

## RESEARCH ARTICLE

# Endo-lysosomal protein concentrations in CSF from patients with frontotemporal dementia caused by *CHMP2B* mutation

Anders Toft<sup>1</sup> | Simon Sjödin<sup>2</sup> | Anja Hviid Simonsen<sup>1</sup> | Patrick Ejlerskov<sup>1</sup> |  
 Peter Roos<sup>1</sup> | Christian Sandøe Musaeus<sup>1</sup> | Emil Elbæk Henriksen<sup>1</sup> |  
 Troels Tolstrup Nielsen<sup>1</sup> | Ann Brinkmalm<sup>3</sup> | Kaj Blennow<sup>3,4</sup> |  
 Henrik Zetterberg<sup>3,4,5,6,7</sup> | Jørgen Erik Nielsen<sup>1</sup>

<sup>1</sup>Neurogenetics Clinic & Research Lab, Danish Dementia Research Centre, Rigshospitalet, Copenhagen, Denmark

<sup>2</sup>Laboratory of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden

<sup>3</sup>Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

<sup>4</sup>Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

<sup>5</sup>Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK

<sup>6</sup>UK Dementia Research Institute at UCL, London, UK

<sup>7</sup>Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China

## Correspondence

Anders Toft, MD, Neurogenetics Clinic & Research Lab, Danish Dementia Research Centre, Rigshospitalet, Section 8008, Inge Lehmanns vej 8, 2100 Copenhagen, Denmark.  
 Email: [anders.toft.01@regionh.dk](mailto:anders.toft.01@regionh.dk)

## Funding information

Swedish Research Council, Grant/Award Numbers: #2017-00915, 2018-02532; the Alzheimer Drug Discovery Foundation (ADDF), Grant/Award Number: #RDAPB-201809-2016615; the Swedish Alzheimer Foundation, Grant/Award Numbers: #AF-930351, #AF-939721, #AF-968270; Hjärnfonden, Grant/Award Numbers: #FO2017-0243, #ALZ2022-0006; ALF-agreement, Grant/Award Numbers: #ALFGBG-715986, #ALFGBG-965240; the European Union Joint Program for Neurodegenerative Disorders, Grant/Award Number: JPND2019-466-236; the National Institute of Health (NIH), Grant/Award Number: #1R01AG068398-01; the Alzheimer's Association 2021 Zenith Award, Grant/Award Number: ZEN-21-848495;

## Abstract

**Introduction:** Increasing evidence implicates proteostatic dysfunction as an early event in the development of frontotemporal dementia (FTD). This study aimed to explore potential cerebrospinal fluid (CSF) biomarkers associated with the proteolytic systems in genetic FTD caused by *CHMP2B* mutation.

**Methods:** Combining solid-phase extraction and parallel reaction monitoring mass spectrometry, a panel of 47 peptides derived from 20 proteins was analyzed in CSF from 31 members of the Danish *CHMP2B*-FTD family.

**Results:** Compared with family controls, mutation carriers had significantly higher levels of complement C9, lysozyme and transcobalamin II, and lower levels of ubiquitin, cathepsin B, and amyloid precursor protein.

**Discussion:** Lower CSF ubiquitin concentrations in *CHMP2B* mutation carriers indicate that ubiquitin levels relate to the specific disease pathology, rather than all-cause neurodegeneration. Increased lysozyme and complement proteins may indicate innate immune activation. Altered levels of amyloid precursor protein and cathepsins have previously been associated with impaired lysosomal proteolysis in FTD.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* published by Wiley Periodicals, LLC on behalf of Alzheimer's Association.

the European Research Council, Grant/Award Numbers: #681712, #101053962; Swedish State Support for Clinical Research, Grant/Award Number: #ALFGBG-71320; the Alzheimer Drug Discovery Foundation (ADDF), Grant/Award Number: #201809-2016862; AD Strategic Fund and the Alzheimer's Association, Grant/Award Numbers: #ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C; the Bluefield Project; the Olav Thon Foundation; the Erling-Persson Family Foundation; Stiftelsen för Gamla Tjänarinnor; he Marie Skłodowska-Curie, Grant/Award Number: 860197; the European Union Joint Programme – Neurodegenerative Disease Research, Grant/Award Number: JPN2021-00694; the UK Dementia Research Institute at UCL, Grant/Award Number: UKDRI-1003; Aase & Ejnar Danielsens Fond, Grant/Award Number: 19-10-0192; P.A. Messerschmidt & Hustrus Fond; Lægeforeningens Forskningsfond

## KEYWORDS

amyloid precursor protein, biomarkers, cathepsin B, cerebrospinal fluid, complement C9, frontotemporal dementia, lysozyme, proteomics, transcobalamin II, ubiquitin

## Highlights

- CSF markers of proteostasis were explored in *CHMP2B*-mediated frontotemporal dementia (FTD).
- 31 members of the Danish *CHMP2B*-FTD family were included.
- We used solid-phase extraction and parallel reaction monitoring mass spectrometry.
- Six protein levels were significantly altered in *CHMP2B*-FTD compared with controls.
- Lower CSF ubiquitin levels in patients suggest association with disease mechanisms.

## 1 | INTRODUCTION

Frontotemporal dementia (FTD) comprises a group of early-onset dementia syndromes characterized by high heritability. Pathogenic variants in a dozen genes have been demonstrated, of which the *C9orf72*, *GRN*, and *MAPT* genes are implicated in more than half of familial cases. A point mutation in the *CHMP2B* gene (*c.532-1G > C*) has been identified as a cause of autosomal dominant dementia in a Danish family with behavioral variant FTD.<sup>1</sup> The *CHMP2B* protein is a core component of the heteromeric 'endosomal sorting complex required for transport III' (ESCRT-III). This protein complex is involved in biogenesis of endocytic multivesicular bodies (MVBs), membrane repair, and regulation of autophagy.<sup>2,3</sup> Most well-described is the role of ESCRT-III in the endo-lysosomal sorting of ubiquitinated cargo: The protein complex binds transiently to endosomal surfaces and is responsible for the scission of membranes budding into the MVB compartment during the formation of intraluminal vesicles. The mutation renders *CHMP2B* and thereby the ESCRT-III unable to dissociate from its binding partners and disassemble from endosomal membranes, where the protein accumulates.<sup>4,5</sup> The result is defective trafficking of endosomes and impaired fusion of endosomes and autophagosomes with lysosomes.<sup>3,6</sup> Correspondingly, enlarged endosomes have been observed in *CHMP2B*-FTD patient brain tissue and fibroblasts, as well as animal and cell models expressing the *CHMP2B* mutation.<sup>3,4,6-9</sup> It has not been fully established whether the organelle malformation itself or mechanisms downstream of it are driving neurodegeneration.<sup>7,8</sup> However, the mutations that cause FTD converge on defects in the autophagy-lysosomal and ubiquitin-proteasomal systems.<sup>10</sup> Disturbed proteostasis particularly affects neurons, presumably owing to their specialized morphology and post-mitotic longevity. A distinct neuronal histopathology has been described in post-mortem tissue from *CHMP2B*-FTD patients, namely the presence of cytoplasmic

ubiquitin and p62 inclusions (FTLD-UPS), in the absence of tau, TDP-43 or FUS deposits.<sup>11,12</sup> This hallmark has been recapitulated in three independent, transgenic mouse models expressing mutant *CHMP2B*.<sup>13-16</sup> Interestingly, reactive gliosis was present in all *CHMP2B*-mice, and one study showed that the endo-lysosomal defects are present in both microglia and neurons,<sup>14,17</sup> indicating involvement of the innate immune system – another common feature of FTD subtypes.

At present, there are no disease-modifying treatment options and no disease-specific biomarkers for FTD that can inform clinicians about expected disease onset, rate of progression, timing of potential treatment, or monitoring of the response to pharmacological intervention. Collection of cerebrospinal fluid (CSF) is essential for establishing such biomarkers. Peptides that reflect brain pathology can reach the CSF as a consequence of neurodegeneration, through secretion of soluble proteins or secretory vesicles, or from the blood across the blood-brain barrier (BBB).<sup>18</sup> We have recently shown that a marker of axonal degeneration, neurofilament light (NfL), starts to increase in CSF and serum several years before debut of clinical *CHMP2B*-FTD.<sup>19,20</sup> Genetic disorders provide ideal settings for investigating this preclinical stage, and the Danish *CHMP2B*-FTD family offers a genetically homogeneous cohort for investigating the underlying pathology in a familial FTD subtype. The *CHMP2B* animal and cell models have shown that endo-lysosomal components such as certain cathepsins, LAMPs and Rab proteins are affected by *CHMP2B* mutation,<sup>4,7,8,14,21</sup> but the mediators of proteostasis have not yet been assessed in CSF from *CHMP2B*-FTD patients. To this end, we quantified a panel of CSF peptides in patients and controls. The targeted mass spectrometry panel was identified in an explorative proteomics study as tryptic peptides of proteins associated with endocytosis, lysosomal function, and the ubiquitin-proteasome system, that could be reliably quantified using solid-phase extraction and parallel reaction monitoring mass spectrometry.<sup>23</sup>

## 2 | METHODS

### 2.1 | Participants

This cross-sectional study included 31 members of the Danish *CHMP2B*-FTD family: 11 symptomatic and 7 presymptomatic *CHMP2B* mutation carriers and 13 non-carrier controls. Patients were diagnosed at the Danish Dementia Research Centre, Rigshospitalet, Denmark, using international consensus criteria for behavioral variant FTD.<sup>24</sup> Members of the *CHMP2B*-FTD family are continuously offered pre-clinical genetic testing and genetic counseling. If consented to, serum and CSF are obtained from each family member for research purposes, without disclosing the genetic status, if so desired by the individual. Family members who do not carry the *CHMP2B* mutation and have no neurological or cognitive deficits on examination serve as the control group. Addenbrooke's Cognitive Examination (ACE) was employed as a measure of cognitive disease burden. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-1-2012-041).

### 2.2 | CSF acquisition

Lumbar punctures were performed according to standard protocol. CSF samples were analyzed in clinical routine at the Department of Clinical Biochemistry, Rigshospitalet. Samples intended for PRM-MS were centrifuged at 2000 g for 10 min, and aliquots of 250  $\mu$ L were immediately stored at  $-80^{\circ}\text{C}$  in the Danish Dementia Biobank, pending biochemical assay at the Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy, University of Gothenburg.

### 2.3 | Pre-analysis sample preparation

The methods have previously been described in detail, see Brinkmalm et al.,<sup>22</sup> Sjödin et al.,<sup>23</sup> and Fernström et al.<sup>25</sup> In short, 100  $\mu$ L CSF was mixed with 25  $\mu$ L internal standard (IS) containing heavy isotope-labeled peptides (C-term  $^{13}\text{C}/^{15}\text{N}$ -labeled K and L, and cysteine carbamidomethylation) corresponding to all peptides included in the analysis (JPT Peptide Technologies and Thermo Fisher Scientific) as well as uniformly  $^{13}\text{C}$ -labeled ubiquitin (Silantes). The samples were reduced and alkylated by the addition of 25  $\mu$ L 30 mM 1,4-dithiothreitol (in 50 mM  $\text{NH}_4\text{HCO}_3$ ; shaking incubation at  $+60^{\circ}\text{C}$  for 30 min and then 30 min at room temperature) and 25  $\mu$ L 70 mM iodoacetamide (in 50 mM  $\text{NH}_4\text{HCO}_3$ ; shaking at room temperature in the dark for 30 min), respectively. Digestion was performed by adding 25  $\mu$ L 0.08  $\mu\text{g}/\mu\text{L}$  sequencing grade modified trypsin (Promega Co.) diluted in 50 mM  $\text{NH}_4\text{HCO}_3$  followed by shaking incubation for 18 h at  $+37^{\circ}\text{C}$  which was ended by the addition of 25  $\mu$ L 10% trifluoroacetic acid. SPE was performed using standard procedure and Oasis HLB 96-well  $\mu$ Elution Plates (2 mg sorbent and 30  $\mu\text{m}$  particle size; Waters Co.). The plate was conditioned ( $2 \times 300 \mu\text{L}$  methanol) and equilibrated ( $2 \times 300 \mu\text{L}$   $\text{H}_2\text{O}$ ). The samples were loaded followed by

### RESEARCH IN CONTEXT

- 1. Systematic review:** PubMed searches were conducted to compare data from biomarker, animal, and cell model studies on impaired proteostasis in neurodegenerative diseases, with focus on frontotemporal dementia (FTD). Knowledge is lacking regarding which components of the endo-lysosomal and ubiquitin-proteasomal systems are affected, and whether such proteins might be utilized as biomarkers.
- 2. Interpretation:** This is the first study to assess CSF levels of proteostatic markers in a genetically homogenous FTD cohort. From mass spectrometry data, we identified six significantly different protein levels when comparing *CHMP2B* mutation carriers with family controls. Particularly interesting was the finding of lower CSF ubiquitin in *CHMP2B*-FTD patients, challenging the view that ubiquitin levels solely reflect neurodegeneration.
- 3. Future directions:** The proposed markers should be included in upcoming studies on fluid biomarkers in familial FTD to confirm the alterations. For certain peptides, for example, ubiquitin, quantification of full-length protein in CSF from *CHMP2B*-FTD patients is relevant.

a wash ( $2 \times 300 \mu\text{L}$   $\text{H}_2\text{O}$ ) and were finally eluted ( $2 \times 100 \mu\text{L}$  methanol). The samples were dried by vacuum centrifugation and frozen at  $-80^{\circ}\text{C}$  pending analysis.

### 2.4 | PRM-MS

The target panel of interest included 47 peptides derived from 20 proteins. Table S1 shows all peptide measurements and protein estimates. Frozen and dried samples were dissolved, and injected and separated using a Dionex UltiMate 3000 standard-LC system (Thermo Fisher Scientific) in reversed phase on a Hypersil GOLD HPLC C18 column (length 200 mm; inner diameter 2.1 mm; particle size 1.9  $\mu\text{m}$ ; Thermo Fisher Scientific). Analysis was performed on a hybrid Q Exactive mass spectrometer (Thermo Fisher Scientific) using electrospray ionization and a HESI-II ionization probe (Thermo Fisher Scientific). The instrument operated in positive ionization mode and data acquisition was performed using a scheduled PRM method with a retention time isolation window of 2 min, an isolation window of  $m/z$  3, a resolution setting of 70k, an automatic gain control target of  $1 \times 10^6$  and a maximum injection time of 300 ms.

Peak detection and area integration were performed using Skyline v3.6,<sup>26</sup> targeting  $[\text{M} + \text{H}]^{1+}$  y-ions from a data-independent acquisition method with a fixed isolation window of  $m/z$  3 and an orbitrap analyzer resolution setting of 70k at  $m/z$  200. The ratio of the sum of the product ion areas of tryptic to isotope-labeled peptide was used for quantification.

**TABLE 1** Patient characteristics.

	Presymptomatic <i>CHMP2B</i> mutation carriers	Symptomatic <i>CHMP2B</i> mutation carriers	Healthy family members	<i>p</i> -value
No. of participants	7	11	13	0.405
Sex, females/males	4/3	3/8	5/8	0.511
Mean age, years (range)	52.0 (32.7–68.1)	62.9 (53.6–73.3)	60.5 (38.1–71.1)	0.060
Mean ACE score (range)	92 (88–95)	74.9 (60–89)	93.3 (87–100)	<0.001

## 2.5 | Immunoprecipitation and PRM-MS

SNAP-25/synaptotagmin-1 were quantified with an in-house assay consisting of enrichment with immunoprecipitation (mouse monoclonal antibodies clone 41.1 (Synaptic Systems) and SMI81 (Biosite)) followed by quantitation with liquid chromatography/tandem mass spectrometry (LC-MS/MS, see section above). The instrument was set to acquire scheduled pairs or triplets of fragmentation scans (PRM scans) in profile mode, allowing simultaneous detection of the CSF peptide and the corresponding IS. LC-MS/MS raw files acquired with Xcalibur software version 2.2 SP1.48 (Thermo Fisher Scientific) were imported into Pinpoint software version 1.3.0 (Thermo Fisher Scientific), and peak areas of the CSF and IS peptides were generated. CSF levels of SNAP-25tot, and synaptotagmin-1 were calculated by multiplying the ratio of the LC-MS peak areas with the concentration of the corresponding IS.

## 2.6 | Statistics

Statistical analyses were carried out using R statistical software, version 1.2.1335. Normal distribution of peptide values was checked with QQ-plots, histograms, and Shapiro-Wilk normality tests. Non-normally distributed data were logarithmically transformed. Comparisons of mean protein or peptide levels in the clinical groups were calculated with ANOVA and post-hoc comparisons were conducted using Tukey test with single-step adjustment as implemented in the *multcomp* toolbox.<sup>27</sup> Since there was a tendency towards significantly different ages in the clinical groups, age was included as a covariate in these comparisons. Correlations between continuous variables were assessed using the Spearman's Rank correlation coefficient. Descriptive statistics were carried out with Fisher's exact test or one-way ANOVA with post-hoc Tukey test. *p*-values below 0.05 were considered significant.

## 3 | RESULTS

### 3.1 | Patient characteristics

Participants' demographics and ACE scores are presented in Table 1. Female-to-male ratios did not significantly differ between groups. Mean age differed between symptomatic and presymptomatic patients by 10.8 years ( $p = 0.055$ , Tukey test). As expected, ACE scores were

significantly lower in symptomatic participants compared with both presymptomatic and healthy participants.

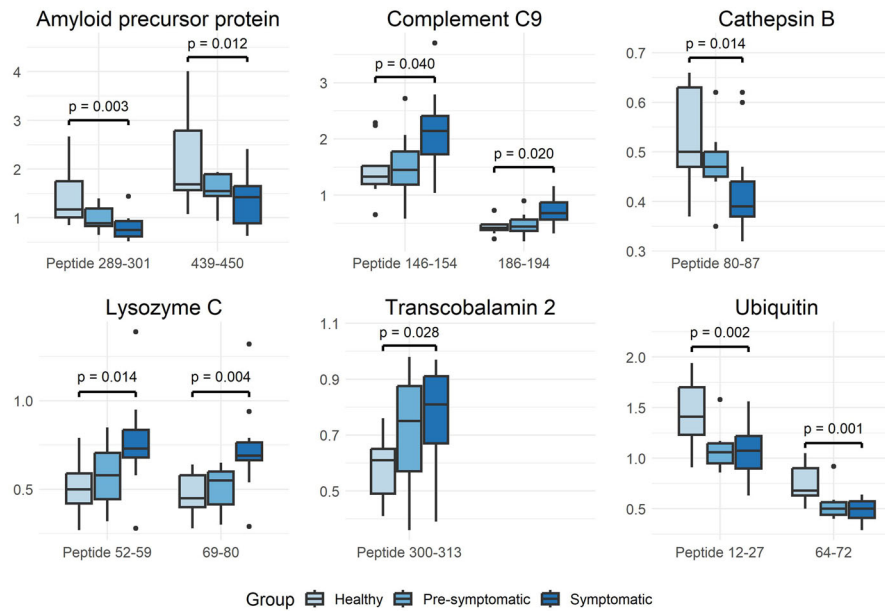
### 3.2 | CSF protein concentrations

Comparing *CHMP2B* mutation carriers with controls revealed significantly increased protein levels of complement C9, lysozyme C (lysozyme), and transcobalamin II (TCN2) in mutations carriers, and significantly lower levels of ubiquitin, amyloid precursor protein (APP) and cathepsin B (CTSB). No significant differences were detected for AP2B1, CTSD, CTSF, CTSL, CTSZ, DPP7, GM2A, HEXB, LAMP1, LAMP2, FUCA1, TPP1, SYT1, or SNAP25 (Table S2). The six protein estimates significantly associated with *CHMP2B* mutation were based on the levels of 14 peptides in total. Peptide pairs and triplets originating from the same protein were highly correlated, except the combination of the pro-peptide CTSB<sub>58-71</sub> and the light chain peptide CTSB<sub>80-87</sub> ( $R_s = 0.38$ ,  $p = 0.062$ ), which were both highly correlated with the heavy chain peptide, CTSB<sub>210-220</sub> ( $R_s = 0.73$ – $0.77$ ,  $p < 0.0001$ ). When dividing the *CHMP2B* mutation carriers into symptomatic and presymptomatic participants, the levels of 10 of the 14 peptides were significantly different among the three clinical groups (Figure 1, Table S2).

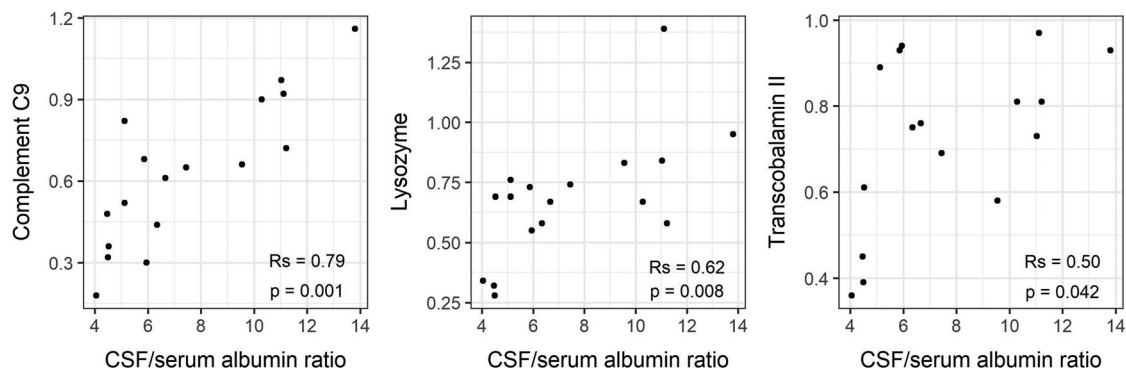
We have recently found an increased level of NfL in CSF from both symptomatic and presymptomatic *CHMP2B* mutation carriers, as well as an increased CSF/serum albumin ratio in symptomatic *CHMP2B*-FTD patients<sup>19,20</sup> – the former being a well-established marker of axonal degeneration, the latter reflecting potential compromise of BBB integrity. In the current study, none of the 10 peptides were significantly correlated with CSF NfL levels in *CHMP2B* mutation carriers (Table S2). The complement C9, lysozyme, and TCN2 peptides correlated significantly with the CSF/serum albumin ratios, as shown in Figure 2. The complement C9 peptides and one of two lysozyme peptides were significantly correlated with age (Table S2). Notably, CSF/serum albumin ratios did not correlate with age in the mutation carrier group ( $R_s = 0.11$ ,  $p = 0.670$ ). No correlations between peptide levels and ACE-scores were observed in mutation carriers (Table S2).

## 4 | DISCUSSION

Increasing evidence implicates proteostatic dysfunction as an early event in the development of neurodegenerative diseases, including



**FIGURE 1** Significantly different CSF peptide levels between the three clinical groups: Symptomatic *CHMP2B* mutation carriers, presymptomatic carriers, and healthy family controls. Y-axis values represent a ratio between tryptic peptide and added internal standard. Calculations were performed using ANOVA with post-hoc Tukey multiple comparisons of means test.



**FIGURE 2** Correlations between CSF/serum albumin ratios and complement C9 (peptide 186–194), lysozyme (peptide 52–59), and transcobalamin II (peptide 300–313) in *CHMP2B* mutation carriers. Y-axis values represent a ratio between tryptic peptide and added internal standard. Calculations were performed using Spearman's correlation. Rs, Spearman's rank correlation coefficient.

FTD and *CHMP2B*-FTD specifically. To explore potential mediators of impaired proteostasis in *CHMP2B*-FTD, this study investigated CSF levels of a panel of peptides functionally involved in endo-lysosomal or ubiquitin-proteasome function. A total of 47 peptides originating from 20 proteins were analyzed and their concentrations compared among 31 members of the Danish *CHMP2B*-FTD family. PRM-MS revealed significantly higher levels of complement C9, lysozyme, and TCN2 in *CHMP2B* mutation carriers compared with controls, and significantly lower levels of ubiquitin, APP and CTSB (Figure 1).

Neuronal inclusions containing ubiquitin and the ubiquitin-binding autophagy receptor p62 remain the only immunohistochemical hallmarks of *CHMP2B*-FTD postmortem brain tissue.<sup>11,12</sup> How the cerebral deposition of ubiquitin is reflected in CSF, has not been investigated. Here, both presymptomatic carriers and symptomatic *CHMP2B*-

FTD patients had significantly lower ubiquitin levels than controls. Two studies have previously included measurements of CSF ubiquitin in FTD patients.<sup>28,29</sup> Both found the concentration of ubiquitin to be at the same level as healthy controls. Patients with AD and Creutzfeldt–Jakob disease (CJD) showed significantly increased levels of CSF ubiquitin, demonstrated across several studies.<sup>29–34</sup> Elevated CSF ubiquitin in AD and particularly in CJD has been linked to the extent of neuroaxonal damage.<sup>28–31</sup> This is supported by correlations between CSF ubiquitin and established markers of neurodegeneration, that is, NfL and total-tau,<sup>28,29,35</sup> as well as observed rise in CSF ubiquitin following traumatic brain injury.<sup>36</sup> However, seemingly unaltered CSF levels in other neurodegenerative disorders such as PD indicate that ubiquitin is not exclusively a biomarker of nonspecific neurodegeneration,<sup>29,33,35,37</sup> but instead suggests some degree

of association with disease mechanisms or affected brain region. Of note, the studies that have analyzed CSF ubiquitin levels in behavioral type FTD did not distinguish between genetic subtypes, which may differ in terms of ubiquitin dynamics, as it might be the case with NfL.<sup>20</sup>

A possible explanation for the lower CSF ubiquitin levels in *CHMP2B* mutation carriers lies in the impairment of the ESCRT machinery, responsible for internalizing ubiquitinated proteins into MVBs, where they are sorted for either lysosomal degradation with accompanying release of ubiquitin or trafficking back to the plasma membrane. We speculate that the accumulated MVBs observed in *CHMP2B* models are maintaining an intracellular reservoir of ubiquitin, not reaching the CSF. One might expect that such neuronal ubiquitin deposits would increase CSF levels as neurodegeneration progresses, similar to levels of NfL in *CHMP2B*-FTD.<sup>19</sup> In this regard, an apparent spatial mismatch between the frontotemporal cortical neurodegeneration and the neuronal ubiquitin inclusions – which are predominantly found in the hippocampus<sup>11</sup> – might partly explain the seemingly non-parallel courses of CSF ubiquitin and NfL in *CHMP2B*-FTD. Another contributing possibility is compromised ESCRT-dependent formation of exosomes, potentially leading to fewer ubiquitinated proteins being transported to the extracellular space. However, silencing one of the direct binding partners of *CHMP2B*, *VPS4B*, in HeLa cells has previously been shown to increase overall exosome secretion, not reduce it.<sup>38</sup> If validated, decreased CSF ubiquitin levels may distinguish *CHMP2B*-FTD or FTD collectively from other neurodegenerative disease entities, as it has only been found significantly reduced in one other neurodegenerative disease cohort.<sup>23</sup>

CSF levels of complement C9 and lysozyme were increased in *CHMP2B*-FTD patients compared with controls. The complement proteins constitute part of the innate immune system and are produced, in the CNS, by neurons and glial cells.<sup>39</sup> They mediate proinflammatory stimulation and chemoattraction between these cell types, as well as clearance of pathogens and damaged cells via the membrane attack complex (MAC), and tagging of synapses for phagocytosis by microglia.<sup>39</sup> Lui et al. have demonstrated that lysosomal defects and complement activation in microglia can lead to neurodegeneration through excessive synaptic pruning in a mouse model of FTD caused by *GRN* mutation.<sup>40</sup> Astrocytes generated from induced pluripotent stem cells from *CHMP2B*-FTD patients have been shown to be more reactive and display increased production of complement C3, which was confirmed in the brains of mice expressing mutant *CHMP2B*.<sup>41</sup> Complement C9, the terminal constituent of the complement cascade and major part of the cytolytic MAC, has not previously been investigated in CSF or in vitro models in FTD, but its elevation in CSF indicates involvement of innate immune function. This is supported by increased levels of CSF lysozyme – a glycan cleaving polypeptide produced by microglia.<sup>42</sup> Findings of higher CSF lysozyme in AD patients than controls have been inconsistent,<sup>22,23,43,44</sup> although a protective effect of the protein has been proposed based on rescue experiments in *Drosophila* models.<sup>43,44</sup> To our knowledge, the role or quantities of lysozyme in FTD have not previously been studied. Importantly, both complement C9 and lysozyme were associated with the CSF/serum

albumin ratio (Figure 2).<sup>20</sup> Correlations between soluble MAC components and BBB dysfunction is known from patients with traumatic brain injury,<sup>45</sup> and the positive correlation between complement C9 and age has previously been observed in AD.<sup>46</sup> Earlier studies have reported increased levels of CSF lysozyme as part of an inflammatory reaction to a variety of CNS pathologies.<sup>47</sup> Passage of lysozyme from blood through an impaired BBB as well as local production by microglia within the CNS, have been suggested as the underlying mechanisms. Thus, it is plausible that complement C9 and lysozyme emanate from serum and contribute to the measured CSF amounts in *CHMP2B*-FTD patients. In relation to this, we have previously observed a systemic immune response in *CHMP2B*-FTD patients.<sup>48</sup> Innate immune activation is also supported by results from transgenic *CHMP2B Drosophila* models<sup>49–51</sup> and *CHMP2B* mouse models, which have all exhibited reactive gliosis.<sup>13–16</sup> One such mouse model revealed that accumulation of endo-lysosomal byproducts was present in microglia as well as neurons.<sup>14</sup> A follow-up study showed an early microglial activation in the transgenic mice, progressing into overt microgliosis with a proinflammatory phenotype, neuronal loss, and behavioral changes.<sup>17</sup> In addition, the authors detected increased levels of lysosomal cathepsin D and LAMP-1,<sup>14</sup> which has also been reported in *Grn* knockout mice and patients with *GRN*-FTD.<sup>52</sup>

In the present study, CSF concentrations of these lysosomal proteins were unchanged, whereas *CTSB* was significantly reduced in *CHMP2B*-FTD patients. Several studies have demonstrated increased CSF levels of *CTSB* in AD, traumatic brain injury, inflammatory neurological diseases, and aging.<sup>23,53,54</sup> Lysosomal leakage of *CTSB* with ensuing unchecked proteolytic activity in the cytosol has been suggested as the deleterious mechanism.<sup>53,54</sup> Since ESCRT-III is involved in endo-lysosomal membrane repair, one might expect *CHMP2B* mutation to increase the concentration of *CTSB* in CSF, rather than reduce it in *CHMP2B*-FTD patients, which we observe. The potential reduction in *CTSB* requires further exploration.

In this study, the quantity of APP was significantly lower in CSF from *CHMP2B*-FTD patients compared with controls. The transmembrane APP protein functions as a cell surface receptor on neurons, involved in neurite growth and synaptogenesis.<sup>55</sup> It is cleaved by secretases into smaller fragments relating to the amyloidogenic or non-amyloidogenic pathways.<sup>55</sup> In AD, the CSF concentrations of soluble APP did not differ between patients and controls in a recent meta-analysis,<sup>56</sup> neither did the levels of the major isoforms of APP in studies using targeted proteomics.<sup>22,23</sup> In FTD, albeit less well studied, findings point to lower levels of soluble APP in patients with different types of FTLD.<sup>57–60</sup> Inside neurons, APP is ubiquitinated and targeted to the intraluminal vesicles of MVBs through interaction with the ESCRT machinery.<sup>61</sup> The internalization of APP and delivery to lysosomes has been shown to parallel that of the epidermal growth factor receptor (EGFR),<sup>61</sup> the degradation of which is delayed in *CHMP2B* mutation models<sup>3,62</sup> – supposedly due to entrapment of EGFR in mutant *CHMP2B* positive compartments.<sup>3</sup> Thus, as described for ubiquitin, APP may be retained in the MVB compartment, reducing the proportion of APP available for cleavage into soluble forms at the plasma membrane, lowering the CSF concentration in *CHMP2B*-FTD patients.

Lastly, TCN2 was significantly elevated in the patient group. It is responsible for transporting vitamin B12 into tissues via its receptor (CD320), where it is degraded in lysosomes, while dissociated B12 is exported into the cytosol.<sup>63</sup> It is unknown whether the ESCRT system is involved in this process, but ubiquitination of CD320 does not appear to occur.<sup>63</sup> A strong correlation between TCN2 levels in plasma and CSF has been demonstrated elsewhere,<sup>64</sup> and it is likely that the bulk of TCN2 in CSF originates from serum. In line with this, TCN2 was significantly associated with the CSF/albumin ratio in our study.

Research into mediators of disrupted proteostasis has been limited in FTD, particularly for genetic subtypes. This study has screened a panel of potential biomarkers associated with endo-lysosomal function in CHMP2B-FTD. Of note, the proteins investigated are multifunctional and hence significantly different CSF levels do not necessarily equate to significant differences in cellular proteostasis. Although the number of statistical comparisons has been attempted to be kept at a minimum, the rarity of the disorder means that the size of our cohort is modest in relation to the number of analytes tested, increasing the risk of false positives. However, the cohort is pathogenetically homogenous, which is necessary for disease entities with such diverse genetic backgrounds as FTD. The findings require validation in an independent cohort and for certain peptides, for example, ubiquitin, further quantification of the full-length protein in CSF from CHMP2B-FTD patients will be relevant. The ubiquitin peptides generated by our enzymatic digestion approach can be derived from both free and conjugated forms of the protein – thus, we cannot assess if intact, free ubiquitin is decreased in CHMP2B-FTD. In AD, however, previous studies have shown that both free and conjugated ubiquitin levels are increased.<sup>65</sup> Furthermore, a recent study employing the same technique as herein could confirm that the levels of these particular ubiquitin peptides are increased in CSF from AD patients.<sup>23</sup> Taken together, the data support different concentrations of CSF ubiquitin in AD, FTD, and healthy controls. The lack of correlation between the studied peptides and CSF NfL suggests that the altered protein levels in mutation carriers are not secondary to axonal neurodegeneration, as expressed by CSF NfL levels, but perhaps represent another part of the disease mechanism.

## ACKNOWLEDGMENTS

K.B. is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721, and #AF-968270), Hjärfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the National Institute of Health (NIH), USA, (grant #1R01AG068398-01), and the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495). H.Z. is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712 and #101053962), Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-

2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, and #ADSF-21-831377-C), the Bluefield Project, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003), Aase & Ejnar Danielsens Fond (19-10-0192), P.A. Messerschmidt & Hustrus Fond, Lægeföreningens Forskningsfond.

## CONFLICT OF INTEREST STATEMENT

K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, and JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). The remaining authors (A.T., S.S., A.H.S., P.E., P.R., C.S.M., E.E.H., T.T.N., A.B., and J.E.N.) have no conflicts of interest to declare. Author disclosures are available in the [supporting information](#).

## REFERENCES

- Roos P, Johannsen P, Lindquist SG, et al. Six generations of CHMP2B-mediated frontotemporal dementia: clinical features, predictive testing, progression, and survival. *Acta Neurol Scand.* 2022;145(5):529-540. doi:10.1111/ane.13578
- Urwin H, Ghazi-Noori S, Collinge J, Isaacs A. The role of CHMP2B in frontotemporal dementia. *Biochem Soc Trans.* 2009;37(Pt 1):208-212. doi:10.1042/bst0370208
- Urwin H, Authier A, Nielsen JE, et al. Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. *Hum Mol Genet.* 2010;19(11):2228-2238. doi:10.1093/hmg/ddq100
- Lee JA, Beigneux A, Ahmad ST, Young SG, Gao FB. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr Biol.* 2007;17(18):1561-1567. doi:10.1016/j.cub.2007.07.029
- Clayton EL, Milioto C, Muralidharan B, et al. Frontotemporal dementia causative CHMP2B impairs neuronal endolysosomal traffic-rescue by TMEM106B knockdown. *Brain.* 2018;141(12):3428-3442. doi:10.1093/brain/awy284
- Filimonenko M, Stuffers S, Raiborg C, et al. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. *J Cell Biol.* 2007;179(3):485-500. doi:10.1083/jcb.200702115
- Lee JA, Gao FB. Inhibition of autophagy induction delays neuronal cell loss caused by dysfunctional ESCRT-III in frontotemporal dementia. *J Neurosci.* 2009;29(26):8506-8511. doi:10.1523/jneurosci.0924-09.2009

8. West RJH, Ugbo C, Fort-Aznar L, Sweeney ST. Neuroprotective activity of ursodeoxycholic acid in CHMP2B(Intron5) models of frontotemporal dementia. *Neurobiol Dis.* 2020;144:105047. doi:10.1016/j.nbd.2020.105047
9. Zhang Y, Schmid B, Nikolaisen NK, et al. Patient iPSC-derived neurons for disease modeling of frontotemporal dementia with mutation in CHMP2B. *Stem Cell Reports.* 2017;8(3):648-658. doi:10.1016/j.stemcr.2017.01.012
10. Root J, Merino P, Nuckols A, Johnson M, Kukar T, Lysosome dysfunction as a cause of neurodegenerative diseases: lessons from frontotemporal dementia and amyotrophic lateral sclerosis. *Neurobiol Dis.* 2021;154:105360. doi:10.1016/j.nbd.2021.105360
11. Holm IE, Englund E, Mackenzie IR, Johannsen P, Isaacs AM. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. *J Neuropathol Exp Neurol.* 2007;66(10):884-891. doi:10.1097/nen.0b013e3181567f02
12. Holm IE, Isaacs AM, Mackenzie IR. Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the CHMP2B gene. *Acta Neuropathol.* 2009;118(5):719-720. doi:10.1007/s00401-009-0593-1
13. Ghazi-Noori S, Froud KE, Mizielinska S, et al. Progressive neuronal inclusion formation and axonal degeneration in CHMP2B mutant transgenic mice. *Brain.* 2012;135(Pt 3):819-832. doi:10.1093/brain/aww006
14. Clayton EL, Mizielinska S, Edgar JR, et al. Frontotemporal dementia caused by CHMP2B mutation is characterised by neuronal lysosomal storage pathology. *Acta Neuropathol.* 2015;130(4):511-523. doi:10.1007/s00401-015-1475-3
15. Vernay A, Therreau L, Blot B, et al. A transgenic mouse expressing CHMP2Bintron5 mutant in neurons develops histological and behavioural features of amyotrophic lateral sclerosis and frontotemporal dementia. *Hum Mol Genet.* 2016;25(15):3341-3360. doi:10.1093/hmg/ddw182
16. Gascon E, Lynch K, Ruan H, et al. Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat Med.* 2014;20(12):1444-1451. doi:10.1038/nm.3717
17. Clayton EL, Mancuso R, Nielsen TT, et al. Early microglial precedes neuronal loss and behavioural impairment in mice with a frontotemporal dementia-causing CHMP2B mutation. *Hum Mol Genet.* 2017;26(5):873-887. doi:10.1093/hmg/ddx003
18. Zlokovic BV, The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron.* 2008;57(2):178-201. doi:10.1016/j.neuron.2008.01.003
19. Rostgaard N, Roos P, Portelius E, et al. CSF neurofilament light concentration is increased in presymptomatic CHMP2B mutation carriers. *Neurology.* 2018;90(2):e157-e163. doi:10.1212/wnl.0000000000004799
20. Toft A, Roos P, Jääskeläinen O, et al. Serum neurofilament light in patients with frontotemporal dementia caused by CHMP2B mutation. *Dement Geriatr Cogn Disord.* 2020;49(6):533-538. doi:10.1159/000513877
21. Skibinski G, Parkinson NJ, Brown JM, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet.* 2005;37(8):806-808. doi:10.1038/ng1609
22. Brinkmalm G, Sjödin S, Simonsen AH, et al. A parallel reaction monitoring mass spectrometric method for analysis of potential CSF biomarkers for Alzheimer's disease. *Proteomics Clin Appl.* 2018;12(1). doi:10.1002/prca.201700131
23. Sjödin S, Brinkmalm G, Öhrfelt A, et al. Endo-lysosomal proteins and ubiquitin CSF concentrations in Alzheimer's and Parkinson's disease. *Alzheimers Res Ther.* 2019;11(1):82. doi:10.1186/s13195-019-0533-9
24. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain.* 2011;134(Pt 9):2456-2477. doi:10.1093/brain/awr179
25. Fernström E, Minta K, Andreasson U, et al. Cerebrospinal fluid markers of extracellular matrix remodelling, synaptic plasticity and neuroinflammation before and after cranial radiotherapy. *J Intern Med.* 2018;284(2):211-225. doi:10.1111/joim.12763
26. MacLean B, Tomazela DM, Shulman N, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics.* 2010;26(7):966-968. doi:10.1093/bioinformatics/btq054
27. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J.* 2008;50(3):346-363. doi:10.1002/bimj.200810425
28. Abu-Rumeileh S, Oeckl P, Baiardi S, et al. CSF ubiquitin levels are higher in Alzheimer's disease than in frontotemporal dementia and reflect the molecular subtype in prion disease. *Biomolecules.* 2020;10(4):497. doi:10.3390/biom10040497
29. Oeckl P, Steinacker P, von Arnim CA, et al. Intact protein analysis of ubiquitin in cerebrospinal fluid by multiple reaction monitoring reveals differences in Alzheimer's disease and frontotemporal lobar degeneration. *J Proteome Res.* 2014;13(11):4518-4525. doi:10.1021/pr5006058
30. Blennow K, Davidsson P, Wallin A, Gottfries CG, Svennerholm L. Ubiquitin in cerebrospinal fluid in Alzheimer's disease and vascular dementia. *Int Psychogeriatr.* 1994;6(1):13-22; discussion 59-60. doi:10.1017/s1041610294001584
31. Kudo T, Iqbal K, Ravid R, Swaab DF, Grundke-Iqbal I. Alzheimer disease: correlation of cerebrospinal fluid and brain ubiquitin levels. *Brain Res.* 1994;639(1):1-7. doi:10.1016/0006-8993(94)91757-4
32. Kandimalla RJ, Anand R, Veeramanikandan R, et al. CSF ubiquitin as a specific biomarker in Alzheimer's disease. *Curr Alzheimer Res.* 2014;11(4):340-348. doi:10.2174/1567205011666140331161027
33. Heywood WE, Galimberti D, Bliss E, et al. Identification of novel CSF biomarkers for neurodegeneration and their validation by a high-throughput multiplexed targeted proteomic assay. *Mol Neurodegener.* 2015;10:64. doi:10.1186/s13024-015-0059-y
34. Steinacker P, Rist W, Swiatek-de-Lange M, et al. Ubiquitin as potential cerebrospinal fluid marker of Creutzfeldt-Jakob disease. *Proteomics.* 2010;10(1):81-89. doi:10.1002/pmic.200900246
35. Sjödin S, Hansson O, Öhrfelt A, et al. Mass spectrometric analysis of cerebrospinal fluid ubiquitin in Alzheimer's disease and parkinsonian disorders. *Proteomics Clin Appl.* 2017;11:11-12. doi:10.1002/prca.201700100
36. Majetschak M, King DR, Krehmeier U, et al. Ubiquitin immunoreactivity in cerebrospinal fluid after traumatic brain injury: clinical and experimental findings. *Crit Care Med.* 2005;33(7):1589-1594. doi:10.1097/01.ccm.0000169883.41245.23
37. Constantinescu R, Andreasson U, Li S, et al. Proteomic profiling of cerebrospinal fluid in parkinsonian disorders. *Parkinsonism Relat Disord.* 2010;16(8):545-549. doi:10.1016/j.parkreldis.2010.06.011
38. Colombo M, Moita C, van Niel G, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *J Cell Sci.* 2013;126(Pt 24):5553-5565. doi:10.1242/jcs.128868
39. Veerhuis R, Nielsen HM, Tenner AJ. Complement in the brain. *Mol Immunol.* 2011;48(14):1592-1603. doi:10.1016/j.molimm.2011.04.003
40. Lui H, Zhang J, Makinson SR, et al. Progranulin deficiency promotes circuit-specific synaptic pruning by microglia via complement activation. *Cell.* 2016;165(4):921-935. doi:10.1016/j.cell.2016.04.001
41. Chandrasekaran A, Dittlau KS, Corsi GI, et al. Astrocytic reactivity triggered by defective autophagy and metabolic failure causes neurotoxicity in frontotemporal dementia type 3. *Stem Cell Reports.* 2021;16(11):2736-2751. doi:10.1016/j.stemcr.2021.09.013
42. Ganz T. Antimicrobial polypeptides. *J Leukoc Biol.* 2004;75(1):34-38. doi:10.1189/jlb.0403150



43. Helmfors L, Boman A, Civitelli L, et al. Protective properties of lysozyme on  $\beta$ -amyloid pathology: implications for Alzheimer disease. *Neurobiol Dis*. 2015;83:122-133. doi:10.1016/j.nbd.2015.08.024
44. Sandin L, Bergkvist L, Nath S, et al. Beneficial effects of increased lysozyme levels in Alzheimer's disease modelled in *Drosophila melanogaster*. *Febs j*. 2016;283(19):3508-3522. doi:10.1111/febs.13830
45. Stahel PF, Morganti-Kossmann MC, Perez D, et al. Intrathecal levels of complement-derived soluble membrane attack complex (sC5b-9) correlate with blood-brain barrier dysfunction in patients with traumatic brain injury. *J Neurotrauma*. 2001;18(8):773-781. doi:10.1089/089771501316919139
46. Loeffler DA, Brickman CM, Juneau PL, Perry MF, Pomara N, Lewitt PA. Cerebrospinal fluid C3a increases with age, but does not increase further in Alzheimer's disease. *Neurobiol Aging*. 1997;18(5):555-557. doi:10.1016/s0197-4580(97)00110-3
47. Hansen NE, Karle H, Jensen A, Bock E. Lysozyme activity in cerebrospinal fluid. Studies in inflammatory and non-inflammatory CNS disorders. *Acta Neurol Scand*. 1977;55(5):418-424. doi:10.1111/j.1600-0404.1977.tb05660.x
48. Roos P, von Essen MR, Nielsen TT, et al. Inflammatory markers of CHMP2B-mediated frontotemporal dementia. *J Neuroimmunol*. 2018;324:136-142. doi:10.1016/j.jneuroim.2018.08.009
49. Ahmad ST, Sweeney ST, Lee JA, Sweeney NT, Gao FB. Genetic screen identifies serpin5 as a regulator of the toll pathway and CHMP2B toxicity associated with frontotemporal dementia. *Proc Natl Acad Sci U S A*. 2009;106(29):12168-12173. doi:10.1073/pnas.0903134106
50. West RJ, Lu Y, Marie B, Gao FB, Sweeney ST. Rab8, POSH, and TAK1 regulate synaptic growth in a *Drosophila* model of frontotemporal dementia. *J Cell Biol*. 2015;208(7):931-947. doi:10.1083/jcb.201404066
51. Lu Y, West RJH, Pons M, Sweeney ST, Gao FB, Ik2/TBK1 and Hook/Dynein, an adaptor complex for early endosome transport, are genetic modifiers of FTD-associated mutant CHMP2B toxicity in *Drosophila*. *Sci Rep*. 2020;10(1):14221. doi:10.1038/s41598-020-71097-5
52. Götzl JK, Mori K, Damme M, et al. Common pathobiochemical hallmarks of progranulin-associated frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis. *Acta Neuropathol*. 2014;127(6):845-860. doi:10.1007/s00401-014-1262-6
53. Hook V, Yoon M, Mosier C, et al. Cathepsin B in neurodegeneration of Alzheimer's disease, traumatic brain injury, and related brain disorders. *Biochim Biophys Acta Proteins Proteom*. 2020;1868(8):140428. doi:10.1016/j.bbapap.2020.140428
54. Hook G, Reinheckel T, Ni J, et al. Cathepsin B gene knockout improves behavioral deficits and reduces pathology in models of neurologic disorders. *Pharmacol Rev*. 2022;74(3):600-629. doi:10.1124/pharmrev.121.000527
55. O'Brien RJ, Wong PC. Amyloid precursor protein processing and Alzheimer's disease. *Annu Rev Neurosci*. 2011;34:185-204. doi:10.1146/annurev-neuro-061010-113613
56. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol*. 2016;15(7):673-684. doi:10.1016/s1474-4422(16)00070-3
57. Illán-Gala I, Alcolea D, Montal V, et al. CSF sAPP $\beta$ , YKL-40, and NfL along the ALS-FTD spectrum. *Neurology*. 2018;91(17):e1619-e1628. doi:10.1212/wnl.0000000000006383
58. Illán-Gala I, Pegueroles J, Montal V, et al. APP-derived peptides reflect neurodegeneration in frontotemporal dementia. *Ann Clin Transl Neurol*. 2019;6(12):2518-2530. doi:10.1002/acn3.50948
59. Alcolea D, Carmona-Iragui M, Suárez-Calvet M, et al. Relationship between  $\beta$ -Secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. *J Alzheimers Dis*. 2014;42(1):157-167. doi:10.3233/jad-140240
60. Gabelle A, Roche S, Gény C, et al. Decreased sA $\beta$ PP $\beta$ , A $\beta$ 38, and A $\beta$ 40 cerebrospinal fluid levels in frontotemporal dementia. *J Alzheimers Dis*. 2011;26(3):553-563. doi:10.3233/jad-2011-110515
61. Edgar JR, Willén K, Gouras GK, Futter CE. ESCRTs regulate amyloid precursor protein sorting in multivesicular bodies and intracellular amyloid- $\beta$  accumulation. *J Cell Sci*. 2015;128(14):2520-2528. doi:10.1242/jcs.170233
62. Jun MH, Han JH, Lee YK, Jang DJ, Kaang BK, Lee JA, TMEM106B, a frontotemporal lobar dementia (FTLD) modifier, associates with FTD-3-linked CHMP2B, a complex of ESCRT-III. *Mol Brain*. 2015;8:85. doi:10.1186/s13041-015-0177-z
63. Gick GG, Arora K, Sequeira JM, Nakayama Y, Lai SC, Quadros EV. Cellular uptake of vitamin B(12): role and fate of TcBlr/CD320, the transcobalamin receptor. *Exp Cell Res*. 2020;396(1):112256. doi:10.1016/j.yexcr.2020.112256
64. Zetterberg H, Nexö E, Regland B, et al. The transcobalamin (TC) codon 259 genetic polymorphism influences holo-TC concentration in cerebrospinal fluid from patients with Alzheimer disease. *Clin Chem*. 2003;49(7):1195-1198. doi:10.1373/49.7.1195
65. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx*. 2004;1(2):213-225. doi:10.1602/neurorx.1.2.213

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Toft A, Sjödin S, Simonsen AH, et al. Endo-lysosomal protein concentrations in CSF from patients with frontotemporal dementia caused by CHMP2B mutation. *Alzheimer's Dement*. 2023;15:e12402. <https://doi.org/10.1002/dad2.12402>