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Title:
Cerebrospinal fluid neurofilament light and cerebral atrophy in younger-onset dementia and primary psychiatric disorders

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Background

Neurofilament light (NfL), a marker of axonal degeneration and injury in the central nervous system (CNS), is establishing itself as a promising biomarker for the intensity of neurodegeneration underpinning a variety of neurodegenerative diseases.[1-4] NfL is typically found in large-calibre, myelinated axons, and forms a major component of the neuronal cytoskeleton. Following axonal injury, NfL accumulates in the cerebrospinal fluid (CSF), where it can be quantified, thereby making it a useful biomarker of subcortical and white matter tract damage.[5,6]

CSF NfL has been shown to correlate with a range of structural CNS changes in many neurodegenerative conditions, such as motor neuron disease, multiple sclerosis, and various dementia aetiologies, illustrating its utility as a marker of neurodegeneration.[3,4,7-11] The relationship between cerebral atrophy and NfL has not been studied as extensively in cohorts of younger-onset dementia (YOD, defined as dementia with symptom onset under 65 years of age).[12] YOD represents a rare subgroup of dementia, often associated with significant diagnostic and prognostic uncertainty due to atypical presentations, highlighting the need for further evaluation of biomarkers of neurodegeneration in YOD.[13]

There is also a growing consensus that primary psychiatric disorders (PSY), such as schizophrenia, may have underlying neurodegenerative processes, as evidenced by structural and atrophic changes identified on MRI brain.[14-16] Whether NfL is related to these MRI changes in this group of patients is yet to be investigated.

Our unit, Neuropsychiatry, The Royal Melbourne Hospital, Victoria, Australia, provides diagnostic and management services for young patients with a wide range of complex psychiatric, cognitive and neurological symptoms. Over a third of patients assessed at the diagnostic inpatient unit are ultimately diagnosed with YOD, and the remainder is frequently diagnosed with PSY.[17] As part of their routine diagnostic work-up, patients undergo MRI brain and CSF sampling. As such, we had available a unique cohort of patients in which we aimed to assess CSF NfL as a marker of neurodegeneration, by comparing it to MRI volume parameters, across a heterogenous group of aetiologies.

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Methods

Study design, setting and participants

This was a retrospective study conducted at Neuropsychiatry, The Royal Melbourne Hospital, Victoria, Australia. Patients received comprehensive multidisciplinary assessments by neuropsychiatrists, neurologists, neuropsychologists, neuroradiologists, speech pathology, and multimodal investigations including structural and functional neuroimaging (e.g. MRI, SPECT/PET brain) and CSF analysis, after which a consensus diagnosis of dementia or PSY was made based on established diagnostic criteria.[18-22]

131 patients referred to Neuropsychiatry for assessment, diagnostic evaluation or management from March 2009 and February 2018 with CSF analysis were identified. Of these, 57 were excluded as they did not have contemporaneous CSF NfL analysis and MRI Brain. Four patients with dementia with onset over the age of 65 years were excluded as this did not meet the definition for YOD.[12]

Ethics approval for this project was obtained through the Human Research Ethics Committees at Melbourne Health (2016.038, 2017.090, 2018.371, 2020.142), University of Melbourne (1341074), and Florey Institute of Neurosciences and Mental Health (1648441.1).

Biomarker analysis

This has previously been described in detail.[1] In brief, CSF samples from patients had been tested for Tau and amyloid-β-42 concentrations since March 2009 at the National Dementia Diagnostic Laboratory, Florey Institute of Neuroscience and Mental Health, Melbourne. CSF samples were stored at -80^{o}C and subsequently analysed for NfL using an enzyme immunoassay (NF-light, ELISA, UmanDiagnostics, Umea, Sweden). Diluted CSF and reconstituted standards were added to the plate in duplicate, incubated and washed according to the manufacturer's protocol. Samples displaying concentration above the highest standard point were further diluted and re-assayed. Two internal

controls of pooled CSF were included in each plate. The mean intra-assay coefficient of variation (CV) was 6.2%, and the inter-assay CV was 11.3%.

Data collection

Patients with CSF sampling were identified from March 2009 to February 2018. Medical records were used to access contemporaneous MRI brain and clinical data including age at CSF collection, sex, CSF investigation results, and cognitive assessment. Cognition was evaluated using the Neuropsychiatry Unit cognitive assessment tool (NUCOG), which assesses five domains of cognition, including attention, visuospatial, memory, executive function and language, (0-20 for each domain, 0-100 total score), with lower scores indicating greater cognitive impairment.[23]

MRI brain volume analysis

Throughout the study period, patients had undergone either fast spoiler gradient echo (FSPGR) or magnetization prepared rapid acquisition gradient echo (MPRAGE) MRI brain sequences. Anonymised T1-weighted MRI brains for each patient were analysed using volBrain, an automated MRI brain volumetry system.[24] Total white matter, grey matter and whole brain volumes were divided by total intracranial volume to obtain a % white matter (WM), grey matter (GM) and whole brain (WB) volumes for statistical analysis.

Statistical Methods

The data was non-normally distributed. As such, non-parametric tests such as Mann-Whitney *U* tests were used to compare two sample groups and Kruskal-Wallis tests were used to compare means across more than two groups. The Bonferroni adjustment was used to correct for inflated type I error associated with post-hoc comparisons. Correlations between continuous variables were assessed using Pearson's correlation coefficient. Statistical significance for correlations was defined as 95% confidence intervals that did not capture the null hypothesis value. Due to possible violation of distributional assumptions, robust bias corrected and accelerated (BCa) confidence intervals were computed via bootstrapping with 1000 replicates. Partial correlations were used to adjust for age.

Different MRI brain sequences, for example FSPGR and MPRAGE, have varying effects on tissue differentiation and estimation of brain atrophy,[25] and were therefore grouped separately for analysis. Statistical analyses were performed using IBM SPSS Statistics version 26.

Results

The final cohort consisted of 70 patients, including 23 PSY and 47 YOD patient. (Table 1). PSY included schizophrenia (n=7) major depressive disorder (n=6), adjustment disorder (n=2), bipolar affective disorder (n=2), conversion disorder (n=2), somatic symptom disorder (n=2), generalised anxiety disorder (n=1) and schizoaffective disorder (n=1). YOD included Alzheimer's dementia (AD, n=18), behavioural variant FTD (bvFTD, n=9), dementia not otherwise specified (where there was a clear neurodegenerative process, but a more specific diagnosis could not be made, D-NOS, n=7) and other dementia (OD, n=12). This latter group included corticobasal syndrome (n=4), vascular dementia (n=2), primary progressive aphasia (n=2), multiple system atrophy (n=2), dementia with Lewy bodies (n=1), frontotemporal dementia with motor neuron disease (n=1) and progressive supranuclear palsy (n=1).

Of the MRI brain sequences, 39 patients had FSPGR whilst 31 had MPRAGE. The average time between CSF NfL sampling and MRI imaging was 1 month (SD=2). In the MPRAGE group, WB was higher in PSY compared to bvFTD (p=.047). No other differences in WM, GM or WB were identified between diagnostic groups in the MPRAGE or FSPGR groups.

PSY had significantly lower NfL levels compared to AD (p<.001), bvFTD (p<.001), D-NOS (p<.001) and OD (p<.001) as previously described.[1] AD and OD patients were older on average compared to bvFTD, D-NOS and PSY. Older age was associated with reduced WM (r=-.364, 95% CI:[-.535, -.159], p=.002) and WB (r=-.368, 95% CI:[-.590, -.102], p=.002), but not GM or NfL levels. There were no differences in brain volume or NfL based on sex. With regards to cognition, low NUCOG scores were associated with low GM and WB (supplementary information), but not WM or CSF NfL.

In the total cohort, whilst controlling for age, high CSF NfL was associated with increased whole brain atrophy in the FSPGR and MPRAGE sequences (r=-.402, 95% CI:[-.593, -.147], p=.008 and r=-

.625, 95% CI:[-.828, -.395], p<.001, respectively) (table 2, figure 1). NfL was associated with reduced GM in the FSPGR group (r= -.385, 95% CI:[-.649, -.014], p=.017). NfL was associated with reduced WM in the MPRAGE group (r=-.650, 95% CI:[-.830, -.307], p<.001). Similar relationships were evident in YOD, but not PSY.

Discussion

This study investigated the relationships between CSF NfL and brain volumetry across a range of YOD and PSY, to determine its utility as a marker of neurodegeneration. We found that higher NfL was associated with a greater degree of whole brain atrophy in the total cohort, and that this relationship persisted in the YOD group. This suggests that CSF NfL is representative of neuroaxonal degeneration in YOD, but not PSY.

Prior studies have identified NfL to be associated with CNS atrophy in a heterogenous group of neurodegenerative conditions. These relationships have been shown in motor neuron disease, multiple sclerosis, spinocerebellar ataxia and various dementia types, highlighting the potential of NfL as a broad biomarker of neurodegeneration intensity.[3,4,7-10,26]

No relationship was identified between NfL and brain volume in PSY. Although prior research suggests a neurodegenerative component to PSY, as evidenced by structural brain changes in patients with psychosis,[14-16], it does not appear that NfL is sensitive to these changes. This is in keeping with other research that has not identified differences in NfL between PSY and healthy controls.[1]

Although NfL has classically been associated with white matter tract damage,[5,6] which is in keeping with the results from the MPRAGE group, NfL had a weak relationship to grey matter volume loss in the FSPGR group. NfL associations with grey matter volume have been demonstrated in multiple sclerosis.[27,28] This may in part be driven by Wallerian degeneration of white matter contributing to loss of GM, or could be driven by an independent process.[29]

A relative strength of our study is the use of bias corrected and accelerated 95% confidence intervals, a conservative measure of significance.[30] Automated brain volumetry with volBrain has demonstrated precision, and has been shown to be superior to other platforms such as Freesurfer in terms of accuracy and reproducibility.[24]

Despite this, automated volumetry is not as reliable as manual segmentation.[24] Although MRI Brain sequences were separated for statistical analysis, the sequences within these groups were not all acquired by an identical MRI scanner. The study is also limited by its retrospective nature and reliance on clinical diagnosis. The small sample sizes prevent meaningful analysis in the individual diagnostic groups. Given different disease states may have varying degrees of NfL association in their underlying pathologic processes, further evaluation in individual diagnostic groups in YOD, with larger sample sizes is necessary. Due to acceptable correlations between CSF and plasma NfL,[31] future research should evaluate the ability of plasma NfL to determine the severity of neurodegeneration in YOD and PSY, as this would provide a less invasive, and more clinically translatable measure to assess disease severity.

Conclusion

CSF NfL was associated with the degree of contemporaneous cerebral atrophy across a heterogenous group of YOD, as measured by automated volumetric analysis of brain imaging. This further supports the use of CSF NfL as a non-specific marker of neurodegeneration which has clinical utility in a range of neurodegenerative processes. Further research with larger sample sizes and longitudinal measures of brain volume are required to consolidate these findings.

References

1. Eratne D, Loi SM, Walia N, et al. A pilot study of the utility of cerebrospinal fluid neurofilament light chain in differentiating neurodegenerative from psychiatric disorders: A 'C-reactive protein' for psychiatrists and neurologists? Aust N Z J Psychiatry. 2019:4867419857811.
2. Eratne D, Loi SM, Li QX, et al. Cerebrospinal fluid neurofilament light chain is elevated in Niemann-Pick type C compared to psychiatric disorders and healthy controls and may be a marker of treatment response. The Australian and New Zealand journal of psychiatry. 2020;54(6):648-9.
3. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nature reviews Neurology. 2018;14(10):577-89.
4. Gaetani L, Blennow K, Calabresi P, et al. Neurofilament light chain as a biomarker in neurological disorders. Journal of Neurology, Neurosurgery & Psychiatry. 2019;90(8):870.
5. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. Journal of the neurological sciences. 2005;233(1-2):183-98.
6. Sjogren M, Blomberg M, Jonsson M, et al. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. Journal of neuroscience research. 2001;66(3):510-6.
7. Filippi P, Vestenická V, Siarnik P, et al. Neurofilament light chain and MRI volume parameters as markers of neurodegeneration in multiple sclerosis. Neuro endocrinology letters. 2020;41(1):17-26.
8. Dhiman K, Gupta VB, Villemagne VL, et al. Cerebrospinal fluid neurofilament light concentration predicts brain atrophy and cognition in Alzheimer's disease. Alzheimers Dement (Amst). 2020;12(1):e12005-e.
9. Menke RA, Gray E, Lu CH, et al. CSF neurofilament light chain reflects corticospinal tract degeneration in ALS. Ann Clin Transl Neurol. 2015;2(7):748-55.
10. Coarelli G, Darios F, Petit E, et al. Plasma neurofilament light chain predicts cerebellar atrophy and clinical progression in spinocerebellar ataxia. Neurobiology of Disease. 2021;153:105311.
11. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology. 2016;87(13):1329-36.
12. Draper B, Withall A. Young onset dementia. Internal medicine journal. 2016;46(7):779-86.
13. Draper B, Cations M, White F, et al. Time to diagnosis in young-onset dementia and its determinants: the INSPIRED study. Int J Geriatr Psychiatry. 2016;31(11):1217-24.
14. Csernansky JG. Neurodegeneration in schizophrenia: evidence from in vivo neuroimaging studies. TheScientificWorldJournal. 2007;7:135-43.
15. Takahashi T, Wood SJ, Yung AR, et al. Progressive gray matter reduction of the superior temporal gyrus during transition to psychosis. Archives of general psychiatry. 2009;66(4):366-76.
16. Walterfang M, McGuire PK, Yung AR, et al. White matter volume changes in people who develop psychosis. The British journal of psychiatry : the journal of mental science. 2008;193(3):210-5.
17. Loi S, Eratne D, Goh A, et al. A 10 year retrospective cohort study of inpatients with younger-onset dementia. International journal of geriatric psychiatry. 2020;36: 294-301.
18. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018;14(4):535-62.
19. Lethin C, Rahm Hallberg I, Renom Guiteras A, et al. Prevalence of dementia diagnoses not otherwise specified in eight European countries: a cross-sectional cohort study. BMC Geriatrics. 2019;19(1):172.
20. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain : a journal of neurology. 2011;134(Pt 9):2456-77.
21. American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force. (2013). Diagnostic and statistical manual of mental disorders : DSM-5. Arlington VAPA.

22. American Psychiatric Association. & American Psychiatric Association. Task Force on DSM-IV. (1994). Diagnostic and statistical manual of mental disorders : DSM-IV. Washington DAP.
23. Walterfang M, Siu R, Velakoulis D. The NUCOG: validity and reliability of a brief cognitive screening tool in neuropsychiatric patients. The Australian and New Zealand journal of psychiatry. 2006;40(11-12):995-1002.
24. Manjón JV, Coupé P. volBrain: An Online MRI Brain Volumetry System. Frontiers in Neuroinformatics. 2016;10(30).
25. Leung KK, Malone IM, Ourselin S, et al. Effects of changing from non-accelerated to accelerated MRI for follow-up in brain atrophy measurement. Neuroimage. 2015;107:46-53.
26. Johnson EB, Byrne LM, Gregory S, et al. Neurofilament light protein in blood predicts regional atrophy in Huntington disease. Neurology. 2018;90(8):e717-e23.
27. Buchmann A, Pirpamer L, Pinter D, et al. Serum Neurofilament Light Levels Correlate with Reduced Gray Matter Volume in Advanced Multiple Sclerosis (2046). Neurology. 2021;96(15 Supplement):2046.
28. Fujimori J, Nakashima I. Serum neurofilament light is a sensitive biomarker that reflects grey matter volume in Japanese patients with multiple sclerosis. Journal of the Neurological Sciences. 2021;427:117528.
29. Kiljan S, Preziosa P, Jonkman LE, et al. Cortical axonal loss is associated with both gray matter demyelination and white matter tract pathology in progressive multiple sclerosis: Evidence from a combined MRI-histopathology study. Multiple sclerosis (Houndmills, Basingstoke, England). 2021;27(3):380-90.
30. Bishara AJ, Hittner JB. Confidence intervals for correlations when data are not normal. Behavior Research Methods. 2017;49(1):294-309.
31. Alagaratnam J, von Widekind S, De Francesco D, et al. Correlation between CSF and blood neurofilament light chain protein: a systematic review and meta-analysis. BMJ Neurology Open. 2021;3(1):e000143.

Table 1: Patient demographics, numbers, cognitive assessment, NfL levels and MRI Brain volume analysis across diagnostic groups

	Total	**AD**	**bvFTD**	**D-NOS**	**OD**	**PSY**	***p***
Age at CSF, y	54(10)	57(6)	50(7)	50(11)	60(7)	50(12)	.011*
n	70	18	9	7	13	23	
Sex (female)	34%	39%	56%	29%	15%	35%	.951
CSF NfL (pg/mL)	1357(1339)	2172(325)	2022(1968)	2064(2273)	2306(1350)	489(213)	<.001*
NUCOG score	72(17)	66(15)	73(22)	70(15)	68(19)	78(15)	.209
Attention	14(4)	12(3)	15(60	14(4)	13(4)	15(3)	.188
Visuospatial	16(4)	15(4)	16(4)	16(4)	15(5)	17(2)	.536
Memory	12(4)	10(3)	15(4)	12(1)1	11(5)	14(5)	.043*
Executive function	12(5)	12(4)	11(7)	13(5)	12(5)	13(6)	.886
Language	17(3)	17(3)	17(4)	16(4)	17(3)	18(2)	.798
MRI Brain							
FSPGR *n*	39	9	6	6	7	11	
WM%	33(3)	32(1)	33(3)	34(4)	31(3)	34(3)	.193
GM%	48(3)	46(2)	48(5)	49(4)	47(2)	49(3)	.251
WB%	81(5)	78(3)	81(6)	82(8)	78(3)	83(5)	.178
MPRAGE *n*	31	9	3	1	6	12	
WM%	34(3)	33(4)	32(2)	30(0)	31(2)	36(2)	.027*
GM%	45(3)	45(2)	41(1)	39(0)	45(3)	47(3)	.054
WB%	79(5)	78(5)	73(3)	69(0)	77(2)	83(4)	.006*

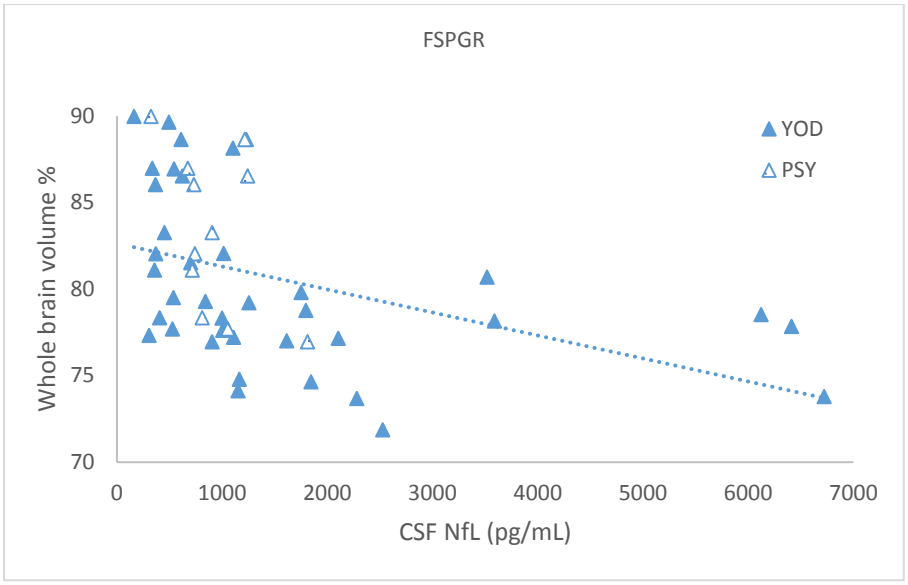
Significant differences between groups as determined by Kruskal-Wallis tests. Data stated as mean(standard deviation) unless otherwise specified.* ***Abbreviations:* *AD=Alzheimer's disease; bvFTD=behavioural variant frontotemporal dementia; D-NOS=dementia not otherwise specified; OD=other dementia or neurodegenerative disorder; PSY=Primary psychiatric disorders; CSF=cerebrospinal fluid; NfL=neurofilament light; tTau=total tau; pTau=phosphorylated tau; Aβ42=amyloid beta peptide 42; NUCOG=Neuropsychiatry unit cognitive assessment tool; MRI=Magnetic resonance imaging; FSPGR=Fast spoiler gradient echo; MPRAGE=Magnetization Prepared Rapid Acquisition Gradient Echo; WM=% White matter volume; GM=% Grey matter volume; WB=% Whole brain volume.*

Table 2: Relationship between CSF NfL and MRI Brain volume in YOD and PSY whilst controlling for age

	WM%			GM%			WBV%		
	r	p	95% CI	r	p	95% CI	r	p	95% CI
FSPGR Total cohort	-.213	.199	-.466, .042	-.385	.017*	-.649, -.014⁷	-.402	.008**	-.593, -.147⁷
YOD	-.237	.234	-.510, .177	-.414	.032*	-.685, -.012⁷	-.413	.032*	-.692, -.034⁷
PSY	-.177	.625	-.693, .648	-.115	.751	-.757, .730	-.269	.452	-.881, .878
MPRAGE Total cohort	-.650	<.001***	-.830, -.307⁷	-.319	.086	-.595, -.114⁷	-.625	<.001***	-.828, -.395⁷
YOD	-.543	.020*	-.777, -.081⁷	.003	.990	-.514, .458	-.413	.089	-.704, -.003⁷
PSY	.126	.712	-.753, .848	-.461	.153	-.941, .269	-.239	.479	-.921, .545

**Significant at p<.05; **significant at p<.01; ***significant at p<.001; ⁷ significant confidence interval*

Abbreviations: *YOD=Younger-onset dementia; PSY=Primary Psychiatric Disorders; FSPGR=Fast spoiler gradient echo; MPRAGE=Magnetization Prepared Rapid Acquisition Gradient Echo; WM=% White matter volume; GM=% Grey matter volume; WB=% Whole brain volume.*



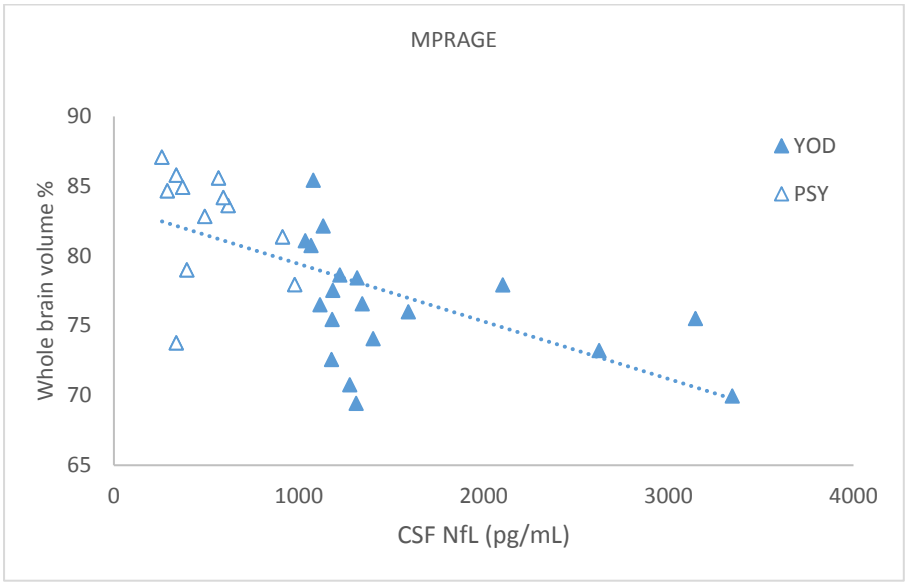


Fig 1 *Relationships between CSF NfL and Whole Brain Volume % in YOD and PSY*
Abbreviations: *CSF=cerebrospinal fluid; NfL=neurofilament light; YOD=younger-onset dementia; PSY=primary psychiatric disorder; FSPGR=Fast spoiler gradient echo; MPRAGE=Magnetization Prepared Rapid Acquisition Gradient Echo*