Apolipoprotein L1 is Increased in Frontotemporal Lobar Degeneration Post-mortem Brain but Not in Ante-mortem Cerebrospinal Fluid

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Research article

Keywords: APOL1, CSF, Brain tissue, FTD, FTLD-Tau, FTLD-TDP

Posted Date: January 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1202306/v1

Abstract

Background: Frontotemporal Dementia (FTD) is a spectrum of heterogeneous disorders caused by frontal-temporal lobar degeneration (FTLD). FTLD is mainly characterized by brain protein aggregates of tau (FTLD-Tau) or TDP-43 (FTLD-TDP), however, the clinicopathological heterogeneity across the FTLD spectrum makes ante-mortem diagnosis of these pathological subtypes challenging. Our unbiased proteomics study showed increased Apolipoprotein L1 (APOL1) levels, a protein involved in lipid metabolism and transport, in CSF of FTD patients, which was more prominent in FTLD-Tau. We aimed to understand APOL1 expression in FTLD post-mortem brain tissue with defined pathology and to validate its potential as a CSF biomarker for FTD and its pathological subtypes.

Methods: APOL1 levels were analyzed in the frontal cortex of FTLD (n = 62, including 23 FTLD-Tau and 29 FTLD-TDP) and non-demented controls (n = 18) by immunohistochemistry, western blot (WB), and a novel prototype ELISA. The association of APOL1 immunoreactivity with phosphorylated Tau (pTau) and TDP-43 (pTDP43) immunoreactivity was assessed. CSF APOL1 was analyzed in confirmed FTD patients (n = 27, including 12 FTLD-Tau and 15 FTLD-TDP) and controls (n = 15) using the same ELISA.

Results: APOL1 levels were significantly increased in FTLD compared to controls as measured by immunohistochemistry ($p < 0.001$), WB ($p < 0.001$), and ELISA ($p < 0.05$), but no differences between the pathological subtypes were observed. APOL1 immunoreactivity was present in neuronal and glial cells. APOL1 immunoreactivity did not specifically co-localize with any of the main FTLD proteinopathies (pTau or pTDP43). CSF APOL1 levels were comparable between FTD patients and controls and between FTLD pathological subtypes.

Conclusion: We show an upregulation of APOL1 in FTLD pathology irrespective of the subtypes, indicating a role of this novel protein in FTD pathophysiology. The APOL1 levels detected in brain tissue were not mirrored in the CSF, limiting its potential as a specific FTD biofluid-based biomarker using our current in-house immunoassay. Future research should explore the role of APOL1 downstream specific FTLD pathologies.

1. Background

Frontotemporal dementia (FTD) is the second most common form of dementia among people under the age of 65 [1]. FTD is a heterogeneous disorder presenting with different clinical phenotypes. It is caused by frontotemporal lobar degeneration (FTLD) in which two main and exclusive protein hallmarks have been identified: aggregates of the microtubulin-associated protein Tau (FTLD-Tau) or inclusions of the TAR DNA-binding protein 43 (TDP-43, FTLD-TDP) [2, 3]. Approximately 10-20% of the FTD cases is caused by genetic mutations that are linked to distinct neuropathologies [4]. Mutations in the microtubule-associated protein tau (*MAPT*) gene are related to FTLD-Tau, while mutations in progranulin (*GRN*) and chromosome 9 open reading frame 72 (*C9orf72*) genes are related to FTLD-TDP pathology [2, 4]. These two proteinopathies likely require distinct pharmacological treatments, and therefore,

discrimination between these pathological subtypes (i.e. FTLD-Tau and FTLD-TDP) is needed. Currently, no fluid biomarkers exist that can identify the underlying pathology in sporadic FTD cases, and thus biomarkers that reflect different aspects of FTD pathophysiology are needed [5–7].

Our previous unbiased proteomics study using cerebrospinal fluid (CSF) from FTD cases with known underlying pathology revealed that Apolipoprotein 1 (APOL1) was increased in FTLD-Tau compared to controls and FTLD-TDP patients (5- and 3-fold, respectively) [8]. Single nucleotide polymorphisms in the ApoL gene family have been associated with schizophrenia and an upregulation of APOL1 expression has also been detected in the prefrontal cortex of schizophrenia patients [9, 10]. This is of interest considering that schizophrenia and FTD have overlapping clinical features [11–13]. APOL1 is part of the high-density lipoprotein (HDL) complex, which plays a role in cholesterol transport and abnormalities in cholesterol turnover have been associated with FTD and other neurodegenerative disorders such as Alzheimer's disease or Niemann-Pick disease [14–17]. Furthermore, overexpression of APOL1 induces lysosomal-dependent autophagic cell death [18–20]. Autophagy is an important mechanism for maintaining cellular homeostasis and is involved in the clearance of misfolded or aggregated proteins. Moreover, mutations in genes associated with lysosomal functioning or trafficking are related to the etiology of FTD (*CHMP2B*) or FTLD-TDP specifically (*C9orf72, GRN* and *TMEM106b*) [21–26], indicating that impaired lysosomal functioning is an important pathological pathway that might be dysregulated in FTD [27, 28]. Whether APOL1 could play a role in FTD or the specific FTLD subtypes remains unknown. We hypothesize that APOL1 is increased in FTD, potentially reflecting lysosomal-related pathological brain changes and could be a novel CSF biomarker aiding in the discrimination of the FTLD pathological subtypes. In this study, we aimed to characterize APOL1 in post-mortem brain tissue of FTLD patients with definite neuropathology (FTLD-Tau and FTLD-TDP) and validate its potential as a discriminatory CSF biomarker for FTLD pathological subtypes. We additionally, assessed the association of APOL1 with YKL-40, a marker also detected in our previous unbiased proteomic study reflecting ongoing neuroinflammation.

2. Methods

2.1 Human samples

2.1.1 Post-mortem human brain tissue

Frozen frontal cortex tissue blocks of non-demented controls (n = 9) and individuals with FTLD pathology (FTLD = 44; FTLD-Tau = 21 and FTLD-TDP = 23) were obtained from the Netherlands Brain Bank (n = 26) and the CIEN Foundation Brain bank (n = 27; BT-CIEN, Madrid, Spain). The FTLD-Tau group included cases with different underlying tauopathies such as progressive supranuclear palsy (PSP, n = 8), pick disease (PiD, n = 4), and corticobasal degeneration (CBD, n = 3). Cases with underlying *MAPT* mutation (n = 6, related to Tau pathology) or *C9orf72* (n = 7) and *GRN* (n = 1) mutations (related to TDP pathology) were also included [21, 29, 30]. Frontal cortex tissue blocks were homogenized with tissue protein extraction reagent (T-PER, 0.1 g/mL, Thermo Fisher Scientific, Waltham, USA) containing EDTA-free

protease inhibitor cocktail (1:25, Roche, Basel, Switzerland) and phosphatase inhibitor (1:10, Roche, Basel, Switzerland). Tissue homogenates were centrifuged at 10.000 *g* for 5 minutes at 4°C. Thereafter, total protein content was measured with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Brain lysates were aliquoted and stored at -80°C until further analysis.

Paraffin-embedded frontal cortex tissue of non-demented controls (n = 9) and individuals with FTLD pathology (FTLD = 18; FTLD-Tau = 12 and FTLD-TDP = 6) were similarly obtained from the Netherlands Brain Bank (n = 9) and BT-CIEN (n = 18). In the FTLD-Tau group, PSP (n = 5), PiD (n = 3), CBD (n = 1) patients and *MAPT* mutation carriers (n = 3) were included. The FTLD-TDP group included individuals with mutation in the *C9orf72* gene (n = 3). Sections of 5-µm thick were mounted on tissue slides (Superfrost® plus, Menzel Glaser, Braunschweig, Germany) and dried overnight at 37°C.

Pathological diagnosis was performed following established guidelines [31, 32]. All donors or their next of kin provided written informed consent for brain autopsy and the use of medical records for research purposes. An overview of patient demographics is presented in Table 1.

Table 1
Demographic data post-mortem tissue

Frozen tissue blocks	Control	FTLD (total)	FTLD-Tau	FTLD-TDP
n (M/F)	9 (5/4)	44 (20/24)	21 (8/13)	23 (12/11)
Age, years (mean ± SD)	69 (11)	68 (9)	69 (11)	67 (8)
PMD, hours (mean ± SD)	6,9 (2,8)	6,3 (3,5)	5,5 (2,1)	6,9 (4,1)
FTLD Subclassifications			8 PSP	7 *C9orf72*
			4 PiD	1 *GRN*
			3 CBD	15 sporadic[$]
			6 *MAPT*	
Paraffin-embedded tissue	Control	FTLD (total)	FTLD-Tau	FTLD-TDP
n (M/F)	9 (3/6)	18 (9/9)	12 (5/7)	6 (4/2)
Age, years (mean ± SD)	69 (9)	69 (10)	67 (10)	72 (9)
PMD, hours (mean ± SD)	5 (3,4)	6 (1,9)	6,2 (2,3)	5,7 (1,6)
FTLD Subclassifications			5 PSP	3 *C9orf72*
			3 PiD	3 sporadic[$]
			1 CBD	
			3 *MAPT*	

$ Sporadic cases were confirmed for TDP pathology by autopsy.

FTLD, frontotemporal lobar degeneration; TDP, TAR DNA-binding protein; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; PiD, Pick Disease; n, number of cases; M, male; F, female; PMD, post-mortem delay; SD, standard deviation

2.1.2 Ante-mortem human CSF

CSF samples were obtained from the Amsterdam dementia cohort (n = 27) [33, 34] and the Erasmus Medical Center (n = 13). The total CSF cohort included controls (subjective cognitive decline, n = 14) and FTD cases with known underlying neuropathology (n = 26, FTLD-Tau = 12, FTLD-TDP = 14). FTLD-Tau was confirmed based on autopsy (n = 6) or *MAPT* mutation (n = 3). The FTLD-Tau group was enriched with patients clinically diagnosed with PSP (n = 3), which is primarily associated with Tau neuropathology [35]. The FTLD-TDP group included autopsy-confirmed cases (n = 9) and patients with *GRN* (n = 1) or *C9orf72* (n = 1) mutations. The FTLD-TDP group was further enriched with FTD cases that presented with amyotrophic lateral sclerosis (FTD-ALS, n = 3), which associates with TDP pathology [36].

Patients underwent cognitive and neurological assessments and FTD diagnosis was determined according to consensus criteria [31, 37]. The control group consisted of cases with subjective cognitive decline, while other neurological or biochemical assessments were normal (CSF total-Tau/Aβ42 ratio < 0.52 [38])and did not meet the criteria for mild cognitive impairment, dementia, or another neurological disorder [39, 40]. Informed consent from all participants in this study was obtained. Patient characteristics are presented in Table 2.

Table 2
Demographic data of CSF samples

	Control	FTD (total)	FTLD-Tau	FTLD-TDP
n (M/F)	14 (6/8)	26 (12/14)	12 (4/8)	14 (8/6)
Age, years (mean ± SD)	63 (9)	60 (9)	59 (12)	60 (5)
FTLD Subclassifications			3 PSP	3 FTD-ALS
			3 *MAPT*	1 *GRN*
			6 sporadic[$]	1 *C9orf72*
				9 sporadic[$]
[$] Sporadic cases were confirmed for FTLD pathology by autopsy.				
FTD, Frontotemporal dementia; FTLD, frontotemporal lobar degeneration; TDP, TAR DNA-binding protein; n, number of cases; M, male; F, female; y, years; PSP, progressive supranuclear palsy; PiD, Pick Disease; ALS, Amyotrophic lateral sclerosis; SD, standard deviation				

2.3 Western Blot

CSF (30 µL), frontal cortex lysates (10 µg) or human APOL1 recombinant protein (5 ng, 1 – 398 aa, SinoBiological, Wayne, USA) were prepared in sample buffer (0.03 M Tris, 2% SDS, 10% glycerol, 50mM DTT, 0.1 mM bromophenol blue) and heated for 5 minutes at 95°C. Electrophoresis was performed with 1.5 mm NuPAGE Novex 4-12% Bis-Tris Protein Gels (Thermo Fisher Scientific) and immunoblotting was performed as previously described (*Y.S Hok-A-Hin et al. submitted*). The following primary antibodies were used: monoclonal mouse anti-human APOL1 antibody (1:20.000, ProteinTech, Manchester, UK), polyclonal rabbit anti-human APOL1 antibody (1:1000, Novus Biologicals, Centennial, USA), or monoclonal mouse anti-human Actin antibody (1:5000, clone AC-40, Sigma-Aldrich, Saint Louis, USA). Frontal cortex lysates were quantified for APOL1 (ProteinTech) and Actin (Sigma-Aldrich) protein signals with the ImageLab^{TM} software version 3.0 (Bio-Rad, Hercules, USA). The specificity of the mouse anti-APOL1 antibody (ProteinTech) was supported by antibody pre-absorption. The anti-APOL1 antibody was incubated 24 hours at 4°C with 200 molar excess of its specific antigenic peptide (1 – 238 aa, ProteinTech), before incubation with the immunoblot.

2.4 Immunohistochemistry

Immunohistochemistry (IHC) of frontal cortex sections was performed as previously described (*Y.S Hok-A-Hin et al. submitted*). In brief, sections were deparaffinized and boiled for 15 minutes in a microwave with sodium citrate buffer (10 mmol/L pH 6.0) to perform antigen retrieval. Sections were incubated overnight at 4°C in a humid environment with mouse anti-human APOL1 antibody (1:1000, ProteinTech) in antibody diluent (Immunologic, Duiven, The Nederlands). After washing with PBS, sections were incubated with EnVision (anti-mouse/rabbit HRP, undiluted; DAKO, Glostrup, Denmark) for 1 hour at room temperature (RT). The colour was developed using 3,3' diaminobenzidine tetrahydrochloride dihydrate (DAB; 0.1 mg/mL, 0.02% H_2O_2, DAKO) for 10 minutes as the chromogen. Nuclei were stained with hematoxylin and section were mounted with Quick-D mounting medium (Klinipath, Duiven, The Nederlands). The specificity of the mouse anti-human APOL1 antibody (ProteinTech) for IHC was also tested by pre-absorption. First, the anti-APOL1 antibody was pre-absorbed for 24 hours at 4°C with 200 molar excess of its specific antigenic peptide (1 – 238 aa, ProteinTech), thereafter, sections were incubated with the pre-absorbed antibody overnight at 4°C.

Double immunohistochemistry experiments were performed to determine the association of APOL1 with phosphorylated Tau (pTau) and phosphorylated TDP-43 (pTDP43). Sections were incubated overnight at 4°C with mouse anti-APOL1 (1:1000, ProteinTech) and either rabbit anti-pTau (AT8, 1:1000, Abcam) or anti-pTDP43 (Ser409 + 410, 1:500, ProteinTech) diluted in antibody diluent (Immunologic). Thereafter, sections were incubated with polyclonal goat anti-mouse HRP (1:150, DAKO) and anti-rabbit biotin (1:300, DAKO) antibodies for 1 hour at RT. Next, a one-hour incubation with streptavidin-alkaline phosphatase (1:100, 1000U, Roche) diluted in antibody diluent (Immunologic) was performed. APOL1 immunoreactivity was first visualized with DAB (0.1 mg/mL, 0.02% H_2O_2, DAKO). Thereafter, sections were emerged in Tris-HCL buffer (0.2M, pH 8.5) and pTau and pTDP43 immunoreactivity were visualized with Liquid permanent red solution (1:100, DAKO). Nuclei were stained with hematoxylin and section were mounted with Aquatex® (Merck, Darmstadt, Germany).

Semi-quantitative analysis of APOL1 immunoreactivity was performed with the QuPath software [41] (version 0.1.2, Queen's University of Belfast, Ireland) including five microscopic areas randomly selected per section, acquired at 200x magnification. APOL1 immunoreactivity was determined by using the DAB positive pixel count (thresholds: down-sample factor = 4, Gaussian sigma = 2, hematoxylin threshold (negative) = 0.1, DAB threshold (positive) = 0.3). This threshold was applied for all images (supplementary figure 1). The analysis was performed by a researcher who was blinded from the diagnosis.

2.5 In-house APOL1 Immunoassay

An immunoassay specific for APOL1 was developed following recommended procedures [42, 43]. High-binding 96-well microplates (Costar, New York, USA) were coated with capture antibody (1µg/mL of mouse anti-human APOL1, ProteinTech) in coating buffer (0.1 M Na_2CO_3, 0.1 M $NaHCO_3$, pH 9.6) and incubated overnight at RT. Next, plates were rinsed with PBS and blocked with 0.5% Casein, 0.1% Gelatin in Tris buffer (20 mM Tris-HCl + 50 mM NaCl, pH 7.5) for 1.5 hours at RT. Thereafter, plates were washed

with washing buffer (Tris buffer containing 0.05% Tween-20) and incubated with samples (brain lysates (1:50) or CSF (1:4)) for 2 hours at RT. The standard curve was prepared with human APOL1 Recombinant Protein (SinoBiological) using the following concentrations: 160, 80, 40, 20, 10, 5, and 2.5 ng/mL diluted in Tris buffer. After a washing step, samples were incubated with a detection antibody (5µg/mL of rabbit anti-human APOL1, Novus Biologicals) for 1 hour at RT. Plates were then washed and incubated with polyclonal swine anti-rabbit IgG/HRP (1:2000, DAKO). After washing, plates were incubated with substrate tetramethylbenzidine/dimethylsulfoxide (TMB/DMSO, 10mg/mL) in substrate buffer (0.1 M $C_6H_8O_7$, 0.1 M NaOAc, pH 4.0) containing 0.03% H_2O_2 for 10 minutes and the reaction was stopped using 1 M H_2SO_4. The absorbance was read at 450 nm. APOL1 concentrations measured in brain lysates were corrected for the total protein content.

Our in-house APOL1 immunoassay was validated for CSF following the international guidelines for immunoassay validation [43]. In brief, parallelism was performed by using a 2-times serial dilution of four CSF samples. Recovery was evaluated by spiking five CSF samples with a low (5 ng/mL), medium (50 ng/mL), or a high (100 ng/mL) spike of the APOL1 recombinant protein (SinoBiological). Dilution linearity was performed by spiking three CSF samples with APOL1 recombinant protein (1600 ng/mL, SinoBiological) following a 4-times serial dilution. The effect of freeze/thawing on APOL1 levels was also assessed. Pooled CSF samples were aliquoted and exposed for 1, 2, 3, 5 and 7 freeze/thaw cycles, samples were thawed for 2 hours at RT and freezing at -80°C for minimal 12 hours. Reference aliquots were stored directly at −80°C. The intra- and inter-assay coefficient variations (CV) were established as < 3% and < 8% for brain lysates and < 4% and < 11% for CSF.

2.6 YKL-40 Immunoassay

CSF protein levels for another FTD-relevant biomarker, YKL-40 were previously measured in a subset of cases (11 controls and 19 FTD patients) using the MicroVue YKL-40 enzyme immunoassay (Quidel Corporation, San Diego, USA) [8, 44]. This assay was validated for analysis in CSF with intra- and inter-assay of CV < 4% and 11% [8, 44].

2.7 Statistical Analysis

Statistical analysis was carried out using IBM SPSS statistics (version 26, IBM, Armonk, NY) and graphs were plotted with GraphPad Prism (version 9.1.0, San Diego, USA). The normality of the data was assessed with the Shapiro-Wilk test. The effect of potential covariates (i.e age, sex and center) were analyzed by Spearman correlation analysis and Mann-Whitney U tests. Differences in APOL1 levels between groups were evaluated either by analysis of covariance (ANCOVA) with log-transformed data including center as a covariate or Kruskal-Wallis test, when applicable. Bonferonni post-hoc analysis was applied. Pearson correlation analysis was performed to analyze the association of CSF APOL1 levels with YKL-40. Values of $p < 0.05$ were considered significant.

3. Results

3.1 APOL1 antibody characterization and immunoassay validation

Mouse and rabbit anti-APOL1 antibodies detected the recombinant APOL1 protein on western blot (WB) around 44 kDa, the expected molecular weight of the protein. A similar protein signal around 44 kDa was also observed in both CSF and post-mortem frontal cortex for the mouse anti-APOL1 antibody. The rabbit anti-APOL1 antibody showed an additional band between 37-50 kDa in post-mortem frontal cortex (Fig. 1A). Antibody preabsorption with its antigenic peptide abolished protein signals in both WB and IHC, supporting the specificity of the mouse APOL1 antibody (Fig. 1B-C). The mouse anti-APOL1 antibody was then used for WB, IHC, and as a capture antibody in the ELISA. Rabbit anti-APOL1 antibody was used as a detection antibody for the prototype assay. Validation of the prototype APOL1 immunoassay for CSF analysis showed that parallelism, recovery and dilution linearity tests were within the acceptance criteria (i.e. 80–120%) (Supplementary figure 2A-C). Furthermore, APOL1 levels in CSF remained stable up to seven freezing and thawing cycles (Supplementary figure 2D).

3.2 APOL1 immunoreactivity and protein levels are increased in FTLD frontal cortex

APOL1 immunoreactivity was detected in controls and FTLD cases within the cytoplasm of neuronal and glial cells, identified based on morphology (Fig. 2A). We observed an increase of APOL1 immunoreactivity specially associated with glial cells in FTLD compared to controls ($p<0.001$, Fig. 2B), which was present in both FTLD-Tau ($p<0.001$) and FTLD-TDP ($p<0.05$) groups, and no differences were detected between these pathological subtypes. Despite the limited sample size, exploratory graphical inspection within FTLD subgroups suggests higher APOL1 immunoreactivity in FTD mutation carriers (i.e. *MAPT* and *C9orf72*) (Fig. 2C). No specific association of APOL1 immunoreactivity to either pTau or pTDP-43 was observed (Fig. 2A and supplementary figure 3). Similar to the immunohistochemistry data, we observed by WB that APOL1 was increased in FTLD cases compared to controls independently of the pathological subtype or any specific FTLD subclassification (e.g *MAPT*, *C9orf72*) (Fig. 3A-C, $p<0.001$). Our novel prototype ELISA confirmed that APOL1 concentrations were increased in FTLD cases (Fig. 3D, $p<0.05$), though this increase was especially driven by the FTLD-TDP group (Fig. 3E, $p<0.01$). A tendency towards an increase of APOL1 in FTLD-Tau was also detected ($p = 0.03$), but differences did not survive correction for multiple testing (Fig. 3E). The significant correlation between APOL1 levels measured by either WB and ELISA (Fig. 3F, $r = 0.634$, $p<0.01$) suggests that similar protein isoforms are measured by these technologies.

3.3 CSF APOL1 levels remain unchanged in FTD patients

To analyze whether APOL1 could be a useful biomarker for FTD or the FTLD subtypes we next analyzed APOL1 in ante-mortem CSF from confirmed FTD patients with our in-house APOL1 sandwich immunoassay. No difference in CSF APOL1 concentrations were detected between controls and FTD

patients (Fig. 4A), nor between pathological subtypes or any FTLD subclassifications (Fig. 4B). We also assessed the association of CSF APOL1 with YKL-40, a marker associated with neuroinflammatory processes, and found that APOL1 showed a moderate negative association with YKL-40 (r = -0.398, $p<0.05$, Fig. 4C).

4. Discussion

In this study, we have characterized APOL1 protein expression in post-mortem human brain tissue using three different methods. We show that APOL1 is expressed in the cytoplasm of both neuronal and glial cells. APOL1 levels are increased in post-mortem frontal cortex of FTLD tissue independently of the pathological subtypes. However, such changes were not mirrored in the CSF, where APOL1 levels were comparable between pathological confirmed FTD patients and controls.

Characterization of the APOL1 antibodies in post-mortem frontal cortex by WB showed several protein bands around 44kDa, which is the expected molecular weight of the APOL1 protein. These signals were abolished after pre-absorption with its specific APOL1 antigenic peptide, supporting the specificity of the monoclonal antibody used across the different methods. The different bands around 44 kDa may represent different protein isoforms or splice variants [14, 45], as also observed in previous studies [46]. Previous studies showed that APOL1 is present in neuronal cells from controls and gene expression analysis showed an up-regulation in schizophrenia patients [9, 10]. In line with these findings, we observed APOL1 neuronal reactivity in non-demented and FTLD cases. However, we also observed APOL1 immunoreactivity in glial-like cells in FTLD pathology, which can be explained by the increased microglial activation and astrogliosis observed in FTD patients [47, 48]. Using three different (semi)-quantitative methods, we showed that APOL1 levels are increased in FTLD post-mortem brain tissue compared to controls, which was independent of the pathological subtypes. Immunohistochemistry characterization suggests that APOL1 reactivity tends to be higher in the FTD mutation carriers but this did not reach statistical significance. These trends were not observed in (semi-) quantitative analyses (i.e WB or ELISA) using a larger sample size, thereby suggesting that APOL1 increase is independent of subclassifications. This is supported by our double IHC analysis that showed no specific association of APOL1 immunoreactivity to that of the main FTLD pathological proteins (i.e pTau or pTDP-43). This suggests that APOL1 is not related to these pathological hallmarks and might be involved in independent molecular processes or in a common process downstream of the FTLD pathology. APOL1 regulates lipid metabolism and cholesterol transport, mechanisms that are involved in FTLD pathophysiology [14, 15, 19, 49]. In addition, APOL1 can induce lysosomal-dependent autophagic cell death [19]. The strong elevated APOL1 levels in FTLD may thus reflect the dysregulation of lipid metabolism or lysosomal processes downstream of the primary effect of the specific pathological aggregations. Interestingly, dysregulation of other lysosomal-related proteins (i.e C9orf72, GRN and TMEM106) causes lysosomal dysfunction, which leads to a defect in the autophagic pathway contributing to FTLD pathology [50–52]. Future experimental studies should define the exact biological processes by which APOL1 changes may contribute to the pathophysiology of FTD.

We observed that CSF APOL1 levels were comparable between FTD and controls using our validated prototype ELISA that was also used for APOL1 tissue level quantification. These results are in contrast with previous mass-spectrometry findings, in which increased APOL1 levels were detected in CSF and serum of FTD patients [8, 53]. Translation of proteomic findings into immunoassays for routine analysis poses great challenges for the development of optimal fluid-based biomarkers [54]. While mass-spectrometry approaches identify peptides from trypsinized proteins, antibody-based technologies detect proteins in their native conformation, which may explain the discrepancies between studies, in terms of detected isoforms and post-translational modifications. In addition, our previous study used a smaller sample size, which could increase the change of false-positive results. Furthermore, here we included additional subclassifications (i.e PSP and FTD-ALS) within the main pathologies that were not included in our original proteomic study potentially increasing heterogeneity, which could also explain observed discrepancies [8]. It is however striking that the APOL1 changes we observed in frontal cortex tissue were not detected in CSF using the same immunoassay. One explanation could be the differences in the sample preparation. A lysis reagent (i.e T-per) was used to prepare the tissue lysates probably extracting proteins from various cellular compartments while in CSF such additional steps were not performed and circulating APOL1 to some extent might still be associated with HDL particles [55] or exosomes [56] and thus the relevant epitopes may be covered. Even though we did not detect differences in CSF APOL1 across clinical groups, we observed a negative correlation with YKL-40, a marker increased in CSF from FTD patients, reflecting ongoing neuroinflammation [57]. Our data could indicate that the dysregulation of CSF APOL1 levels affects or is influenced by ongoing inflammatory processes.

This study is not without limitations. FTD is a heterogeneous disease with a range of clinical and pathological phenotypes. Here, to increase statistical power, different tauopathies and mutations associated with a specific FTLD pathological group (i.e. Tau and TDP) were grouped. This is not trivial considering that differences between genetic and sporadic FTD cases may exist [58]. Exploratory visual inspection suggests that APOL1 changes are not associated with a specific FTD subclassification (e.g. specific mutation carriership, sporadic, etc) but a larger sample size is needed to confirm that subtype-specific changes are not masked by FTLD heterogeneity. Nevertheless, the APOL1 changes detected in brain were not mirrored in the CSF, limiting its potential as a specific FTD fluid-based biomarker using the developed prototype immunoassay. Additionally, we were not able to include post-mortem brain and ante-mortem CSF from the same patients, as these samples are rare. This also highlights the need to develop large well-characterized FTD cohorts and calls for strong collaborative work across different centers. The strengths of our study are the use of well-characterized cohorts including both post-mortem brain tissue and ante-mortem CSF and the use of complementary methods supporting the reliable measurement of APOL1 protein.

5. Conclusion

This study detected increased APOL1 levels in the post-mortem frontal cortex of FTLD cases independent of the underlying pathological hallmark, indicating that APOL1 is a novel unifying protein involved in FTD pathophysiology. The lack of bodyfluid biomarkers for FTD or FTLD-related pathologies remains of

importance to improve clinical diagnosis, calling for additional studies to identify and validate novel FTD-specific biomarkers.

Abbreviations

Analysis of covariance = ANCOVA

Amyotrophic lateral sclerosis = ALS

Apolipoprotein 1 = APOL1

Chromosome 9 open reading frame 72 = *C9orf72*

Corticobasal degeneration = CBD

Cerebrospinal fluid = CSF

Coefficient variations = CV

Diaminobenzidine tetrahydrochloride dihydrate = DAB

Frontotemporal Dementia = FTD

Frontal-temporal lobar degeneration = FTLD

TAR DNA-binding protein 43 = TDP-43

Progranulin = GRN

High-density lipoprotein = HDL

Microtubule-associated protein tau = MAPT

Pick disease = PiD

Progressive supranuclear palsy = PSP

Phosphorylated Tau =pTau

Phosphorylated TDP-43 = pTDP43

Tissue protein Extraction reagent = T-PER

Declarations

Acknowledgments

Not applicable.

Funding

This project was funded by the memorable project PRODIA (project number 733050206), supported by ZonMw (The Netherlands) and the PRIDE project, supported by Alzheimer Nederland (WE.03-2018-05). MdC is supported by the attraction talent fellowship from Comunidad de Madrid (2018-T2/BMD-11885) and San Pablo CEU University.

Availability of data and materials

The datasets used and or analyzed in the current study are available from the corresponding author upon reasonable request

Ethics approval and consent to participate

This study was approved by the ethical review boards from Amsterdam UMC and CIEN Foundation Brain bank. Informed consent was obtained from all subjects or their authorized representative.

Competing interest

Not applicable.

Authors contributions

YSH contributed to the study design and was responsible for acquisition, statistical analysis, interpretation of the data and drafting the manuscript. AD, AR, HS, JH, JS, and YP provided material. All authors participated in data interpretation and critically revised the manuscript. CET and MC conducted the study concept and design, data acquisition and interpretation, critically revised the manuscript and supervised the study. All authors read and approved the final manuscript.

References

1. Onyike CU, Diehl-Schmid J. The epidemiology of frontotemporal dementia. Int Rev Psychiatry. 2013;25(2):130–7.
2. Irwin DJ, et al. Frontotemporal lobar degeneration: defining phenotypic diversity through personalized medicine. Acta Neuropathol. 2015;129(4):469–91.
3. Mann DMA, Snowden JS. Frontotemporal lobar degeneration: Pathogenesis, pathology and pathways to phenotype. Brain Pathol. 2017;27(6):723–36.

4. Lashley T, et al. Review: an update on clinical, genetic and pathological aspects of frontotemporal lobar degenerations. Neuropathol Appl Neurobiol. 2015;41(7):858–81.
5. Josephs KA, et al. Neuropathological background of phenotypical variability in frontotemporal dementia. Acta Neuropathol. 2011;122(2):137–53.
6. Swift IJ, et al. Fluid biomarkers in frontotemporal dementia: past, present and future. J Neurol Neurosurg Psychiatry. 2021;92(2):204–15.
7. van der Ende EL, van Swieten JC, *Fluid Biomarkers of Frontotemporal Lobar Degeneration*, in *Frontotemporal Dementias: Emerging Milestones of the 21st Century*, B. Ghetti, et al., Editors. 2021, Springer International Publishing: Cham. p. 123-139.
8. Teunissen CE, et al. Novel diagnostic cerebrospinal fluid biomarkers for pathologic subtypes of frontotemporal dementia identified by proteomics. Alzheimers Dement (Amst). 2016;2:86–94.
9. Mimmack ML, et al., *Gene expression analysis in schizophrenia: Reproducible up-regulation of several members of the apolipoprotein L family located in a high-susceptibility locus for schizophrenia on chromosome 22*. PNAS, 2002. 99.
10. Takahashi S, et al. Association of SNPs and haplotypes in APOL1, 2 and 4 with schizophrenia. Schizophr Res. 2008;104(1-3):153–64.
11. Cooper JJ, Ovsiew F. The relationship between schizophrenia and frontotemporal dementia. J Geriatr Psychiatry Neurol. 2013;26(3):131–7.
12. Momeni P, et al. Familial early onset frontotemporal dementia caused by a novel S356T MAPT mutation, initially diagnosed as schizophrenia. Clin Neurol Neurosurg. 2010;112(10):917–20.
13. Velakoulis D, et al. Frontotemporal dementia presenting as schizophrenia-like psychosis in young people: clinicopathological series and review of cases. Br J Psychiatry. 2009;194(4):298–305.
14. Duchateau PN, et al., *Apolipoprotein L, a New Human High Density Lipoprotein Apolipoprotein Expressed by the Pancreas*. The Journal Of Biological Chemistry, 1997. 272.
15. Kim WS, et al. Altered High Density Lipoprotein Composition in Behavioral Variant Frontotemporal Dementia. Front Neurosci. 2018;12:847.
16. Hottman DA, et al. HDL and cognition in neurodegenerative disorders. Neurobiol Dis. 2014;72 Pt A:22–36.
17. Brauer AU, et al., *Current Challenges in Understanding the Cellular and Molecular Mechanisms in Niemann-Pick Disease Type C1*. Int J Mol Sci, 2019. 20(18).
18. Wan G, et al. Apolipoprotein L1, a novel Bcl-2 homology domain 3-only lipid-binding protein, induces autophagic cell death. J Biol Chem. 2008;283(31):21540–9.
19. Zhaorigetu S, et al., *ApoL1, a BH3-only lipid-binding protein, induces autophagic cell death*. Autophagy, 2008. 4(8).
20. Hu CA, Klopfer EI, Ray PE. Human apolipoprotein L1 (ApoL1) in cancer and chronic kidney disease. FEBS Lett. 2012;586(7):947–55.

21. Baker M, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature. 2006;442(7105):916–9.
22. Hu F, et al. Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron. 2010;68(4):654–67.
23. Clayton EL, et al. Frontotemporal dementia caused by CHMP2B mutation is characterised by neuronal lysosomal storage pathology. Acta Neuropathol. 2015;130(4):511–23.
24. Urwin H, et al. Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. Hum Mol Genet. 2010;19(11):2228–38.
25. Brady OA, et al. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. Hum Mol Genet. 2013;22(4):685–95.
26. Farg MA, et al. C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking. Hum Mol Genet. 2014;23(13):3579–95.
27. Budini M, et al. Autophagy and Its Impact on Neurodegenerative Diseases: New Roles for TDP-43 and C9orf72. Front Mol Neurosci. 2017;10:170.
28. Root J, et al. Lysosome dysfunction as a cause of neurodegenerative diseases: Lessons from frontotemporal dementia and amyotrophic lateral sclerosis. Neurobiol Dis. 2021;154:105360.
29. Renton AE, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72(2):257–68.
30. Spillantini MG, Van Swieten JC, Goedert M. Tau gene mutations in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Neurogenetics. 2000;2:12.
31. Lund T. Clinical and neuropathological criteria for frontotemporal dementia. Journal of Neurology Neurosurgery Psychiatry. 1994;57:2.
32. Cairns NJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 2007;114(1):5–22.
33. van der Flier WM, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. J Alzheimers Dis. 2014;41(1):313–27.
34. van der Flier WM, Scheltens P. Amsterdam Dementia Cohort: Performing Research to Optimize Care. J Alzheimers Dis. 2018;62(3):1091–111.
35. Ingelsson M, et al. Increase in the relative expression of tau with four microtubule binding repeat regions in frontotemporal lobar degeneration and progressive supranuclear palsy brains. Acta Neuropathol. 2007;114(5):471–9.
36. Neumann M, et al., *Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis*. Science, 2006. 314.
37. Neary D, et al., *Frontotemporal lobar degeneration. A consensus on clinical diagnosic criteria*. Neurology, 1998. 51.

38. Duits FH, et al. The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? Alzheimers Dement. 2014;10(6):713–23 e2.
39. Sperling RA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280–92.
40. Jessen F, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement. 2014;10(6):844–52.
41. Bankhead P, et al. QuPath: Open source software for digital pathology image analysis. Sci Rep. 2017;7(1):16878.
42. Del Campo M, et al. Facilitating the Validation of Novel Protein Biomarkers for Dementia: An Optimal Workflow for the Development of Sandwich Immunoassays. Front Neurol. 2015;6:202.
43. Andreasson U, et al. A Practical Guide to Immunoassay Method Validation. Front Neurol. 2015;6:179.
44. Del Campo M, et al. Novel CSF biomarkers to discriminate FTLD and its pathological subtypes. Ann Clin Transl Neurol. 2018;5(10):1163–75.
45. Duchateau PN, et al. Apolipoprotein L gene family: tissue-specific expression, splicing, promoter regions; discovery of a new gene. J Lipid Res. 2001;42(4):620–30.
46. Kumar V, et al. Disrupted apolipoprotein L1-miR193a axis dedifferentiates podocytes through autophagy blockade in an APOL1 risk milieu. Am J Physiol Cell Physiol. 2019;317(2):C209–25.
47. Woollacott IOC, et al. Microglial burden, activation and dystrophy patterns in frontotemporal lobar degeneration. J Neuroinflammation. 2020;17(1):234.
48. Hartnell IJ, et al., *Glial cells and adaptive immunity in frontotemporal dementia with tau pathology*. Brain, 2021.
49. Bain HDC, et al. The role of lysosomes and autophagosomes in frontotemporal lobar degeneration. Neuropathol Appl Neurobiol. 2019;45(3):244–61.
50. Feng T, et al. Loss of TMEM106B and PGRN leads to severe lysosomal abnormalities and neurodegeneration in mice. EMBO Rep. 2020;21(10):e50219.
51. Zhou X, et al. Loss of Tmem106b exacerbates FTLD pathologies and causes motor deficits in progranulin-deficient mice. EMBO Rep. 2020;21(10):e50197.
52. Deng Z, et al. Is amyotrophic lateral sclerosis/frontotemporal dementia an autophagy disease? Mol Neurodegener. 2017;12(1):90.
53. Katzeff JS, et al. Altered serum protein levels in frontotemporal dementia and amyotrophic lateral sclerosis indicate calcium and immunity dysregulation. Sci Rep. 2020;10(1):13741.
54. Teunissen CE, et al. White paper by the Society for CSF Analysis and Clinical Neurochemistry: Overcoming barriers in biomarker development and clinical translation. Alzheimers Res Ther. 2018;10(1):30.
55. Madhavan SM, O'Toole JF. The biology of APOL1 with insights into the association between APOL1 variants and chronic kidney disease. Clin Exp Nephrol. 2014;18(2):238–42.

56. Chiasserini D, et al. Proteomic analysis of cerebrospinal fluid extracellular vesicles: a comprehensive dataset. J Proteomics. 2014;106:191–204.
57. Alcolea D, et al., *CSF sAPPb, YKL-40, and neurofilament light in frontotemporal lobar degeneration*. Neurology, 2017. 89(2).
58. Bottero V, et al. Transcriptomic and Network Meta-Analysis of Frontotemporal Dementias. Front Mol Neurosci. 2021;14:747798.

Figures

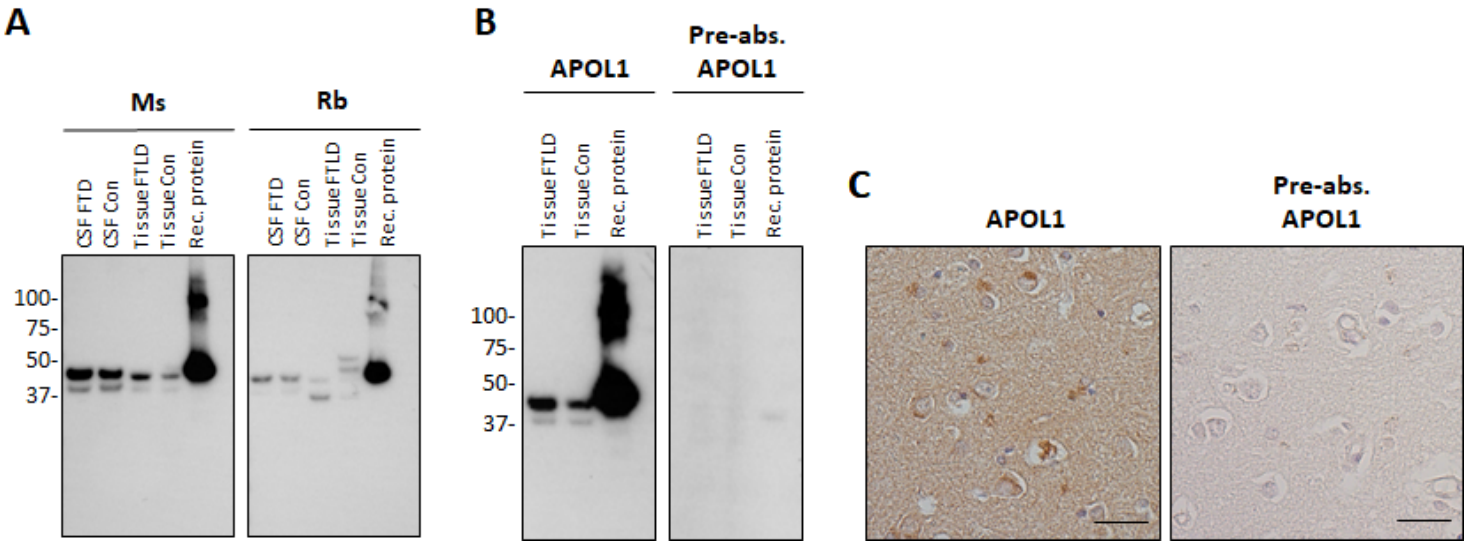

Figure 1

APOL1 antibody characterization.

(A) Mouse (Ms) anti-APOL1 antibody detects similar protein bands around 44 kDa for CSF, frontal cortex tissue and the recombinant protein. Rabbit (Rb) anti-APOL1 antibody shows a protein band around 44 kDa in the CSF and the recombinant protein while in tissue lysates multiple protein bands between 37- 50 kDa were detected (B). Mouse anti-APOL1 antibody was pre-absorbed (Pre-abs.) with a 200 molar excess of the antigenic peptide. APOL1 immunoreactivity in FTLD and control frontal cortex and the APOL1 recombinant protein was abolished in western blot (A) and immunohistochemistry (C) after pre-absorption of the antibody with its antigenic peptide. Scale bars indicate 50 µM. Abbreviations: FTLD, Frontal temporal lobar degeneration; FTD, Frontotemporal dementia; Con, control; CSF, cerebrospinal fluid

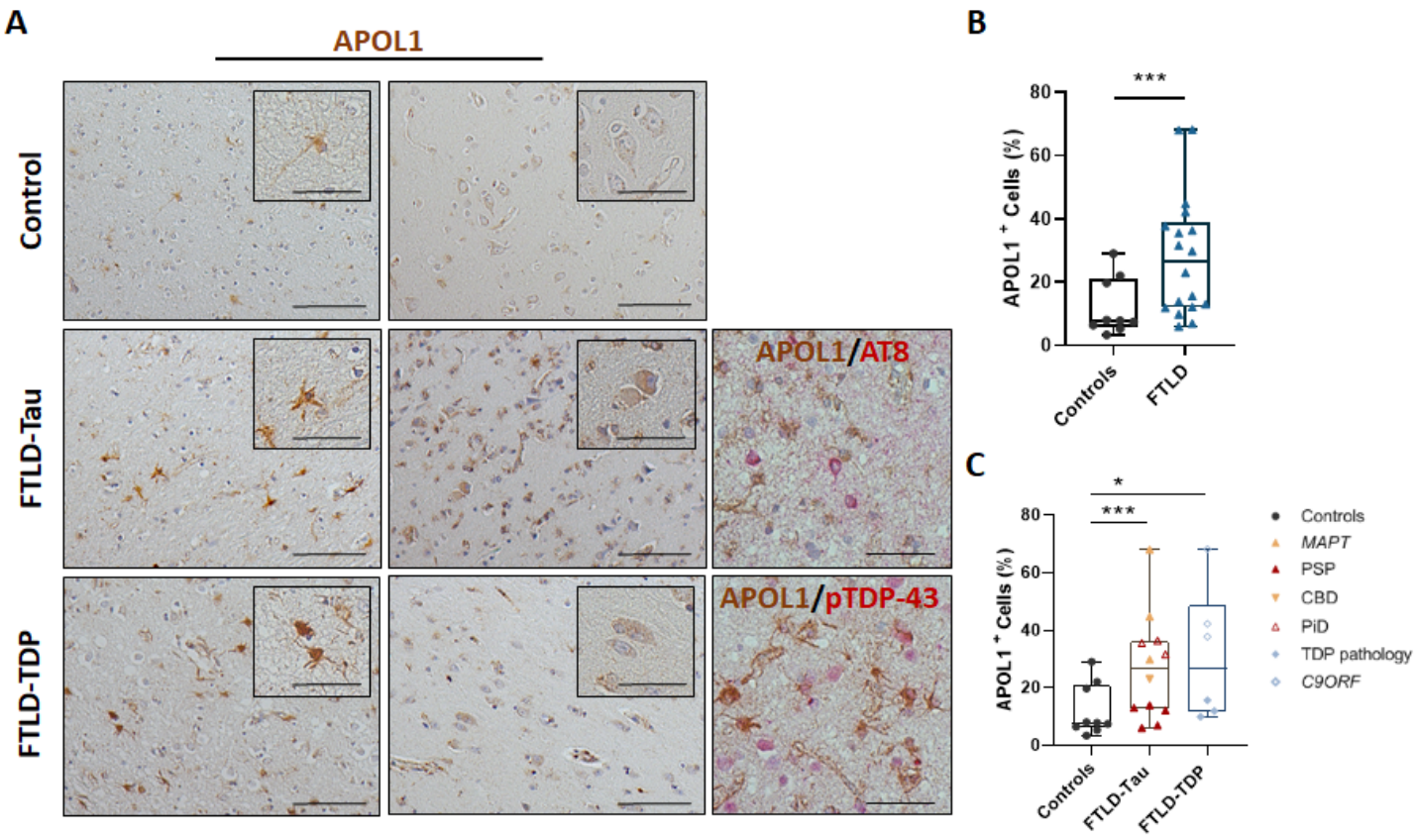

Figure 2

APOL1 immunoreactivity increased in FTLD frontal cortex.

(A) Representative image of APOL1 immunoreactivity in frontal cortex sections of controls and FTLD (FTLD-Tau and FTLD-TDP) cases showed association of APOL1 to glia and neuronal-like cells but not with pTau (AT8) or pTDP-43. (B) Single stained slides were quantified and APOL1 immunoreactivity was shown to be increased in FTLD cases. (C) Stratification of FTLD into the pathological subtypes showed higher APOL1 in both FTLD-TDP and FTLD-Tau compared to controls, but not between FTLD pathological subtypes. Boxplots represent median with interquartile range. *** $p < 0.001$, * $p < 0.05$. Scale bars indicate 100 μM, scale bars in the inserts and double staining represent 50 μM.

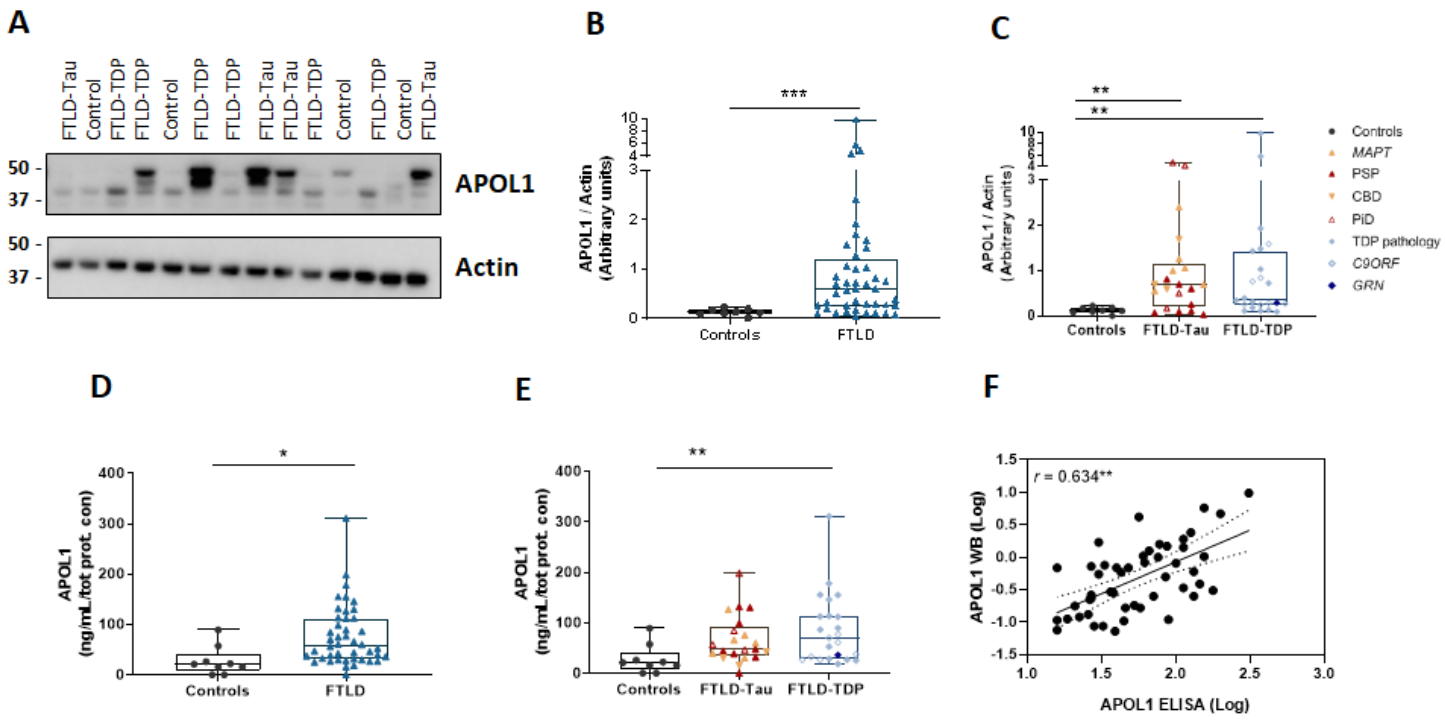

Figure 3

APOL1 protein levels are increased in FTLD frontal cortex tissue.

(A) Representative western blot of APOL1 (44kDa) and Actin (42kDa) protein, tested in tissue lysates from controls and FTLD (including FTLD-Tau and FTLD-TDP subtypes) cases. (B) APOL1 reactivity was quantified and corrected for levels in actin and show that APOL1 reactivity was increased in FTLD compared to controls. (C) APOL1 was higher in both FTLD-Tau and FTLD-TDP compared to controls and no difference was observed between FTLD pathological subtypes. (D) APOL1 levels were measured by our in-house immunoassay and corrected for the total protein content (tot prot. con). (E) We show increased APOL1 levels in FTLD cases compared to controls which was specific for FTLD-TDP. (F) A positive correlation between the APOL1 protein measured by western blot and our in-house immunoassay is detected. Dotted lines represent the 95% confidence bands. Boxplots represent the median ± interquartile range. *** p <0.001, ** p <0.01, * p <0.05

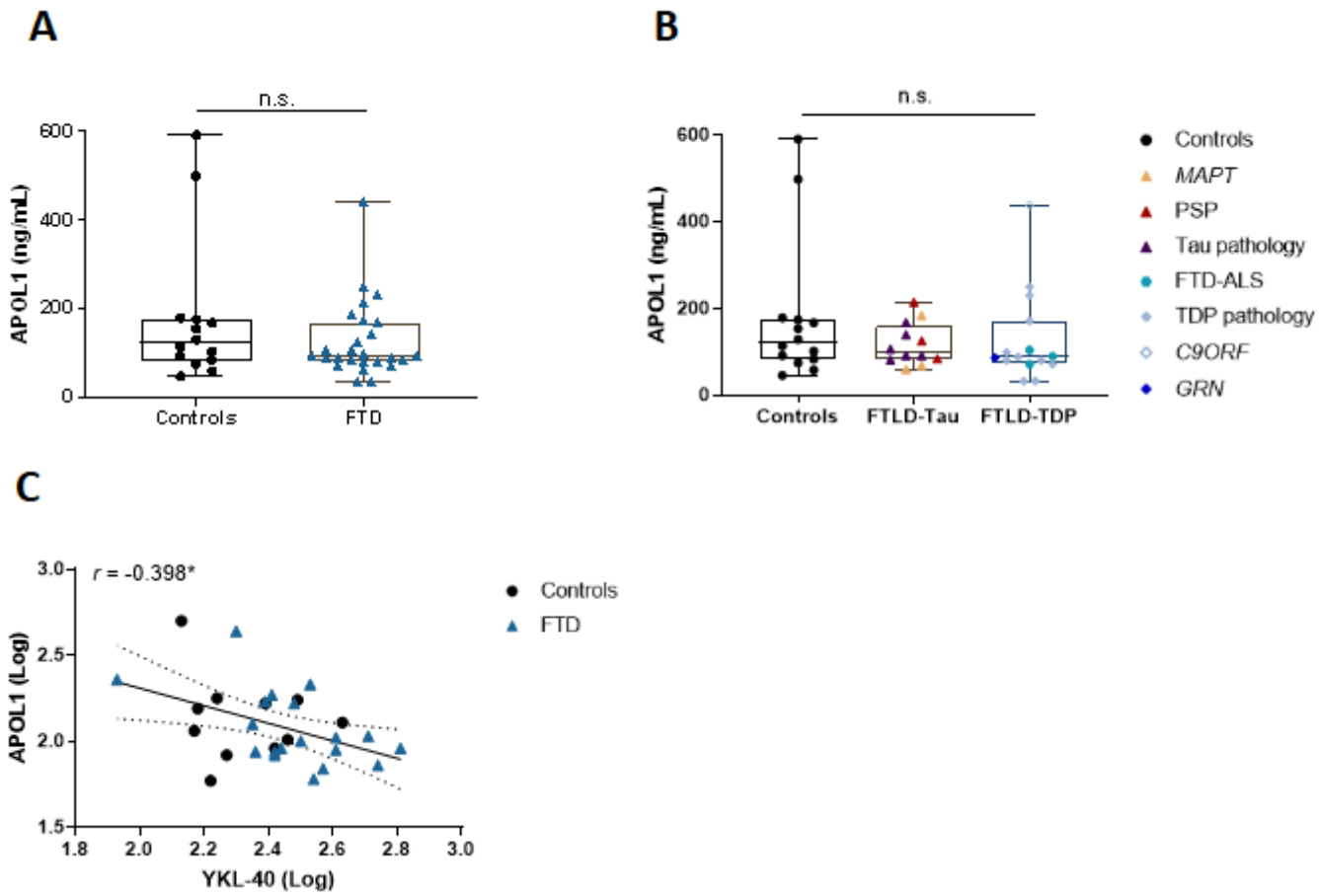

Figure 4

APOL1 levels in CSF remained comparable between FTD patients and controls.

CSF APOL1 was analyzed in controls and FTD patients (with defined Tau or TDP pathology) with our in-house immunoassay. (A) APOL1 levels remained similar across clinical groups and no differences were observed between the pathological subtypes (B). (C) CSF APOL1 levels showed a negative correlation with CSF YKL-40 levels (r = -0.398). Boxplots represent the median ± interquartile range. n.s. indicates a non-significant difference between groups. Dotted lines represent the 95% confidence bands. * p <0.05

Supplementary Files

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