From protein biomarkers to proteomics in dementia with Lewy Bodies

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ABSTRACT

Dementia with Lewy Bodies (DLB) is the second most common neurodegenerative dementia. Despite considerable research progress, there remain gaps in our understanding of the pathophysiology and there is no disease-modifying treatment. Proteomics is a powerful tool to elucidate complex biological pathways across heterogeneous conditions. This review summarizes the widely used proteomic methods and presents evidence for protein dysregulation in the brain and peripheral tissues in DLB. Proteomics of post-mortem brain tissue shows that DLB shares common features with other dementias, such as synaptic dysfunction, but retains a unique protein signature. Promising diagnostic biomarkers are being identified in cerebrospinal fluid (CSF), blood, and peripheral tissues, such as serum Heart-type fatty acid binding protein. Research is needed to track these changes from the prodromal stage to established dementia, with standardized workflows to ensure replicability. Identifying novel protein targets in causative biological pathways could lead to the development of new targeted therapeutics or the stratification of participants for clinical trials.

1. Introduction

Lewy body dementia (LBD) comprises of Dementia with Lewy Bodies and Parkinson’s disease dementia (PDD). DLB is the second most common neurodegenerative dementia in older people, following Alzheimer’s disease (AD) (Vann Jones and O’Brien, 2014). The core clinical features are fluctuating cognition, recurrent visual hallucinations, Rapid Eye Movement (REM) sleep behaviour disorder, and Parkinsonism. There is a range of supportive features including autonomic and psychiatric symptoms. Indicative biomarkers are reduced dopamine uptake in the basal ganglia on positron emission tomography (PET) or Single-photon emission computed tomography (SPECT), low uptake on myocardial scintigraphy, and polysomnographic evidence of REM sleep without atonia. Using the current criteria, probable DLB is diagnosed with two core features, and possible DLB with either a single core feature or indicative biomarker (McKeith et al., 2017). Meta-analysis of the previous consensus criteria showed that clinical diagnosis predicts post-mortem Lewy Body pathology at autopsy with 79.9% accuracy (Rizzo et al., 2018). DLB is frequently misdiagnosed as AD, however combing clinical assessment with neuroimaging biomarkers based on protein targets has improved diagnostic accuracy (McKeith et al., 2007).

The dopamine transporter scan is able to distinguish DLB from AD with an overall 87.5% accuracy. PDD occurs in 80% of PD cases within 20 years of disease onset (Emre et al., 2007). The common pathology across LBDs is the accumulation of phosphorylated α-synuclein in Lewy bodies and Lewy neurites in neurons (Fujisawa et al., 2002; Takahashi et al., 2002). α-synuclein is a presynaptic protein involved in neurotransmission and neuronal signalling (Bendor et al., 2013; Savica et al., 2016) although its role is not fully understood. Some degree of AD co-pathology, comprising neuritic plaques of amyloid β peptide and neurofibrillary tangles of tau protein, is frequently present in LBD (Irwin et al., 2017). Amyloid burden, as measured by the PET tracer Pittsburgh compound B, is higher in DLB compared to PDD and controls (Gomperts et al., 2012; Gomperts et al., 2016). AD pathology is associated with higher levels of cortical synuclein, and in PD is associated with accelerated development of dementia, increased morbidity, and mortality (Irwin et al., 2017; Irwin and Hurtig, 2018). Whilst new mechanisms of disease are being identified, such as inflammation (Surendranathan et al., 2018), there is a need to improve the diagnosis and prognosis with accessible biomarkers, and there are currently no disease-modifying therapies.

Abbreviation: PD, Parkinson’s disease; AD, Alzheimer’s disease; PDD, Parkinson’s disease dementia; DLB, Dementia with Lewy bodies; LBD, Lewy body dementia; FTD, Frontotemporal dementia; PSP, Progressive supranuclear palsy; CJD, Creutzfeldt-Jakob disease; SNCA, α-Synuclein; LB, Lewy bodies; LN, Lewy neurites; CGI, Glial cytoplasmic inclusions.

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1568-1637/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Proteomics is the large-scale study of proteins. It has the potential to revolutionize clinical practice in a range of fields such as endocrinology, immunology, oncology, and neurodegeneration. The primary aims are improving screening, diagnosis, and moving to wards personalized medicine (Kim et al., 2019; Nyman et al., 2017; Li et al., 2019; Shruthi et al., 2016). Identifying protein markers in body fluids, such as the use of tumour markers such as prostate-specific antigen (PSA) and carcinoembryonic antigen (CEA) are of substantial value in diagnosis and prognosis. Discoveries such as the human epidermal growth factor receptor 2 (HER2) have led to stratified treatment of breast cancer (Mitri et al., 2012). In dementia, stratification is primarily based on dementia subtyping, supported by genetic or imaging investigations (Sidhom et al., 2020). The field is advancing with the measurement of pathological proteins such as amyloid and tau by PET, CSF assays, and most recently highly sensitive assays in plasma (Choullaras et al., 2022). Technological advances are moving the field beyond single genes or proteins to large-scale complex systems. Genomics has identified a range of risk alleles in DLB, namely APOE, SNCA, and GBA (Guerreiro et al., 2018). This ‘omics’ approach has highlighted pathways involved in disease and potential therapeutic targets, although there are further opportunities within proteomics. There are differences between the genome, what is transcribed to the transcriptome, and finally translated to the proteome. Changes in gene expression, alternative splicing, and mRNA degradation all affect the final protein products (Siuti and Kelber, 2007), and this is compounded further by post-translational modifications. Finally, whilst the genome is relatively fixed, the proteome could reflect a specific cell type, disease stage, progression, or treatment response (Fu et al., 2017).

Thus, identifying the LBD proteome could provide biomarkers for diagnosis, stage, or response to a new treatment. Proteomics can also identify disrupted pathways that could provide targets for new or repurposed therapeutics with greater relevance than those identified at the genomic or transcriptomic level. Individuals may have a range of pathways disrupted with heterogeneity within the diagnostic group or due to disease stage, with proteomics allowing biologically defined stratification for clinical trials. The field of proteomics in DLB is at an early but rapidly growing stage, and this review aims to summarize the current techniques being used, potential fluid biomarkers being identified, and the early stage of advances in monitoring therapeutic response.

### 2. Methods

PubMed was used to identify relevant articles through to August 2022. The initial search was performed in October 2020, with updates in March and August 2022. The following search terms “Lewy body dementia” or “dementia with Lewy bodies” and “Parkinson’s dementia” and “proteomics” “proteins” “protein analysis” and “biomarker” were used with searches in UniProtKB (Magrane and Consortium, 2011), NCBI Protein database (Sayers et al., 2020), The Human Protein Atlas (Uhlen et al., 2010), Protein Data Bank (Berman et al., 2000) and MobiDB (Di Domenico et al., 2012). Studies and database entries were screened by title, abstract, or full text and included if they measured one or more proteins in the brain or peripheral tissues in DLB as the focus or comparator. Articles were supplemented with those known to the authors and exemplars from key proteomic studies in other fields that demonstrated relevant techniques or future research directions. New therapeutic trials were identified by searching ‘Dementia with Lewy Bodies’ or ‘Lewy Body Dementia’ at [https://clinicaltrials.gov/](https://clinicaltrials.gov/). (Table 1).

### 3. Current proteomic techniques

The most widespread technique for measure proteins in biological samples is the enzyme-linked immunosorbent assay (ELISA). ELISA allows highly sensitive and specific analyses of proteins using enzymatic antibody labelling and can provide quantitative and qualitative results. ELISA is easy to perform, cost-effective, and widely accessible (Sakamoto et al., 2018). For these reasons, ELISA has been a mainstay of dementia biomarker studies (Mukaetova-Ladinska et al., 2010). The electro-chemiluminescent immune assay (ECLIA) has advanced sensitivity, achieving the excitation of the conjugated antibody via an electric field and allowed multiplexing (measuring multiple protein targets in the same sample at the same time) (Leng et al., 2008). Single-molecule array (SIMOA) is a new technique offering up to 1000 times sensitivity compared to conventional ELISA, allowing protein identification and quantification in lower concentrations than previously possible (Chang et al., 2012). The processes couples antibodies with paramagnetic particles which bind to protein targets. The sample is concentrated by magnetic separation and the purified sample is loaded into an array with femtolitre-sized wells for detection.

Western blotting is another widely accessible, sensitive, and specific technique to identify protein targets, in which the protein mixture is separated via gel electrophoresis based on the different molecular weights (Mahmood and Yang, 2012). Traditionally this was semi-quantitative, although there have been advances in quantitative western blotting (Taylor and Posch, 2014).

Multiparametric protein analysis provides more stable and accurate results compared to single-core biomarkers, as the findings become more resistant to outliers and single protein fluctuations among different samples. Two-dimensional polyacrylamide gel electrophoreses (2D-PAGE) can relative quantification from multiple samples and is able to resolve about 5000 different proteins. 2D-PAGE is also capable of separating isomers and post-translational modifications, making it a valuable tool for protein identification and metabolomics (Meleady, 2018). The similar technique of Two-dimensional differential gel electrophoresis (2D-DIGE) uses the same principals as 2D-PAGE. After labelling the protein of interest, differences are made visually comparing the results, increasing reproducibility and requiring fewer samples for each analysis. This high resolution of 2D-PAGE and 2D-DIGE allows a more accurate picture of the proteome, although at the increased expense and technical requirements (Meleady, 2018; Aslam et al., 2017).

Tissue Microarray was designed for high throughput detection of molecular targets from hundreds of different specimens. A paraffin block can contain multiple different samples with analysis performed under identical conditions (Jawhar, 2009). Combing Tissue Microarrays with immunoblotting and immunostaining, it provides quantitative and qualitative results (Chung et al., 2007). This method has been used for proteomic analysis of neuropathology specimens and combined with MS (Groseljose et al., 2008; Wilson et al., 2021). The advances in techniques for protein identification from single epitope immunoassays to multiplex and mass spectrometry of intact proteins allows powerful and detailed

### Table 1
Description of databases used to identify studies.

| Database       | Description                            | URL                                      |
|----------------|----------------------------------------|------------------------------------------|
| UniProtKB      | Integrated protein information from multiple sources | [https://www.uniprot.org/](https://www.uniprot.org/) |
| NCBI Protein database | Large protein database from multiple sources | [https://www.ncbi.nlm.nih.gov/](https://www.ncbi.nlm.nih.gov/) |
| The Human Protein Atlas | Public database of protein expression data | [https://www.proteinatlas.org/](https://www.proteinatlas.org/) |
| Protein Data Bank | Database of protein structures | [https://www.wwpdb.org/](https://www.wwpdb.org/) |
| MobiDB Database   | Database of intrinsically disordered proteins | [https://pubmed.ncbi.nlm.nih.gov/32237329/](https://pubmed.ncbi.nlm.nih.gov/32237329/) |
Identification of the protein composition of biological samples. This has the potential to identify novel and unexpected pathways beyond the traditional dementia biomarkers, whilst also presenting challenges of ever more complex data.

The advanced, unbiased technique of mass spectrometry is a powerful tool for quantitative analysis and protein identification. Mass spectrometry (MS) provides a detailed mapping of the proteome in the fluid or tissue of interest, providing data for analyzing network interactions and biomarker profiles which maximize diagnostic accuracy. The first step involves the transformation of proteins into gas-phase ions which are then separated based on their mass/charge \((m/z)\) values (Aslam et al., 2017). This allows rapid and accurate protein analysis at a relatively low cost with high reproducibility (Nilsson et al., 2010). There are a number of ionization methods. These have been developed to improve detection sensitivity and reduce sample fragmentation, or for compatibility with new technologies. Electron ionization (EI) was among the first methods used, in which a vaporized sample is radiated by a beam of energetic electrons in order to remove an electron from the molecule, creating a positive ion (Dass, 2007). Chemical ionization (CI) uses ion-molecule reactions in place of electron beaming (Munson, 2000). Field ionization (FI) is similar to EL, with the electrons removed directly from the molecule by tunnelling through a strong electric field.

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The next step is to analyse the mass of the ionized molecules or molecule fragments. In the magnetic sector mass spectrometer, the ions travel in a curved magnetic field. In electrostatic sector mass spectrometer, an electric field is used instead. Smaller ions are deflected more towards the detector at the end of a curved field, while bigger ones are less prone to deflection due to their high inertia (Rubakhin and Sweedler, 2010). Time of flight (TOF) analysers measure the amount of time required for the ions to travel a certain distance. Pulse acceleration through a homogenous electric field provides greater acceleration to smaller ions due to smaller inertia (Boesl, 2017). Quadrupole mass spectrometers make use of four parallel rods that emit oscillating electric fields allowing only specific \(m/z\) molecules to pass through the detector. The Quadrupole ion trap (QIT) has the beneficial effect of containing ions through collision with a buffer gas (such as helium). Contained ions are able to be released later enabling multistage tandem MS/MS analysis (Chong et al., 2018). Another method of ion trap is the Orbitrap, which achieves ion confinement by forcing ions onto an orbiting spiral through a radial electric field, akin to the planetary orbit around the sun, allowing passage of ions with a specific \(m/z\) ratio (Zubarev and Makarov, 2013). Alternative methods, such as the Fourier-transform ion cyclotron resonance (FT-ICR), base ion separation based on rotation during cyclotron resonance whilst moving in a fixed magnetic field. From the cyclotron frequency, the \(m/z\) can be calculated (Nagornov et al., 2022). Ionization and measurement techniques are then combined – such as MALDI-TOF, where the \(m/z\) ratio is calculated by measuring the time duration of flight of the ionized molecule to travel the length of a flight tube (Singhal et al., 2015).

Controlled fragmentation of ions offers higher resolution MS and is very valuable in post-translational analysis. Electron-transfer dissociation (ETD) can be used between stages of tandem MS/MS analysis. After first ionization, an electron is transferred to the formed cation, cleaving it along the peptide backbone. This offers structural and post-translational information on the target protein through controlled fragmentation (Riley and Coon, 2018). Ultraviolet photodissociation (UVPD) similarly uses photons for ion fragmentation, providing another useful tool to analyse post-translation modifications (Brodbelt et al., 2020). Both of these tools have improved top-down MS analysis.

MS can be used in tandem with different labelling methods for quantitative proteomics analysis. Chemical labelling such as Isotope-Coded Affinity Tag (ICAT) and isobaric Tags for Relative and Absolute Quantification (ITRAQ), or metabolic labelling such as Stable Isotope Labelling of Amino acids in Cell culture (SILAC) allowed reduced machine times and strict study conditions improve reproducibility (Anand et al., 2017). ICAT and ITRAQ make use of isotope tags with different molecular weight to bind proteins at different sites (ICAT tags bind cysteine whilst ITRAQ tags bind N-terminus and lysine residues).

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**Fig. 1.** Pipeline from sample preparation to analysis in mass spectrometry.
Another difference is ICAT is mainly performed on intact proteins while ITraq is performed on peptides (Yu et al., 2007; Dong et al., 2016). Differential tagging of pathological and control tissue prior to MS allows post hoc quantification of proteins, and these differences can inform pathway analysis. One method uses ‘light’ or ‘heavy’ tags, the heavy tags with deuterium in place of hydrogen. Metabolic labelling, such as SILAC, uses nitrogen isotopes to separate control and test cultures prior to protein extraction (Ong and Mann, 2007). Protein selection parameters allow the study of protein-protein interactions, link proteomics with metabolomics (the system-wide study of metabolite levels and transformation in biological samples) and analysis of post-translational modifications (Chokkathukalam et al., 2014).

MS can be coupled with reverse-phase chromatography, and the improved reverse-phase high-performance liquid chromatography (RP-HPLC). This uses a pump to create flow in the soluble sample through a solvent gradient. The components of the sample interact differentially with the solvent, resulting in distinct flows reflecting the components. This achieves elution of multiple mixtures with multiple components increasing the resolution of MS (Joscic and Kovac, 2010). In normal phase phased HLC there is a non-polar mobile phase, followed by a polar stationary phase, while in RP-HPLC there is a polar mobile phase followed by a non-polar stationary phase. The latter technique is more suited to organic analytes (Bird, 1989).

MS relies on separation by m/z ratio, but ion mobility spectrometry (IMS) uses the movement of ions through an inert gas in an electric field as a parametric function of temperature and pressure (Dodds and Baker, 2019). Combining both techniques (IMS-MS) offer multidimensional capabilities for protein analysis. A variety of ion mobility devices have further advanced ion separation (Yates, 2019). Some of the most commonly used instruments are Drift Tube Ion Mobility Spectrometry (DTIMS), Traveling Wave Ion Mobility Spectrometry (TWIMS) and Trapped Ion Mobility Spectrometry (TIMS) (Dodds and Baker, 2019). DTIMS measures the time ions take to pass through a uniform electric field at a specific pressure. TWIMS use non-uniform drifting electric fields, effectively ‘pushing’ the ions through the gas. In TIMS ions remain stationary while the gas in the chamber moves. The ions are controllably released according to their mobility (Meier et al., 2021).

Beyond protein identification is the study of the three-dimensional structure of the protein obtained through X-ray crystallography or with Nuclear Magnetic Resonance (NMR) spectroscopy (Aslam et al., 2017). Monomolecular structural analysis with X-ray crystallography uses diffraction patterns and intensities to determine the structural map of the molecule following crystallisation (Huxford, 2013). NMR spectroscopy measures the magnetic properties of atomic nuclei inside a strong magnetic field. Electromagnetic radiation causes nuclei excitation, and when the radiation is stopped energy is released from the nuclei. This signal can be used for structural analysis (Zia et al., 2019).

Choice of tissue sample depends on the aims of the study. Studying brain tissue is useful to identify pathophysiology and target identification, but it may not be suitable to identify biomarkers for widespread use, such as screening. CSF and blood proteomics are increasingly popular for identifying biomarkers to improve diagnostic accuracy for neurodegenerative diseases (Ganash, 2017). CSF is a compromise; a more direct relationship to the pathological tissue although obtained through a more invasive procedure. Blood proteomics may be limited in their reflection of neuropathology, or with lower concentrations of relevant proteins, but collection is minimally invasive and safe. Other biofluids like saliva, tears, and urine could allow sample collection without any invasive procedure, but whether these biofluids have use in DLB is yet to be explored. Irrespective of the tissue source, there is a focus on standardization of laboratory techniques and bioinformatic analysis to improve reliability and reproducibility for translation into clinical practice (Hood et al., 2012). (Table 2).

4. Profiling the brain proteome in DLB

Proteomics have been used in DBL research to better understand the composition of Lewy bodies, as they contain a constellation of proteins beyond α-synuclein. Beyond this, proteomic profiles of post-mortem brain tissue from patients with DLB have been compared to cognitively unimpaired controls and other dementia groups. One of the largest studies (52 DLB, 34 PDD, 18 AD and 25 control participants) identified reduced levels of the synaptic markers Ras-related protein Rab-3A (Rab3A), Synaptosomal-Associated Protein 25 (SNAP25), and Neurogranin (NRG) in dementia groups compared to controls across multiple brain regions, without distinguishing between different dementias (Bereczki et al., 2016). In DLB, Rab3A levels correlated with α-synuclein levels in the inferior parietal lobe. Cognitive decline (assessed by MMSE) correlated with regional-specific synaptic markers in both AD and DLB. Reduced Rab3A in the inferior parietal lobe and reduced SNAP25 in the prefrontal cortex correlated with increased rate of cognitive decline. Neurogranin levels correlated positively with MMSE scores. A post-mortem study using a multiplex immunoassay for brain-enriched proteins in the CSF found no difference in NRG (or

| Tissue          | Advantages                                                                 | Disadvantages                                                                 | Example protein markers identified                                  |
|-----------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------------------------|
| Brain           | Direct source of pathology, Multiple regions can be analysed separately.      | Rarely accessible prior to post mortem. Studies often rely on smaller sample sizes. | Synaptic proteins e.g SNAP25 (Bereczki et al., 2016)               |
| CSF             | Continuous with the central nervous system. Less likely to be impacted by systemic co-morbidity or source of protein. | Requires invasive procedure to obtain, often performed in more specialist clinics | Synuclein, H-FABP, markers of AD copathology (p-tau, Abeta) (Chaisson et al., 2017) |
| Blood           | Easily accessible. Can be obtained much more widely than CSF with minimally invasive procedure. | Blood brain barrier selectively permeable so may not reflect state of CNS. Influenced by peripheral source of proteins and haemolysis. | Markers of AD copathology (e.g p-tau) and neurodegeneration (e.g NIL) (Chouliaras et al., 2022) |
| Skin/submandibular gland | Potentially early identification of pathological proteins                        | Still requires procedure to obtain.                                              | Synuclein (Donadio et al., 2017)                                 |
| Saliva          | Easily obtainable. Results can be inconsistent depending on sample handling. | No studies of urine proteome in DLB                                           |                                                                   |
| Urine           | Very easily accessible without any specialised procedure                       | Low concentration of protein combined with urea and salts can be difficult to detect. Highly variable within and between individuals. | No studies of urine proteome in DLB                               |

Table 2: Advantages and disadvantages of different proteome sources.
synaptic protein GAP43) between AD and DLB groups (Remn茅st茅 et al., 2016).

A follow-up study of a subset of the same cohort identified 10,325 differentially expressed proteins in the prefrontal cortex associated with LBD using mass spectrometry (Bereczki et al., 2018). For the DLB patients, 1010 proteins were differentially expressed (448 upregulated and 562 downregulated compared to non-demented controls). However, as was the case for the previous study, DLB could not be reliably discriminated from either PDD or AD. These studies are suggestive of a common pathway of synaptic dysfunction associated with neurodegenerative disease (Taoufik et al., 2018).

Olfactory deficits are prominent in Lewy Body disease and Lewy pathology has been identified in the olfactory system (Lachen-Montes et al., 2020). Olfactory involvement may reflect an early or prodromal stage. Post-mortem olfactory bulbs from DLB cases (n = 4) and controls (n = 4) were examined by mass spectrometry with 99 proteins (out of 23214) showing significant alternations in DLB. These proteins formed networks including protein translation, synaptic vesicles, and the innate immune system with significant enrichment in synopsis-related pathways. By combining published olfactory bulb proteomic datasets (including the Lachen-Montes data) across neurodegenerative disorders (DLB, AD, PD, mixed dementia, FTD, PSP) Cartas-Cartas-Tejedo et al. (2021) identified pathways disrupted specifically in DLB and those shared across disorders. Globally disrupted pathways included synaptic signalling, exocytosis, and those related to vascular endothelial growth factor (VEGF), DBL-specific changes localized to the excitatory synapse, transmembrane transporter complex, and cytosolic small ribosomal subunits. This study combined data sets with different workflows, so it remains to be seen if the results identified were due to pathogenic differences between disorders or the methodologies used to generate the data.

E3 Ubiquitin ligases promote ubiquitination and degradation via the proteasomal or lysosomal pathways (Zheng and Shabek, 2017). Parkin, an E3 ubiquitin ligase associated with Lewy Body pathology (Dawson and Dawson, 2010) was shown to colocalize with Lewy Bodies in DLB and PD. This study combined sporadic, Parkin and inherited α-synuclein linked PD, Parkin linked PD with Lewy Bodies and Lewy Body Dementia. Using immunohistochemistry Parkin was identified in LBDs in the substantia nigra, anterior cingulate, and entorhinal cortices of two DLB cases (Schloessmacher et al., 2002). Levels of TRIM9, a brain-specific E3 ubiquitin ligase, were decreased in the temporal cortex and hippocampus in DLB (n = 3) but not in AD (n = 3) or control subjects (n = 3) (Tanji et al., 2010). These results were confirmed using western blotting, showing that levels of TRIM9 were reduced by up to 70% in DLB compared to controls.

Ubiquitin C-terminal hydrolyase 1 (UCHL-1) provides ubiquitins to the proteasome and loss of function is strongly linked to the accumulation of α-synuclein (Shimshek et al., 2012). Protein synthesis and mRNA expression were downregulated in DLB in a study comparing 7 DLB cases with 6 PD, and 6 DLB with Alzheimer’s copathology, with 6 controls (Barrachina et al., 2006). Western blots showed decreased protein levels in the cerebral cortex in DBL, but not in PD, compared with controls. Protein levels were decreased in the medulla oblongata in PD, and there was a decrease in the substantia nigra in both DBL and PD. This suggests a role for UCHL-1 in LB pathology, possibly related to proteasome dysfunction. Proteasome dysregulation exacerbates protein aggregation, whilst α-synuclein leads to proteasome impairment (Bi et al., 2021). This creates a feedback loop of pathology.

In a study with brain tissue from 10 DLB, 5 AD, and 5 controls, using immunohistochemistry, synphilin-1 was found localized in LBs in α-synuclein positive brainstems, specifically the substantia nigra and locus coeruleus, along with well-defined LBs in the cortex (Iseki et al., 2002). Ill-defined LBs and LB neurites were negative for synphilin-1, suggesting an indirect association between synphilin-1 and α-synuclein. Synphilin-1 is linked to synaptic function and the ubiquitin-proteasome system (Krüger, 2004).

Using tissue microarrays, Ras analogy in brain 39B (RAB39B) protein levels were decreased in DLB cases compared to AD and controls (10 DLB, 12 AD, 12 controls). RAB39B is a vesicular trafficking protein. RAB39B was colocalized with amyloid plaques, cortical and subcortical Lewy Bodies, and decreased in the pre-frontal, frontal, cingulate, insular, motor, entorhinal, temporal, parietal and occipital cortices.

5. CSF proteomics

CSF proteomic profiling has yielded promising results in the search for LBD biomarkers. Some studies have found reduced CSF α-synuclein in LBD when compared with AD and controls, although these findings have not been widely replicated (Mollenhauer et al., 2008; Wennstron et al., 2013; Spies et al., 2009). A study measuring α-synuclein, β-synuclein, and γ-synuclein across neurodegenerative disorders (LBD = 10, PDD = 17, PD = 23, Progressive supranuclear palsy (PSP) = 20, Corticobasal syndrome (CBS) = 10, AD = 19, Creutzfeldt-Jakob disease (CJD) = 10, Control = 37) found higher levels of α-synuclein in AD and CJD but not synucleinopathies. There was an increased β-synuclein/α-synuclein ratio in PDD compared to PD, and β-synuclein correlated with CSF tau (Ocelki et al., 2016). Separating α-synuclein into total, oligomeric, and phosphorylated, van Steenoven observed that whilst total α-synuclein was lower in CSF of patients with DLB (n = 42) and PD (n = 46) than AD (n = 39) or controls (n = 78), oligomeric α-synuclein was higher in DLB and PD compared to AD and controls. No changes were found in phosphorylated α-synuclein between groups. Additionally, there was a strong correlation between total α-synuclein, tau, and phosphorylated tau. A combined biomarker model including Aβ42, tau, total α-synuclein, oligomeric α-synuclein, age, and sex to differentiated DLB from controls, with an area under curve value (AUC) of 0.90 (van Steenoven et al., 2018).

Wennstron et al. also compared the CSF levels of α-synuclein and neurosin (a protease that degrades α-synuclein in vitro and is expressed in the brain) in 33 DLB, 46 AD, 38 PD and 52 control participants using ELISA. CSF neurosin and α-synuclein were lower in DLB and PD compared to AD and controls. AD participants had increased concentrations of α-synuclein but no changes in neurosin compared to controls. CSF α-synuclein and neurosin were highly correlated with CSF phosphorylated tau (p-tau) across groups (Wennstron et al., 2013).

AD biomarkers have been studied in LBD CSF samples. Phosphorylated tau at threonine 181 (p-tau181) and at 231 (p-tau231) are significantly higher in AD than DLB (Buckley, 2006; Vanderstichele et al., 2006; Wada-Isoe et al., 2007; Hampel et al., 2004). CSF levels of total Aβ are inconsistent and unable to differentiate between AD, DLB, and controls without being included in multiparametric biomarker evaluations (Gómez-Tortosa et al., 2003; Wiltfang et al., 2002; Bibl et al., 2006; Parnetti et al., 2001; van Steenoven et al., 2019). Note-worthy is the detection of a novel peptide Aβ40, an oxidized α-helical form of Aβ1-40, found to be increased by 100% in DLB (n = 22) compared to PDD (n = 21) (Bibl et al., 2006). Aerts et al. measured CSF levels of the neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenethylenglycol (MHPG), alongside total tau (t-tau), p-tau, and Aβ42, in 45 AD and 23 DLB participants. MHPG, t-tau, and p-tau were lower in DLB compared to AD, while Aβ42 was higher in DLB. Using the combination of p-tau, t-tau, Aβ42, and MHPG they achieved high accuracy for the discrimination between DLB and AD (AUC = 0.99). There was no control to comparisons (Aerts et al., 2011). Bousiges et al. (2016) measured CSF p-tau181, t-tau, Aβ42 and Aβ40 in 35 prodromal DLB, 39 prodromal AD, 30 DLB, 31 AD, and mixed DLB and AD participants along with 15 controls and were able to distinguish DLB from AD with high accuracy (AUC 0.93–0.95) outside of the mixed pathology groups. Across nine neurodegenerative disorders Delaby et al. (2020) found that CSF Neurofilament Light chain (NFL) levels were higher in DLB patients compared to controls, with higher NFL in DLB patients with co-existing AD pathology. In the same cohort Delaby found
that a low Aβ42/Aβ40 ratio was associated with increased tau across disorders regardless of Aβ42 levels (Delaby et al., 2022). These studies show the potential of combining α-synuclein with established and emerging dementia biomarkers to improve diagnostic recognition of LBD but will need to be replicated in independent cohorts.

Heart-type fatty acid-binding protein is a promising biomarker candidate for DLB with reproducible results among a range of studies. H-FABP was initially isolated from cardiac myocytes and serum levels were used as a biomarker of myocardial infarction (Yabuki et al., 2020). It has since been found to be expressed in nervous tissue and proposed as a biomarker for stroke. In mouse models fatty acid-binding proteins (FABP) are highly expressed within dopaminergic neurons and interact with α-synuclein. Knockout or inhibition of FABP reduced α-synuclein spreading and alleviated motor and cognitive impairments. A study measured the H-FABP levels, as well as the levels of α-synuclein, Aβ42, t-tau, and p-tau181 using ELISA, among 48 AD, 40 DLB, 20 PDD and 54 PD participants and 46 controls without cognitive impairment. They observed significantly elevated levels of H-FABP in AD and DLB when compared to non-demented controls (p < 0.001). Combining the measurements of H-FABP and p-tau181 yielded the highest diagnostic accuracy between AD and DLB (AUC 0.92) (Chiasseri et al., 2017).

Schultz et al. (2009) introduced the Cocaine and Amphetamine-regulated transcript (CART), a neuropeptide highly expressed in the hypothalamus, as a possible biomarker for Dementia. This was based on the hypothesis of increased hypothalamic pathology in DLB compared to AD. Using a 1251 radioimmunoassay they measured CART levels in 12 DLB, 14 AD, and 12 controls and observed significantly reduced CSF CART levels in DLB. This contrasts with a multiplex proteomics study of 5 DLB, 10 AD, 10 PD, and 10 controls (Abdi et al., 2006). Using iTRAQ and multidimensional chromatography followed by mass spectrometry identified over 1500 proteins in the CSF, with 101 showing quantitative changes in DLB. Functional classification showed these proteins were enriched in neuronal activity, cell cycle and structure, metabolism, extracellular matrix, and immunity. The results included CART, but with increased levels (in contrast to the Schultz study). Not all the identified markers could be confirmed by Western blotting, and no single marker could differentiate DLB from controls and non-DLB cases. A combination of the lipoprotein ApoC1 and neuroendocrine protein Chromogranin B was the best combination (AUC = 0.92 to differentiate DLB versus all others). Using label-free proteomics a study measured over 1000 CSF proteins in 10 patients with DLB and 16 controls, identifying 38 proteins with significant altered expression in DLB. These results were combined with previous studies to create a multiplex assay validated in a cohort of 17 LBD, 16 AD, 7 PD, and 15 controls. Nineteen raised proteins overlapped with those raised in AD and four were unique to DLB (ectonucleotide pyrophosphatase, lysosome-associated membrane protein 1, pro-orexin, and transthyretin) (Heywood et al., 2015). Using 2D fluorescence gel electrophoresis followed by mass spectrometry Dieks et al. (2013) identified a number of differentially expressed proteins in DLB, from 8 DLB, 8 AD, 8 PD, and 8 controls. The greatest changes were in Inter alpha trypsin inhibitor heavy chain (ITIH4) and calysntenin1 which had a 60-fold increase versus controls, making them promising biomarkers for exploration.

Bruggink et al. used an approach combining mass spectrometry of Aβ associated proteins to guide biomarker confirmation by ELISA. This identified Dickkopf-related protein 3 (a signalling protein in the Wnt pathway). Whilst it correlated with CSF Aβ40 it did not distinguish between AD, DLB and controls (Bruggink et al., 2015). Mass spectrometry can also identify differences in specific protein isoforms and post-translational modifications. A small study found a predominance of the 1 N3 R isoform in AD that was not present in DLB or PSP (AD = 11, DLB = 4, PSP = 5). This approach could identify specific isoforms of pathogenic proteins to be translated into diagnostic assays (Barthelemy et al., 2016).

6. Blood proteomics

Blood testing is less invasive than CSF collection and could provide more accessible clinical biomarkers for a larger population. Recent technological advances have allowed a greater detection of a range of dementia biomarkers in plasma and serum.

Laske et al. (2011) found a reduction in α-synuclein levels in DLB patients compared to both AD and controls, although much like the results in CSF the use of ELISA for measuring serum α-synuclein levels has proven inconsistent. Across synucleinopathies, ELISA has found α-synuclein levels to be either increased, decreased, or unchanged between patients and controls (Atik et al., 2016). Koehler et al. used ELISA to measure serum IgG levels to monomeric α-synuclein and found these were increased in both DLB (n = 19) and AD (n = 15) when compared to healthy controls (n = 16). Antibody levels in young DLB patients were considerably higher compared to older patients. In controls, antibody levels conversely showed an increase with age, leading to the observed difference between cases and controls diminishing in ages greater than 75. Antibody levels in DBL patients decreased with disease duration, peaking in the early stages, while in AD patients there was a strong and significant increase with disease duration (Koehler et al., 2013).

Serum H-FABP was found to be elevated in DLB compared to AD and controls in a study of 16 DLB, 14 CJD, 18 AD and 16 controls. Serum H-FABP did not correlate with CSF H-FABP levels (although independent studies have identified raised H-FABP in CSF of DLB participants as discussed above) (Steinacker et al., 2004). Two further studies replicated these findings. One compared 17 DLB, 63 PD, 23 AD and 10 controls showing increased serum H-FABP in DLB and PD compared to AD controls (Wada-Isoe et al., 2008). The other measured serum and CSF H-FABP levels in 33 DLB and 25 PDD participants compared to 51 controls and 35 AD participants. This study found increased levels of H-FABP in both blood and CSF of DLB participants versus controls (Mollenhauer et al., 2007).

Dementia biomarkers are now able to be detected in plasma using highly sensitive SIMOA. A recent study analysed plasma phosphorylated tau at threonine-181 (p-tau181), Aβ42, Aβ40, NfL and glial fibrillary acidic protein (GFAP) in 300 participants (110 Dementia with Lewy Bodies, 7 Parkinson’s disease Dementia, amyloid positive mild cognitive impairment (MCI+) and AD (MCI+ = 63, FTDT 28, PSP 19, and controls= 73). GFAP was elevated in LBD and AD compared to controls, and all dementia groups had higher levels of NfL. Plasma biomarkers showed limited ability in differentiating LBD from MCI+ (AUC τ-ant = 0.67, Aβ42/40 = 0.42, NfL = 0.55, GFAP = 0.61) and were unable to distinguish PET Aβ positive with PET Aβ negative LBD (Choularias et al., 2022). Another study found that a combination of plasma p-tau181, Aβ, and NfL could distinguish amyloid positive from amyloid negative in a group of DLB and frontotemporal lobar degeneration (AUC = 0.87) but did not report DLB results separately (Alcolea et al., 2021). A cohort study including participants from the European-DLB Consortium cohort, with a total of 1122 participants (DLB = 371, PD = 203, AD = 207, healthy controls = 205) showed that p-tau181 and p-tau231 were significantly higher in DLB than healthy controls, lower than in AD, and no significant difference was observed between DLB and PD (Gonzalez et al., 2022). Both p-tau markers were increased in DLB subgroups with abnormal CSF levels of Aβ42 compared to DLB with normal levels. P-tau levels of both biomarkers were inversely correlated with MMSE scores in DLB.

Using MS 146 peptides were identified in a training set of 30 DLB, 30 AD, and 28 healthy control participants. Four peptides were selected that discriminated the DLB group best, and this was taken into a testing cohort of 20 DLB, 30 AD, and 14 healthy controls with a sensitivity of 90% and specificity of 89%. Out of the six peptides fragments, they identified four of the parent proteins; Complement C4A, Protein Wnt-2b, Fibrogenen alpha chain (FIBA), and Lipopolysaccharide binding protein (LBP) (Suzuki et al., 2015).

Using ECLIA in a cohort of 57 DLB, 32 PD, and 56 control...
participants, panels of proteins were identified, including several cyto-
kines, that could differentiate DLB and PD from controls (91% sensitivity
and 86% specificity), and DLB from PD (94% sensitivity and 88%
specificity) (O’Bryant et al., 2019). Surendranathan et al. (2018)
assayed cytokines using ELISA and ECLIA and observed increased levels
of macrophage inflammatory protein 3α (MIP-3α), IL-17A and IL-2 as
well as lower levels of IL-8 in 19 DLB compared to 26 controls. This is
evidence of a peripheral immune response associated with DLB.

Loss of function mutations in the PARK7 gene that encodes the
protein DJ-1 are linked to Lewy Body pathology (Repici and Giorgini,
2019). Waragai et al. (2007) found that DJ-1 protein levels were higher
in 30 DLB (and 103 PD) participants compared to 28 controls.

A new and promising technique is the analysis of extracellular ves-
icles (EVs). EVs contain proteins and nucleic acids surrounded by a lipid
bilayer and are released from almost every cell type (Serrano-Pertierra
et al., 2019). Analysing the protein component of plasma-derived EVs
with mass spectrometry Gamez-Valero et al. (2019) found gelsolin
(GSN) and butyrylcholinesterase (BChe) to be differentially expressed
between 19 DLB and 20 controls. GSN is an actin-binding protein and a
key regulator of actin filament assembly that has been identified as a
component of Lewy Bodies and accelerates α-synuclein aggregation in
vitro (Welander et al., 2011).

One of the challenges with mass spectrometry is assigning protein
labels from the identified peptides based on databases of known se-
quences. This leads to missing unknown sequences or post-translational
modifications. Using mass spectrometry and an enrichment approach on
the serum proteome Lundström et al. identified novel peptide sequences
not previously recognised. Splitting participants into a training (DLB =
24, AD = 24) and test group (DLB = 23, AD = 73) to identify the pro-
teinomic signature of each dementia did not lead to an improvement of
diagnostic accuracy (AUC = 0.85) compared to the original protein
model but at the peptide level accuracy was improved from AUC =
0.84-0.94 (Lundström et al., 2017).

7. Peripheral tissue proteomics

Peripheral tissues may also provide more accessible samples for
biomarkers or represent involvement at an early disease stage. For
instance, α-synuclein has been identified in the deposits of skin nerves
of patients with PD and DLB (Ikemura et al., 2008; Wang et al., 2013).
Donadio et al. (2014) found α-synuclein positive deposits in nerves from

cervical skin biopsies in all 18 DLB patients tested, whilst none were
found in 23 patients with non-synucleinopathy dementias. Salivary
glands have been found to contain α-synuclein pathology, with sub-
mandibular Lewy type synucleinopathy identified in 89% of 46 PD cases
and 71% of 28 DLB cases tested, compared to none of the 79 controls
(Beanch et al., 2016).

8. Other biofluids

Urine is an easily accessible biofluid that may allow for the more
widespread use and larger volumes of collection. There have been no
published studies on urine biomarkers in DLB. Urinary proteins, nucleic
acids, and metabolites have been analysed in the urine of AD and PD
cases suggesting there may be a role in DLB in the future (Seol et al.,
2020). Faecal markers of intestinal inflammation were identified in PD
patients, and given the similarities in inflammatory pathways between
PD and DLB this could be another potential source of biomarkers
(Dumitrescu et al., 2021). Tear protein assays recently identified alter-
ations which could be used diagnostically or relating to ocular pathology
in PD (Boerger et al., 2019). The presence of ocular amyloid in AD pat-
ients further suggests the eye as a window to brain biomarkers in
neurodegenerative disorders (Singh and Verma, 2020).

9. Post-translational modifications

Post-translational modifications (PTMs) are at the core of neurode-
genereative pathologies, with extensive PTM of α-synuclein, including
phosphorylation, ubiquitination, nitration, truncation, O-linked-N-acet-
ylglucosaminylation contributing to the aggregation and accumulation
of Lewy Body pathology in PD (Zhang et al., 2019; He et al., 2021).
α-synuclein phosphorylation is linked to synaptic toxicity in DLB, with
phosphorylated α-synuclein identified in both pre and postsynaptic
terminals. These changes were greater in presynaptic regions localized
in small aggregates, leading to changes in the synaptic morphology and
size (Colom-Cadena et al., 2017). α-synuclein ubiquitination has simi-
larly been identified in brain tissue in DLB. Increases in ubiquitinated
α-synuclein were not the result of proteasomal impairment, indicative of
PTMs elsewhere in the α-synuclein aggregation process (Tofaris et al.,
2003). Nitrated α-synuclein aggregates have also been identified in DLB
(Giasson, 2000). Nitration is shown to mediate oxidative stress and
promote nitric oxide production, leading to cellular dysfunction
α-synuclein truncation is shown to promote aggregation (Sorrentino and Giasson, 2020). Truncated α-synuclein has been identified in the amygdala and substantia nigra of DLB patients in a pattern distinguishable from AD (Hass et al., 2021). Targeting PTMs that lead to protein aggregation may be a therapeutic target at the early stages of pathology. There are promising results that suggest interruptions to mechanisms promoting aggregation are beneficial in animal models (Carter and Schaffert, 2020). This area remains in its early stages in DLB but could provide new therapeutic targets, as in the emerging AD and PD field.

10. Therapeutics

(Fig. 2) Clinical trials in DLB have been guided by identifying disease-associated proteins and using protein assays to monitor target engagement and disease response (Table 3). The main approach has been targeting aggregated α-synuclein focusing on the removal of α-synuclein or lowering gene expression (Menon et al., 2022). Given the common pathological pathways among synucleinopathies, there is clear benefit for DLB from introducing these therapeutic agents into clinical trials. Targeted research for DLB therapeutics however remains under-developed. Bosutinib, a dual Abelson/Src inhibitor reduces α-synuclein and induces the immune response in preclinical models of PD. It has subsequently been shown to reduce CSF α-synuclein and dopamine catabolism in patients with DLB in a double-blind clinical trial, possibly attenuating pathology progression (Pagan et al., 2022). Beyond α-synuclein, newly identified inflammatory pathways are being targeted with repurposed drugs. Neflamapimod, a p38 mitogen-activated protein kinase alpha (p38 MAPKα) inhibitor, is under investigation in patients with DLB in a phase 2 clinical trial (NCT04001517). Although awaiting full results, reports suggest a differential response dependent on p-tau181 levels. Fosgonimeton (ATH-1017) is undergoing phase 2 clinical trials for its treatment effects on PDD and DLB (NCT04831281). Ambroxol, a surfactant synthesis stimulator, is also under investigation in a double-blind study for its effectiveness in treating LBD (NCT04405596). Glucocerebrosidase concentration, α-synuclein, Tau, p-tau, Aβ42 in CSF and plasma are secondary outcome measures. Another agent is NYX-458, currently under investigation in MCI due to PD or LBD (NCT04148391). Nilotinib, a tyrosine kinase Abelson inhibitor, was shown to have possible beneficial effects on motor and cognitive outcomes in PD and DLB. Also, there was a decrease in CSF α-synuclein, with not proven significance given the lack of a placebo group (Pagan et al., 2016) and is currently in a phase two trial with CSF HVA as a secondary outcome.

11. Discussion

Current proteome research provides good evidence for protein level changes in DLB; however, the findings are often inconclusive and require additional large-scale research to validate and replicate results. Increasing sample size with consistent methodologies and case definitions will be vital for the development of an LBD proteomics library across tissues. The use of proteomics to improve diagnosis may help to identify prodromal and established α-synuclein pathology, as well as identify pure and mixed pathologies in DLB. Other fields, such as oncology, are benefiting greatly from proteomics with the identification of clinically relevant biomarkers that contribute to therapeutics (Kwon et al., 2021). Combining genome sequencing with protein quantitative trait loci has yielded new insights into both mechanisms and potential therapeutics in other neurological disorders (Png et al., 2021).

One of the main challenges of LBD research is the lack of post-mortem confirmation of diagnosis. DLB often is misdiagnosed as AD, while patients with mixed AD and DLB pathology are rarely distinguished from patients with pure AD or pure DLB pathology (Outeiro et al., 2019). It is common for studies to use different methods of diagnosis including clinical assessment, imaging, and other biomarkers. This introduces heterogeneity that makes it difficult to compare results in meta-analysis (Shinogle, 2012). Measuring peripheral circulating proteins may provide inexpensive and easily accessible biomarkers to support clinical and neuroimaging diagnostic criteria. Fluid biomarkers discussed in this paper demonstrate high diagnostic accuracy (> 80% for both sensitivity and specificity). Using different biomarkers with a range of sensitivities and specificities may be suited to a range of clinical scenarios from supporting a clinical diagnosis to screening (Miller et al., 2017).

Brain proteomic studies in LBD are in the early stages, with small sample sizes and lacking statistical power, often without independent replication. Overcoming these challenges could lead to significant contributions to our understanding of the pathophysiology of LBD and provide a starting point for the development of therapeutics, by highlighting dysregulated proteins and pathways. It could also lead to a greater understanding of the development of mixed pathologies. Both DLB and AD are linked to the dysregulation of protein catabolic pathways. Disruption of autophagy, UPS catabolic pathways, and the ER unfolded protein response are linked with both DLB and AD (Berwick et al., 2015). The Wnt signalling cascade (involved in neurogenesis) has been strongly associated with both AD and synucleinopathies (Berwick and Harvey, 2012) and synaptic dysfunction is a common pathway in DLB and AD. These overlapping pathological pathways may be linked to the common occurrence of mixed pathology or suggest transdiagnostic

### Table 3

| Therapeutic | Trial design | Stage | Protein measures | Identifier |
|-------------|--------------|-------|-----------------|------------|
| Bosutinib   | Phase two randomised placebo-controlled study for mild to moderate Lewy Body dementia | Recruitment completed, awaiting results | Plasma: HVA, DOPAC, Abeta40/42, total tau, p-tau213/181, total α-synuclein, oligomeric α-synuclein | NCT03888222 |
| Neflamapimod | Phase two randomised placebo-controlled study in DLB | Results reported November 2021 | Plasma: p-tau181; patients with < 2.2 pg/ml suggesting less AD pathophysiology had greater treatment effect | NCT04001517 |
| Fosgonimeton | Phase two randomised placebo-controlled study in PDD or DLB | Recruiting | Not reported | NCT04831281 |
| Ambroxol    | Phase one/two randomised placebo-controlled trial in Lewy Body disease | Not yet recruiting | Blood: White blood cell GCase | NCT04405596 |
| NYX-458     | Phase two randomised placebo-controlled trial in Lewy Body disease, mild cognitive impairment, mild dementia, and PD | Recruiting | Not reported | NCT04148391 |
| Nilotinib   | Phase two randomised placebo-controlled trial in Dementia with Lewy Bodies | Recruiting | CSF: HVA PET: amyloid (Florbetaben) | NCT04002674 |
Mass Spectrometry proteomics presents a number of challenges. The need for protein solubilization without uncontrolled peptide fragmentation, especially membrane proteins, is required for MS methods to be applicable (Melby et al., 2021). These proteins often have relevance in neurodegenerative disorders. New methods of liberating membrane proteins and incorporation into membrane mimetic systems without compromising the protein itself, whilst increased solubility, is one technique now being used to address this (Ratkeviciute et al., 2021). The immense dynamic range and complexity of the human proteome can make it challenging to perform adequately powered neuropathology studies as tissue is often limited. Implementing MS with protein separation techniques, such as MALDI-TOF MS with Liquid chromatography can provide high-quality data for top-down proteomics (Fernández-puenteb, 2014). Labelling methods such as ICAT are able to reduce protein solubilization without uncontrolled peptide fragmentation, whilst increased solubility, is one technique now being used to address this (Ratkeviciute et al., 2021).

Computational complexity is rising alongside new bioinformatics, with statistical enrichment analysis and machine learning approaches being implemented. These may improve protein network and pathological pathway analysis, whilst upstream protein analysis is now increasingly reliable and straightforward (Chen et al., 2020).

Proteomic studies have moved from measuring single proteins to large multiplex assays giving an insight into the biology of LBD beyond α-synuclein, amyloid and tau. Studies have highlighted synaptic dysfunction as a common mechanism between neurodegenerative disorders, but also unique aspects of LBDs with and without co-pathology. Large-scale proteomics brings new challenges. To create replicable results, larger sample sizes and standardisation of analytic pathways are required. Putative biomarkers from large-scale proteomics will need to be confirmed with targeted assays in new cohorts. LBD develops over time, and it is likely different pathways predominate across the time course, so longitudinal studies from prodromal to established dementia could highlight optimal timing for trialling interventions. Combining proteomics with genomics, transcriptomics will detail the pathway from risk gene to disrupted protein pathway and combining with clinical rating scales and neuroimaging may lead to prioritisation of target proteins and finally bridge the gap between molecular pathophysiology and clinical outcomes. Relative quantification studies provide valuable information yet remain dimensionless. Next-generation MS techniques introduce more accurate quantification evaluating the absolute levels of proteins across neuropathologies (Altelaar et al., 2013). Proteome profiling is now possible with next-generation MS, being able to perform top-down evaluation of biomarkers, whilst simultaneously recognizing drug targets.

### Table 4

| Brain Region | Study Cohort | Methodology | Observations | Reference |
|--------------|--------------|-------------|--------------|-----------|
| Prefrontal, temporal, anterior cingulate and parietal cortex | 52 DLB | ELISA, Western blotting, Immuno-histochemistry | Reduced levels of RAB3A, SNAP25, and NRGN across brain regions in dementia groups compared to controls. | Bereczki et al. (2016) |
| | 34 PDD | | Significant differences in synaptic proteins in DLB compared to controls. | |
| | 18 AD | | | |
| | 25 controls | | | |
| Prefrontal cortex | 25 DLB | Mass spectrometry, Western blotting, ELISA | Significant differences in proteins in the prefrontal cortex (BA9) in DLB compared to controls: ↓SNAP47 (0.78 fold), ↓SV2C (0.76 fold), ↓LRNF2 (0.68 fold), ↓SYT2 (0.76 fold) | Bereczki et al. (2016) |
| | 24 FDD | | | |
| | 18 AD | | | |
| | 24 controls | | | |
| Olfactory bulb | 4 DLB | Mass spectrometry | 99 proteins (out of 3214) showing significant alternations in DLB | Lachin-Montes et al. (2020) |
| | 4 controls | | | |
| Olfactory bulb | 4 DLB | Mass spectrometry | Differentially expressed protein localized to the excitatory synapse, transmembrane transporter complexes and cytosolic small ribosomal subunits. | Cartas-Cejudo et al., (2021) |
| | 13 AD | | | |
| | 21 PD | | | |
| | 8 FTD | | | |
| | 6 mixed | | | |
| | 12 ALS | | | |
| | 34 controls | | | |
| Substantia nigra, anterior cingulate cortex, entorhinal cortex | 2 DLB | Immunohistochemistry | Parkin colocalized α-synuclein within brainstem and cortical LBs. | (Schlossmacher et al., 2002) |
| | 3 PD | | | |
| | 1 asynuclein-linked PD | | | |
| | 2 controls | | | |
| Temporal cortex, hippocampus | 3 DLB | Immunohistochemistry, Western blotting | TRIM9 decreased in DLB but not in AD and controls. | (Tanji et al., 2010) |
| | 3 AD | | | |
| | 3 controls | | | |
| Cerebral cortex, substantia nigra | 7 pure DLB | Western blotting | Decreased UCHL-1 in cerebral cortex in DLB, and decreased levels in substantia nigra in both PD and DLB. | (Barrachina et al., 2006) |
| | 6 DLB with AD pathology | | | |
| | 6 PD | | | |
| | 6 controls | | | |
| Substantia nigra, locus coeruleus, cerebral cortex | 10 DLB | Immunohistochemistry | Synphilin-1 colocalized α-synuclein in brainstem and well-defined cortical LBs. | (Isaki et al., 2002) |
| | 5 AD | | | |
| | 5 controls | | | |
| Prefrontal, frontal, cingulate, insular, motor, entorhinal, temporal, parietal, occipital cortex | 10 DLB | Tissue microarrays | RAB9B colocalized cortical LBs and amyloid plaques in DLB cases. | (Köss et al., 2021) |
| | 12 AD | | | |
| | 12 controls | | | |
| Cingulate cortex, putamen | 5 DLB | Tissue microarrays, Immunofluorescence | Pre and post-synaptic accumulation of phosphorylated α-synuclein in and LBs and LNs in DLB | (Colom-Cadena et al., 2017) |
| | 5 AD | | | |
| | 5 controls | | | |
| Frontal cortex, substantia nigra | 3 DLB | Immunohistochemistry | Accumulation of ubiquinated α-synuclein in DLB brain regions | (Tofaris et al., 2003) |
| | 3 PD | | | |
| | 3 AD | | | |
| Substantia nigra, amygdala | 5 LBD | ELISA, Immunohistochemistry | Truncated carboxy terminal α-synuclein pattern specific accumulation in in LBs and LNs in LBD | (Hass et al., 2021) |
| | 7 MSA | | | |
| | 3 AD | | | |
| | 4 controls | | | |
Table 5

CSF proteomics studies in Dementia with Lewy Bodies.

| Sample origin | Study Cohort | Methodology | Observations | Diagnostic value | Reference |
|---------------|--------------|-------------|--------------|------------------|-----------|
| Serum, CSF    | 16 DLB       | ELISA       | ↑ serum levels of H-FABP in DLB than AD and controls. | ↑ Serum-H-FABP to CSF-H-FABP ratio DLB vs AD: 100% sensitivity, 63% specificity | Steinacker et al. (2004) |
|               | 18 AD        |             | ↑ H-FABP CSF levels in AD and DLB than controls. | | |
|               | 14 CJD       |             | ↑ H-FABP serum levels in DLB compared to PDD, and in PDD and DLB compared to controls. | ↑ Serum-H-FABP to CSF tau protein levels ratio DLB vs AD: 91% sensitivity, 66% specificity | Mollenhauer et al. (2007) |
|               | 16 controls  |             | ↑ CSF H-FABP in DLB, AD, PDD compared to controls. | | |
| Serum, CSF    | 33 DLB       | ELISA       | ↑ CSF t-α-synuclein in DLB and PD compared to AD and controls | Combining amyloid-β1-42, tau, total α-synuclein, oligomeric α-synuclein, age, and sex, AUC = 0.90 in DLB vs controls | van Steenoven et al. (2018) |
|               | 37 DLB       | Western blotting, ELISA | ↑ levels of CSF α-synuclein in patients DLB, PD, PDD compared to AD and controls | ↑ oligomeric α-synuclein in DLB and PD when compared to AD | Vanderstichele et al. (2006) |
|               | 42 DLB       | ELISA       | ↑ levels of t-tau in AD compared to DLB and controls | Not calculated | |
|               | 46 PD        |             | ↑ levels of Aβ42 in AD and DLB compared to controls | | |
|               | 39 AD        |             | No significant difference in Aβ42 between DLB and AD | | |
|               | 78 controls  |             | | | |
| CSF           | 33 DLB       | Western blotting, ELISA | ↑ p-tau181 in AD compared to DLB and controls | p-tau181, DLB vs AD: 80% sensitivity, 79% specificity | Vanderstichele et al. (2006) |
|               | 38 PD        |             | ↑ levels of Aβ42 in AD compared to PD and controls | | |
|               | 22 PDD       |             | ↑ levels of Aβ42 in AD and DLB compared to controls | | |
|               | 46 AD        |             | No significant difference in Aβ42 between DLB and AD | | |
|               | 52 controls  |             | | | |
| CSF           | 60 DLB       | ELISA       | ↑ p-tau181, p-tau199 and p-tau231 in DLB compared to AD | DLB vs AD: p-tau181: 85% sensitivity, 68% specificity | Hampel et al., 2004 |
|               | 94 AD        |             | ↑ p-tau231 in DLB compared to control | p-tau231: 85% sensitivity, 64% specificity | |
|               | 60 controls  |             | ↑ p-tau181, p-tau199 and p-tau231 in AD compared to controls and the other groups. | p-tau-199: 85% sensitivity, 50-64% specificity | |
| CSF           | 22 DLB       | ELISA       | ↑ p-tau181 in AD compared to DLB and controls | DLB vs AD: p-tau181: 68.2% sensitivity, 82.4% specificity | Wada-Isoe et al. (2007) |
|               | 108 AD       |             | ↑ p-tau181 and p-tau231 in DLB compared to AD | p-tau181/Aβ42 ratio: 72.7% sensitivity, 70.6% specificity | |
|               | 7 VaD        |             | ↑ p-tau181 in DLB compared to control | | |
|               | 24 FTD       |             | | | |
|               | 22 other neurologic disorder | | | | |
|               | 23 controls  |             | ↑ p-tau181 in AD compared to DLB and controls | | |
| CSF           | 22 DLB       | ELISA       | ↑ p-tau181 in AD compared to DLB and controls | DLB vs AD: p-tau181: 68.2% sensitivity, 82.4% specificity | Wada-Isoe et al. (2007) |
|               | 34 AD        |             | ↑ Aβ42 in AD and DLB compared to controls | | |
|               | 37 controls  |             | | | |
| CSF           | 72 DLB       | ELISA       | ↑ Aβ40, Aβ42 in DLB compared to controls | Not calculated | van Steenoven et al., 2019 |
|               | 38 AD        |             | ↑ Aβ38 levels in DLB compared to AD. Lower levels of Aβ were associated with increased cognitive impairment. | | Bibl et al. (2006) |
|               | 38 controls  |             | | | |
| CSF           | 21 DLB       | Western blotting | ↑ Aβ42 in DLB, AD, and PDD compared to controls. Increased Aβ40 * in DLB compared to PDD. | Aβ42/37 ratio, DLB vs AD: 74% sensitivity, 71% specificity | Aerts et al. (2011) |
|               | 21 PDD       |             | | | |
|               | 23 AD        |             | | | |
|               | 23 controls  |             | | | |
| CSF           | 23 DLB       | ELISA       | ↑ CSF MHPG, t-tau, and p-tau in DLB compared to AD. | Combining of p-tau, t-tau, Aβ42, and MHPG for DLB vs AD: 97.6% sensitivity, 95% specificity. | | |
|               | 45 AD        |             | ↑ 5-HIAA (p < 0.01) and ↑ HVA. | | |
|               |             |             | ↑ Aβ42 in DLB compared to AD. | | |
| CSF           | 30 DLB       | ELISA       | No significantly altered levels of t-tau and p-tau181 DLB and pDLB compared to controls. | pDLB vs pAD: t-tau: AUC = 0.93 | Bousiges et al. (2016) |
|               | 35 pDLB      |             | ↑ Aβ42 and Aβ40 levels in DLB compared to controls and prodromal DLB. | p-tau181: AUC = 0.94 | |
|               | 31 AD        |             | | | |
|               | 39 pAD       |             | ↑ Aβ40 and Aβ42 levels in DLB compared to controls. | Aβ40/42: AUC = 0.95 | |
|               | 21 AD+DLB    |             | | | |
|               | 15 controls  |             | | | |
| CSF           | 37 DLB       | ELISA       | ↑ NFL levels in all groups (except DS) compared to controls. | Positive correlation between CSF NFL levels and cognitive impairment in AD and pDLB | Delaby et al. (2020) |
|               | 26 pDLB      |             | ↑ NFL levels in DLB compared to controls. | | |
|               | 116 AD       |             | | | |
|               | 47 DS        |             | | | |
|               | 50 DS+AD     |             | | | |

(continued on next page)
## Table 5 (continued)

| Sample origin | Study Cohort | Methodology | Observations | Diagnostic value | Reference |
|---------------|--------------|-------------|--------------|------------------|-----------|
| CSF 40 DLB    | ELISA        | ↑ H-FABP in AD and DLB when compared to neurological controls. Positive correlation between H-FABP and α-synuclein. A positive correlation between H-FABP and cognitive impairment in the cohort. | H-FABP and p-tau181, DLB vs AD: 95% sensitivity, 76% specificity. H-FABP, α-synuclein, and p-tau181, DLB vs PD: 80% sensitivity, 95% specificity | Chiasserini et al. (2017) |
| CSF 12 DLB    | 125I radioimmunoassay, ELISA | ↑ CART levels in DLB compared to AD and controls. CART levels correlated significantly with levels of p-tau and t-tau. | Not calculated | Schultz et al. (2009) |
| CSF 10 DLB    | Mass spectrometry | Elevated proteins in DLB and AD relative to controls: IGF2, CPE, Proaposin, Malate dehydrogenase, UCHL1, Ubiquitin, Clusterin, Transferrin, CHI3L1, CNDP1, TIMP1, Serum Amyloid A4, IIβP, TREM2, VitrDBP, Cystatin C, ApoL, GM2 activator protein, S100B. Elevated proteins in AD but not in DLB: ENPP2, Transhyretin, Pro-orexin, LAMP1. | Not calculated | Heywood et al. (2015) |
| CSF 5 DLB     | tTRAQ, multidimensional chromatography, mass spectrometry, Western blotting | Greater than 1.5 fold change in DLB vs control: ↑PLRB, ↑AGT, ↑APOC2, ↑CART, ↑CUTA, ↑HPX, ↑ZG168, 15LCL9A10, 1LBP4, 1LYZ, ↑EPOR1, ↑NG3, ↑MYO1F, ↑H4GAT1, ↑PCS2, ↑NUCB1, 1PVALB, ↑PGF2, ↑IFN2, ↑FAM3C, ↑ISLENOV, ↑ITG3, ↑ITGAV, ↑RELN, ↑SOD1, ↑ITR [KFBP1A, ↓PDE12, ↑APOC1, ↓CD99L2, ↓FS, ↓CHRD1L1, ↓DPT, ↓PAF2, ↓ROR5D9, ↓ZYG11B, ↓LAMC1, ↓KIF7, ↓MGAT3, ↓NAMC1, ↓NPTXR, ↓IPENK, ↓NRSN2, ↓ARHGA10, ↓SCM1, ↓SHDGL2B, ↓SST, ↓SORT1, ↓IBG, ↓PPTPN2, ↓CO6AA2, ↓ENPP2, ↓EPHA5, ↓GCDH1, ↓GRM8, ↓SLC24A2, ↓SULF2, ↓CDH13, ↓ZNF277 | DLB vs all others: ApoC1 and Chromogranin B: 50% sensitivity, 95% specificity. ApoC1 and VitrDBP: 50% sensitivity, 95% specificity | Abdi et al. (2006) |
| CSF 8 DLB     | 2D fluorescence difference gel electrophoresis, mass spectrometry | Increased in DLB: ITH4, Calyceatin, COA4, C3, CGA, Prostaglandin-H2, PGD5S, GPX3, TRIB3, CNTN1, TTHY, ENOA, APOA4, ALDOC, KIF2B, B3GNT1. Reduced in DLB: ALBU, ANCT, | Not calculated | Dieks et al. (2013) |
| CSF 37 DLB    | ELISA        | CHI3L1, sTREM2 and progranulin levels did not differ between DLB groups and controls. ↑ CHI3L1 in AD and pAD compared to controls, DLB and pDLB. DLB with a CSF profile suggestive of AD pathology had increased CHI3L1 compared to those without. CHI3L1 correlated with t-tau and p-tau in pDLB and DLB | Not calculated | (Morenas-Rodríguez et al., 2019) |
| CSF 40 DLB/PDD| ELISA, Western blotting, immunohistochemical analysis | ↑ CHI3L1 levels in AD but not DLB/PDD compared to controls. Positive correlation between CHI3L1 levels and tau but not Aβ42. | CHI3L1, DLB/PDD vs controls: 80% sensitivity, 60% specificity | (Llorens et al., 2017) |
| CSF LBD 10    | Multiple reaction monitoring | ↑ α-synuclein in AD and CJD vs controls. Increased β-amyloid/α-syn ratio in PDD compared to PD, and β-syn correlated with CSF tau. | Not calculated | Oeckl et al. (2016) |
| CSF DBL 18    | Suspension bead array | No difference in NRG or GAP43 between DLB, AD or controls. | Not calculated | (Rønnestad et al., 2016) |
### Table 6
Blood proteomics studies in Dementia with Lewy Bodies.

| Sample origin | Study Cohort | Methodology | Observations | Diagnostic value | Reference |
|---------------|--------------|-------------|--------------|-------------------|-----------|
| Serum, CSF    | 16 DLB       | ELISA       | ↑ serum levels of H-FABP in DLB than AD and controls. | Serum-H-FABP to CSF-H-FABP ratio DLB vs AD: 100% sensitivity, 63% specificity | Steinacker et al. (2004) |
|               | 18 AD        | ELISA       | ↑ H-FABP CSF levels in AD and DLB than controls. | | |
|               | 14 CJD       | ELISA       | ↑ H-FABP serum levels in DLB compared to PDD, and in PDD and DLB compared to controls. | Serum-H-FABP to CSF tau protein levels ratio DLB vs AD: 91% sensitivity, 66% specificity | Mollenhauer et al. (2007) |
|               | 16 controls  | ELISA       | ↑ CSF H-FABP in DLB, AD, PDD compared to controls. | Serum-H-FABP to CSF tau protein levels ratio DLB vs AD: 91% sensitivity, 66% specificity | Mollenhauer et al. (2007) |
| Serum         | 25 PDD       | ELISA       | ↑ a-synuclein in DLB compared to AD and controls. | AD vs DLB: 70% sensitivity and 65% specificity | Laske et al. (2011) |
|               | 35 AD        | ELISA       | ↑ IgG anti-a-synuclein antibody in DLB and AD compared to controls. Antibody levels in DLB decreased with disease duration. | DLB vs controls: AUC = 0.714 | Koehler et al. (2013) |
|               | 45 PD        | ELISA       | ↑ DJ-1 in DLB vs controls and early stages of PD | | |
|               | 51 controls  | ELISA       | ↑ GFAPI in LBD and AD vs controls, ↑ NfL in all dementia groups LBD from MCI + AD (AUC p-tau-181 = 0.67, AUC p-tau-231 = 0.67, AUC NfL = 0.55, GFAPI = 0.61) | LBD vs MCI + AD AUC p-tau-181 = 0.67, AUC p-tau-231 = 0.67, AUC NfL = 0.55, GFAPI = 0.61 | Chouliaras et al. (2022) |
| Plasma        | 30 DLB       | SIMOA       | ↑ NfL in controls | | |
|               | 103 PD       | SIMOA       | ↑ DJ-1 in DLB vs controls and early stages of PD | Not calculated | Waragai et al. (2007) |
| Plasma        | 7 90 AD      | SIMOA       | ↑ NfL in all dementia groups LBD from MCI + AD (AUC p-tau-181 = 0.67, AUC p-tau-231 = 0.67, AUC NfL = 0.55, GFAPI = 0.61) | LBD vs MCI + AD AUC p-tau-181 = 0.67, AUC p-tau-231 = 0.67, AUC NfL = 0.55, GFAPI = 0.61 | Chouliaras et al. (2022) |
| Serum         | 371 DLB      | SIMOA       | ↑ NfL in controls, lower than AD, and similar with PD | Not calculated | Gonzalez et al., 2022 |
| Serum         | 203 PD       | ELISA       | ↑ serum-H-FABP in DLB and PD compared to AD and controls. | DLB vs AD: 47.1% sensitivity, 91.3% specificity | Wada-Isoe et al. (2008) |
| Serum         | 53 AD        | Mass spectrometry | DLB compared to controls: ↑Fibrinogen p1462, p1613, p2860 peptides ↑Protein Wnt-2b ↑Collagen alpha-1 (IV) chain ↑Fibrinogen p2988 ↑Complement C4A | 4 peptide model (Wnt-2b, lipopolysaccharide-binding protein, p4090, p5002) DLB vs other, 93.3% sensitivity and 87.9% specificity, 2 peptide model (Complement C4Bn p5002) DLB vs AD, 95% sensitivity and 93.3% specificity | Suzuki et al. (2015) |
|               | 200 AD       | ELISA       | ↑ serum-H-FABP in DLB and PD compared to AD and controls. | DLB vs AD: 47.1% sensitivity, 91.3% specificity | Wada-Isoe et al. (2008) |
|               | 205 healthy controls | | | | |
| Plasma        | 57 DLB       | QuickPlex electrochemiluminescence | ↑ vCAM1 (1.2 fold) ↑ B2M (1.09 fold) ↑ AdipoQ (1.27 fold) ↑ Eotaxin3 (3.5 fold) ↑ MIP1 (1.22 fold) ↑ IL1 (1.1 fold) ↑ IL5 (1.24 fold) ↑ IL1 (1.29 fold) | DLB vs PD controls (using age, TNC, B2M, SAA, IL6, TPO, AdipoQ, A2M, TARC, Eotaxin3): 93.9% sensitivity and 93.3% specificity | O’Bryant et al. (2019) |
|               | 32 PD        | QuickPlex electrochemiluminescence | ↑ vCAM1 (1.2 fold) ↑ B2M (1.09 fold) ↑ AdipoQ (1.27 fold) ↑ Eotaxin3 (3.5 fold) ↑ MIP1 (1.22 fold) ↑ IL1 (1.1 fold) ↑ IL5 (1.24 fold) ↑ IL1 (1.29 fold) | DLB vs PD controls (using age, TNC, B2M, SAA, IL6, TPO, AdipoQ, A2M, TARC, Eotaxin3): 93.9% sensitivity and 93.3% specificity | O’Bryant et al. (2019) |
| Serum         | 19 DLB       | ELISA, electrochemiluminescence | ↑ MIP-3α ↑ IL17A ↑ IL2 ↑ IL8 | DLB vs controls using (MIP-3α, IL8, IL2, IL13, VEGF, CHEL1, and IL16): 71% sensitivity, 87% specificity | Surendranathan et al. (2018) |
| Serum         | 26 controls  | Mass spectrometry | ↑ Transthyreren ↑ Multiple PDZ domain protein ↑ Serum amyloid P ↑ Apolipoprotein D | Peptide model AUC = 0.94 | Lundstrom et al. (2017) |
| Serum         | Training     | Mass spectrometry | ↑ Transthyreren ↑ Multiple PDZ domain protein ↑ Serum amyloid P ↑ Apolipoprotein D | Peptide model AUC = 0.94 | Lundstrom et al. (2017) |

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more dysregulated proteins, and this already is being used in PD research (Dong et al., 2019). (Tables 4–7).

Competing interests

Unrelated to this work, JTOB has received honoraria for work as DSMB chair or member for TauRx, Axon, Eisai, has acted as a consultant for Roche, and has received research support from Alliance Medical and Merck.

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