleinberger G, Madore C, Daria A, Werner G, et al. TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. EMBO Rep 2017; 18: 1186–98.

Medawar E, Benway TA, Liu W, Hanan TA, Haslehurst P, James OT, et al. Effects of rising amyloidβ levels on hippocampal synaptic transmission, microglial response and cognition in APPswe/PSEN1M146V transgenic mice. EBioMedicine 2019; 39: 422–35.

Merlini M, Rafalski VA, Rios Coronado PE, Gill TM, Ellisman M, Muthukumar G, et al. Fibrinogen induces microglia-mediated spine elimination and cognitive impairment in an Alzheimer’s disease model. Neuron 2019; 101: 1099–108 e6.

Miller JA, Horvath S, Geschwind DH. Divergence of human and mouse brain transcriptome highlights Alzheimer disease pathways. Proc Natl Acad Sci USA 2010; 107: 12698–703.

Moskvina V, O’Dushlaine C, Purcell S, Craddock N, Holmans P, O’Donovan MC. Evaluation of an approximation method for assessment of overall significance of multiple-dependent tests in a genomewide association study. Genet Epidemiol 2011; 35: 861–6.

Nam KN, Wolfe CM, Fitz NF, Letronne F, Castriano EL, Mounier A, et al. Integrated approach reveals diet, APOE genotype and sex affect immune response in APP mice. Biochim Biophys Acta Mol Basis Dis 2018; 1864: 152–61.

Oldham MC, Horvath S, Geschwind DH. Conservation and evolution of gene coexpression networks in human and chimpanzee brains. Proc Natl Acad Sci USA 2006; 103: 17973–8.

Olmos-Alonso A, Schetters ST, Sri S, Askew K, Mancuso R, Vargas-Caballero M, et al. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer’s-like pathology. Brain 2016; 139: 891–907.

Ramasamy A, Trabzuni D, Guelfi S, Vargheshe V, Smith C, Walker R, et al. Genetic variability in the regulation of gene expression in ten regions of the human brain. Nat Neurosci 2014; 17: 1418–28.

Sala Frigerio C, Wolfs L, Fantonelli N, Thrupp N, Voyeruk I, Schmidt I, et al. The major risk factors for Alzheimer’s disease: age, sex, and genes modulate the microglia response to Abeta plaques. Cell Rep 2019; 27: 1293–306 e6.

Salih DA, Bayram S, Guelfi MS, Reynolds RH, Shaoi M, Ryten M, et al. Genetic variability in response to Aβ deposition influences Alzheimer’s risk. bioRxiv 2018; doi: 10.1101/437657.

Satoh J, Asahina N, Kitano S, Kino Y. A comprehensive profile of ChIP-Seq-based PU.1/sp1 target genes in microglia. Gene Regul Syst Biol 2014; 8: 127–39.

Sierksma A, Lu A, Salta E, Mancuso R, Zoco J, Blum D, et al. Novel Alzheimer risk genes determine the microglia response to amyloid-β but not to TAU pathology. bioRxiv 2019; doi: 10.1101/491902.

Sims R, van der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, et al. Rare coding variants in PLCG2, Aβ3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer’s disease. Nat Genet 2017; 49: 1373–84.

Song WM, Joshita 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.

Srinivasan K, Friedman BA, Larson JL, Laufer BE, Goldstein LD, Appling LL, et al. Untangling the brain’s neuroinflammatory and neurodegenerative transcriptional responses. Nat Commun 2016; 7: 11295.

Wang Y, Cellia M, Mallinson K, Ulrich JD, Young KL, Robinette ML, et al. TREM2 lipid sensing sustains the microglial response in an Alzheimer’s disease model. Cell 2015; 160: 1061–71.

Wirz KTS, Bossers K, Stargardt A, Kamphuis W, Swaab DF, Hol EM, et al. Cortical beta amyloid protein triggers an immune response, but no synaptic changes in the APPswe/PS1dE9 Alzheimer’s disease mouse model. Neurobiol Aging 2013; 34: 1328–42.

Zhang B, Gaiteri C, Bodea LG, 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.

Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol 2005; 4: e17. doi: 10.2202/1544-6155.1128.