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Synaptic and cytoskeletal CSF signatures of motor neuron disease: the role of cyclase-associated protein 2

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Cyclase-associated protein 2 (CAP2) is a synaptic actin-binding protein involved in cofilin-mediated spine remodelling, Alzheimer's Disease synaptic failure and myofibril maintenance, indicating its potential involvement in motor neuron disease (MND). This study examined cerebrospinal fluid (CSF) levels of CAP2 in 60 patients with MND and 40 healthy controls (HC) to assess its diagnostic and prognostic value and its relationship with neuronal, glial and synaptic markers. Glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), phosphorylated and total tau (p-Tau 181, t-Tau), CAP2 and synaptosomal-associated protein 25 (SNAP-25) were quantified using ELISA, Lumipulse and SIMOA platforms. MND patients displayed increased GFAP, NfL, t-Tau, p-Tau 181 levels and CAP2 while SNAP-25 was reduced. CAP2 correlated with tau markers, but not with NfL or GFAP. Unlike NfL, which was higher in upper motor neuron–predominant cases and predicted faster progression and poorer survival, CAP2 did not vary with disease subtypes or severity. The study showed that CAP2 is associated with MND independently from neuronal, glial and presynaptic dysfunction. Integrating CAP2 into multi-marker panels could enhance understanding of synaptic pathology in MND.

Keywords Motor neuron disease (MND), Synaptic biomarkers, Neurofilament light chain (NfL), Cerebrospinal fluid (CSF)

Synaptic dysfunction is increasingly recognized as a critical early event in the pathogenesis of neurodegenerative diseases[1]. Long before neuronal death becomes evident, synaptic alterations disrupt communication within neural circuits, contributing to neurological impairments. These insights have spurred extensive efforts to identify CSF biomarkers reflecting synaptic integrity, including neurogranin and SNAP-25[2,3].

Synaptic pathology in MND has been less investigated[4,5]. Nevertheless, a growing body of research highlights that synaptic degeneration is a prominent and early feature of MND. Human and animal studies consistently show a loss of presynaptic proteins such as synaptophysin, SNAP-25, and syntaxin in the spinal cord and neuromuscular junction[6]. Changes in excitatory and inhibitory neurotransmission, marked by imbalances in glutamate and GABA receptor subunits and by the synaptic mislocalization of proteins such as fused in sarcoma (FUS) and TAR DNA-binding protein 43 (TDP-43), further underscore the critical contribution of synaptic dysfunction to disease progression[5].

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Despite these findings, few fluid biomarkers have been established to reflect neuronal and synaptic pathology in MND. Most studies to date have focused on axonal damage markers such as NfL and phosphorylated neurofilament heavy chain (pNfH), which consistently demonstrate high sensitivity and prognostic value[7,8]. Synaptic proteins in CSF, including SNAP-25 and neurogranin appeared to be less studied in MND, with still discussed findings[9,10]. Nevertheless, the recent evidence that the neuronal pentraxins might represent a valid readout for therapy responsiveness in MND increased the potential clinical relevance of synaptic biomarkers in MND[11].

Within this framework, CAP2 represents a particularly compelling candidate. Although it can be categorized as a synaptic protein, CAP2 is not merely a marker of synaptic presence. As a member of the cyclase-associated protein family, CAP2 plays a central role in actin cytoskeleton regulation, a process that is critical for dendritic spine stability, synaptic plasticity, and axonal integrity. CAP2 belongs to the cyclase-associated protein (CAP) family, actin-binding proteins that regulate actin dynamics. Whereas CAP1, the other member of CAP family, is ubiquitously expressed, CAP2 expression showed a more restricted expression pattern, limited to a few organs, including the brain and skeletal muscles[12,13]. In skeletal muscle, CAP2 emerges as a critical regulator of skeletal muscle development and function, primarily through its role in actin cytoskeleton dynamics and myofibril differentiation[14]. In neurons, CAP2 is a postsynaptic protein that modulates dendritic spine morphology and facilitates cofilin-mediated spine remodeling during synaptic plasticity events[15].

On this basis, we hypothesized that CSF CAP2 levels may reflect synaptic and cytoskeletal alterations in MND, potentially providing complementary biological information to established neurodegeneration markers. We therefore measured CSF CAP2 concentration in patients with MND compared to healthy controls and explored its relationship with CSF markers such as phospho-Tau, total Tau, neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP). Furthermore, the association with clinical subtypes of the disease and the prognostic value of CAP2 levels were assessed longitudinally in the MND cohort.

Methods

Study population

Patients with Gold Coast criteria for MND were consecutively enrolled at the outpatient Neuromuscular Clinic at Brescia University Hospital[16]. Individuals were included according to the following exclusion criteria: (1) age < 55 years; (2) dementia or significant cognitive changes reported; (3) abnormal cognitive screening according to Montreal Cognitive Assessment (MoCA < 26); (4) medical conditions potentially associated with cognitive deficits or movement/gait alterations; (5) major psychiatric disorders (6) recent acute fever/inflammation/concussion.

For each subject, the following data were collected: (1) demographic details and clinical characteristics, including the region of symptom onset; (2) presence of upper motor neuron (UMN) signs (e.g., hyperreflexia, spasticity, and pseudobulbar features) and lower motor neuron (LMN) signs (e.g. muscle wasting, fasciculations, and hyporeflexia).

Clinical assessment

At baseline, all suspected MND patients underwent a comprehensive clinical and diagnostic work-up including CSF. All patients underwent brain and spinal cord MRI, electromyography and motor/sensory evoked potential, as well as biochemical screening, according to current standard of care and MND diagnosis[17]. Assessment of UMN and LMN was defined by neurological examination and supported by electrodiagnostic studies[16]. The Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) was used to assess disease severity[18]. DPR was defined as (48-ALSFRS-R)/disease duration (points per month) and calculated at first visit, as well as 12 months follow-up[19].

For biomarker comparison, an age-matched group of controls who underwent CSF analyses for isolated persistent headache, but any other neurological symptom or MRI/EEG/CSF alteration, was included and considered as negative controls, as previously described[20]. The study was approved by the local ethics committee (NP 1471, DMA, Brescia) and was performed in conformity with the Helsinki Declaration; informed consent was obtained from each study participant or their legally authorized representative.

CSF collection and analysis

CSF collection was performed in fasting condition according to the standardized protocol of the outpatient clinic, from 09:00 to 11:00 in the morning, after clinical informed written consent was obtained. CSF was collected in sterile polypropylene tubes and gently mixed to avoid gradient effects. CSF was centrifugated and firstly processed for standard biochemical analyses, whereas two milliliters of CSF were stored in cryotubes at −80 °C before biomarkers testing. As by the guidelines delivered by the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry, CSF samples were subjected to a maximum of two freeze–thaw cycles[21]. Only patients with normal routine measures were included in further analyses.

CSF neuronal, glial and synaptic markers

CSF p-Tau181, and total tau were measured using the Lumipulse G assays (Fujirebio) on the LUMIPULSE G600II for diagnostic standard analyses performed at Central Chemical Analysis Laboratory, as previously reported[22]. All CSF samples were additionally analyzed for neuronal (NfL), glial (GFAP) and synaptic markers, namely SNAP-25 and CAP2. All CSF analyses were conducted by researchers who were blind to the origin of plasma biomarkers, at the Laboratory of Advanced Biological Markers at the University of Brescia (SIMOA/Lumipulse G600II) and Department of Pharmacological and Biomolecular Sciences, University of Milan (ELISA). SIMOA analyses were performed using the SR-X platform and the Neurology 2-Plex Advantage Kits (NfL/GFAP) and

SNAP-25 kit (single-plex) from Quanterix, Billerica, MA. Samples were measured in duplicate using one batch of reagents from the same lot in one round of experiments. Intra-assay coefficients of variation were below 10%. CAP2 was measured using enzyme-linked immunosorbent assays (ELISA) performed at the Department of Pharmacological and Biomolecular Sciences (University of Milan), as previously reported[23]. Briefly, the CSF samples were diluted at 1:20, and their CAP2 concentration was determined using a commercially available ELISA (catalog number IK5163; Immunological Sciences, Rome, Italy). This assay has high sensitivity and specificity for CAP2 detection; no significant cross-reactivity or interference between CAP2 and its analogs was observed. The ELISA CAP2 intra-assay coefficient of variability was 5,73%, and the inter-assay coefficient of variability was 12%. The mean of duplicate assessment was used for final analysis.

Statistical analyses

Continuous variables are reported as median (interquartile range), and categorical variables are reported as numbers and percentages (*n*, %). The normality of distributions was assessed using the Shapiro–Wilk test and Q–Q plots. Depending on data distribution and variance homogeneity, between-group comparisons (MND vs HC and between MND subgroups) were performed using the Mann–Whitney U test for continuous variables, and the Chi-squared test for categorical variables. Multivariable logistic regression analyses were performed to assess the independent association of CAP2 with MND status after adjustment for CSF biomarkers. For prospective analyses, Cox proportional hazards models were used with death as the primary outcome. Models were adjusted for age, sex and baseline ALSFRS-R score and each biomarker was tested separately as the main predictor. Repeated measures ANOVA were conducted for DPR over time, adjusting for their respective baseline values, to assess longitudinal changes at the biomarker level and the influence of baseline characteristics. Statistical significance was set at $p < 0.05$ for all tests. Data analyses were performed using R version 4.3.1 and JASP.

Results

Participants characteristics and CSF markers at baseline

The study enrolled 100 subjects, namely 60 MND patients with confirmed diagnosis and 40 age-matched controls. Demographic and clinical characteristics, as well as CSF biomarkers distribution are highlighted in Table 1 and Fig. 1. Motor neuron disease patients exhibited higher levels of GFAP, NfL, phosphorylated tau and total tau (p-Tau 181 and t-Tau) compared to HC. Levels of the synaptic protein CAP2 were higher in MND compared to HC whereas levels of SNAP-25 protein did not differ between MND and HC. At baseline, ALSFRS-R and DPR did not correlate with CAP2, SNAP-25, or any other CSF biomarker; only ALSFRS-R showed significant correlations with NfL and t-Tau.

The correlation matrix, corrected for age and sex, showed that CAP2 behaves differently in MND compared to HC. CAP2 levels were significantly correlated with tau biomarkers in MND (p-Tau 181 and t-Tau respectively $\rho = 0.349$, $p < 0.01$; $\rho = 0.448$, $p < 0.01$), as shown in Fig. 2. MND subjects with predominant upper motor neuron involvement exhibited higher NfL but comparable CAP2 increased levels compared to subjects with lower motor neuron involvement (Supplementary Table 1).

Moreover, based on these findings, multivariable logistic regression analyses were performed. When included together with CAP2 and NfL, neither t-Tau nor *p*-Tau 181 retained an independent association with MND status. In the final model including CAP2 and NfL as covariates, both biomarkers were independently associated with diagnosis (CAP2: $p = 0.002$; NfL: $p < 0.001$) (Supplementary Table 2).

Predictive progression value of NfL, CAP2

Prospective 12-month follow-up data were available for all 60 patients included in the study. To assess the prognostic impact of NfL and CAP2 on survival in patients with MND, a Cox proportional hazards regression analysis was performed, adjusting for age, sex, and baseline ALSFRS-R scores. For each biomarker, patients were

	HC ($n = 40$)	MND ($n = 60$)	*p*-value
Age	65.10(15.61)	67.32(16.97)	0.184[a]
Sex (F)	22(55%)	20(33.3%)	0.043[b]
Disease duration	–	1.000(1.000)	–
CSF biomarkers			
GFAP [pg/mL]	4339.03(3562.68)	10,202.58(6607.11)	< 0.001[a]
NfL [pg/mL]	604.53(456.74)	3082.62(3156.47)	< 0.001[a]
p-Tau 181 [pg/mL]	24.50(19.10)	36.70(19.60)	< 0.001[a]
t-Tau [pg/mL]	192.00(123.00)	312.00(178.00)	< 0.001[a]
CAP2 [pg/mL]	24.49(12.28)	35.92(13.66)	< 0.001[a]
SNAP-25 [pg/mL]	53.71(30.53)	55.52(31.56)	0.189[a]

Table 1. Demographics and CSF biomarkers for HC and MND. Continuous variables are reported as median (IQR), and categorical variables are reported as numbers and percentages (*n*, %). [a]Mann–Whitney U test, [b]χ^2 Chi-squared test. HC, healthy controls; MND, motor neuron disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-Tau 181, phosphorylated Tau 181; t-Tau, total Tau; CAP2, cyclase-associated protein 2; SNAP-25, synaptosomal-associated protein 25; IQR = interquartile range (Q3–Q1).

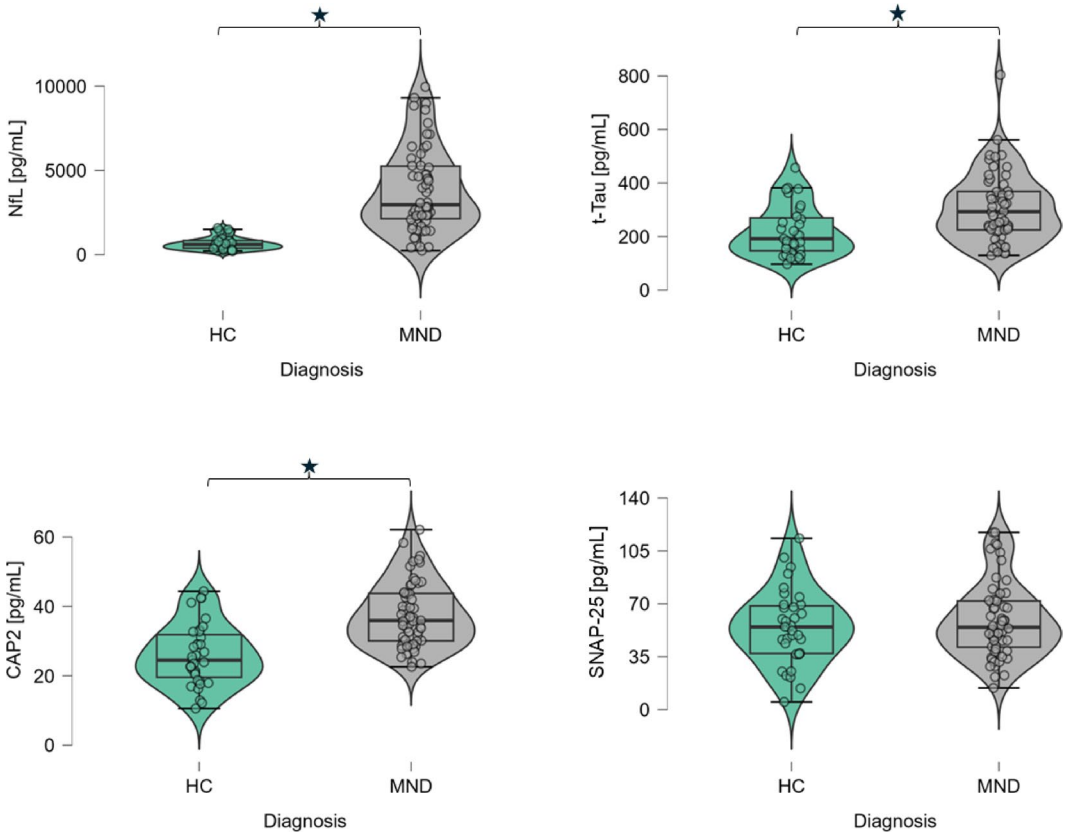

Fig. 1. Violin plots showing CSF levels of synaptic markers CAP2 and SNAP-25 in HC and MND patients. A significant increase in biomarker levels in MND is indicated by the star. HC, healthy controls; MND, motor neuron disease; NfL, neurofilament light chain; t-Tau, total Tau; CAP2, cyclase-associated protein 2; SNAP-25, synaptosomal-associated protein 25.

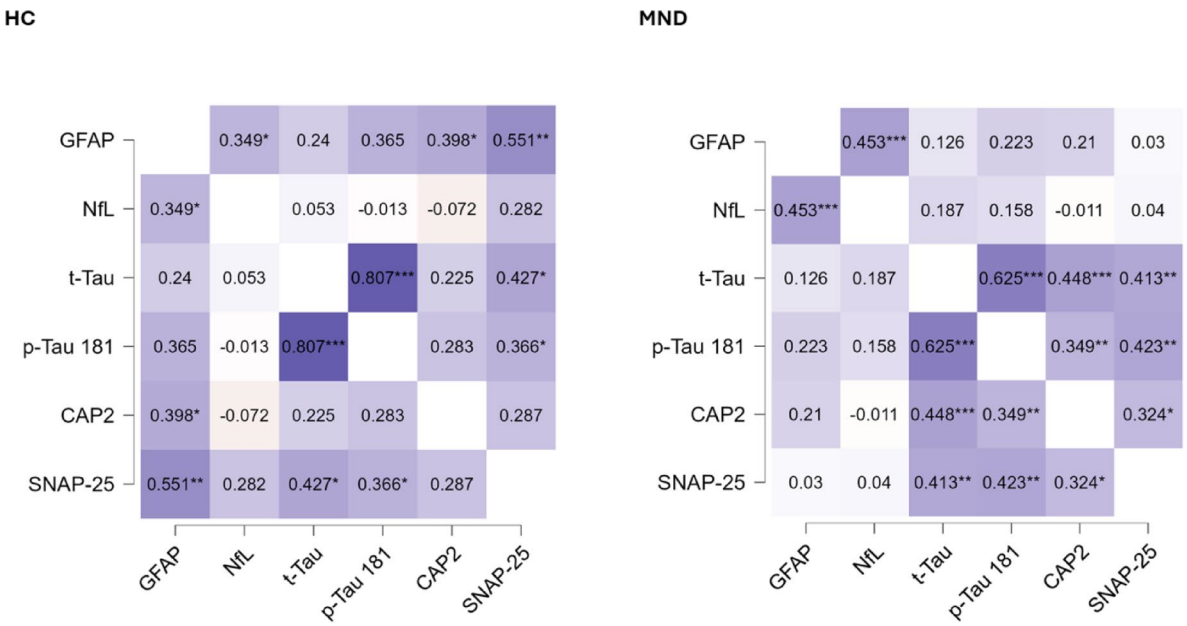

Fig. 2. Spearman's correlation matrix corrected for age and sex including all the CSF biomarkers analysed in the cohort. HC, healthy controls; MND, motor neuron disease; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-Tau 181, phosphorylated Tau 181; t-Tau, total Tau; CAP2, cyclase-associated protein 2; SNAP-25, synaptosomal-associated protein 25.

stratified into two groups, high and low, based on median values. The analysis revealed that higher baseline NfL levels were significantly associated with worse survival outcomes (hazard ratio [HR] = 2.51, 95% confidence interval [CI] 1.2–5.1, $p = 0.01$), whereas CAP2 baseline levels alone were not significantly associated with clinical outcomes ($p = 0.55$).

A repeated measures ANOVA was performed with DPR (i.e. the rate of progression of ALSFRS from onset) as the within-subject factor (baseline and 12-month follow-up) and CSF biomarker levels as the dependent variable. Baseline DPR was included as a covariate to control interindividual variability in disease progression at baseline. A significant interaction was found between time and baseline NfL group (dichotomized by median value) ($p < 0.001$), indicating that patients with higher baseline NfL levels exhibited a different longitudinal trajectory in disease progression compared to those with lower levels. The same analytical approach was applied to CAP2, using NfL and baseline DPR as covariates, with no relevant interaction ($p = 0.510$).

Discussion

In this study, we report for the first time that CSF levels of the postsynaptic protein CAP2 are significantly increased in patients with MND, whereas the levels of the presynaptic marker SNAP-25 remain unchanged. This contrasting pattern of synaptic protein alterations in MND may reflect differences in subcellular localization, disease-stage vulnerability, and pathophysiological mechanisms.

Markers of axonal degeneration, such as NfL, have been extensively validated in MND[8]. In contrast, validated biomarkers of synaptic impairment in vivo are still lacking, despite growing recognition of synaptic dysfunction as a critical contributor to disease progression[4]. Our findings add to emerging evidence that synaptic dysfunction is an early and concomitant feature of MND, supporting the hypothesis that disturbances in synaptic physiology are closely linked to neuronal loss in neurodegenerative disorders.

This is particularly relevant considering the limited availability of large clinical biomarkers data, yet mounting neuropathological and experimental evidence demonstrates presynaptic loss, dendritic spine remodeling, and excitatory/inhibitory imbalances in MND models and post-mortem tissues[24].

CAP2 is a postsynaptic actin-binding protein controlling the translocation of cofilin into dendritic spine in response to long-term potentiation, a process essential for actin-mediated spine remodeling[15,25]. In contrast, SNAP-25 is a presynaptic SNARE protein essential for vesicle docking and neurotransmitter release[26,27]. The distinct compartmentalization of these proteins may differentially influence their presence in CSF. It can be hypothesized that postsynaptic proteins such as CAP2 could be released in the context of dendritic spine remodeling or cytoskeletal stress, whereas SNAP-25 levels may more closely reflect alterations at the presynaptic terminal.

Synaptic pathology in MND is region- and stage-specific[28]. Post-mortem and experimental studies consistently have demonstrated early presynaptic protein loss in spinal cord and neuromuscular junction, including alterations in synaptophysin, synapsin, and SNAP-25[27]. On the other hand, cortical synaptic compartments often show preserved or even increased excitatory spine density during early disease stages[29]. Considering its prominent postsynaptic localization CAP2 may capture early cortical remodeling processes that are not tracked by presynaptic SNAP-25[30,31].

In this study, we show that CSF CAP2 levels are elevated in MND compared to HC and do not merely reflect neuronal degeneration, as indicated by the lack of correlation with NfL and the independent association with MND in logistic regression analyses. Of note, CAP2 levels were unrelated to the predominant involvement of upper versus lower motor neurons, clinical severity or survival, but, in MND patients only, were associated with p-Tau 181 and t-Tau pathology, both recognized features of the MND spectrum[19]. The significant correlation between CAP2 and tau biomarkers (p-Tau181 and t-Tau) observed in MND further supports a mechanistic link with cytoskeletal pathways. CAP2 and tau both contribute to actin–microtubule crosstalk, a process disrupted in MND and other neurodegenerative conditions[32,33]. In Alzheimer's disease, CAP2 elevation has been reported in early stages and shown to correlate with tau pathology independently of amyloid burden[23], suggesting a conserved pathophysiological association between tau dysregulation and actin-remodeling proteins across disorders. The disease-specific correlation between CAP2 and tau biomarkers in MND, absent in controls, points to selective engagement of disease-specific postsynaptic response. The independence of CAP2 from NfL and GFAP levels raises the possibility that CAP2 elevation reflects compensatory synaptic plasticity or maladaptive sprouting[9,34]. Such mechanisms may contribute to "motor reserve," buffering the clinical impact of neuronal loss, while presynaptic markers like SNAP-25 remain more closely tied to terminal degeneration[35,36].

In line with these findings, a multivariable logistic regression analysis showed that CAP2 was independently associated with MND status after adjustment for NfL. This result suggests that CAP2 captures disease-related information not fully explained by markers of axonal degeneration. The lack of association between CAP2 levels and clinical measures of disease severity, progression, or survival suggests that CAP2 may reflect synaptic integrity or adaptive synaptic responses rather than downstream neuronal degeneration. While this raises the possibility of a compensatory synaptic plasticity mechanism in MND, this interpretation remains speculative and requires confirmation through longitudinal and mechanistic studies.

Although our findings are promising, several limitations warrant discussion. First, the sample size, while adequate for initial analysis, limits stratification by MND subtype (including bulbar vs spinal onset subtypes of disease other than UMN vs LMN-predominant forms), which may differ in their synaptic vulnerability. Second, CAP2 was quantified using a commercial ELISA, and further standardization is necessary for broader clinical application. Third, we did not assess other synaptic markers, which would have allowed comparative profiling of pre- and postsynaptic components. For these reasons, an ongoing multicenter validation study also including preclinical monogenic cases is ongoing, in order to validate and further address the clinical and biological signature associated with CAP2 elevation in MND. Future studies will investigate the association with imaging biomarkers of either cortical atrophy or synaptic density (e.g., SV2A PET). Furthermore, exploring the

association between CAP2 and a broader panel of synaptic proteins beyond SNAP-25, including NPTX2 and neurogranin, may help clarify its mechanistic role in MND synaptic pathology[37,38].

An important future direction is the extension of CAP2 to blood-based measurements. Reliable detection of CAP2 in plasma or serum would enhance the translational and clinical relevance of this biomarker and provide a less invasive alternative to CSF sampling. However, synaptic proteins are challenging to assess in peripheral biofluids[39,40]. Ongoing efforts are therefore focused on developing sensitive and specific assays capable of detecting circulating CAP2 and capturing CSF-associated changes. Until such validation is achieved, the present findings should be considered CSF-specific, representing a limitation of the study.

In conclusion, this study seems to indicate that MND is accompanied by an ongoing synaptic rearrangement which is closely related to cytoskeletal proteins, such as CAP2 and tau. These findings support a broader conceptual framework in which synaptic dysfunction and compensation might play key roles in MND subtypes definition and may open avenues for targeted therapeutic strategies aimed at preserving synaptic integrity.

Data availability

The data are available from the corresponding author upon reasonable request.

Received: 23 October 2025; Accepted: 4 February 2026
Published online: 16 February 2026

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Acknowledgements

The authors wish to thank the study participants who took part in this research. We are very grateful for the assistance received from all the nurses of the Neurology department of the Spedali Civili of Brescia. We want to thank especially the laboratory technicians of the Central Clinical Laboratory for their amazing availability.

Author contributions

APa and APi contributed to the conceptualization and design of the study. APi and CT contributed to drafting the text or preparing the figures. AP, SP, CT, LF, LP, CT, IG, BL, EM contributed to the acquisition and analyses of data; APi, CT contributed to statistical analyses. AP, SP, CT, LF, LP, AC, BL, FG, LDA, RS, MdL EM and AP commented and revised the manuscript. All authors read and approved the final manuscript.

Funding

APi has been supported by grants of Airalzh Foundation AGYR2021 Life-Bio Grant, The LIMPE-DISMOV Foundation Segala Grant 2021, the Italian Ministry of University and Research PRIN COCOON (2017MYJ5TH), PRIN 2022PNJS5Z and PRIn PNRR (P20224ZHM9), DIGI-BRAIN the H2020 IMI IDEA-FAST (ID853981), Italian Ministry of Health, Grant/Award Number: RF-2018-12366209, PNRR-Health PNRR-MAD-2022-12376110 and PNRR- MCNT2-2023-12378387, The MJFF Foundation Grant 022343, #NEXTGENERATIONEU (NGEU) funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006)—a multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022)—subproject DIGI-BRAIN; the European Union. SP received funding from the *Piano di Sostegno alla Ricerca (PSR)*, *Università degli Studi di Milano* (PSR2023_SPELU; PSR2025_SPELU). AC receives fundings from the LifeArc foundation, the Karin Christiane Conradi Stiftungsfonds, the Deutsche Gesellschaft für Muskelkranke e.V. (DGM; German Society for Muscle Diseases; project Ca2/1), the Frick-Foundation for ALS research, the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation; projects CA 2915/4 – 1, CA 2915/10 – 1, and A01 within the SFB1506). MDL received funding from Italian Ministry of Research and University (MUR) (PRIN 20202THZAW and PRIN2022 PNRR P2022TKN8C, Fondo Italiano per la Scienza FIS00000560—Stone to MDL). EM received funding from Italian Ministry of Research and University (MUR) (PRIN202039WMFP, PRIN2022 PNRR P2022R2E8N to EM), from the Giovanni Armenise Harvard Foundation and AIRALZH ONLUS (2023 Armenise Harvard-AIRALZH Mid-Career Award in Neurodegenerative Diseases—AHA MCA). APa has been supported by grants of the Italian Ministry of University and Research PRIN 2021 RePlast (20202THZAW), Prin 2022 EGADi (P2022TKN8C) the H2020 IMI IDEA-FAST (ID853981), #NEXTGENERATIONEU (NGEU) funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006)—a multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022)—subproject DIGI-BRAIN Grant/Award Number: RF-2018-12366209, RF-2019-12369272 and PNRR-Health PNRR-MAD-2022-12376110, the Next Generation EU—NRRP M6C2—Investment 2.1 Enhancement and strengthening of biomedical research in the NHS- PNRR—PNRR PNRR-Health PNRR-MAD-2022-12376110.

Declarations

Competing interests

Andrea Pilotto received consultancy/speaker fees from Abbvie, Angelini, Bial, Eli Lilly, Lundbeck, Roche and Zambon pharmaceuticals. He acts as consultant as part of advisory Board of Angelini Pharma and BIAL pharmaceutics. Silvia Pelucchi declares no conflict of interest. Chiara Trasciatti declares no conflict of interest. Lucia Ferullo declares no conflict of interest. Loris Poli declares no conflict of interest. Chiara Tolassi declares no conflict of interest. Alberto Catanese declares no conflict of interest. Irene Girotto declares no conflict of interest. Beatrice Labella declares no conflict of interest. Federica Gorla declares no conflict of interest. Laura D'Andrea declares no conflict of interest Ramona Stringhi declares no conflict of interest Monica di Luca received advisory board fees from Roche Elena Marcello received speaker fees from Eli Lilly and GE Healthcare, advisory board fees from Roche, teaching fees from Eisai Alessandro Padovani received personal compensation as a consultant/scientific advisory board member for Biogen, Eisai Eli Lilly, General Healthcare (GE), Lundbeck, Nestlè, Roche.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-39274-0.

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