SUPPLEMENTAL MATERIAL

Supplemental Methods

Participants

The study included 394 participants in the ALLFTD consortium. ALLFTD represents a harmonized merger of two complementary familial FTLD studies initiated in 2014: The Advancing Research and Treatment of Frontotemporal Lobar Degeneration (ARTFL) and Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects (LEFTTDS) studies. The ALLFTD consortium currently includes 23 centers across the United States and Canada (allftd.org) with 18 contributing data to this project. Sites enroll individuals based on a family history suggestive of familial FTLD, usually identified through interview of a symptomatic proband. Once a family member with a pathogenic variant is identified, other members of the family are contacted and invited to participate. Enrollees do not need to know their genetic status. Only patients with pathogenic C9orf72, GRN, or MAPT variants, or non-carrier family members, were included in the analyses reported here. Genetic screening for the three major autosomal dominant pathogenic variants plus apolipoprotein E genotyping methods are described in detail elsewhere[1].

The 394 study participants included 251 FTLD pathogenic variant carriers (C9orf72=117, GRN=62, MAPT=72) and 143 functionally normal, non-carrier family members with at least one of the three inflammation proteins (IL-6, YKL-40, TNFα) analyzed at baseline. All non-carriers were functionally normal at baseline based on a global score of 0 using the Clinical Dementia Rating scale plus National Alzheimer’s Coordinating Center (NACC) FTLD module (CDR®+NACC FTLD; see below). Clinical phenotype frequency for each genetic group is shown in Table 1.

Converters vs. Non-converters

To inform clinical utility of inflammatory biomarkers, we used longitudinal clinical data (minimum 2 study visits) to define cohorts at baseline based on their future disease trajectory. Clinical disease severity was defined using the CDR+NACC FTLD global score[2]. Asymptomatic non-converter pathogenic variant carriers were clinically normal (CDR+NACC FTLD Global=0) at all study visits (N=90). Asymptomatic converters were clinically normal at baseline and exhibited at least mild behavioral or cognitive changes (CDR+NACC FTLD Global>0) at their last study visit (N=19).

Plasma Collection and Protein Measurement

Blood samples were collected and stored following standardized procedures for the ALLFTD consortium. Plasma IL-6, YKL-40, and TNFα concentrations were quantified using Meso Scale Discovery (MSD) chemiluminescence assays. IL-6 and TNFα were measured via automated multiplex (UCSF Gladstone Institute; completed June 2019) and selected from the larger set of markers for the present study given their broad relevance within systemic inflammatory pathways and both analytes were consistently quantified in the linear range of detection. YKL-40 was measured via singleplex (San Francisco VA hospital core lab; completed December 2019). All samples underwent two freeze thaw cycles during the analyte measurement process. Batch effects were minimized through sample randomization by the National Centralized...
Repository for Alzheimer’s Disease and Related Dementias (NCRAD) prior to shipment to UCSF.

All 394 participants had at least one inflammatory biomarker measured (IL-6, N=375; YKL-40, N=394; TNFα, N=389). Samples with coefficient of variation (CV%) >25% were excluded from all analyses (IL-6, N=35; YKL-40, N=0; TNFα, N=21). After exclusion of samples with high CV%, mean±SD CV% were 8.7±6.4% (IL-6), 1.8±1.8% (YKL-40), and 8.7±4.6% (TNFα).

Through a separate study, plasma NfL concentrations were measured with single-molecule array technology (Simoa) using the commercially available NF-light digital immunoassay kit (Quanterix, Lexington, MA), as described elsewhere[3]. Plasma samples were thawed at room temperature (1 cycle), mixed thoroughly, and centrifuged at 14,000g for 3 minutes. The supernatant was loaded onto a Quanterix HD-1 Analyzer with 1:4 specified dilution. Samples were measured in duplicate with an 8-point calibration curve tested in triplicate and 2 controls tested in duplicate. Plasma concentrations were interpolated from the calibration curve within the same batch and corrected for the dilution. All samples were quantifiable within the dynamic range of 0.69-2,000 pg/mL with average coefficient of variation of 6.2±4.3%. The samples from the subset of participants with plasma NfL data included in this study all had CV<20%.

Functional, Behavioral, and Cognitive Assessments

Primary Clinical Outcome: Clinical Disease Severity

Our primary longitudinal clinical outcome was based on the CDR+NACC FTLD rating scale. The CDR+NACC FTLD is a measure of clinical disease severity optimized for FTD spectrum cohorts[2, 4]. It can be used to generate a “Global” score that represents a weighted average of eight functional domain scores to categorize each patient as asymptomatic (CDR+NACC FTLD Global=0), prodromal mild cognitive or behavioral symptoms of neurodegenerative disease[4] (“prodromal”; CDR+NACC FTLD Global =0.5), or clear functionally impairing symptoms consistent with dementia (“overtly symptomatic;” CDR+NACC FTLD Global ≥1). For analyzing longitudinal clinical disease severity, we used the CDR+NACC FTLD Sum of Boxes (SB) score (range 0 to 24; higher scores indicate worse severity).

Secondary Clinical Outcomes: Socioemotional Sensitivity and Cognition

We evaluated longitudinal changes in socioemotional sensitivity using the Revised Self-Monitoring Scale (RSMS) total score[5]. The RSMS is a validated 13-item questionnaire that measures sensitivity and responsiveness to subtle emotional expressions during face-to-face interactions. The questionnaire has good internal consistency, retest reliability, and construct validity[6, 7]. The RSMS was completed by an informant who was a first-degree family member or friend who had known the participant for ≥5 years. Informants rated patients on each item on a 6-point Likert scale ranging from “certainly, always false” to “certainly, always true,” with lower scores representing more severe dysfunction (i.e., less socioemotional sensitivity). The RSMS has been shown to be sensitive to progression of both socioemotional symptoms and salience network atrophy in patients with familial and non-familial forms of the behavioral variant phenotype of FTLD[8, 9]. Behavioral variant phenotypes are the most common symptom presentation in the ALLFTD cohort.

We defined cognition using the National Alzheimer's Coordinating Center Uniform Data Set (v3.0) executive function composite score (UDS3-EF)[10]. The UDS3-EF is an item response
theory-based composite derived from 7 total UDS3-EF test scores: category fluency (animals and vegetables; total correct), lexical fluency (F and L words; total correct), number span backward (total correct trials), Trail Making Test parts A and B (correct lines per minute). The cognitive domains that factor into the UDS3-EF score (e.g., mental set-shifting, verbal fluency, attention/working memory) support the potential utility as a single composite measure of the common cognitive changes observed across a range of clinical phenotypes in FTLD spectrum patients. Higher UDS3-EF scores suggest better executive functioning, and a validation study showed significantly lower scores in bvFTD patients compared to controls and other patient groups[10]. Additionally, the Montreal Cognitive assessment (MoCA) was analyzed to help characterize global cognition in the study cohort at baseline.

Voxel-Based Morphometry

Volumetric MPRAGE sequences acquire T1-weighted images of the entire brain (sagittal slice orientation; slice thickness = 1.2 mm; in-plane resolution = 1.0x1.0 mm; TR, TE and flip angle are vendor specific according to the LEFFTDS recommendation[11]. Before any prepossessing of the images, all T1-weighted images were visually inspected for quality control. Images with excessive motion or image artifact were excluded. T1-weighted images underwent bias field correction using N3 algorithm, and segmentation was performed using SPM12 (Wellcome Trust Center for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm) unified segmentation[12]. An intra-subject template was created by non-linear diffeomorphic and rigid-body registration proposed by the symmetric diffeomorphic registration for longitudinal MRI framework[13]. The intra-subject template was segmented also using SPM12’s unified segmentation. A within-subject modulation was applied by multiplying the timepoints’ Jacobian determinant with the intra-subject averaged tissues[14]. A customized group template was generated from the within-subject average gray and white matter tissues and cerebrospinal fluid by non-linear registration template generation using Large Deformation Diffeomorphic Metric Mapping framework[15]. Modulated intra-subject gray and white matter were geometrically normalized to the group template and then smoothed using approximately 8mm full width half maximum Gaussian kernel in the group template. Every step of the transformation was carefully inspected from the native space to the group template. For statistical purposes, linear and non-linear transformations between the group template space and International Consortium of Brain Mapping (ICBM) were applied[16]. For the cross-sectional analysis, the baseline time points were modulated by taking the determinant of the Jacobian composed transformations from the native space to the group template. We used the same smoothing kernel as previously described.

Data Analyses

Analyses were performed using SPSS (IBM Corporation; Versions 25.0 and 27.0). Group differences in potentially confounding variables (age, sex, education) between pathogenic variant carriers and non-carriers were analyzed with linear regression models. We further assessed differences in global cognition (MoCA), CDR+NACC FTLD SB, RSMS total score, and UDS3-EF score, and frequency of asymptomatic status (CDR+NACC FTLD Global=0) between the three genetic groups at baseline. We evaluated histograms and Q-Q plots of residuals for all regression models. Dependent variables were log transformed when there were clear departures from normality.

Cross-sectional Group Comparisons
We performed linear regressions to evaluate baseline plasma inflammation differences between pathogenic variant carriers and non-carriers and between the three genetic groups (dummy coded to interpret pairwise genetic group differences) controlling for age, sex, and years of education. To determine whether presence of a pathogenic variant (versus disease severity) was associated with differences in inflammatory protein levels, we compared asymptomatic non-converter pathogenic variant carriers to non-carrier family members who also had 2+ study visits (N=112). We also compared inflammatory protein levels among genetic groups (C9orf72 vs. GRN vs. MAPT) while additionally controlling for disease severity (CDR+NACC FTLD SB). We then specifically evaluated the effect of disease severity on plasma inflammatory proteins among all pathogenic variant carriers (N=251; CDR+NACC FTLD Global=0 vs. 0.5 vs. 1+). Effect sizes were interpreted based on standardized beta (β) estimates: 0.1 = small, 0.3 = medium, 0.5 = large.

**Baseline Inflammation and Rate of Functional, Socioemotional, and Cognitive Changes**

We used linear mixed effects models (restricted maximum likelihood estimation) with random slopes and intercepts (unstructured covariance) to evaluate the association between baseline inflammation levels and longitudinal changes in our primary (CDR+NACC FTLD SB) and secondary (RSMS total score, UDS3-EF score) clinical outcomes. Each participant’s baseline was defined as the first study visit with an available plasma inflammation biomarker measurement. Longitudinal models excluded pathogenic variant carriers with baseline CDR+NACC FTLD Global>1 to limit ceiling effects associated with severe impairment at baseline.

Our primary goal was to determine whether baseline inflammation levels were associated with rates of change in clinical outcomes among pathogenic variant carriers. We therefore evaluated the interaction between baseline inflammation level and time since baseline visit and present unstandardized regression estimates controlling for baseline age, sex, and years of education among known pathogenic variant carriers. Each genetic group was analyzed in separate models and interpreted using Benjamini-Hochberg adjusted alpha levels (false discovery rate p<.05)[17].

Secondarily, we aimed to inform whether significant associations between baseline inflammation and longitudinal clinical outcomes were specific to pathogenic variant carriers. We incorporated healthy non-carriers into our models and evaluated the three-way interaction between baseline inflammation level, time since baseline, and pathogenic variant status (pathogenic variant carriers vs. non-carriers). A significant three-way interaction would indicate that the association between baseline inflammation and longitudinal clinical outcomes observed in pathogenic variant carriers was significantly stronger (or weaker) than the effect of baseline inflammation observed in non-carriers. These analyses were only performed when a significant association between baseline inflammation and longitudinal clinical outcomes was first observed among pathogenic variant carriers.

**Neuroimaging Analyses**

The image analyses were conducted using FSL[18] for the voxel based morphometry cross sectional analysis. The familywise error correction was performed using 5000 permutations with threshold free cluster enhancement[19]. The effect, at baseline, of the inflammation markers was corrected for age, gender and total intracranial volume. The longitudinal analyses were performed in the Bayesian linear mixed-effect model framework[14]. The interaction between
rate of change and inflammation marker at base line was threshold using the posterior probability maps with an alpha = 5%. Neuroimaging analyses were restricted to pathogenic variant carriers and stratified by gene group given regional atrophy pattern differences expected between groups[20-22].

**Identifying Asymptomatic Converter Pathogenic Variant Carriers**

Among pathogenic variant carriers, we compared plasma inflammatory markers between asymptomatic non-converters and asymptomatic converters. Inflammatory proteins that significantly differed were then evaluated using area under the receiver operating characteristic curve analyses (AUC/ROC) in the same participants to further inform the clinical relevance of observed group-level associations. These analyses were repeated in a subset of participants who also had plasma NfL levels measured previously. Binary logistic regression models additionally adjusting for age, sex, and education were used to evaluate the added value of plasma inflammatory markers beyond the expected prognostic utility of NfL[3, 23].


Supplemental Results

Descriptive Comparisons and Baseline Differences

Pathogenic variant carriers were older than non-carriers. There were no statistically significant differences in the proportion of female participants, years of education, or frequency of apolipoprotein E e4 carriers between carriers and noncarriers. As expected, pathogenic variant carriers had significantly lower MoCA total score, higher CDR+NACC FTLD SB, lower RSMS total score, and lower UDS3-EF score than non-carriers. Within pathogenic variant carriers, age differed between genetic groups (GRN > C9orf72 > MAPT). There were no statistically significant differences across genotypes in proportion of female participants, years of education, apolipoprotein E e4 carrier frequency, MoCA total score, CDR+NACC FTLD SB, RSMS total score, or proportion of symptomatic participants at baseline (CDR+NACC FTLD Global>0). C9orf72 carriers had lower baseline UDS3-EF scores than MAPT carriers (p=.04).

Cross-Sectional Group Comparisons of Plasma Inflammation Levels

Asymptomatic non-converter pathogenic variant carriers had significantly lower baseline levels of plasma TNFα (β=-.16 [-.31, -.02], p=.03) and IL-6 (β=-.21 [-.34, -.06], p=.006) than non-carrier family members (data not shown). Among all pathogenic variant carriers at baseline, MAPT carriers had higher levels of IL-6 than C9orf72 (β=.17 [.01, .32], p=.03) and GRN carriers (β=.16 [.001, .33], p=.048).

Among pathogenic variant carriers, baseline inflammation levels differed based on clinical disease severity (Supplemental Figure 1). Prodromal carriers (CDR+NACC FTLD Global=0.5) had statistically significantly higher plasma TNFα levels than asymptomatic carriers (β=.16 [.02, .30], p=.02). Pathogenic variant carriers with dementia (CDR+NACC FTLD Global ≥ 1) had higher plasma IL-6 than asymptomatic carriers (β=.22 [.06, .37], p=.006).
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**Supplemental Table 1:** Number of participants with all three plasma inflammatory markers measured and eligible for analyses (N=334; 85% of total sample with at least one inflammatory marker). Across non-carrier kindred and pathogenic variant carriers, 120/143 (84%) of non-carrier kindred and 214/251 (85%) of pathogenic variant carriers had all three inflammatory markers. Refer to Table 1 for frequency of participants with at least one available inflammatory markers across individual clinical phenotypes (Clinically Normal, bvFTD, MBI, etc.).
Supplemental Figure 1: Comparison of baseline plasma inflammatory proteins among pathogenic FTLD gene variant carriers. Prodromal participants (CDR+NACC FTLD Global score = 0.5) had significantly higher plasma TNFα than asymptomatic participants (CDR+NACC FTLD Global score = 0). For plasma IL-6, overtly symptomatic participants (CDR+NACC FTLD Global score ≥ 1.0) had significantly higher concentrations than asymptomatic participants. No group differences in plasma YKL-40 were observed between asymptomatic, prodromal, and overtly symptomatic participants. Separate box-and-whisker plots are shown for each gene group for visualization only. Gene groups were combined for analyses.
Supplemental Figure 2: Inter-correlations between plasma inflammatory markers and their association with participant age (baseline). (A) All associations between plasma inflammatory markers at baseline were statistically significant (p < .03) with small to moderate effect sizes. (B) All associations between plasma inflammatory markers and participant age were statistically significant (p < .001) and ranged from small (.17, TNFα) to relatively large (.47, YKL-40) effect sizes. These relationships were similar or slightly stronger when restricting only to pathogenic variant carriers.
Supplemental Figure 3: Associations between baseline plasma inflammatory marker concentrations and longitudinal clinical outcomes (CDR+NACC FTLD Sum of Boxes, RSMS total score, UDS3-EF score) for pathogenic variant carriers (all gene groups combined). Longitudinal trend lines are shown for participants with low (<25th percentile, green line) or high (>75th percentile, blue line) inflammatory marker concentrations for each of the three markers evaluated (TNFα, IL-6, YKL-40). Statistically significant associations (* = p < .05) indicate where high baseline plasma inflammation was associated with more rapid clinical decline.

CDR+NACC FTLD SB = Clinical Dementia Rating plus National Alzheimer’s Coordinating Center Frontotemporal Lobar Degeneration module Sum of Boxes, RSMS = Revised Self-Monitoring Scale, UDS3-EF = Uniform Data Set version 3 Executive Function composite score.
Supplemental figure 4: Multi-slice axial views showing the association of baseline plasma TNFα concentrations with brain volume among pathogenic MAPT variant carriers. (A) At baseline (cross-sectionally), voxel-based morphometry analysis revealed that higher TNFα was associated with lower brain volume in a small region of the left anterior temporal lobe (orange = p<.05 after family-wise error correction using 5,000 permutations with threshold-free cluster enhancement). (B) Longitudinally, Bayesian linear mixed-effects (BLME) analyses revealed widespread areas where higher baseline plasma TNF-alpha was associated with significantly faster atrophy rates (warmer colors = steeper atrophy slopes; threshold using posterior probability maps and p<.05 alpha). These multi-slice images correspond with the selected representative slices presented in main Figure 1.
Supplemental figure 5: Multi-slice axial views showing the association of baseline plasma IL-6 concentrations with brain volume among pathogenic C9orf72 variant carriers. At baseline (cross-sectionally), voxel-based morphometry analyses revealed regions with a trend towards an association of higher IL-6 with lower brain volume predominantly in lateral parietal and medial parietal/precuneus regions (red = p<.10 after family-wise error correction using 5,000 permutations with threshold-free cluster enhancement). There was no significant association of baseline plasma IL-6 concentrations with the rates of brain atrophy longitudinally. These multi-slice images correspond with the representative slices presented in main Figure 2.
Supplemental figure 6: Multi-slice axial views showing the association of baseline plasma IL-6 concentrations with brain volume among pathogenic GRN variant carriers. Longitudinally, Bayesian linear mixed-effects (BLME) analyses revealed scattered areas in subcortical, temporal, insula, and dorsal parietal/precuneus regions where higher baseline plasma IL-6 was associated with significantly faster atrophy rates (yellow = areas of statistical significance after threshold using posterior probability maps and p<.05 alpha). We did not observe associations of baseline plasma IL-6 and brain volume cross-sectionally at baseline using voxel-based morphometry analyses, and plasma IL-6 did not relate significantly to clinical outcomes in this sample.
Supplemental Figure 7: Baseline TNFα associations with longitudinal clinical outcomes (CDR+NACC FTLD Sum of Boxes, RSMS total score, UDS3-EF score) stratified by each pathogenic variant group. Longitudinal trend lines are shown for participants with low (<25th percentile, green line) or high (>75th percentile, blue line) plasma TNFα concentrations. Statistically significant associations (* = p < .05) indicate where high baseline plasma TNFα was associated with more rapid clinical decline. Analyses that further incorporated non-carrier family members showed that each of these significant relationships also were specific to the pathogenic variant carriers.

CDR+NACC FTLD SB = Clinical Dementia Rating plus National Alzheimer’s Coordinating Center Frontotemporal Lobar Degeneration module Sum of Boxes, RSMS = Revised Self-Monitoring Scale, UDS3-EF = Uniform Data Set version 3 Executive Function composite score
Supplemental Figure 8: Baseline IL-6 associations with longitudinal clinical outcomes (CDR+NACC FTLD Sum of Boxes, RSMS total score, UDS3-EF score) stratified by each pathogenic variant group. Longitudinal trend lines are shown for participants with low (<25th percentile, green line) or high (>75th percentile, blue line) plasma IL-6 concentrations. Statistically significant associations (* = p < .05) indicate where high baseline plasma IL-6 was associated with more rapid clinical decline. Analyses that further incorporated non-carrier family members showed that the significant relationship was specific to the pathogenic variant carriers.

CDR+NACC FTLD SB = Clinical Dementia Rating plus National Alzheimer’s Coordinating Center Frontotemporal Lobar Degeneration module Sum of Boxes, RSMS = Revised Self-Monitoring Scale, UDS3-EF = Uniform Data Set version 3 Executive Function composite score
Supplemental Figure 9: Baseline YKL-40 associations with longitudinal clinical outcomes (CDR+NACC FTLD Sum of Boxes, RSMS total score, UDS3-EF score) stratified by each pathogenic variant group. Longitudinal trend lines are shown for participants with low (<25th percentile, green line) or high (>75th percentile, blue line) plasma YKL-40 concentrations. Statistically significant associations (* = p < .05) indicate where high baseline plasma YKL-40 was associated with more rapid clinical decline. Analyses that further incorporated non-carrier family members showed that the significant relationships were specific to the pathogenic variant carriers.

CDR+NACC FTLD SB = Clinical Dementia Rating plus National Alzheimer's Coordinating Center Frontotemporal Lobar Degeneration module Sum of Boxes, RSMS = Revised Self-Monitoring Scale, UDS3-EF = Uniform Data Set version 3 Executive Function composite score