Differences in peripheral immune system gene expression in frontotemporal degeneration

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Abstract

The peripheral immune system has a key pathophysiologic role in Frontotemporal degeneration (FTD). We sought a comprehensive transcriptome-wide evaluation of gene expression alterations unique to the peripheral immune system in FTD compared to healthy controls and amyotrophic lateral sclerosis. Nineteen subjects with FTD with 19 matched healthy controls and 9 subjects with amyotrophic lateral sclerosis underwent isolation of peripheral blood mononuclear cells (PBMCs) which then underwent bulk ribonucleic acid sequencing.

There was increased expression in genes associated with CD19+ B-cells, CD4+ T-cells, and CD8+ T-cells in FTD participants compared to healthy controls. In contrast, there was decreased expression in CD33+ myeloid cells, CD14+ monocytes, BDCA4+ dendritic cells, and CD56+ natural killer cells in FTD and healthy controls. Additionally, there was decreased expression seen in associated with 2 molecular processes: autophagy with phagosomes and lysosomes, and protein processing/export. Significantly downregulated in PBMCs of FTD subjects were genes involved in antigen processing and presentation as well as lysosomal lumen formation compared to healthy control PBMCs.

Our findings that the immune signature based on gene expression in PBMCs of FTD participants favors adaptive immune cells compared to innate immune cells. And decreased expression in genes associated with phagosomes and lysosomes in PBMCs of FTD participants compared to healthy controls.

Abbreviations: ALS = amyotrophic lateral sclerosis, DEGs = differentially expressed genes, FTD = frontotemporal degeneration, GO = gene ontologies, KEGG = Kyoto Encyclopedia of Genes and Genomes, PBMCs = peripheral blood mononuclear cells, RNA = ribonucleic acid.

Keywords: amyotrophic lateral sclerosis, frontotemporal dementia, genetics:dementia, neurodegeneration:inflammation, transcriptomics

1. Introduction

Frontotemporal degeneration (FTD) is a progressive neurodegenerative disorder that is characterized clinically by marked behavior and/or language disturbance. It is the second most common younger-onset dementia syndrome, after Alzheimer disease (AD), and accounts for 10% to 15% of all dementia cases.[1] Given the younger onset of FTD, approximately 60% of FTD patients are 45 to 64 years old at age of onset,[1,2] the economic cost of FTD is highest among the dementia subtypes.[3] FTD also has substantial overlap with amyotrophic lateral sclerosis (ALS), a fatal neurological disorder characterized by motor neuron degeneration in the primary motor cortex, brainstem, and spinal cord.[4] Approximately 15% of FTD patients demonstrate motor neuron degeneration consistent with ALS.[5] Furthermore intraneuronal TAR DNA-binding protein 43 inclusions are common pathologic findings in FTD and ALS at autopsy.[6]

Inflammation is becoming increasingly appreciated as a contributor to FTD. Inflammatory markers in blood, cerebrospinal fluid, and identified in neuropathology support a central and peripheral inflammatory response in FTD-associated neurodegeneration.[7] Increased rates of autoimmune disease, including autoimmune thyroid disease, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, and chronic lymphocytic colitis in FTD subtypes compared to healthy controls provided early evidence of systemic differences in the immune system in FTD.
OR = 3.27, 95% CI: 1.009-10.6) and AD (OR = 3.73, 95% CI: 1.1-12.9).\[8,9\] This was reinforced by molecular studies that demonstrated higher levels of inflammatory cytokines: autoantibody IgG-GA1, IL-6, Tumor Necrosis Factor-alpha, Transforming Growth Factor-beta in serum and cerebrospinal fluid in FTD compared to healthy controls.\[10-14\] Genome wide association studies have demonstrated an immune-mediated genetic enrichment in FTD, suggesting that for a subset of patients, immune dysfunction may contribute to FTD risk.\[15,16\] Furthermore, FTD has a unique circulating immune cell population compared to AD and healthy controls.\[17\] Thus, the peripheral immune system may have a key pathophysiologic role in FTD and may have potential roles as a diagnostic biomarker, prognostic biomarker, and potential therapeutic target in FTD.

Previous evaluations of the peripheral immune system have focused on cytokines or genomics. Instead, we sought a comprehensive transcriptome-wide evaluation of gene expression alterations unique to FTD compared to healthy controls as well as compared to ALS, which frequently shares underlying TAR DNA-binding protein 43 pathology. To elucidate the unique patterns of immune expression in the peripheral immune system of FTD subjects, we performed ribonucleic acid (RNA)-sequencing (RNA-seq) of peripheral blood mononuclear cells (PBMCs), cells of interest for immune function and mechanisms of inflammation. This preliminary work sought to determine differences in gene expression within the peripheral immune system in FTD compared to healthy aging. Additionally, we sought to whether gene expression favored the rapid and nonspecific innate immune system, or the more specific memory cell-based immunity seen in the adaptive immune system.\[18\]

2. Materials and methods

2.1. Standard protocol approvals, registrations, and patient consents

The protocol for this study was approved by the University of Cincinnati institutional review board, approval # 2018-7713. Informed consent was obtained from all research subjects. Assent was given by guardian/power-of-attorney for FTD and ALS subjects. Enrollment was completed between March 2018 and November 2018; enrollment was based on patient consent during the time frame.

2.2. Subjects

Subjects with FTD had a diagnosis of probable or definite FTD based on Rascovsky criteria for behavioral variant FTD or Gorno-Tempini criteria for primary progressive aphasia.\[19,20\] FTD subjects were excluded if they had concurrent AD, Parkinson disease, or Lewy Body disease based on the diagnostic review of 2 behavioral neurologists at our center (RPS, RSS).

Healthy controls were recruited from the Genetic and Environmental Risk Factors for Hemoeragic Stroke study being conducted at University of Cincinnati. All controls were over the age of 18 years old and matched to an FTD subject by age (± 5 years), race/ethnicity (by self-identification), and sex. For healthy controls, no participant or informant could report subjective cognitive decline in the prior year, and there must be no evidence from screening visit suggesting a neurodegenerative disorder. Additionally, individuals with a family history (3 degrees) of autosomal dominant neurodegenerative or neuropsychiatric disease, and individuals harboring a known disease mutation, were excluded from the study. Diabetes Mellitus, smoking, and body mass index have been shown to alter the peripheral immune system and were included in the analysis as covariates. ALS subjects were included if they met the El Escorial diagnostic criteria for probable or definitive ALS and had no clinical features consistent with FTD based on review by 2 behavioral neurologists at the time of enrolment.\[21\] FTD and ALS subjects underwent commercial genetic testing through PreventionGenetics, LLC incorporated to evaluate presence of pathogenic mutations in 25 genes associated with FTD including Chromosome 9 open reading frame 72 repeat expansions, granulin gene, and microtubule associated protein tau. This testing was used to categorize but not exclude FTD/ALS subjects.

2.3. Specimen collection and RNA sequencing

Freshly collected whole blood in EDTA-coated tube was inverted 10 times before putting the tube on ice, and immediately sent to the lab for PBMC isolation using a Sepmate-15 tube according to the manufacturer’s instruction (Stemcell Technologies, Vancouver, Canada). Once the cells were pelleted in the last step, 1 mL of Lysis Buffer from mirVana microRNA Isolation Kit (Thermo Fisher, Grand Island, NY) was added and vortexed to lyse the cells, and then stored in −80 °C until total RNA extraction.

Total RNA was extracted according to the protocol from mirVana microRNA Isolation Kit and was eluted with 60 μL elution buffer. The RNA concentration was measured by Nanodrop (Thermo Scientific, Wilmington, DE) and its integrity was determined by Bioanalyzer with Agilent RNA 6000 Nano Kit (Agilent, Santa Clara, CA). To enrich polyA RNA for RNA-seq, NEBNext Poly(A) mRNA Magnetic Isolation Module (New England BioLabs, Ipswich, MA) was used with 1 μg of high-quality total RNA as input. SMARTer Apollo NGS library prep system (Takara, Mountain View, CA) was used for automated polyA RNA isolation via PrepX PolyA protocol. The library for RNA-seq was prepared by using NEBNext Ultra II Directional RNA Library Prep kit (New England BioLabs, Ipswich, MA) according to the vendor’s instructions. In the indexing step, the polymerase chain reaction cycle number was set to 8 and the amplified libraries were cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA) according to the standard protocol. After Bioanalyzer quality control using Agilent High Sensitivity DNA Kit followed by library quantification using NEBNext Library Quant Kit (New England BioLabs), the libraries at the final concentration of 15 pM were clustered onto a single read (SR) flow cell v3 using TruSeq SR Cluster kit v3 (Illumina, San Diego, CA) and sequenced to 51 bp using TruSeq SBS kit v3 (Illumina) on HiSeq sequencer (Illumina).

2.4. Bioinformatics

The RNA-seq data was analyzed following a standard, previously described pipeline.\[22\] Sequence reads were aligned to the current reference genome (GRCh38). The reads aligned to each known gene were counted using the STAR aligner\[23\] and the latest GENCODE definitions of gene features.\[24\] The quality control of raw and aligned reads was performed using FastQC,\[25\] RNA-SeQC,\[26\] and summarized using MultiQC\[27\] software. Differentially expressed genes (DEGs) were identified based on the false discovery rate-adjusted P values\[28\] obtained by

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fitting generalized linear model based on negative-binominal distribution of read counts as implemented in the edgeR Bioconductor package. The patterns of gene expression across different sample groups were summarized and visualized using in-house developed Bayesian Infinite Mixture Models cluster analyses methods.

The functional characteristics of DEGs were studied by Gene Set Enrichment Analysis as implemented in the R package fgsea and empirical P values based on 100,000 randomly sampled genes. Gene sets analyzed included publicly available databases such as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, genes associated to Gene Ontologies (GO), ImSig markers of immune cells in blood and tissue transcriptomics data, and previously described lists of cell type markers derived from gene atlas dataset.

2.5. Data availability statement

Raw data was generated at University of Cincinnati. Derived data supporting the findings of this study are available from the corresponding author on request.

3. Results

Forty-seven subjects were enrolled in the study; their demographic information is available in Table 1. Because healthy controls were matched to the FTD subjects based on age, sex, and race/ethnicity, there was no difference between the 2 groups in these variables. The ALS group was not matched based on age and was younger overall than the FTD group (2-tailed P value equal to .0079, 95% CI –14.71 to –2.43). No differences in frequency of diabetes mellitus, smoking, and body mass index were noted between the FTD and healthy control groups. Of the 19 FTD subjects, 1 subject had a pathogenic mutation in TANK Binding Kinase 1, 1 subject had a pathogenic mutation in Senataxin, 1 subject had a pathogenic mutation in Fused in Sarcoma, 1 subject had a pathogenic mutation in Progranulin, and 1 subject had a pathogenic mutation in Microtubule associated protein Tau. Of the ALS subjects, there were 2 subjects with pathogenic expansions in chromosome 9 open reading frame-72 and 1 pathogenic Annexin A11 mutation.

In total, 15,782 genes were expressed in the PBMCs in our study. There were 341 DEG’s in the FTD group compared to the healthy control group, with a false discovery rate <0.1. Utilizing public database ImSig, we sought to determine whether gene expression within PBMCs of FTD compared to PBMCs of healthy controls favored specific cell lines. There was increased expression in genes associated with CD19+ B-cells (adjusted P value = .0002, normalized enrichment score = 2.17), CD4+ T-cells (adjusted P value = .0002, normalized enrichment score = 2.05), and CD8+ T-cells (adjusted P value = .0002, normalized enrichment score = 1.83) in FTD participants compared to HC. In contrast, there was decreased expression in CD33+ myeloid cells (adjusted P value = .0002, normalized enrichment score = –2.89), CD14+ monocytes (adjusted P value = .0002, normalized enrichment score = –2.75), BDCA4+ dendritic cells (adjusted P value = .0002, normalized enrichment score = –2.25), and CD56+ natural killer cells (adjusted P value = .0002, normalized enrichment score = –1.90) in FTD and healthy controls.

To determine which biologic functions and molecular pathways are affected in PBMCs of FTD compared to healthy controls, we completed KEGG analysis and GO. During KEGG analysis, 57 pathways were identified with an adjusted P value <.05. The majority of KEGG pathways were downregulated in the PBMCs of the FTD group compared to the HC group. Table 2 demonstrates the KEGG pathways with the highest absolute normalized effect size with a P value <.05. The 3 KEGG pathways with both adjusted P value of <.05 and a normalized effect size >1.5, are listed in Table 2 as well. Decreased expression is seen in KEGG pathways associated with 2 molecular processes: autophagy with phagosomes and lysosomes, and protein processing/export. GO analysis uncovered 295 GO processes with an adjusted P value <.05. Significantly downregulated in PBMCs of FTD subjects were genes involved in antigen processing and presentation as well as lysosomal lumen formation compared to healthy control PBMCs. Purine receptor activity and T-cell proliferation/apoptosis were significantly upregulated in FTD PBMCs compared to healthy controls according to GO. These results are summarized in Table 3.

Only a single gene was differentially expressed between FTD and ALS samples, though after adjusting for multiple comparisons, it did not meet statistical significance. The ALS group had 8 DEG’s compared to the healthy control group, which are listed in Table S1, Supplemental Digital Content, http://links.lww.com/MD2/A848. Utilizing the public database ImSig, there was increased expression in genes associated with T-cells (adjusted P

| Table 1 |
| --- |
| **Demographics.** | **FTD** | **HC** | **ALS** | **P value** |
| # Subjects | 19 | 19 | 9 |  |
| %Female | 42.1% | 42.1% | 22.2% | .31<sup>4</sup> |
| Mean age (yrs) | 70.6 (5.9) | 69.9 (6.4) | 62 (9.1) | .74 |
| % Caucasian | 100% | 100% | 88.9% | .32<sup>4</sup> |
| % Diabetes | 15.8% | 31.6% | 11.1% | .25 |
| % Smoker | 10.5% | 11.1% | 11.1% | .55 |
| % Genetic | 26.3% | 0% | 33.3% | .70<sup>4</sup> |
| Mean BMI | 29.2 (7.2) | 28.6 (4.7) | 27.8 (6.6) | .78 |
| Mean CDR | 0.95 (0.4) | 2.17 (0.3) | 2.5 (0.4) | .18 |

Genetic refers to a pathogenic autosomal dominant mutation resulting in FTD or ALS.
ALS = amyotrophic lateral sclerosis, BMI = body mass index, CDR = clinical dementia rating scale, CDR-SOB = clinical dementia rating scale sum of boxes, FTD = frontotemporal dementia, HC = healthy control.

<sup>1</sup> Two FTD subjects subsequently developed motor neuron symptoms after sample collection and inclusion in the study. Their data was included in the FTD group as they were originally assigned.

<sup>4</sup> P value calculated as FTD compared to ALS subjects.
value = .004, normalized enrichment score = 1.4) and B-cells (adjusted P value = .0003, normalized enrichment score = 2.35) within PBMCs of ALS compared to PBMCs of healthy controls. There was also decreased gene expression in genes associated with neutrophils (adjusted P value = .0003, normalized enrichment score = -2.42), monocytes (adjusted P value = .0003, normalized enrichment score = -2.13), and macrophages (adjusted P value = .0003, normalized enrichment score = -2.09).

During KEGG analysis, 13 pathways were identified with an adjusted P value < .05 in ALS PBMCs compared to healthy control PBMCs. Like the FTD PBMCs, ALS PBMCs had decreased expression of genes involved in KEGG pathways associated with autophagy (adjusted P value = .0004, normalized enrichment score = -1.63) and lysosomes (adjusted P value = .002, normalized enrichment score = -1.56). See Table S2, Supplemental Digital Content, http://links.lww.com/MD2/A849 for complete list of KEGG pathways in ALS PBMCs compared to healthy control PBMCs with adjusted P value < .05. GO analysis found 149 GO processes with an adjusted P value < .05 in ALS PBMCs vs healthy control PBMCs. Table S3, Supplemental Digital Content, http://links.lww.com/MD2/A850 lists the 10 GO processes with the highest normalized enrichment scores and the 10 GO processes with the lowest normalized enrichment scores. In ALS PBMCs there was increased expression in genes associated with translation: elongation (adjusted P value = .002, normalized enrichment score = 2.49), termination (adjusted P value = .002, normalized enrichment score = 2.48), and initiation (adjusted P value = .002, normalized enrichment score = 2.39) compared to healthy control PBMCs.

4. Discussion
Our study uncovered significant differences in gene expression within the peripheral immune system in FTD and ALS compared to healthy controls. The findings suggest dysregulation in pathways involved in autophagy and lysosomal function, as well as changes in gene expression associated with translation and protein processing. These alterations may provide insights into the immune response in frontotemporal degeneration and amyotrophic lateral sclerosis and contribute to our understanding of the disease processes in these conditions.
to healthy aging. Although our study examined PBMCs collectively, rather than filtering them into cellular subgroups, there was significantly increased expression in genes associated with adaptive immune cells (CD4+ and CD8+ T-cells as well as CD19+ B-cells) in FTD. Additionally, there was decreased expression in genes associated with innate immune cells in FTD compared to healthy aging: CD33+ myeloid cells, CD14+ monocytes, BDCA4+ dendritic cells, and CD56+ natural killer cells. Similar results were seen in the ALS group compared to the healthy controls, PBMCs of ALS participants had greater gene expression in genes favoring B-cells and T-cells compared to healthy controls, PBMCs of ALS participants had greater gene expression in genes favoring B-cells and T-cells compared to healthy aging. Within PBMCs there is upregulation of genes associated with the innate immune system in FTD and ALS, or if these are independent events. Our findings that the immune signature based on gene expression in PBMCs favors adaptive immune cells compared to innate immune cells, differs from previous work which demonstrated no difference in the number of memory T-cells, monocytes, and natural killer cells in FTD compared to healthy controls based on flow cytometry. Although these contradictory findings may be a result of methodological differences (gene expression vs flow cytometry) or smaller sample sizes (19 FTD subjects in our study vs 12 in Susse et al'), an alternative explanation is that gene expression within the adaptive immune system is significantly more altered in FTD compared to healthy aging. Genome wide association data supports our findings, which favored the adaptive immune system activation. GWAS has shown FTD risk genes in HLA regions and RAB38/CTSC loci in FTD. Additionally, previous studies of mouse models of FTD demonstrated infiltration of the brain parenchyma by CD8+ cells, which was not observed in healthy aging controls.

Beyond PBMC expression patterns favoring an adaptive immune response in FTD compared to healthy aging, there was decreased expression in genes associated with phagosomes and lysosomes in PBMCs of FTD participants compared to healthy controls. Concordantly, there was also decreased gene expression in the autophagy pathway, of which phagosomes and lysosomes are key players. Genes associated with lysosomes, as well as lysosomal dysfunction, have been implicated in FTD through GWAS, human tissue, induced pluripotent stem cells, and animal models. Thus, it is unsurprising that we observed decreased gene expression in genes associated with lysosomes in FTD compared to healthy aging within PBMCs. This may represent a genetic predisposition in FTD for decreased lysosomal function, occurring in both brain tissue as well as the immune system. Alternatively, because lysosomes function primarily in the innate immune system, this may represent further downregulation of the innate immune system in FTD. Autophagy-lysosome pathways may also be potent regulators of inflammatory responses within the immune system. Altered gene expression in autophagy-lysosome genes within PBMCs may promote altered systemic inflammatory responses in FTD compared to healthy aging.

We observed no significant differences between gene expression within PBMCs of FTD and ALS participants. Although this is likely the result of the relatively small sample size, 19 FTD participants and 9 ALS participants, it could also be the result of similar changes in gene expression within the immune system. Supporting the latter idea is the downregulation in genes associated with autophagy and lysosomes KEGG pathways in ALS participants compared to healthy controls, like the FTD participants discussed above. Unsurprisingly, autophagy and lysosomes have been implicated in ALS pathogenesis.58,59

Our findings indicate differences in gene expression within the peripheral immune system in FTD and ALS compared to healthy aging. This could be driven by a response to tissue damage in the brain, initially sensed by resident glia then amplified and propagated by the peripheral immune cells; or an intrinsically altered peripheral immune system in ALS and FTD patients driven by genetic differences. Supporting the latter concept, several autosomal dominant mutations resulting in FTD and ALS already have evidence linking their dysfunction to altered immunity. Additionally, altered autophagy and lysosome function, as was seen in our study, could modify the immunologic tone toward a pro-inflammatory state. Thus targeting autophagy and lysosomal pathways in the immunologic system has the potential to be a therapeutic target in FTD and ALS.

Although our limited sample size of 19 FTD subjects and 19 matched healthy controls limits our generalizability, this work serves as a building block for studying differences in the peripheral immune system in FTD compared to healthy aging.

5. Conclusions

There are significant differences in the gene expression within the peripheral immune system in FTD and ALS compared to healthy aging. Within PBMCs there is upregulation of genes associated with the adaptive immune response (B-cells and T-cells) with downregulation of genes associated with the innate immune system (monocytes, neutrophils, natural killer cells, and macrophages) in FTD and ALS. This may be the related to altered expression of genes related to autophagy and lysosome function within the immune system of FTD and ALS. Thus, targeting autophagy and lysosome function and altering immunologic tone may represent a therapeutic target within FTD and ALS.

Author contributions

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