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Abstract

Objectives

The objective of this study was to investigate the expression of genes in Alzheimer's disease (AD) and dementia with Lewy bodies (DLB), both at the mild cognitive impairment (MCI) and dementia stages, to improve our understanding of disease pathophysiology and investigate the potential for diagnostic and prognostic biomarkers based on mRNA expression.

Design

Cross-sectional observational study.

Setting

University research centre.

Participants

People with MCI with Lewy bodies (MCI-LB, n=55), MCI-AD (n=19), DLB (n=38), AD (n=24) and a cognitively unimpaired comparison group (n=28).

Measurements

RNA sequencing of whole blood. Differentially expressed genes (DEGs) were identified and gene set enrichment analysis was carried out.

Results

Compared with the cognitively unimpaired group, there were 22 DEGs in MCI-LB/DLB and 61 DEGs in MCI-AD/AD. DEGS were also identified when comparing the two disease groups. Expression of ANP32A was associated with more rapid cognitive decline in MCI-AD/AD.

Gene set enrichment analysis identified downregulation in gene sets including MYC targets and oxidative phosphorylation in MCI-LB/DLB; upregulation of immune and inflammatory responses in MCI-AD/AD; and upregulation of interferon-α and -γ responses in MCI-AD/AD compared with MCI-LB/DLB.

Conclusions

This study identified multiple DEGs in MCI-LB/DLB and MCI-AD/AD. One of these DEGs, ANP32A, may be a prognostic marker in AD. Genes related to mitochondrial function were downregulated in MCI-LB/DLB. Previously reported upregulation of genes associated with inflammation and immune responses in MCI-AD/AD was confirmed in this cohort. Differences in interferon responses between MCI-AD/AD and MCI-LB/DLB suggest that there are key differences in peripheral immune responses between these diseases.

Introduction

The importance of genetic influences in dementia has become increasingly recognised in recent years, with more than 40 genetic loci identified as risk factors for Alzheimer's disease (AD) (1). These genes are involved in pathways such as tau binding proteins, amyloid precursor protein metabolism, immunity and lipid metabolism (2). Genetic risk factors have also been identified for dementia with Lewy bodies (DLB), including polymorphisms in *APOE*, *GBA*, *SNCA* and *BIN1 and TMEM175* (3).

Whilst genetic differences are static, pathophysiology may differ across different disease stages in dementia. For example, we have demonstrated raised peripheral inflammatory cytokines in mild cognitive impairment (MCI), but not in the dementia phase of DLB and AD (4). mRNA sequencing (RNAseq) allows the analysis of relative quantities of mRNA transcripts, providing insights into differential expression of transcripts that may underlie key pathogenic pathways. Whilst RNAseq of brain tissue tends to report late-stage disease, RNAseq of other tissues, such as blood, allows the investigation of gene expression throughout the disease course. This has the potential to improve our understanding of disease pathophysiology and facilitate the identification of novel diagnostic and prognostic biomarkers.

RNAseq has been applied to whole blood in AD, identifying 1102 differentially expressed genes (DEGs) (5). Gene set enrichment analysis identified upregulation of genes involved in haem metabolism, interferon-α and -γ responses, and downregulation of E2F targets and genes downregulated in response to ultraviolet light.

Very few studies have applied RNAseq in DLB and none have investigated mild cognitive impairment with Lewy bodies (MCI-LB) (6). One study performed RNA sequencing of serum small extracellular vesicles in 10 DLB subjects compared with 10 cognitively unimpaired participants (7) but no individual DEGs were identified following false discovery rate (FDR) correction. Pathway analysis found that genes associated with Huntington's disease signalling, regulation of eIF4 and p70S6K and glucocorticoid receptor signalling were upregulated, whereas genes related to proinflammatory

pathways including interferon signalling were downregulated. A recent systematic review of gene expression studies in Lewy body dementia found that most studies had used qPCR to measure specific genes and their expression (6). Analysis of the DEGs identified by these studies found enrichment in gene sets related to movement disorders of basal ganglia, schizophrenia, immune response of brain, neuronal death and survival, tauopathy, neuronal morphology and synaptic transmission. To our knowledge, no study has previously compared RNAseq in DLB and AD.

The aim of this study was to investigate the expression of genes in AD and DLB, both at the MCI and dementia stages, to improve our understanding of disease pathophysiology and investigate the potential for diagnostic and prognostic biomarkers based on mRNA expression.

Material and methods

Participants

This study analysed blood from participants with probable mild cognitive impairment with Lewy bodies (MCI-LB, n=58), MCI due to Alzheimer's disease (MCI-AD, n=22), DLB (n=40), AD (n=25) and a cognitively unimpaired comparison group (n=30). Venous blood samples were obtained between April 2013 and June 2018 as part of the LewyPro (Research Ethics Committee Reference Number 12/NE/0290), AMPLE (13/NE/0064), MIDAS (15/NE/0034) and SUPErB (15/NE/0420) studies. Recruitment and assessment procedures have been described in detail elsewhere (8-11). Briefly, participants with MCI and dementia, ≥60 years old were recruited through Old Age Psychiatry, Elderly Medicine and Neurology clinics in North-East England and Cumbria, or research volunteer registers. A cognitively unimpaired comparison group was recruited through a research volunteer register or were partners of participants. Participants were diagnosed based on consensus criteria (12-15). Diagnosis was confirmed by an expert consensus panel of three clinicians based on the most recent available clinical data. Where the first two raters did not agree, the third made a final

decision. MCI-LB was diagnosed based on the presence of two core features (visual hallucinations, cognitive fluctuations, spontaneous parkinsonism or REM sleep behaviour disorder) or the presence of one core feature along with either an abnormal FP-CIT SPECT or cardiac MIBG scan.

Participants with capacity gave their written informed consent to take part in the study. For those who lacked capacity, their participation in the study was discussed with a consultee in accordance with the UK Mental Capacity Act 2005. All studies were approved by a research ethics committee and were conducted in accordance with the Declaration of Helsinki.

Clinical assessment and diagnosis

All participants had a comprehensive cognitive and clinical assessment. Diagnosis was confirmed by a three-rater panel. AD (14) and DLB (12) were diagnosed based on established criteria. Participants in LewyPro and SUPErB fulfilled criteria for all cause MCI (13) i.e. subjective and objective cognitive impairment with generally maintained independence of function in daily life, with minimal aids or assistance. MCI subtype was defined based on criteria for probable MCI-LB (15) and MCI-AD (13). 136/164 (83%) of participants had 1-year follow-up data available. For participants with follow-up data, the MCI and dementia subtype diagnosis was based on the most recent clinical data.

Blood sampling, storage and processing

A total of 2.5mL of venous blood was acquired at baseline assessment in PAXgene tubes (PreAnalytix, BD Diagnostics) to preserve RNA integrity. After two hours at room temperature, samples were stored at -80°C until processing. Isolation of RNA was carried out through PAXgene Blood RNA Kit IVD (Qiagen) followed by globin mRNA depletion with GLOBINClear (Invitrogen). Globin mRNA-depleted total RNA was quality assessed using a TapeStation 4200 (Agilent).

RNA Sequencing and statistical analysis

Stranded mRNA sequencing libraries were prepared using the TruSeq Stranded mRNA kit (Illumina, San Diego, USA) and IDT for Illumina TruSeq RNA UD Index adapters (Illumina, San Diego, USA) following the manufacturer's protocol. Libraries were quantified using a Tapestation 4200 and Qubit 4 (Thermo Fisher, Massachusetts, USA) and equimolar pooled into two batches and sequenced at ~30 million (2 x 150 bp) per sample on a NovaSeq 6000 using two lanes of an S4 300 cycle flow cell (Illumina, San Diego, USA). Data for individual samples was demultiplexed into separate FASTQ files using Illumina's bcl2fastq software. Fastqc (v0.11.7) and MultiQC (v1.8) (16) were used to establish raw sequencing quality. Transcript quantification estimates were generated using Salmon (v0.14.1) (17) and Gencode (release 32) (18). Gene-level count tables were produced using tximport (v1.12.3) (19). Counts were normalised using the trimmed mean of M values method implemented by DESeq2 (v1.24.0) (20). Normalised counts were analysed for differential expression using Wald's test as implemented by DESeq2 (20). Statistical significance was determined by genes passing an FDR corrected p-value of less than 0.05.

The following potential confounding factors were compared with the top five principal components of gene expression: age, sex, Addenbrooke's Cognitive Examination score, sample collection date, RIN, sequencing batch and sequencing lane. Sex, RIN and sequencing batch showed a significant correlation with gene expression. For each grouped comparison, a correction for age, sex, sequencing batch and sample RIN value was applied.

Combined MCI-AD/AD and MCI-LB/DLB groups were each compared with the cognitively unimpaired comparison group to investigate differences in gene expression in each disease. Following this, comparisons were made between the disease groups, to identify differences between Alzheimer's and Lewy body disease. Upstream regulators of DEGs were identified using Ingenuity Pathway Analysis (Ingenuity, USA).

DEGs identified in this study were cross referenced with those that have been identified previously. DEGs in AD were compared with a recent single RNAseq analysis in blood in AD (n=1,102 DEGs) (5) and a meta-analysis of blood-based microarray gene expression profiles in AD (n=5 DEGs) (21). DEGS in DLB/MCI-LB were compared with those identified in a recent systematic review as DEGS identified in post mortem brain tissue and in the periphery in Lewy body dementia (6), after confirming the original publication undertook correction for multiple comparisons.

DEGs identified in this study that had previously been reported were investigated for diagnostic utility and associations with disease progression. Diagnostic utility was investigated using discriminant analysis. The relevant DEGs were entered into the discriminant analysis and each participant was classified by the functions derived from all participants other than that individual. Association with disease progression was investigated by correlating gene expression with change in Addenbrooke's Cognitive Examination – Revised over one year using Spearman's correlation. To ensure results were not affected by age and sex of participants, significant results were re-analysed using the general linear model with age and sex as covariates.

Gene set enrichment analysis (22) was carried out using the Molecular Signatures Database Hallmark Gene Set collection, a group of 50 gene sets representing specific biological processes (23). Gene set enrichment analysis was run using the fgsea R package (https://bioconductor.org/packages/release/bioc/html/fgsea.html) with 10,000 permutations. All genes were ranked by fold change, with no prefiltering. Gene sets with a Benjamini-Hochberg false discovery rate corrected p<0.05 were considered significant.

Co-expression analysis was carried out using Co-Expression Modules Identification Tool (CEMiTool) (24).

Results

DEGs

mRNA was extracted from 175 samples. 11 samples failed quality control prior to sequencing. A total of 164 samples were included in the statistical analysis: n=55 MCI-LB, n=19 MCI-AD, n=38 DLB, n=24 AD and n=28 cognitively unimpaired.

Supplementary Table 1 shows the demographic details of participant groups. There were no statistically significant differences in age or sex, though the MCI-AD group had a numerically greater proportion of females than the four other groups. This was controlled for in all statistical analyses. The two dementia groups and two MCI groups were well balanced for degree of cognitive impairment.

Tables 1-2 and Supplementary Tables 2-6 list the genes that showed differential expression in pairwise comparisons between diagnostic groups. Compared with cognitively unimpaired group, there were 22 DEGs in the combined MCI-LB/DLB group, 61 DEGs in the combined AD/MCI-AD group, 24 DEGs in MCI-LB, 17 DEGs in DLB, 5 DEGs in MCI-AD and 66 DEGs in AD. There were 4 DEGs in MCI-AD compared with MCI-LB, 18 DEGs in AD compared with DLB and 2 DEGs in the combined AD/MCI-AD group compared with the DLB/MCI-LB group.

INSERT TABLES 1 AND 2 HERE

Comparison with previous reports

DEGs identified in DLB and MCI-LB were compared with a recent meta-analysis (6). Following this, the original publication was accessed to ensure the data was corrected for multiple comparisons. One previously identified DEG, TMEM145, was upregulated in the combined DLB/MCI-LB group compared with cognitively unimpaired participants. This was previously reported as upregulated in the anterior cingulate cortex in DLB (25). This gene was also upregulated in AD compared with

cognitively unimpaired participants in our cohort. None of the DEGs identified in DLB and MCI-LB were reported in previous AD cohorts (5, 21)

DEGS identified in AD and MCI-AD were compared with DEGs previously identified in blood of people with AD by Griswold et al. (5). Four DEGs in the combined MCI-AD/AD group (MRVI1, SLPI, NRDC and FCRL3) and five DEGs in the AD group (SLPI, NRDC, OSTF1, ANP32A and FCRL3) were also found by Griswold et al. The direction of change in expression was in concordance in all six genes: MRVI1, NRDC, SLPI, OSTF1 and ANP32A were upregulated and FCRL3 was downregulated in both cohorts. There was no overlap in the reported DEGS in MCI-AD compared with cognitively unimpaired participants and the AD cohort reported by Griswold et al. (5). None of the five DEGs reported in a meta-analysis of blood-based microarray gene expression in AD were found to be differentially expressed in this cohort (21).

Discriminant analysis

The six DEGs identified above were entered into a discriminant analysis to identify MCI-AD/AD compared with cognitively unimpaired participants. The analysis was able to classify 72% of cases correctly (sensitivity 67%, specificity 79%).

Association with disease progression

We investigated the correlation between six DEGs that were also identified in previous study of AD blood (MRVI1, NRDC, SLPI, OSTF1, ANP32A and FCRL3) (5) and disease progression measured by the Addenbrooke's Cognitive Examination – Revised (ACE-R) in MCI-AD/AD. Greater ANP32A expression was associated greater reduction in cognition over one year (Spearman's r=-0.41 p=0.01). This remained significant when re-analysed using the general linear model, including age and sex as covariates.

There was no correlation between disease progression and TMEM145 in MCI-LB/DLB.

Gene Set Enrichment Analysis

Tables 3-5 and Supplementary Tables 7-12 list the gene sets demonstrating upregulation and downregulation in each diagnostic group identified by Gene Set Enrichment Analysis (22). All sets with an adjusted p<0.10 are listed. Supplementary Figures 1-3 illustrate networks of leading edge genes (p<0.25) in the primary comparisons. Upstream regulators identified by IPA are listed in Supplementary Table 13.

The MCI-LB/DLB group demonstrated downregulation in gene sets associated with MYC targets, oxidative phosphorylation, epithelial mesenchymal transition and KRAS signalling (Table 3).

Four of the five significant gene sets (p_{adj}<0.05) in the MCI-AD/AD group were related to upregulation of immune/inflammatory responses (interferon-α and -γ responses, inflammatory response and TNF-α signalling via NFκB) in addition to downregulation of haem metabolism (Table 4). Interferon alpha and gamma responses were upregulated and haem metabolism was downregulated in both MCI-AD and AD groups independently, compared with cognitively unimpaired participants.

When MCI-AD/AD was compared to MCI-LB/DLB, 12 gene sets showed differences in expression, with upregulation in MCI-AD/AD of interferon alpha and gamma demonstrating the greatest difference in normalised enrichment score (Table 5). Five of these gene sets demonstrated differences in expression when comparing MCI-AD and MCI-LB (Supplementary Table 11) and two demonstrated differences when comparing AD and DLB (Supplementary Table 12). TNF-α signalling via NFκB, MYC targets version 2, Inflammatory Response, and Genes Downregulated by KRAS signalling were gene sets common to both MCI-AD/AD and MCI-LB/DLB patients (Table 3 and Table 4). The results of the co-expression analysis are displayed in Supplementary Figure 4.

INSERT TABLES 3-5 HERE

Discussion

Summary

Multiple DEGs in blood in the MCI and dementia stages of both AD and DLB were identified, including one gene, ANP32A, that correlated with disease progression in AD. Gene Set Enrichment Analysis identified gene sets that may be involved in the pathophysiology of these diseases, including upregulation in gene sets associated with inflammation and immune responses in AD.

DEGS

A single DEG was identified in blood that has previously been reported as upregulated in DLB brain tissue, TMEM145. TMEM145 is associated with the G protein-coupled receptor pathway and is primarily found in the brain (GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)). We could find no evidence of an established pathophysiological link with DLB, and transcript expression was unaltered in a DLB brain RNAseq dataset (unpublished data). This gene was also upregulated in AD compared with cognitively unimpaired participants, and therefore may not be specific to Lewy body disease.

Six DEGs were identified that have previously been reported in AD (5), although none of these DEGs corresponded with a previously published meta-analysis of blood gene expression changes in AD (21). Greater expression of one of these DEGs identified in the current study, ANP32A, was correlated with a more rapid decline in cognition in AD/MCI-AD measured by the Addenbrooke's cognitive examination. ANP32A is an inhibitor of protein phosphatase-2A and is widely expressed

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throughout the human body. It is overexpressed in the brain in AD and has been associated with tau hyperphosphorylation (26). Downregulation of ANP32A in an AD mouse model was associated with reduced tau hyperphosphorylation, reduced synapse damage and relatively preserved memory function (27). The inhibition of ANP32A has therefore been suggested as a potential therapeutic strategy in AD. Our data suggest that peripheral expression of ANP32A is altered in AD and this may have prognostic value. This requires validation in other cohorts.

The six DEGs identified in blood in AD in our study and a previous report were entered into a post-hoc discriminant analysis and demonstrated only 75% accuracy in differentiating AD from the comparison group. Therefore, we did not identify any potential diagnostic biomarker genes in this analysis.

Four DEGs were identified when comparing MCI-AD with MCI-LB, 18 when comparing AD with DLB and two when comparing MCI-AD/AD with MCI-LB/DLB (Supplementary Table 6). RNA5-8SN2, a ribosomal RNA, was upregulated in DLB compared with AD and the cognitively unimpaired comparison group. There were no other DEGs when comparing AD and DLB groups that were also differentially expressed when comparing either disease group to the cognitively unimpaired comparison group.

Gene Set Enrichment Analysis

Five gene sets were significantly downregulated in the MCI-LB/DLB group: Myc Targets (versions 1 and 2), oxidative phosphorylation, epithelial mesenchymal transition and genes downregulated by KRAS activation. These results may be related to mitochondrial dysfunction in MCI-LB/DLB. Myc targets are implicated in mitochondrial regulation and biogenesis (28). Lewy body formation is associated with disruption to oxidative phosphorylation in vitro (29) and we have previously

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demonstrated reduction in oxidative phosphorylation in nucleus basalis of Meynert cholinergic neurons in DLB (30).

Only Myc Targets (version 2) was downregulated at both the MCI and dementia stages of DLB. This set was also downregulated in MCI-AD/AD, approaching statistical significance (p=0.05) therefore, the gene downregulation may be related to neurodegeneration in general, rather than being specific to MCI-LB/DLB. These gene sets did not show significant overlap with pathways identified in DLB brains in a recent meta-analysis of gene expression studies (6).

In contrast to previous reports of downregulation of inflammatory genes in DLB brains (31), gene sets associated with inflammatory response and TNF-alpha signalling via NFκB were upregulated in MCI-LB/DLB, approaching statistical significance (p_{adj}=0.06). These conflicting findings may be related to differences between central and peripheral inflammatory activity in DLB or differences related to disease stage. Recent post-mortem studies using cortical brain tissue have reported no increase in microglial numbers in DLB using immunohistochemistry (32) and downregulation of cytokines and chemokines in brain tissue using RNAseq (31). Post-mortem studies inevitably tend to report end-stage disease and there is a growing body of evidence supporting the presence of peripheral inflammatory processes during life and central immune activity in early disease. Raised plasma cytokine levels have been reported in MCI-LB (4) and DLB (33, 34), though downregulation of proinflammatory genes has been reported in extracellular vesicles isolated from serum in DLB (7). PET imaging using ligands for the translocator protein, which is expressed by microglia and astrocytes, has demonstrated increased binding in mild DLB, but not moderate to severe DLB (33). The interplay between peripheral and brain inflammatory responses in DLB remains to be elucidated and further work is required to understand the diagnostic potential of inflammatory markers in DLB.

In MCI-AD/AD we found upregulation of interferon-α and -γ responses, inflammatory responses and TNFα signalling via NFκB, along with downregulation of haem metabolism. Interferon-α and -γ responses have previously been reported as significantly upregulated in the blood of people with

AD, with TNFα signalling via NFκB and inflammatory response also upregulated in the African American subset of the same cohort (5). This adds to a growing body of research demonstrating immune system and inflammatory changes both in the central nervous system and periphery in AD (35-37). Type I interferons such as interferon-α may be important inflammatory mediators in AD (38). Increased interferon-γ has been found in the blood of people with AD in a meta-analysis (35). TNF-α has been associated with increased neuronal production of amyloid-β and α-synuclein in vitro (39), and TNF-α blockers are associated with a lower risk of AD in people with inflammatory diseases (40).

Genes associated with TNFα signalling via NFκB were significantly upregulated in the AD and mixed MCI-AD/AD cohort, but the increase in expression in the MCI-AD cohort was not significant (NES=1.0, p_{adj}=1). Interestingly, we found evidence of reduced plasma TNF-α in MCI-AD and normal TNF-α levels in AD compared with cognitively unimpaired participants in a cohort that included many of the samples analysed in this manuscript (4). This suggests that alterations in this pathway may not be related to the levels of expression of TNF-α itself. The same cohort had raised levels of interferon gamma in both MCI-AD and AD, but neither of these reached statistical significance. These findings demonstrate the power of transcriptomic studies to identify changes in physiological processes that may not be detected in traditional analyses. Overall, our findings support the presence of inflammation and immune system changes in both MCI-AD and AD.

Whilst our cohort demonstrated downregulation of haem metabolism, the converse was found in another AD cohort (5). The reason for these conflicting findings is not clear. The findings are not explained by the presence of MCI-AD cases in our cohort, as haem metabolism was significantly downregulated in both the MCI-AD and AD groups when examined independently.

There were differences in immune/inflammatory responses between AD and Lewy body disease, with interferon-α and -γ responses upregulated in AD compared with Lewy body disease at both the MCI and dementia stages. This highlights a key difference in peripheral inflammatory profiles

between the two diseases. Differences in B and T cell function between DLB and AD have recently been reported (34). The association of mixed pathology with immune and inflammatory profiles remains to be established.

Strengths and limitations

This is the first cohort of MCI-LB and the largest cohort of DLB to undergo RNAseq. Diagnostic group sizes were weighted towards DLB/MCI-LB due to the relative paucity of evidence in these diseases. All participants underwent a thorough clinical evaluation, and their diagnosis was confirmed by an expert panel. The groups were well balanced for age, sex and cognitive impairment. DEGs were identified following FDR correction to reduce the risk of false positive results. Following this, we cross referenced our findings with previously published research to identify DEGs that had previously been reported in at least one other study. Using these DEGs, we were unable to identify effective diagnostic biomarkers. However, we were able to identify one gene associated with disease progression, with a plausible biological pathway based on evidence from brain gene expression and transgenic mice. Gene set enrichment analysis allowed us to analyse physiological processes associated with altered gene regulation in AD and Lewy body disease. Our results in AD supported previous findings of peripheral changes in immune and inflammatory responses.

The workflow for the analysis of the RNAseq data was chosen due to previous evidence that using transcript level abundance estimates leads to improved differential gene expression results – in particular, gene level counting underestimates the expression of a particular subset of genes (those that have multi-mapping reads) (19). Reads with ambiguous bases were included in the analysis to avoid the introduction of bias through extensive trimming procedures (41). 0.07% of reads contained ambiguous bases.

Using the sample size calculation for RNA sequencing developed by Hart et al. (42), the required sample size to detect a 2-fold change with 80% power in this data would be 50 cases per group. As such, not all genes that differ between these groups will have been identified in our analysis. Nevertheless, this is the largest cohort of RNAseq of blood reported in DLB and the first cohort in MCI-LB, representing a significant contribution to the literature.

RNAseq in blood enables the examination of gene expression across different disease stages and correlation between gene expression and later disease progression. However, gene expression in blood will be affected by activity in a range of tissues and reflects RNA from blood cells, extracellular vesicles and RNA bound to plasma proteins. In addition, differing proportions of blood cell types between participants may affect gene expression, which we were not able to control for in this analysis. There is substantial variability in gene expression between individuals. We accounted for age and sex in our analyses, but other variables such as blood pressure, cholesterol levels, fasting glucose and body mass index can affect gene expression and were not accounted for in our analysis (43).

MCI and dementia groups in Alzheimer's and Lewy body disease were combined for the primary analysis to increase statistical power. However, there may be differences in disease pathophysiology at different disease stages, therefore comparisons were also reported for the MCI and dementia groups independently. The FDR was adjusted for the number of genes assessed but not the number of different comparisons between diagnostic groups, increasing the risk of Type I error.

Conclusions

We identified differences in gene expression in AD and DLB both at the dementia and MCI stages of disease. Whilst these changes do not appear to offer effective diagnostic biomarkers, ANP32A may be a marker of disease prognosis in AD. Gene set enrichment analysis confirmed the presence of

upregulation of interferon-α and -γ responses, inflammatory responses and TNF-α signalling via NFκB in AD. We did not find the previously reported downregulation of inflammatory and immune responses in DLB, but interferon-α and -γ responses were upregulated in AD compared with DLB in both the MCI and dementia stages of disease, highlighting a key difference in peripheral inflammatory responses between the diseases.

Author Contributions

PCD: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Writing - original draft; Writing - review & editing.

SJC: Data curation; Formal analysis; Investigation; Methodology; Resources; Writing - review & editing.

CMR: Investigation; Methodology; Writing - review & editing.

JC: Investigation; Methodology; Writing - review & editing.

JK: Resources; Funding acquisition; Writing - review & editing.

DE: Writing - review & editing.

DK: Writing - review & editing.

JPT: Writing - review & editing.

CMM: Conceptualization; Funding acquisition; Writing - review & editing.

JTO'B: Conceptualization; Funding acquisition; Methodology; Writing - review & editing.

AJT: Conceptualization; Funding acquisition; Methodology; Writing - review & editing.

Data Statement: Data are available via the Dementias Platform UK Portal

https://www.dementiasplatform.uk/.

Supplemental Digital Content

Supplementary Tables 1-6.docx

Supplementary Tables 7-13.docx

Supplementary Figures 1-4

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Tables

Table 1. DEGS in combined MCI-LB/DLB group compared with the cognitively unimpaired group

Ensembl Gene ID	Gene	Description	log$_2$ FC	p	p$_{adj}$
ENSG00000154620	TMSB4Y	Thymosin beta 4 Y-linked	-1.40	1.32×10^{-7}	0.001
ENSG00000260197	AC010889.1	Novel transcript	-1.54	8.38×10^{-8}	0.001
ENSG00000185818	NAT8L	N-acetyltransferase 8 like	-0.98	3.72×10^{-7}	0.002
ENSG00000228430	AL162726.3	SPTLC1 pseudogene	-3.03	4.01×10^{-7}	0.002
ENSG00000215580	BCORP1	BCL6 corepressor pseudogene 1	-1.25	5.57×10^{-7}	0.003
ENSG00000124196	GTSF1L	Gametocyte specific factor 1 like	-1.41	1.67×10^{-6}	0.006
ENSG00000102854	MSLN	Mesothelin	-2.25	2.41×10^{-6}	0.007
ENSG00000149575	SCN2B	Sodium voltage-gated channel beta subunit 2	1.53	2.29×10^{-6}	0.007
ENSG00000228463	AP006222.1	Ribosomal protein L23a (RPL23A) pseudogene	0.78	2.97×10^{-6}	0.007
ENSG00000224650	IGHV3-74	Immunoglobulin heavy variable 3-74	-1.32	6.98×10^{-6}	0.016
ENSG00000167619	TMEM145	Transmembrane protein 145	0.76	9.25×10^{-6}	0.019
ENSG00000225630	MTND2P28	MT-ND2 pseudogene 28	1.98	1.40×10^{-5}	0.026
ENSG00000268240	AC123912.2	Novel transcript	0.94	2.34×10^{-5}	0.041
ENSG00000167748	KLK1	Kallikrein 1	-0.96	3.50×10^{-5}	0.049
ENSG00000173898	SPTBN2	Spectrin beta, non-erythrocytic 2	-1.88	3.29×10^{-5}	0.049

ENSG00000175985	PLEKHD1	Pleckstrin homology and coiled-coil domain containing D1	-0.93	3.55×10^{-5}	0.049
ENSG00000183160	TMEM119	Transmembrane protein 119	0.70	4.37×10^{-5}	0.049
ENSG00000240563	L1TD1	LINE1 type transposase domain containing 1	-1.46	4.32×10^{-5}	0.049
ENSG00000254477	AP000640.1	Novel transcript	0.53	4.47×10^{-5}	0.049
ENSG00000277452	RN7SL473P	RNA, 7SL, cytoplasmic 473, pseudogene	1.06	3.87×10^{-5}	0.049
ENSG00000280399	AC022497.1	TEC	0.59	4.51×10^{-5}	0.049
ENSG00000117862	TXNDC12	Thioredoxin domain containing 12	0.35	4.77×10^{-5}	0.049

FC: fold change, P_{adj}: false discovery rate corrected p value from Wald z-statistic.

Table 2. DEGs in combined MCI-AD/AD group compared with cognitively unimpaired group

Ensembl Gene ID	Gene	Description	log$_2$ FC	p	p$_{adj}$
ENSG00000228430	AL162726.3	SPTLC1 pseudogene	-4.07	5.04×10^{-9}	1.23×10^{-4}
ENSG00000109684	CLNK	Cytokine dependent hematopoietic cell linker	-2.64	8.52×10^{-8}	8.64×10^{-4}
ENSG00000214708	AC116407.1	Novel transcript, antisense to RHOT1	1.26	1.07×10^{-7}	8.64×10^{-4}
ENSG00000260197	AC010889.1	Novel transcript	-1.70	2.74×10^{-7}	0.002
ENSG00000173898	SPTBN2	Spectrin beta, non-erythrocytic 2	-2.62	4.32×10^{-7}	0.002
ENSG00000277246	AL157762.1	Novel transcript, sense intronic to TNFSF13B	0.78	5.43×10^{-7}	0.002
ENSG00000215580	BCORP1	BCL6 corepressor pseudogene 1	-1.42	7.07×10^{-7}	0.002
ENSG00000240563	L1TD1	LINE1 type transposase domain containing 1	-1.99	9.44×10^{-7}	0.003
ENSG00000124196	GTSF1L	Gametocyte specific factor 1 like	-1.63	1.31×10^{-6}	0.003
ENSG00000154620	TMSB4Y	Thymosin beta 4 Y-linked	-1.48	1.38×10^{-6}	0.003
ENSG00000170390	DCLK2	Doublecortin like kinase 2	-2.00	2.45×10^{-6}	0.005
ENSG00000241163	LINC00877	Long intergenic non-protein coding RNA 877	0.54	2.30×10^{-6}	0.005
ENSG00000160856	FCRL3	Fc receptor like 3	-0.90	3.92×10^{-6}	0.007
ENSG00000232860	SMG7-AS1	SMG7 antisense RNA 1	1.08	4.06×10^{-6}	0.007

ENSG00000229246	LINC00377	Long intergenic non-protein coding RNA 377	-2.97	5.79×10^{-6}	0.009
ENSG00000168386	FILIP1L	Filamin A interacting protein 1 like	-1.63	7.74×10^{-6}	0.012
ENSG00000188487	INSC	INSC spindle orientation adaptor protein	1.07	1.02×10^{-5}	0.014
ENSG00000268240	AC123912.2	Novel transcript	1.10	9.75×10^{-6}	0.014
ENSG00000143061	IGSF3	Immunoglobulin superfamily member 3	-2.25	1.39×10^{-5}	0.016
ENSG00000233214	AC002511.2	Novel transcript	0.66	1.37×10^{-5}	0.016
ENSG00000256377	AC009509.1	Novel transcript	2.31	1.43×10^{-5}	0.016
ENSG00000271815	AC008897.3	Novel transcript	0.72	1.26×10^{-5}	0.016
ENSG00000167748	KLK1	Kallikrein 1	-1.14	1.71×10^{-5}	0.018
ENSG00000259138	AL049780.2	Novel transcript, antisense to NEK9 and TMED10	-1.36	1.84×10^{-5}	0.019
ENSG00000271344	AC018638.6	Novel transcript	0.56	2.54×10^{-5}	0.025
ENSG00000008853	RHOBTB2	Rho related BTB domain containing 2	-0.72	2.71×10^{-5}	0.025
ENSG00000154262	ABCA6	ATP binding cassette subfamily A member 6	-2.13	3.04×10^{-5}	0.026
ENSG00000214946	TBC1D26	TBC1 domain family member 26	-1.99	3.03×10^{-5}	0.026
ENSG00000092853	CLSPN	Claspin	0.43	3.30×10^{-5}	0.027
ENSG00000099953	MMP11	Matrix metallopeptidase 11	-0.90	3.37×10^{-5}	0.027

ENSG00000164096	C4orf3	Chromosome 4 open reading frame 3	0.31	3.74×10^{-5}	0.029
ENSG00000120051	CFAP58	Cilia and flagella associated protein 58	0.68	4.20×10^{-5}	0.032
ENSG00000124107	SLPI	Secretory leukocyte peptidase inhibitor	0.81	4.41×10^{-5}	0.033
ENSG00000141753	IGFBP4	Insulin like growth factor binding protein 4	-1.19	5.10×10^{-5}	0.033
ENSG00000185818	NAT8L	N-acetyltransferase 8 like	-0.89	4.98×10^{-5}	0.033
ENSG00000212743	LINC02656	Long intergenic non-protein coding RNA 2656	0.68	4.81×10^{-5}	0.033
ENSG00000228463	AP006222.1	RPL23A pseudogene	0.77	4.77×10^{-5}	0.033
ENSG00000248455	LINC02217	Long intergenic non-protein coding RNA 2217	1.00	5.14×10^{-5}	0.033
ENSG00000171435	KSR2	Kinase suppressor of ras 2	-2.00	5.76×10^{-5}	0.036
ENSG00000167751	KLK2	Kallikrein related peptidase 2	-1.67	5.96×10^{-5}	0.036
ENSG00000163421	PROK2	Prokineticin 2	0.67	7.58×10^{-5}	0.044
ENSG00000253314	LINC00293	Long intergenic non-protein coding RNA 293	2.05	7.61×10^{-5}	0.044
ENSG00000224610	AC108879.1	Novel transcript	-3.07	7.82×10^{-5}	0.044
ENSG00000072952	MRVI1	Murine retrovirus integration site 1 homolog	0.57	8.33×10^{-5}	0.045
ENSG00000105926	MPP6	Membrane palmitoylated protein 6	-0.72	9.30×10^{-5}	0.045
ENSG00000108379	WNT3	Wnt family member 3	-2.20	9.17×10^{-5}	0.045

ENSG00000145819	ARHGAP26	Rho GTPase activating protein 26	0.46	8.96×10^{-5}	0.045
ENSG00000188158	NHS	NHS actin remodeling regulator	0.60	9.08×10^{-5}	0.045
ENSG00000188585	CLEC20A	C-type lectin domain containing 20A	-1.74	8.87×10^{-5}	0.045
ENSG00000267365	KCNJ2-AS1	KCNJ2 antisense RNA 1	0.61	9.17×10^{-5}	0.045
ENSG00000078618	NRDC	Nardilysin convertase	0.29	1.02×10^{-4}	0.048
ENSG00000253519	AC106801.1	Novel transcript	1.15	1.02×10^{-4}	0.048
ENSG00000105426	PTPRS	Protein tyrosine phosphatase receptor type S	-0.55	1.08×10^{-4}	0.048
ENSG00000167619	TMEM145	Transmembrane protein 145	0.75	1.05×10^{-4}	0.048
ENSG00000175003	SLC22A1	Solute carrier family 22 member 1	0.60	1.14×10^{-4}	0.048
ENSG00000203814	HIST2H2BF	Histone cluster 2 H2B family member f	0.64	1.12×10^{-4}	0.048
ENSG00000231233	CFAP58-DT	CFAP58 divergent transcript	0.66	1.12×10^{-4}	0.048
ENSG00000280399	AC022497.1	TEC	0.63	1.12×10^{-4}	0.048
ENSG00000115155	OTOF	Otoferlin	1.19	1.18×10^{-4}	0.049
ENSG00000126262	FFAR2	Free fatty acid receptor 2	0.56	1.23×10^{-4}	0.049
ENSG00000170873	MTSS1	MTSS I-BAR domain containing 1	-0.40	1.24×10^{-4}	0.049

FC: fold change, P_{adj}: false discovery rate corrected p value from Wald z-statistic.

Table 3. Gene Set Enrichment Analysis: MCI-LB/DLB v cognitively

unimpaired

Pathway	p_{adj}	NES
MYC targets version 1	**0.02**	-1.68
MYC targets version 2	**0.02**	-1.86
Oxidative phosphorylation	**0.03**	-1.50
Epithelial mesenchymal transition	**0.05**	-1.44
Genes downregulated by KRAS signalling	**0.05**	-1.54
TNF-α signalling via NFκB	0.06	1.45
Apical junction	0.06	-1.40
Inflammatory response	0.06	1.44
Xenobiotic metabolism	0.06	-1.39

P_{adj}: FDR corrected p value, NES: normalised enrichment score. Significant

results in bold.

Table 4. Gene Set Enrichment Analysis: MCI-AD/AD v cognitively unimpaired

Pathway	p_{adj}	NES
Interferon-α response	**0.004**	2.29
Interferon-γ response	**0.004**	2.07
Inflammatory response	**0.004**	1.76
Haem metabolism	**0.004**	-1.82
TNF-α signalling via NFκB	**0.05**	1.48
MYC targets version 2	0.05	-1.62
Genes downregulated by KRAS signalling	0.07	-1.46

P_{adj}: FDR corrected p value, NES: normalised enrichment score.

Significant results in bold.

Table 5. Gene Set Enrichment Analysis: MCI-AD/AD v MCI-LB/DLB.

Pathway	p$_{adj}$	NES
Interferon-α response	**0.006**	2.53
Interferon-γ response	**0.006**	2.30
Haem metabolism	**0.006**	-1.77
G2/M Checkpoint	**0.009**	1.55
Oestrogen response (late)	**0.009**	1.52
Apical junction	**0.009**	1.52
Epithelial mesenchymal transition	**0.01**	1.50
E2F targets	**0.01**	1.49
Xenobiotic metabolism	**0.03**	1.43
Inflammatory response	**0.04**	1.39
Glycolysis	**0.04**	1.38
Mitotic spindle	**0.05**	1.37

P$_{adj}$: FDR corrected p value, NES: normalised enrichment score. Significant

results in bold.