Extracellular Vesicles as Biomarkers for Neurodegenerative Diseases

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Abstract

For the development of therapies for neurodegenerative diseases, it is essential to have validated biomarkers for diagnosis and monitoring disease progression. Finding relevant biomarkers is difficult because molecules secreted by the cell types involved in neurodegenerative diseases are often diluted and unstable in body fluids such as cerebrospinal fluid and blood. For this purpose, extracellular vesicles (EVs) may be of use. EVs are small particles that are comprised of a membrane which envelops cell-derived biomolecules. EVs are secreted by all cell types, and cargo found in EVs varies according to their origin. EV cargo may reflect the pathological state of central nervous system (CNS) specific cell types in neurodegenerative diseases. EVs from CNS specific cell types can be isolated from body fluids, making EVs a promising tool to detect pathological changes in neurodegenerative diseases.

This review explores the present evidence for the use of extracellular vesicle cargo as potential biomarkers for neurodegenerative diseases such as amyotrophic lateral sclerosis, Huntington's disease, and Parkinson's disease. Evidence is revised on several disease-associated proteins and RNAs that may be used to diagnose neurodegenerative diseases and monitor disease progression during clinical trials. Further validation and standardization are needed.

Layman's summary

Neurodegenerative diseases are brain conditions that cause gradual damage and loss of nerve cells in the brain and/or other parts of the nervous system, leading to problems with cognitive functions and movement. In the search for ways to diagnose and track the progression of neurodegenerative diseases like amyloid lateral sclerosis, Huntington's disease, and Parkinson's disease, scientists are looking for specific markers in the body that can indicate the presence and severity of these diseases. The challenge is that these markers are hard to find because they break down easily and are scarce in body fluids such as blood and brain fluid.

One promising solution to this problem involves using tiny particles called extracellular vesicles (EVs), which are capsules that carry molecules secreted by cells in the body. These EVs can be isolated from body fluids and may contain specific molecules that provide information about the state of specific brain cells involved in these diseases.

This review examines the current evidence for using the content of EVs as potential markers for neurodegenerative diseases. It is found that changes in the content of EVs, such as proteins and RNA molecules, in the body fluids of patients can be used to diagnose and monitor disease severity. However, more research is needed to confirm these findings and standardize their use.

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1. Introduction

Despite decades of research, effective treatments for neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and Parkinson's disease (PD) remain to be developed (Sudhakar and Richardson, 2019). These diseases are characterized by a progressive degeneration of neurons mainly in the central nervous system (CNS), leading to cognitive decline and motor problems. One of the hallmarks that is shared by these diseases is the accumulation of aggregated proteins within neurons (Peng et al., 2020). When developing disease-modifying treatments for neurodegenerative diseases, it is essential to monitor the progression of the disease. For this purpose, biomarkers which allow to accurately perform early diagnosis, that may serve as inclusion criteria in clinical trials, and that allow monitoring treatment outcomes and disease progression (in parallel with clinical monitoring) are a great unmet medical need. Halting the progression of the disease earlier can be of vital importance to patients (Bib, 2005).

An example of a general biomarker of neurodegenerative diseases is neurofilament light chain (NfL; Gaetani et al., 2019). NfL is a subunit of the neurofilament cytoskeleton and has a role in structural stability of neurons. When axons are damaged, they release increased amounts of NfL, which can be monitored in cerebrospinal fluid (CSF) and blood. However, NfL is not a specific marker for any particular type of neurodegenerative disease. For this purpose, other specific and reliable biomarkers for the different neurodegenerative diseases are needed (Bib, 2005). Proteins that are prone to aggregate may be detected in CSF and blood, but concentrations are extremely low, making them less reliable (Thompson et al., 2016). Nucleic acids such as microRNAs (miRNAs) could also be used as biomarkers as they can be amplified using polymerase chain reaction (PCR), but concentrations can still be too low for detection (Thompson et al., 2016).

A solution to the problem of biomarker detection may be through extracellular vesicles (EVs). EVs are small cell-derived particles comprised of a membrane that envelops biomolecules. EVs are involved in intercellular communication, transport of molecules and secretion of waste products. Two main categories of EVs exist: exosomes and microvesicles (Cano et al., 2023). Exosomes are formed intracellularly by invagination of the membrane of multivesicular endosomes (MVE), creating intraluminal vesicles, which are usually 30 to 100 nm in diameter (Cano et al., 2023). They are released extracellularly when MVEs fuse with the plasma membrane. Microvesicles are formed by direct budding of the plasma membrane and are usually around 50 to 1000 nm in diameter (Cano et al., 2023).

Seeing that EVs are involved in intercellular communication, much of the metabolism within a cell is reflected in the cargo of EVs, making them great candidates for the use of biomarkers. It has already been shown that EVs play a significant role in the pathophysiology of neurodegenerative diseases such as ALS, HD and PD (Cano et al., 2023). Furthermore, EVs can be isolated from many body fluids, such as blood and CSF, allowing for enrichment of target molecules at protein or miRNA level (Thompson et al., 2016). Surface markers on EVs can be recognized to identify the cell type origin of the EVs. Lastly, isolated EVs are highly stable if stored properly, thus shielding their contents from degradation (Görgens et al., 2022).

This literature review will cover the recent literature on EV biogenesis and function, and the involvement of EVs in neurodegenerative diseases. This review further explores the potential role of EVs as biomarkers in the context of diagnosing and monitoring neurodegenerative diseases.

2. Extracellular vesicles biogenesis and cargo loading

Previously, secreting EVs was regarded merely as a mechanism of waste disposal pathways. Now, EVs are recognized as essential parts of intercellular communication. EV research has shifted the focus towards the mechanisms by which EVs are employed to exchange molecules intercellularly, such as proteins, nucleic acids and lipids, how cells use EVs to signal to other cells and how these mechanisms are involved and altered in pathological conditions (Lo Cicero et al., 2015; van Niel et al., 2018). EV is a broad term encompassing many lipid bilayer-enclosed membrane vesicles with heterogeneous sizes of 30-2000 nm in diameter. As stated above, EVs can be subdivided by origin into two main categories: exosomes and microvesicles, but other categories of EVs also exist such as apoptotic bodies, which are formed when cells undergo apoptosis, and migrasomes, which are MVBs releasing their vesicles during cell migration (Battistelli and Falcieri, 2020; Ma et al., 2015). Isolation of EVs from bodily fluids or cell culture medium, results in a heterogeneous mix of different subtypes of EVs (Willms et al., 2016). This complicates distinguishing between the different origins of specific EVs. Research into the biogenesis and cargo selection pathways of different EV subtypes provides answers to how EVs can be categorized (Colombo et al., 2014). This chapter will cover how cells orchestrate formation and secretion of exosomes and microvesicles, and how different cargoes such as proteins, RNAs and lipids are loaded into EVs.

Exosomes and microvesicles are formed through different mechanisms, but both start with trafficking of cargo to the membrane; for exosomes this is the endosomal membrane and for microvesicles the plasma membrane. Cargo is then enriched in patches at the membrane, which undergoes bending followed by fission and release of the vesicle. The type and quantity of cargo selected for EV secretion is cell-type specific and dependent on the molecular mechanisms at play in healthy and diseased cells (van Niel et al., 2018).

2.1 Exosome biogenesis

Exosome biogenesis is started by inward budding of ILVs into the lumen of endosomes, creating MVEs, which fuse with the plasma membrane to secrete exosomes. Many proteins that are involved in exosome formation have been found (Dixson et al., 2023). The main exosome biogenesis pathway is the endosomal sorting complex required for transport (ESCRT) pathway. This pathway broadly consists of 4 complexes involved in cargo selection, budding and scission of the forming exosome, named ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, which are recruited sequentially towards the endosome (Dixson et al., 2023). ESCRT-0 and ESCRT-I recruit ubiquitylated transmembrane proteins such as epidermal growth factor receptor (EGFR) and other cargo towards the endosomal membrane (Raiborg et al., 2001). Furthermore, ESCRT-0 recruits clathrin to the early endosome, which allows for clustering ESCRT-0 together with cargo to the forming ILV (Raiborg et al., 2006). Additionally, ESCRT-I and ESCRT-II are involved in endosome membrane invagination to create the ILVs (Henne et al., 2011). Next, ESCRT-II recruits ESCRT-III polymers that remodel the membrane, leading to scission and release of the ILV into the endosome interior, creating MVEs (Bertin et al., 2020). Lastly, vacuolar protein sorting-associated protein 4 (VPS4) removes the ESCRT-III polymers from the membrane and may have a role in scission of the ILV as well (Adell et al., 2017). Another variation on the ESCRT-pathway involves syntenin and the ESCRT accessory protein ALG-2 interacting protein X (ALIX; Baietti et al., 2012). These proteins recruit distinct cargo into clusters, after which ESCRT-III is recruited by ALIX, leading to scission of the ILV.

Knock-down of several key ESCRT proteins does not prevent MVE formation indicating redundancy in exosome formation (Stuffers et al., 2009). We now know that several other ESCRT-independent pathways of exosome formation exist. One of these pathways uses neutral type II sphingomyelinase (nSMAse2, or SMPD3) to hydrolyze sphingomyelin to ceramide, both of which are lipid molecules

found in membranes (Goñi and Alonso, 2009). Ceramide possesses a conical shape which can form curvatures in the membrane leading to ILV formation (Goñi and Alonso, 2009). Furthermore, ceramide is metabolized to sphingosine 1-phosphate, which is the ligand for the inhibitory G protein (Gi)-coupled sphingosine 1-phosphate (S1P) receptors, which are involved in exosomal MVE maturation and in cargo sorting into ILVs (Kajimoto et al., 2013). The nSMAse2 is regulated by neutral sphingomyelinase activation associated factor (NSMAF, also known as FAN), which interacts via its own LIR-domain with lipidated LC3 (LC3-II). This pathway became known as LC3-Dependent Extracellular vesicle Loading and Secretion (LDELS; Leidal et al., 2020; Leidal and Debnath, 2020).

Another class of proteins that is involved in the biogenesis of exosomes is the family of tetraspanins. Tetraspanins are transmembrane proteins that contain four transmembrane helices that are oriented through the membrane (Charrin et al., 2014). Multiple tetraspanins, such as CD9, CD63, CD37, CD81 and CD82 are enriched in EVs and are even used as EV biomarkers (Andreu and Yáñez-Mó, 2014). Tetraspanins are able to interact with each other, with other transmembrane and cytosolic proteins, and with some lipids, forming tetraspanin-enriched microdomains (TEMs) on cell membranes. The shape of tetraspanins, like ceramide, is conical, allowing for clusters of tetraspanins to bend the membrane and promote EV biogenesis (Umeda et al., 2019; Zimmerman et al., 2016). Furthermore, tetraspanins have a role in cargo selection to exosomes. By analyzing the interactome of main components of TEMs, it was found that almost half of the proteins found in exosomes are part of this interactome (Perez-Hernandez et al., 2013).

2.2 Microvesicle biogenesis

Microvesicles are formed by outward budding of the plasma membrane, after which they undergo scission and are released. Microvesicles have a large range of sizes of around 50 to 1000 nm in diameter, indicating that different mechanisms are at play in the biogenesis of microvesicles. Similar to exosome biogenesis, a lot of the same ESCRT machinery and tetraspanins have a role in microvesicle formation. For example, proteins from the ESCRT-I complex, notably TSG101, are recruited by arrestin domain-containing protein 1 (ARRDC1) to the plasma membrane to initiate formation of ARRDC1-mediated microvesicles (ARMMs), a subtype of microvesicles (Wang and Lu, 2017). Furthermore, VPS4 is also required in this pathway to finalize microvesicle formation (Nabhan et al., 2012). Tetraspanins CD9 and CD81 interact with EWI-2 and EWI-F, which link to the actin cytoskeleton, allowing for plasma membrane reorganization and budding into classical microvesicles (Sala-Valdés et al., 2006; Umeda et al., 2020).

Other mechanisms involved in the biogenesis of microvesicles are related to rearrangements of the plasma membrane by changes in Ca^{2+} levels and consequently lipid composition (van Niel et al., 2018). Flippases, floppases, scramblases and calpain are able to move phospholipids between the inner and outer membranes, which is dependent on Ca^{2+} (Bricogne et al., 2019). The movement of phospholipids leads to enrichment of phosphatidylserine on the outside of the plasma membrane, which can cause bending and microvesicle formation. In addition to lipid changes, the cytoskeleton plays an important role in the formation of microvesicle. Small GTPase RhoA regulates actin polymerization on the inside of the plasma membrane, followed by contraction of actomyosin, which causes large microvesicle formation (Antonyak et al., 2012).

As shown here, there are a large amount of mechanisms that work together or independently to attribute to the biogenesis of exosomes and microvesicles. Depending on the cell type, cell metabolism and cargo, different EV biogenesis pathways can be instructed (van Niel et al., 2018).

2.3 Cargo loading of RNA

As described, protein cargo is loaded into EVs by the ESCRT pathway, through lipid domains on membranes and via interaction with tetraspanins. Besides proteins, nucleic acids, such as messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA (lncRNA) and DNA, can also be found inside EVs (Fabbiano et al., 2020). For the most part, the RNA content in the cytoplasm is reflected in the EVs coming from the cell. For example, overexpression of certain targets of miRNA decreased the amount of these miRNA in EVs, because of their target association (Squadrito et al., 2014). Furthermore, overexpression of miRNAs, lead to more availability of miRNAs in EVs (Squadrito et al., 2014). Nevertheless, some RNAs seem to be relatively more common in EVs than other RNAs, and different types of EVs contain different RNA contents. Overall, this indicates that at least some RNA cargo loading is regulated in a selective manner (Fabbiano et al., 2020; Willms et al., 2016).

The main mechanism of RNA cargo loading in forming EVs is via association with RNA binding proteins (RBPs). Many regulators of RNA cargo loading have been identified, such as heterogeneous nuclear ribonucleoproteins (hnRNPs), Y-box binding protein 1 (YBX1), Human antigen R (HuR), Argonaute 2 (AGO2) and IGF2BP1 (Fabbiano et al., 2020). These proteins have RNA-binding domains that can bind to specific sequences in RNAs. Furthermore, many of these proteins can selectively be loaded into EVs through interactions with exosome or microvesicle machinery (Fabbiano et al., 2020). Besides cargo loading through interactions with RBPs, RNAs can also directly interact with certain lipid formations in the membrane. Through electrostatic interactions, RNA is directed to lipid rafts high in sphingomyelin, cholesterol and ceramide (Janas et al., 2006).

3. EVs in the central nervous system

In the CNS, EVs are secreted by both neurons and glial cells. High resolution tandem mass spectrometry of EVs isolated from human CSF has identified EVs that contain neuronal or glial cell markers (Chiasserini et al., 2014). Exchange of EVs by neurons and glial cells plays a big role in cellular communication in the brain (Basso and Bonetto, 2016; Budnik et al., 2016; Lizarraga-Valderrama and Sheridan, 2021). Communication via EVs is important for promoting neuronal homeostasis and survival, maintenance of myelination, and synaptic plasticity (Antonucci et al., 2012; Krämer-Albers et al., 2007). EV secretion is controlled by neurotransmitter signaling and thus electrical activity of neurons. The excitatory neurotransmitter glutamate secreted from neurons can instigate the release of EVs by neurons and oligodendrocytes (Frühbeis et al., 2013; Lachenal et al., 2011). Furthermore, ATP, which is released from synapses, activates glial P2X7 receptor, which in turn leads to EV release from microglia and astrocytes (Antonucci et al., 2012; Bianco et al., 2009). It is important to readily distinguish EVs from synaptic vesicles, as the latter have a different biogenesis and function (Takamori, 2009), although both types of vesicles can co-exist at the axon terminal (Janas et al., 2016).

Besides communication within the CNS, neurons and glial cells are also able to exchange EVs with the periphery. For this exchange, transport over the protecting barrier of the brain or the blood-brain barrier (BBB) is needed. The BBB selectively ensures shuttling of molecules and particles between the bloodstream and the CSF. Furthermore, it protects the brain from damage by immune cells, bacteria and viruses and other sources. It has been shown that EVs from the CNS and the periphery can cross the blood-brain barrier (BBB), but how EVs are able to cross is still uncertain (Banks et al., 2020). Under healthy physiological conditions, most EVs from several sources are generally able to be taken up from the blood into the CSF (Banks et al., 2020). However, via which mechanism and how efficiently they cross the BBB varies between EV cell origin and inflammatory status. A study by Chen et al. used an *in vitro* model of a BBB showing that EVs can cross the BBB through receptor-mediated transcytosis, lipid raft-mediated transport and micropinocytosis under stroke like conditions (Chen et

al., 2016). There is even less understanding on how EVs are transported from the CNS towards the periphery. Evidence suggests that EVs can cross arachnoid granulations from the CSF towards the bloodstream (Glimcher et al., 2008). Furthermore, inflammation in the CNS can lead to EV release from astrocytes and brain endothelial cells into the periphery (Couch et al., 2017; Dickens et al., 2017). Lastly, neurodegenerative diseases are known to make the BBB more permeable, leading to, among other things, more release of EVs from the CNS (Hill, 2019).

4. EVs in neurodegenerative diseases and prion-like spread of aggregates

Apart from their vital physiological roles in the CNS, EVs are believed to play a role in the development of neurodegenerative diseases. There are many mechanisms at play that lead to neurodegenerative diseases, but in the pathophysiology of amyotrophic lateral sclerosis ALS, HD and PD, among others, aggregation of proteins is a shared cause. Protein aggregates are clusters of misfolded or fragmented proteins that have agglomerated and undergone liquid-liquid phase separation (LLPS), leading to disruptions in cellular function via multiple mechanisms (Zbinden et al., 2020). Protein aggregates cause loss of function of the aggregated proteins, but also toxic gain-of-function, such as impairment of protein degradation pathways, mitochondrial dysfunction, axonal transport and synaptic toxicity (Hipp et al., 2014; Moon and Paek, 2015; Terwel et al., 2002; Vanden Broeck et al., 2014). In contrast, protein aggregation could also be an effect of other upstream causes and may even be a neuroprotective factor in some cases (Chen et al., 2018).

Evidence suggests that EVs are involved in spreading of the protein aggregates from cell to cell in neurodegenerative diseases. The first disease type that was discovered to make use of EVs to spread aggregates in the CNS were prion diseases, such as kuru and Creutzfeldt-Jakob disease (Liu et al., 2017). Prion diseases are characterized by infectious misfolded prion proteins (PRPs). Misfolded PRPs can induce other ubiquitously expressed PRPs to be misfolded as well, in a chain reaction that allows for the formation of large aggregates. Symptoms from PRP aggregates in the CNS are characterized by astrogliosis and spongiform degeneration. Misfolded and aggregated PRP proteins are actively loaded into exosomes for secretion (Arellano-Anaya et al., 2015; Fevrier et al., 2004). Furthermore, stimulating or inhibiting exosome secretion in a transwell system leads to respectively increased or decreased PRP spreading (Guo et al., 2016). Similar to prion diseases, neurodegenerative diseases, such as ALS, HD and PD display formation of misfolded protein aggregates. Moreover, evidence shows that misfolded proteins and aggregates spread between cells via EVs in a prion-like manner (Alpaugh et al., 2022; Danzer et al., 2012; Münch et al., 2011) (**Figure 1**).

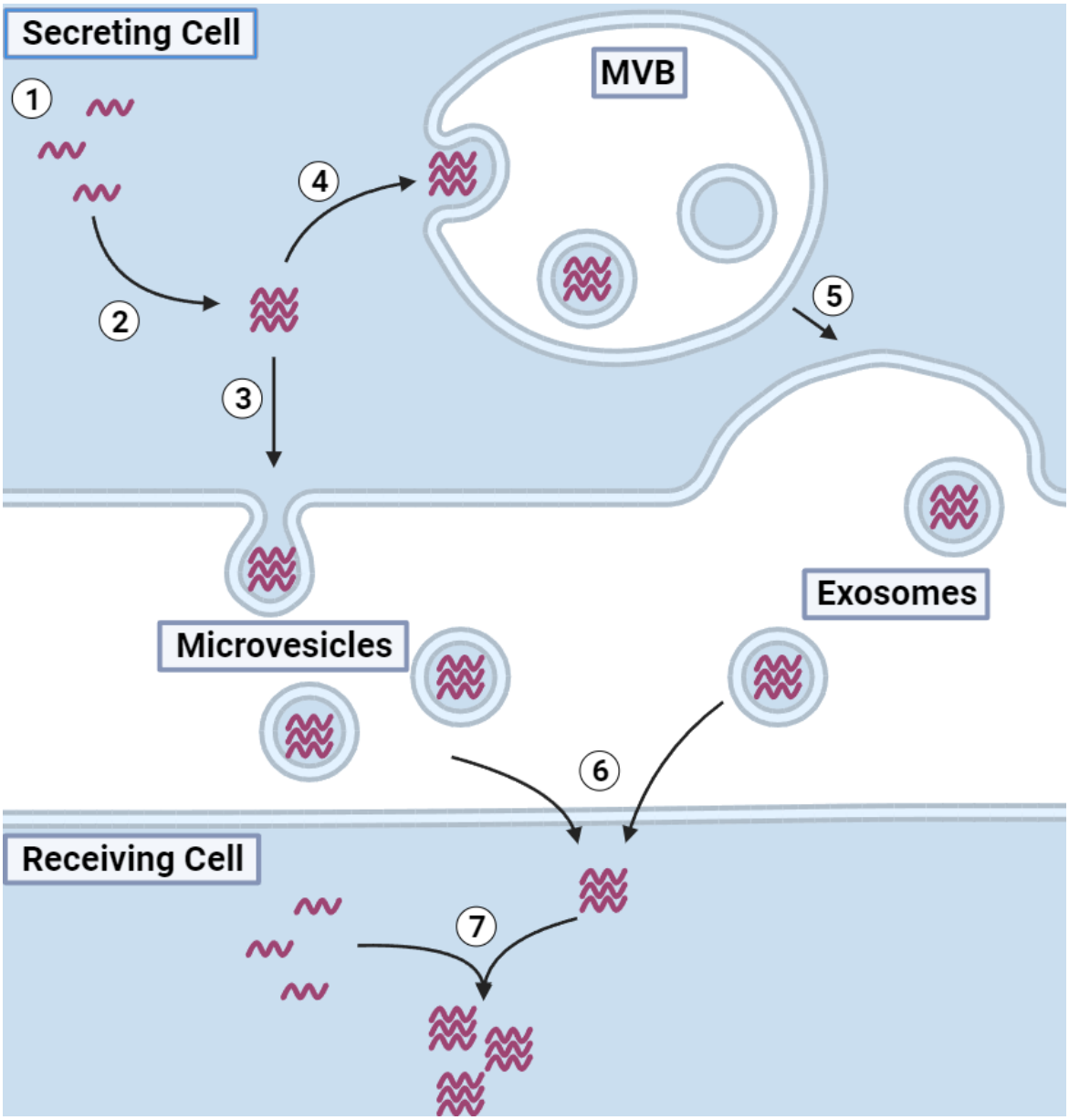

Figure 1. Prion-like spreading of misfolded proteins via extracellular vesicles (EVs). *Proteins involved in neurodegeneration (1), such as TDP43, mutant HTT and α-syn, become misfolded and aggregate (2). Misfolded protein aggregates are loaded into microvesicles, which bud outwards from the plasma membrane (3), or into exosomes in multivesicular bodies (MVBs; 4), which fuse with the plasma membrane to release their contents (5). Microvesicles and exosomes can be taken up by other cells through, for example, fusion with the plasma membrane, releasing the protein aggregates into the receiving cell (6). The misfolded protein aggregates act as a template for natively expressed proteins to aggregate, leading to prion-like spread of neurodegenerative diseases (7).*

5. EVs as biomarkers for neurodegenerative diseases

In the development of disease modifying treatments for neurodegenerative diseases, in addition to clinical symptoms monitoring (for example, motor symptoms), it is necessary to study the disease progression of patients carefully using biomarkers to monitor treatment target engagement and its downstream effects. However, the CNS is a very sensitive organ, which makes taking biopsies for disease monitoring not feasible (Deverman et al., 2018). Biofluids on the other hand can be contaminated and relevant molecules are more likely to be diluted and non-detectible (Thompson et al., 2016). Because EVs have been loaded with cargo directly from the cytoplasm, it means that they more closely reflect the metabolism of the cell. They are also secreted from the CNS into the CSF

from which they can be transferred to the bloodstream, where they are highly stable. The EV cargo is protected from the environment by the double membrane, which means that the cargo can be stored without degrading for a longer time (Görgens et al., 2022). They can be isolated from less invasive liquid biopsies of the CSF or blood to gain information about the pathophysiology in the CNS (**Figure 2**). Taking a liquid biopsy from blood is less invasive than taking CSF from patients and would therefore be preferred. However, the ratio of CNS-derived to periphery-derived EVs will be much lower in the blood. To circumvent this, if needed, different neuronal and astrocytic markers can be used to purify the CNS EVs from blood using immunopurification (Katsu et al., 2019). EVs reflect biological changes in neurodegenerative diseases already from early stages, allowing for the use of EVs as a diagnostic tool or as a way of monitoring disease progression (Thompson et al., 2016).

Understanding which molecules are differentially loaded into EVs in patients with neurodegenerative diseases is the key to developing biomarkers based on isolated EVs. Recently, several different proteins and RNAs have been discovered to be increased in EVs isolated from CSF and blood in ALS, HD, and PD. The next chapter will cover which EV-based biomarkers could be used to diagnose or monitor disease progression of these neurodegenerative diseases.

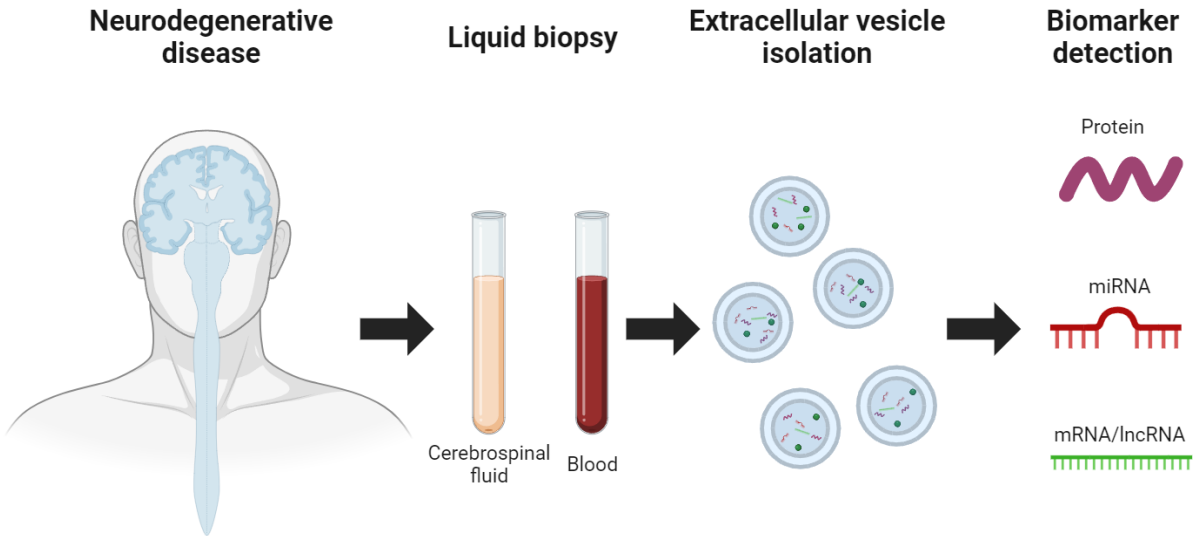

Figure 2. Simplified pipeline of extracellular vesicles (EVs) isolation for biomarkers. *To diagnose or monitor neurodegenerative disease progression, liquid biopsies from the cerebrospinal fluid (CSF) or blood can be taken. EVs are isolated from CSF and blood via, for example, differential ultracentrifugation, size-exclusion chromatography, or poly-ethylene glycol-based precipitation. Cargo of EVs, such as proteins, microRNAs (miRNAs), messenger RNAs (mRNAs) or other long non-coding RNAs (lncRNAs) can be quantified to measure differences between patients and healthy individuals.*

6. EVs as biomarkers in ALS

ALS is a neurodegenerative disease characterized by rapid progressive degeneration of the upper motor neurons in the motor cortex and the lower motor neurons of the spinal cord, leading to paralysis and an accelerated death (Dhasmana et al., 2022). ALS can be subdivided into two main categories, familial ALS (fALS), which occurs due to inherited specific genetic mutations, and sporadic ALS (sALS), which occurs without a specific known genetic mutation, but can be developed due to multiple genetic or environmental factors. Only 5-10% of ALS cases are fALS, whereas 90-95% are sALS (Dhasmana et al., 2022). The main genes that are involved in fALS are copper- and zinc-containing antioxidant superoxide dismutase 1 (SOD1), TAR DNA-Binding Protein 43 (TDP43), fused in sarcoma (FUS), ubiquilin 2 (UBQLN2), and C9ORF72, but many more genetic factors exist as well (Vijayakumar et al., 2019). sALS cases are thought to come from environmental risk factors, DNA damage and epigenetic modifications, and/or changes in multiple genes. In motor neurons, these

factors contribute to degeneration, for example, by protein aggregation, prion-like spread of aggregates, glutamate excitotoxicity, autophagy inhibition, mitochondrial dysfunction, nucleolar stress and oxidative stress (Barbo and Ravnik-Glavač, 2023).

A lot of attention has been given to developing biomarkers for ALS. However, only a few validated biomarkers are available. Some biomarkers with prognostic value from the CSF include neurofilament accumulation, tau expression, oxidative stress biomarkers, and miRNAs (Dhasmana et al., 2022). Blood biomarkers also exist, including C9ORF71, SOD1, TDP43, increased DNA methylation, and inflammatory markers (Dhasmana et al., 2022).

6.1 EV number and size as biomarker for ALS

The first EV-based biomarkers of ALS derived from the CSF was an increase in leukocyte-derived microvesicles (Zachau et al., 2012). Later, it was found that the amount of leukocyte-derived microvesicles was correlated with a slow disease progression, but not with fast disease progression (Sproviero et al., 2019). Furthermore, the mean size for microvesicles and exosomes extracted from blood plasma increased in ALS patients compared to control (Sproviero et al., 2018). However, in this study no difference in number of EVs was found (Sproviero et al., 2018). Another study found no difference in number of EVs or EV size in CSF from ALS patients (Thompson et al., 2020). These contradictory results may be caused by differences in EV isolation methods, size measurements and ALS patient populations.

6.2 Protein cargo of EVs as biomarker for ALS

One of the most commonly mutated genes in patients with fALS is SOD1. SOD1 is an enzyme that functions as an antioxidant (Peggion et al., 2022). Mutations lead to loss-of-function of SOD1 and can also cause protein aggregation (Peggion et al., 2022). SOD1 does not contain a secretion signal, but nonetheless is secreted by cells. It has been found that SOD1 is associated with exosomes in EV isolates from ALS cellular models (Gomes et al., 2007). Mutant SOD1 aggregates are able to enter neurons to act as seeds for further aggregation in a prion-like manner (Münch et al., 2011). Besides micropinocytosis of SOD1 aggregates from dying cells, the prion-like propagation of SOD1 aggregates can also happen via exosome-dependent pathways (Grad et al., 2014).

TDP-43 is one of the major proteins impaired in almost all forms of fALS and sALS (except for SOD1-related fALS) and is required for RNA stability, mRNA translation and RNA splicing (Riku et al., 2021). TDP-43 is normally mostly located inside the nucleus. However, in ALS, TDP-43 mislocalizes to the cytoplasm where it forms aggregates (Blokhuis et al., 2013). The C-terminal region of TDP-43 contains a similar glutamine/asparagine domain that is also found in PrP, revealing that TDP-43 aggregation may spread in a similar way (Guo et al., 2011). Indeed, introducing insoluble TDP-43 aggregates to cell lines, lead to more TDP-43 aggregation within these cells (Nonaka et al., 2013). Furthermore, the same study found TDP-43 in exosomes isolated from these cells (Nonaka et al., 2013). Another study found that TDP-43 is secreted through exosomes in glioblastoma Neuro2a cells and that inhibition of exosome secretion pathway increased aggregation within these cells (Iguchi et al., 2016). Additionally, exosomal TDP-43 and C-terminal fragments of TDP-43 are upregulated in exosomes from human ALS brains (Iguchi et al., 2016).

In EVs isolated from the CSF of ALS patients, multiple proteins have been found to be differentially expressed. As of yet, SOD1 has not been found in EVs isolated from the CSF, even though it is present in the CSF of ALS patients (Grad et al., 2014; Kim et al., 2022). On the other hand, TDP-43 and C-terminal fragments of TDP-43 have been found in EVs isolated from CSF of ALS-frontotemporal dementia (FTD) patients (Ding et al., 2015). Another study used liquid chromatography-tandem mass spectrometry proteomics to identify differentially expressed biomarkers in isolated EVs from sALS-

derived CSF (Hayashi et al., 2020). The analysis revealed three proteins that were increased in CSF-derived exosomes and thirteen that were decreased compared to control. The level of Novel Inhibitor of histone acetyltransferase (INHAT) Repressor (NIR) was increased in exosome fractions from the CSF of ALS patients and the localization in exosomes was confirmed with immunoelectron microscopy (Hayashi et al., 2020). They also found that NIR was mislocalized from the nucleus in motor neurons of ALS patients, indicating that NIR may play a role in pathophysiology through nucleolar stress. Using similar proteomics methodology, Thompson et al. showed that Bleomycin hydrolase (BMH) is increased in EVs isolated from ALS-derived CSF compared with control, whereas several proteasome core complex proteins were decreased (Thompson et al., 2020). BMH and proteasome core complex proteins are involved in protein degradation and thus may be implicated in ALS.

Sproviero et al. showed that ALS associated proteins SOD1, TDP-43, phosphorylated TDP-43 (pTDP-43) and FUS were enriched in microvesicles isolated from ALS patient-derived plasma, but not in exosomes, compared to control plasma (Sproviero et al., 2018). Pasetto et al. found a significant enrichment of phosphorylated TDP-43 in isolated EVs from ALS patients plasma (Pasetto et al., 2021). However, the phosphorylated TDP-43 was observed outside of the EVs via Transmission Electron Microscopy (TEM) with gold staining. This suggested to the researchers that phosphorylated TDP-43 may associate with other proteins stuck to the outside of EVs. The amount of phosphorylated TDP-43 associated with EVs was not significantly different between ALS patients and control (Pasetto et al., 2021). Chen et al. did find an increase in TDP-43 in plasma-derived exosomes in ALS patients (Chen et al., 2020). Patients participating in this study were followed up for 1, 3, 6 and 12 months, with patients showing increase in exosomal TDP-43 during disease progression (Chen et al., 2020). It is worthwhile mentioning that these studies had different methodologies of vesicle isolation, the study of Chen et al. focused specifically in CD63+/TDP43+ EVs analyzed FACS-based techniques after isolation by immunoaffinity purification methods, while the study of Pasetto et al. isolated EVs via ultracentrifugation and nickel-based isolation techniques.

Coronin-1a (CORO1A) is an actin binding protein, involved in cytoskeleton regulation in immune cells and neurons (Zhou et al., 2022). In exosomes extracted from the plasma of ALS patients, a 5.3 fold increase of CORO1A was found that correlated with disease progression, making CORO1A a potential biomarker for ALS (Zhou et al., 2022).

6.3 miRNA cargo of EVs as biomarker for ALS

Besides proteins, several miRNAs have been found to be alternatively expressed in EVs from ALS patients. The advantage of using miRNAs as biomarkers over proteins is the increased sensitivity of the methods, with PCR allowing amplification of signals. One study that used EVs isolated from CSF found miR-124–3p to be increased in male ALS patients and this increase correlated with the ALSFRS-R score, which measures disease stage (Yelick et al., 2020).

Many studies have investigated using miRNA in EVs isolated from blood of ALS patients as potential biomarkers. Several miRNAs were found to be either upregulated or downregulated in patients compared to control (Banack et al., 2020; Katsu et al., 2019; Saucier et al., 2019; Sproviero et al., 2021). However, little overlap between the studies' results was found, some being contradictory. A possible explanation for the studies' variability could be the small patient groups used (6 to 14 patients). In a follow-up study by Banack et al. they used a larger group of 45 sporadic ALS patients to determine their previous study's repeatability (Banack et al., 2022). Neuronal EVs were isolated by immunoaffinity purification from blood plasma samples using the L1 cell adhesion molecule

(L1CAM). They found two upregulated miRNAs miR-151a-5p and miR-146a-5p, and three downregulated miRNAs miR-4454, miR-10b-5p, and miR-29b-3p (Banack et al., 2022).

6.4 mRNA cargo of EVs as biomarker for ALS

Using RNA sequencing techniques, exosomes isolated from CSF of ALS patients revealed 543 altered mRNAs compared to healthy control CSF (Otake et al., 2019). The main gene transcript that was only detected in ALS samples but not in healthy controls was CUE domain-containing protein-2 (CUEDC2). This protein is related to the ubiquitin-proteasome pathway, which is impaired in ALS. CUEDC2 may therefore be a biomarker for ALS (Otake et al., 2019).

7. EVs as biomarkers in HD

HD is an autosomal dominant hereditary neurodegenerative disease. Patients suffer progressive motor disorders, behavioral problems and cognitive decline, eventually leading to death (Dayalu and Albin, 2015). HD is caused by an expansion of the CAG repeats in the first exon of the huntingtin gene (*HTT*). Penetrance, that is, the degree of certainty of HD development during one's lifetime, depends on CAG repeat number. While penetrance is rare for carriers of 36 to 39 repeats, carriers of 40 or more repeats have full penetrance. Furthermore, disease severity and onset are correlated to the expansion length, with childhood-onset HD occurring in carriers with longer CAG repeats. Moreover, the CAG repeat length in HTT exon-1 (HTT-ex1) is prone to somatic instability, leading to further expansion. The expansion leads to a misfolded mutated HTT (mHTT) protein with an expanded poly-glutamine (polyQ) stretch that can aggregate (van der Bent et al., 2022). Additionally, the expansion leads to missplicing of mRNA, causing the formation of mHTT-ex1 protein fragments, which also form aggregates (Sathasivam et al., 2013). mHTT-ex1 fragments can also be formed through proteolytic cleavage of the full length mHTT (Landles et al., 2010). These mHTT-ex1 fragments were shown to be more toxic than the full-length mHTT protein (Barbaro et al., 2015). Through toxic gain-of-function, but possibly also via loss-of-function, several processes are disturbed. These include transcriptional interference, cytoskeletal disruption, inhibition of proteosome and autophagy proteolysis, mitochondrial stress and excitotoxicity, leading to degeneration of, primarily, the medium spiny neurons (MSNs) of the striatum (Dayalu and Albin, 2015).

Diagnosis of HD is usually straightforward as family history and genetic testing for the CAG expansion can give insight into the disease progression at an early age (Stoker et al., 2022). Disease progression is monitored through The Unified Huntington's Disease Rating Scale, which is a standardized tool that can assess the different symptoms of HD. Magnetic resonance imaging (MRI) can be used to determine the shrinkage of the striatum, which is the most affected area in HD (Przybyl et al., 2021). However, the pathology already starts before symptom onset and atrophic changes in the brain can be determined. Furthermore, these changes can only be stopped, but cannot be reversed. This means that for the development of disease-modifying treatments, it is important to diagnose as early as possible. During and after treatment, disease progression must be monitored to confirm treatment effectiveness. For this purpose, biomarkers are used. Some biomarkers of HD include more general neurodegenerative disease markers such as NFL and oxidative stress markers, immune system biomarkers, and mHTT levels, the latter being especially important for targeted therapies (Przybyl et al., 2021). Some evidence also argues in favor of using EVs from CSF or blood as biomarkers of HD progression.

7.1 Protein cargo of EVs as biomarker for HD

Recently, much attention was given to studying mechanisms of spreading of mHTT and/or aggregates with prion-like properties between cells via EVs. A study by Pecho-Vrieseling et al. was one of the first to detect spreading of mHTT between neurons (Pecho-Vrieseling et al., 2014). Human stem cell-

derived neurons were added to organotypic brain slices of R6/2 mice (a mouse model of HD) and found that mHTT was transferred from the mouse tissue to the human neurons. Furthermore, they showed *in vivo* mHTT spreading to grafted neurons inside the cortex of R6/2 mice (Pecho-Vrieseling et al., 2014). In a subset of neurons in the brain of a *Drosophila* model of HD, human mHTT was expressed, leading to accumulation in the synapses of these neurons, and eventually spreading throughout the whole brain (Babcock and Ganetzky, 2015). Additionally, a study analysed post-mortem brains of HD patients that had received allografts of non-diseased fetal striatal neurons around a decade earlier (Cicchetti et al., 2014). mHTT aggregates were found in the grafted tissue. These results combined indicate that mHTT is transmitted between neurons, but transfer is not necessarily evidence for EV involvement alone.

Using a HEK293T mHTT-ex1 overexpression model, Zhang et al. showed that both the mHTT-ex1 protein with a green fluorescent protein (GFP) tag and its RNA could be found in EVs isolated from the culture (Zhang et al., 2016). Primary striatal mouse cells were exposed to these EVs, which allowed for transfer of the mHTT-ex1 and its RNA (Zhang et al., 2016). In a study by Jeon et al. EVs were isolated from the medium of HD patients' fibroblasts with different CAG repeat lengths (Jeon et al., 2016). mHTT was found in EVs stained for CD63. Furthermore, EVs were isolated from induced pluripotent stem cell (iPSC)-derived neuronal precursor cells (NPCs) with 143 CAG repeats, and these EVs were added to a culture of mouse neurons. mHTT aggregates were found in the murine neurons after exposure to these EVs (Jeon et al., 2016). The same EVs from the HD iPSC-derived NPCs were injected into the ventricle of neonatal mice, which lead to HD phenotype, shown by impairments in the swim test and rotarod test. Moreover, mHTT aggregates were found in neurons of the striatum (Jeon et al., 2016). Diaz-Hidalgo et al. used a mouse embryonic fibroblast (MEF)-based model of HD that expresses mHTT-ex1 with 84 CAG repeats (Diaz-Hidalgo et al., 2016). They found that mHTT-ex1 was recruited to exosomes and that this recruitment is dependent on tissue transglutaminase (TG2), which forms a protein complex together with mHTT-ex1, ALIX, TSG101 and BCL2-associated athanogene 3 (BAG3), a co-chaperone involved in clearance of protein aggregates through the proteasome and/or autophagy (Diaz-Hidalgo et al., 2016). Bonavita et al. found a novel role of a complex of heat shock protein family B [small] member 1 (HSPB1) and p62 (also known as sequestosome 1 (SQSTM1)), which act as an EV cargo loading platform of mHTT (Bonavita et al., 2023). HSPB1 interacts preferably with mHTT compared to normal HTT, increasing spread of mHTT towards other cells. These studies together provide evidence that mHTT is loaded into EVs and can spread in a prion-like manner between cells.

With the knowledge that mHTT is loaded in EV, mHTT in EV isolates from bodily fluids may be a potential biomarker for HD. Of all blood cell types, platelets of HD patients have the highest concentration of mHTT. Therefore, it was investigated whether platelet-derived EVs could be used as a biomarker for HD (Denis et al., 2018). No difference was found between the amount of EVs released from the platelets between HD patients and healthy donors, and mHTT was not found within EVs derived from platelets of HD patients, indicating that these particular EVs are not a valuable biomarker for HD (Denis et al., 2018). In another study, two pig models of HD were used, a transgenic and a knock-in pig model, of which plasma was taken and EVs were isolated (Ananbeh et al., 2022). They also isolated EVs from HD patient-derived plasma. They found that full length HTT co-isolates with EVs from human and pig plasma. A significant increase in the amount of HTT was found in plasma of HD compared to wildtype transgenic pig model. An increase was also found in the knock-in pig model and in HD patient's plasmas, although not significant. The difference between the models may be the different CAG lengths that were expressed: 145 CAG repeats for the transgenic model, 85 for the knock-in model and 40-47 for the human HD patients (Ananbeh et al., 2022). A different strategy is measuring mHTT in isolates from the CSF, which would in theory be less diluted

with other peripheral EVs. A highly sensitive mHTT detection method was developed using single-molecule counting immunoassay to detect mHTT in CSF (Wild et al., 2015). Recently, the assay has been better characterized and optimized for use in clinical trials (Vauleon et al., 2023). Specificity of the assay has been validated for 36 to 73 CAG repeats. In the future, the assay may be used on EV isolates from CSF to decrease background signal even more.

Proteomic analysis of urinary EVs revealed enrichment of endolysosomal proteins related to PD, Alzheimer's disease (AD), and HD (Wang et al., 2019). This means that urinary EVs may be a valid source of biomarkers with use for HD.

7.2 miRNAs cargo of EVs as biomarkers for HD

No studies have yet been performed investigating specifically miRNAs from isolated EVs as biomarkers for HD. Some studies did find differences in miRNA expression in CSF of HD patients. Díez-Planelles et al. found 13 significantly increased miRNA in plasma from symptomatic HD patients with 40-45 CAG repeats (Díez-Planelles et al., 2016). In CSF, six miRNAs were significantly increased in mHTT gene carriers compared to control. Furthermore, all six miRNAs increase correlated with the different risk categories of HD, providing evidence that they could be used as biomarkers for disease progression (Reed et al., 2018). Future studies should be performed that look at EV-specific miRNA biomarkers.

8. EVs as biomarkers in PD

PD is a progressive neurodegenerative disease caused by degeneration of the nigrostriatal dopaminergic network. Loss of dopaminergic innervation of the locus coeruleus, leads to the characteristic symptoms of PD such as tremors and slowness of movement or bradykinesia (Hayes, 2019). Other symptoms include cognitive decline, sleep disturbances, olfactory dysfunction, depression, and pain. The primary risk factor for PD is the *SNCA* gene, which encodes for the protein α-synuclein (α-syn; Polymeropoulos et al., 1997). Several different mutations of α-syn lead to an autosomal dominant inheritance of PD. α-syn is the main protein contributing to Lewy bodies, protein aggregates found in the nigrostriatal dopaminergic neurons in brains of PD patients (Mor et al., 2016). Interestingly, Lewy bodies including α-syn are also found in sporadic cases of PD, without any mutation in α-syn itself. Oligomers of α-syn lead to impairment of protein degradation pathways, stress in mitochondria and endoplasmic reticulum, and disruption of neuronal signaling (Ingelsson, 2016).

Development of biomarkers for PD is very important. Because PD is usually only detected in more advanced stages of the disease after neurodegeneration has caused multiple symptom onset, it may already be too late for treating patients (Lotankar et al., 2017). For the development of treatments, it is again important to be able to monitor the progression of the disease after treatment. Biomarkers in use include imaging of the brain and general neurodegenerative biomarkers such as inflammation and oxidative stress (Lotankar et al., 2017).

8.1 EVs and their cargo as biomarkers for PD

An increase in the number of neuron-derived EVs was found in the plasma of PD patients, but no change was found in oligodendrocyte or astrocyte-derived EVs (Ohmichi et al., 2019).

In a similar manner to proteins involved in ALS and HD, evidence has emerged that α-syn and its oligomers are able to spread between cells, acting as seeds for protein aggregates. In a post-mortem brain of a PD patient with grafted embryonic nigral transplants, Lewy bodies were found in the grafted tissue (Kordower et al., 2008). Lewy bodies transmit between different cell types, such as neurons, astrocytes, and microglia (Desplats et al., 2009; Guo et al., 2020; Lee et al., 2010). It is

known that α-syn is loaded into EVs, leading to spreading towards other cells, indicating that α-syn in EV isolates from body fluids could be used as a biomarker for PD (Danzer et al., 2012; Guo et al., 2020; Harischandra et al., 2019).

Indeed, it was reported that α-syn was found in exosomes isolated from CSF of early-stage PD patients, but in lower amounts compared to healthy controls (Stuendl et al., 2016). Interestingly, the opposite result was found in plasma-derived neuronal LCAM1 positive EVs (Shi et al., 2014). Shi et al. reported that in EVs isolated from PD patients, the amount of α-syn was increased compared to healthy control. The same study also showed that EVs containing α-syn were readily secreted from the CSF to the bloodstream in mice, which may explain the differences between these two studies. Another study measured amounts of α-syn in neuronal EVs from plasma of patients with early-stage PD and advanced PD (Niu et al., 2020). They found a significant increase of α-syn in plasma-derived EVs from PD patients compared to healthy controls. α-syn levels in EVs were correlated to the several PD symptom scoring scales, such as the Movement Disorders Society Unified Parkinson's Disease Rating Scale III and the Non-Motor Symptom Questionnaire scores. Furthermore, during follow-up of early-stage PD patients visits over 22 months, it was found that increased neuronal EV-derived α-syn levels were correlated with a higher risk of motor symptom progression (Niu et al., 2020). More studies found significant differences in α-syn levels in serum-derived neuronal EVs between PD patients and healthy controls (Jiang et al., 2020; Si et al., 2019). The results from these studies indicate that α-syn levels in EVs could be used as a biomarker for PD.

Other potential EV-derived protein biomarkers for PD have been found. In addition to α-syn, tau protein may also be involved in PD, contributing to cognitive deterioration (Zhang et al., 2023). Isolation of L1CAM-positive neuronal exosomes from plasma, revealed an increase in tau in the PD patient group compared to healthy controls, which correlated with disease progression (Shi et al., 2016). Another study found an increase in PrP in exosomal isolates from plasma of PD patients with cognitive impairment, but not in patients without cognitive impairment, compared to healthy controls. The increase of PrP had a correlation with cognitive deterioration (Leng et al., 2020). Proteomic analysis of plasma-derived exosomes from plasma of PD patients, showed decrease of three proteins: clusterin (CLU), complement C1r subcomponent (C1r), and apolipoprotein A1 (Apo-A1), compared to healthy controls (Kitamura et al., 2018). Furthermore, Apo-A1 significantly decreased when comparing PD patients in stage II and stage III of the Hoehn and Yahr PD scale.

To this date only one study has investigated the potential of using RNAs in EVs from body fluids as biomarkers for PD, and it found 16 exosomal miRNAs upregulated and 11 downregulated in EVs isolated from PD patient's CSF, compared to healthy controls (Gui et al., 2015). Additionally, mRNA, such as α-syn, tau, and NFL were differentially expressed in CSF-derived exosomes from PD patients.

9. Discussion

EVs play a vital role in shuttling components between cells. Many pathways are involved in the biogenesis of EVs, leading to the heterogeneous pool of EVs that are produced (van Niel et al., 2018). EVs are especially relevant in the CNS where they are important for many neuronal functions and intercellular communication. However, the EV system usage by CNS cells to exert protein quality control (Pink et al., 2021) may backfire in neurodegenerative diseases, allowing for the spread of pathogenic prion-like proteins, such as TDP-43 in ALS, mHTT in HD, and α-syn in PD (Alpaugh et al., 2022; Danzer et al., 2012; Münch et al., 2011).

Diagnosis and monitoring of disease progression of neurodegenerative diseases remains a challenge (Thompson et al., 2016). EVs from body fluids are a promising low invasive technique that could provide a window into the pathophysiology of neurodegenerative diseases. EVs from CNS cells shield their inner cargo components from degradation and can be shuttled from CSF to the peripheral bloodstream, where they are detectable (Banks et al., 2020). CNS specific EVs can be isolated from body fluids and their cargo can be quantified to diagnose neurodegenerative disorders and/or to monitor target engagement during drug development and disease progression in clinical trials, factors to be taken into consideration for regulatory requirements (Committee for Advanced Therapies, 2018).

Because research into the usage of EVs as biomarkers for neurodegenerative diseases is still recent, very little EV-based biomarkers have been validated to be used in clinical trials. In general, validation of biomarkers includes investigation of the sensitivity and specificity of the biomarker and taking regard of the predictive value (Hunter et al., 2010). There is a myriad of EV isolation techniques, some based on size, others based on surface markers. A large heterogeneity of EV population is obtained in isolates, especially if EVs are isolated from biofluids that are in contact with many cell types (Zhao et al., 2021). It is essential that the techniques for EV isolation and cargo quantification are standardized, comparable, and reproducible to produce valid biomarkers. Efforts have been made to improve standardization by making guidelines for EV research that cover EV isolation techniques, quantification, characterization and functional readouts (Théry et al., 2018).

In this literature review, it was shown that research into EV-based biomarkers can lead to differing results. For example, Chen et al. found an increase of TDP in plasma-derived exosomes in ALS patients, but Pasetto et al. did not find any TDP-43 inside of EVs (Chen et al., 2020; Pasetto et al., 2021). The differences between the studies may be explained due to different isolation techniques being used. Chen et al. used beads with EV marker CD63 targeting antibodies, whereas Pasetto et al. used ultracentrifugation and nickel-based beads. It is known that different isolation techniques can give different EV yield, with differences in number of EVs, subtype of EVs and purity of sample (Sidhom et al., 2020). Indeed, it has been shown that EVs of the same origin, if isolated by different techniques will have different characteristics (Veerman et al., 2021). Standardization of isolation techniques will vastly improve the ability of EVs as biomarkers.

To isolate CNS derived EVs in peripheral fluid biopsies, EV surface markers are used in immunoaffinity assays. L1CAM is often used as a marker to differentiate between neuronal and peripheral EVs. However, although L1CAM has the highest expression in neurons, other cell types such as oligodendrocytes, immune cells, and endothelial cells may also express L1CAM (Gomes and Witwer, 2022). In certain types of cancer, L1CAM can also be overexpressed. Lastly, antibodies used in L1CAM EV isolation cross-react with α-syn, possibly explaining the reported enrichment of this protein in EVs (Norman et al., 2021). Better characterization of L1CAM-positive EVs is necessary when used in biomarker development.

Another consideration for biomarker development is the heterogeneity of neurodegenerative diseases. ALS and PD especially are the result of highly variable genetic and environmental factors, which deregulate distinct molecular pathways, possibly leading to alternative EV cargo profiles. In HD, the differences in CAG repeat length on EV cargo should also not be underestimated. Efforts should be made to correlate EV cargo to different subtypes of neurodegenerative diseases. For this purpose, multiomics could be deployed to find EV cargo signatures of disease subtypes, taking into account protein and RNA levels (Catanese et al., 2023).

10. Literature

Adell, M.A.Y., Migliano, S.M., Upadhyayula, S., Bykov, Y.S., Sprenger, S., Pakdel, M., Vogel, G.F., Jih, G., Skillern, W., Behrouzi, R., Babst, M., Schmidt, O., Hess, M.W., Briggs, J.A., Kirchhausen, T., Teis, D., 2017. Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding. eLife 6, e31652. https://doi.org/10.7554/eLife.31652

Alpaugh, M., Denis, H.L., Cicchetti, F., 2022. Prion-like properties of the mutant huntingtin protein in living organisms: the evidence and the relevance. Mol. Psychiatry 27, 269–280. https://doi.org/10.1038/s41380-021-01350-4

Ananbeh, H., Novak, J., Juhas, S., Juhasova, J., Klempir, J., Doleckova, K., Rysankova, I., Turnovcova, K., Hanus, J., Hansikova, H., Vodicka, P., Kupcova Skalnikova, H., 2022. Huntingtin Co-Isolates with Small Extracellular Vesicles from Blood Plasma of TgHD and KI-HD Pig Models of Huntington's Disease and Human Blood Plasma. Int. J. Mol. Sci. 23, 5598. https://doi.org/10.3390/ijms23105598

Andreu, Z., Yáñez-Mó, M., 2014. Tetraspanins in Extracellular Vesicle Formation and Function. Front. Immunol. 5.

Antonucci, F., Turola, E., Riganti, L., Caleo, M., Gabrielli, M., Perrotta, C., Novellino, L., Clementi, E., Giussani, P., Viani, P., Matteoli, M., Verderio, C., 2012. Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. EMBO J. 31, 1231–1240. https://doi.org/10.1038/emboj.2011.489

Antonyak, M.A., Wilson, K.F., Cerione, R.A., 2012. R(h)oads to microvesicles. Small GTPases 3, 219–224. https://doi.org/10.4161/sgtp.20755

Arellano-Anaya, Z.E., Huor, A., Leblanc, P., Lehmann, S., Provansal, M., Raposo, G., Andréoletti, O., Vilette, D., 2015. Prion strains are differentially released through the exosomal pathway. Cell. Mol. Life Sci. CMLS 72, 1185–1196. https://doi.org/10.1007/s00018-014-1735-8

Babcock, D.T., Ganetzky, B., 2015. Transcellular spreading of huntingtin aggregates in the Drosophila brain. Proc. Natl. Acad. Sci. U. S. A. 112, E5427–E5433. https://doi.org/10.1073/pnas.1516217112

Baietti, M.F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., Zimmermann, P., David, G., 2012. Syndecan–syntenin–ALIX regulates the biogenesis of exosomes. Nat. Cell Biol. 14, 677–685. https://doi.org/10.1038/ncb2502

Banack, S.A., Dunlop, R.A., Cox, P.A., 2020. An miRNA fingerprint using neural-enriched extracellular vesicles from blood plasma: towards a biomarker for amyotrophic lateral sclerosis/motor neuron disease. Open Biol. 10, 200116. https://doi.org/10.1098/rsob.200116

Banack, S.A., Dunlop, R.A., Stommel, E.W., Mehta, P., Cox, P.A., 2022. miRNA extracted from extracellular vesicles is a robust biomarker of amyotrophic lateral sclerosis. J. Neurol. Sci. 442, 120396. https://doi.org/10.1016/j.jns.2022.120396

Banks, W.A., Sharma, P., Bullock, K.M., Hansen, K.M., Ludwig, N., Whiteside, T.L., 2020. Transport of Extracellular Vesicles across the Blood-Brain Barrier: Brain Pharmacokinetics and Effects of Inflammation. Int. J. Mol. Sci. 21, 4407. https://doi.org/10.3390/ijms21124407

Barbaro, B.A., Lukacsovich, T., Agrawal, N., Burke, J., Bornemann, D.J., Purcell, J.M., Worthge, S.A., Caricasole, A., Weiss, A., Song, W., Morozova, O.A., Colby, D.W., Marsh, J.L., 2015. Comparative study of naturally occurring huntingtin fragments in Drosophila points to exon 1 as the most pathogenic species in Huntington's disease. Hum. Mol. Genet. 24, 913–925. https://doi.org/10.1093/hmg/ddu504

Barbo, M., Ravnik-Glavač, M., 2023. Extracellular Vesicles as Potential Biomarkers in Amyotrophic Lateral Sclerosis. Genes 14, 325. https://doi.org/10.3390/genes14020325

Basso, M., Bonetto, V., 2016. Extracellular Vesicles and a Novel Form of Communication in the Brain. Front. Neurosci. 10.

Battistelli, M., Falcieri, E., 2020. Apoptotic Bodies: Particular Extracellular Vesicles Involved in Intercellular Communication. Biology 9, 21. https://doi.org/10.3390/biology9010021

Bertin, A., de Franceschi, N., de la Mora, E., Maity, S., Alqabandi, M., Miguet, N., di Cicco, A., Roos, W.H., Mangenot, S., Weissenhorn, W., Bassereau, P., 2020. Human ESCRT-III polymers assemble on positively curved membranes and induce helical membrane tube formation. Nat. Commun. 11, 2663. https://doi.org/10.1038/s41467-020-16368-5

Bianco, F., Perrotta, C., Novellino, L., Francolini, M., Riganti, L., Menna, E., Saglietti, L., Schuchman, E.H., Furlan, R., Clementi, E., Matteoli, M., Verderio, C., 2009. Acid sphingomyelinase activity triggers microparticle release from glial cells. EMBO J. 28, 1043–1054. https://doi.org/10.1038/emboj.2009.45

Bib, M., 2005. Issues for Clinical Drug Development in Neurodegenerative Diseases. Drugs 65, 2463–2479. https://doi.org/10.2165/00003495-200565170-00004

Blokhuis, A.M., Groen, E.J.N., Koppers, M., van den Berg, L.H., Pasterkamp, R.J., 2013. Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol. (Berl.) 125, 777–794. https://doi.org/10.1007/s00401-013-1125-6

Bonavita, R., Scerra, G., Di Martino, R., Nuzzo, S., Polishchuk, E., Di Gennaro, M., Williams, S.V., Caporaso, M.G., Caiazza, C., Polishchuk, R., D'Agostino, M., Fleming, A., Renna, M., 2023. The HSPB1-p62/SQSTM1 functional complex regulates the unconventional secretion and transcellular spreading of the HD-associated mutant huntingtin protein. Hum. Mol. Genet. 32, 2269–2291. https://doi.org/10.1093/hmg/ddad047

Bricogne, C., Fine, M., Pereira, P.M., Sung, J., Tijani, M., Wang, Y., Henriques, R., Collins, M.K., Hilgemann, D.W., 2019. TMEM16F activation by Ca2+ triggers plasma membrane expansion and directs PD-1 trafficking. Sci. Rep. 9, 619. https://doi.org/10.1038/s41598-018-37056-x

Budnik, V., Ruiz-Cañada, C., Wendler, F., 2016. Extracellular vesicles round off communication in the nervous system. Nat. Rev. Neurosci. 17, 160–172. https://doi.org/10.1038/nrn.2015.29

Cano, A., Ettcheto, M., Bernuz, M., Puerta, R., Esteban de Antonio, E., Sánchez-López, E., Souto, E.B., Camins, A., Martí, M., Pividori, M.I., Boada, M., Ruiz, A., 2023. Extracellular vesicles, the emerging mirrors of brain physiopathology. Int. J. Biol. Sci. 19, 721–743. https://doi.org/10.7150/ijbs.79063

Catanese, A., Rajkumar, S., Sommer, D., Masrori, P., Hersmus, N., Van Damme, P., Witzel, S., Ludolph, A., Ho, R., Boeckers, T.M., Mulaw, M., 2023. Multiomics and machine-learning identify novel transcriptional and mutational signatures in amyotrophic lateral sclerosis. Brain 146, 3770–3782. https://doi.org/10.1093/brain/awad075

Charrin, S., Jouannet, S., Boucheix, C., Rubinstein, E., 2014. Tetraspanins at a glance. J. Cell Sci. 127, 3641–3648. https://doi.org/10.1242/jcs.154906

Chen, C.C., Liu, L., Ma, F., Wong, C.W., Guo, X.E., Chacko, J.V., Farhoodi, H.P., Zhang, S.X., Zimak, J., Ségaliny, A., Riazifar, M., Pham, V., Digman, M.A., Pone, E.J., Zhao, W., 2016. Elucidation of Exosome Migration across the Blood-Brain Barrier Model In Vitro. Cell. Mol. Bioeng. 9, 509–529. https://doi.org/10.1007/s12195-016-0458-3

Chen, J.Y., Parekh, M., Seliman, H., Bakshinskaya, D., Dai, W., Kwan, K., Chen, K.Y., Liu, A.Y.C., 2018. Heat shock promotes inclusion body formation of mutant huntingtin (mHtt) and alleviates mHtt-induced transcription factor dysfunction. J. Biol. Chem. 293, 15581–15593. https://doi.org/10.1074/jbc.RA118.002933

Chen, P.-C., Wu, D., Hu, C.-J., Chen, H.-Y., Hsieh, Y.-C., Huang, C.-C., 2020. Exosomal TAR DNA-binding protein-43 and neurofilaments in plasma of amyotrophic lateral sclerosis patients: A longitudinal follow-up study. J. Neurol. Sci. 418. https://doi.org/10.1016/j.jns.2020.117070

Chiasserini, D., van Weering, J.R.T., Piersma, S.R., Pham, T.V., Malekzadeh, A., Teunissen, C.E., de Wit, H., Jiménez, C.R., 2014. Proteomic analysis of cerebrospinal fluid extracellular vesicles: a comprehensive dataset. J. Proteomics 106, 191–204. https://doi.org/10.1016/j.jprot.2014.04.028

Cicchetti, F., Lacroix, S., Cisbani, G., Vallières, N., Saint-Pierre, M., St-Amour, I., Tolouei, R., Skepper, J.N., Hauser, R.A., Mantovani, D., Barker, R.A., Freeman, T.B., 2014. Mutant huntingtin is

present in neuronal grafts in huntington disease patients. Ann. Neurol. 76, 31–42. https://doi.org/10.1002/ana.24174
Colombo, M., Raposo, G., Théry, C., 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu. Rev. Cell Dev. Biol. 30, 255–289. https://doi.org/10.1146/annurev-cellbio-101512-122326
Committee for Advanced Therapies Guideline on the quality, 2018. Non-clinical and clinical aspects of gene therapy medicinal products. European Medicines Agency
Couch, Y., Akbar, N., Roodselaar, J., Evans, M.C., Gardiner, C., Sargent, I., Romero, I.A., Bristow, A., Buchan, A.M., Haughey, N., Anthony, D.C., 2017. Circulating endothelial cell-derived extracellular vesicles mediate the acute phase response and sickness behaviour associated with CNS inflammation. Sci. Rep. 7, 9574. https://doi.org/10.1038/s41598-017-09710-3
Danzer, K.M., Kranich, L.R., Ruf, W.P., Cagsal-Getkin, O., Winslow, A.R., Zhu, L., Vanderburg, C.R., McLean, P.J., 2012. Exosomal cell-to-cell transmission of alpha synuclein oligomers. Mol. Neurodegener. 7, 42. https://doi.org/10.1186/1750-1326-7-42
Dayalu, P., Albin, R.L., 2015. Huntington Disease: Pathogenesis and Treatment. Neurol. Clin., Movement Disorders 33, 101–114. https://doi.org/10.1016/j.ncl.2014.09.003
Denis, H.L., Lamontagne-Proulx, J., St-Amour, I., Mason, S.L., Weiss, A., Chouinard, S., Barker, R.A., Boilard, E., Cicchetti, F., 2018. Platelet-derived extracellular vesicles in Huntington's disease. J. Neurol. 265, 2704–2712. https://doi.org/10.1007/s00415-018-9022-5
Desplats, P., Lee, H.-J., Bae, E.-J., Patrick, C., Rockenstein, E., Crews, L., Spencer, B., Masliah, E., Lee, S.-J., 2009. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. Proc. Natl. Acad. Sci. U. S. A. 106, 13010–13015. https://doi.org/10.1073/pnas.0903691106
Deverman, B.E., Ravina, B.M., Bankiewicz, K.S., Paul, S.M., Sah, D.W.Y., 2018. Gene therapy for neurological disorders: progress and prospects. Nat. Rev. Drug Discov. 17, 641–659. https://doi.org/10.1038/nrd.2018.110
Dhasmana, S., Dhasmana, A., Narula, A.S., Jaggi, M., Yallapu, M.M., Chauhan, S.C., 2022. The panoramic view of amyotrophic lateral sclerosis: A fatal intricate neurological disorder. Life Sci. 288, 120156. https://doi.org/10.1016/j.lfs.2021.120156
Diaz-Hidalgo, L., Altuntas, S., Rossin, F., D'Eletto, M., Marsella, C., Farrace, M.G., Falasca, L., Antonioli, M., Fimia, G.M., Piacentini, M., 2016. Transglutaminase type 2-dependent selective recruitment of proteins into exosomes under stressful cellular conditions. Biochim. Biophys. Acta 1863, 2084–2092. https://doi.org/10.1016/j.bbamcr.2016.05.005
Dickens, A.M., Tovar-y-Romo, L.B., Yoo, S.-W., Trout, A.L., Bae, M., Kanmogne, M., Megra, B., Williams, D.W., Witwer, K.W., Gacias, M., Tabatadze, N., Cole, R.N., Casaccia, P., Berman, J.W., Anthony, D.C., Haughey, N.J., 2017. Astrocyte-shed extracellular vesicles regulate the peripheral leukocyte response to inflammatory brain lesions. Sci. Signal. 10, eaai7696. https://doi.org/10.1126/scisignal.aai7696
Díez-Planelles, C., Sánchez-Lozano, P., Crespo, M.C., Gil-Zamorano, J., Ribacoba, R., González, N., Suárez, E., Martínez-Descals, A., Martínez-Camblor, P., Álvarez, V., Martín-Hernández, R., Huerta-Ruíz, I., González-García, I., Cosgaya, J.M., Visioli, F., Dávalos, A., Iglesias-Gutiérrez, E., Tomás-Zapico, C., 2016. Circulating microRNAs in Huntington's disease: Emerging mediators in metabolic impairment. Pharmacol. Res. 108, 102–110. https://doi.org/10.1016/j.phrs.2016.05.005
Ding, X., Ma, M., Teng, J., Teng, R.K.F., Zhou, S., Yin, J., Fonkem, E., Huang, J.H., Wu, E., Wang, X., 2015. Exposure to ALS-FTD-CSF generates TDP-43 aggregates in glioblastoma cells through exosomes and TNTs-like structure. Oncotarget 6, 24178–24191.
Dixson, A.C., Dawson, T.R., Di Vizio, D., Weaver, A.M., 2023. Context-specific regulation of extracellular vesicle biogenesis and cargo selection. Nat. Rev. Mol. Cell Biol. 24, 454–476. https://doi.org/10.1038/s41580-023-00576-0

Fabbiano, F., Corsi, J., Gurrieri, E., Trevisan, C., Notarangelo, M., D'Agostino, V.G., 2020. RNA packaging into extracellular vesicles: An orchestra of RNA-binding proteins? J. Extracell. Vesicles 10, e12043. https://doi.org/10.1002/jev2.12043

Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H., Raposo, G., 2004. Cells release prions in association with exosomes. Proc. Natl. Acad. Sci. U. S. A. 101, 9683–9688. https://doi.org/10.1073/pnas.0308413101

Frühbeis, C., Fröhlich, D., Kuo, W.P., Amphornrat, J., Thilemann, S., Saab, A.S., Kirchhoff, F., Möbius, W., Goebbels, S., Nave, K.-A., Schneider, A., Simons, M., Klugmann, M., Trotter, J., Krämer-Albers, E.-M., 2013. Neurotransmitter-Triggered Transfer of Exosomes Mediates Oligodendrocyte–Neuron Communication. PLoS Biol. 11, e1001604. https://doi.org/10.1371/journal.pbio.1001604

Gaetani, L., Blennow, K., Calabresi, P., Di Filippo, M., Parnetti, L., Zetterberg, H., 2019. Neurofilament light chain as a biomarker in neurological disorders. J. Neurol. Neurosurg. Psychiatry 90, 870–881. https://doi.org/10.1136/jnnp-2018-320106

Glimcher, S.A., Holman, D.W., Lubow, M., Grzybowski, D.M., 2008. Ex vivo model of cerebrospinal fluid outflow across human arachnoid granulations. Invest. Ophthalmol. Vis. Sci. 49, 4721–4728. https://doi.org/10.1167/iovs.08-2238

Gomes, C., Keller, S., Altevogt, P., Costa, J., 2007. Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. Neurosci. Lett. 428, 43–46. https://doi.org/10.1016/j.neulet.2007.09.024

Gomes, D.E., Witwer, K.W., 2022. L1CAM-associated extracellular vesicles: A systematic review of nomenclature, sources, separation, and characterization. J. Extracell. Biol. 1, e35. https://doi.org/10.1002/jex2.35

Goñi, F.M., Alonso, A., 2009. Effects of ceramide and other simple sphingolipids on membrane lateral structure. Biochim. Biophys. Acta 1788, 169–177. https://doi.org/10.1016/j.bbamem.2008.09.002

Görgens, A., Corso, G., Hagey, D.W., Jawad Wiklander, R., Gustafsson, M.O., Felldin, U., Lee, Y., Bostancioglu, R.B., Sork, H., Liang, X., Zheng, W., Mohammad, D.K., van de Wakker, S.I., Vader, P., Zickler, A.M., Mamand, D.R., Ma, L., Holme, M.N., Stevens, M.M., Wiklander, O.P.B., El Andaloussi, S., 2022. Identification of storage conditions stabilizing extracellular vesicles preparations. J. Extracell. Vesicles 11, e12238. https://doi.org/10.1002/jev2.12238

Grad, L.I., Yerbury, J.J., Turner, B.J., Guest, W.C., Pokrishevsky, E., O'Neill, M.A., Yanai, A., Silverman, J.M., Zeineddine, R., Corcoran, L., Kumita, J.R., Luheshi, L.M., Yousefi, M., Coleman, B.M., Hill, A.F., Plotkin, S.S., Mackenzie, I.R., Cashman, N.R., 2014. Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. Proc. Natl. Acad. Sci. 111, 3620–3625. https://doi.org/10.1073/pnas.1312245111

Gui, Y., Liu, H., Zhang, L., Lv, W., Hu, X., 2015. Altered microRNA profiles in cerebrospinal fluid exosome in Parkinson disease and Alzheimer disease. Oncotarget 6, 37043–37053.

Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products, n.d.

Guo, B.B., Bellingham, S.A., Hill, A.F., 2016. Stimulating the Release of Exosomes Increases the Intercellular Transfer of Prions. J. Biol. Chem. 291, 5128–5137. https://doi.org/10.1074/jbc.M115.684258

Guo, M., Wang, J., Zhao, Y., Feng, Y., Han, S., Dong, Q., Cui, M., Tieu, K., 2020. Microglial exosomes facilitate α-synuclein transmission in Parkinson's disease. Brain 143, 1476–1497. https://doi.org/10.1093/brain/awaa090

Guo, W., Chen, Y., Zhou, X., Kar, A., Ray, P., Chen, X., Rao, E.J., Yang, M., Ye, H., Zhu, L., Liu, J., Xu, M., Yang, Y., Wang, C., Zhang, D., Bigio, E.H., Mesulam, M., Shen, Y., Xu, Q., Fushimi, K., Wu, J.Y., 2011. An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. Nat. Struct. Mol. Biol. 18, 822–830. https://doi.org/10.1038/nsmb.2053

Harischandra, D.S., Rokad, D., Neal, M.L., Ghaisas, S., Manne, S., Sarkar, S., Panicker, N., Zenitsky, G., Jin, H., Lewis, M., Huang, X., Anantharam, V., Kanthasamy, A., Kanthasamy, A.G., 2019. Manganese promotes the aggregation and prion-like cell-to-cell exosomal transmission of α-synuclein. Sci. Signal. 12, eaau4543. https://doi.org/10.1126/scisignal.aau4543

Hayashi, N., Doi, H., Kurata, Y., Kagawa, H., Atobe, Y., Funakoshi, K., Tada, M., Katsumoto, A., Tanaka, K., Kunii, M., Nakamura, H., Takahashi, K., Takeuchi, H., Koyano, S., Kimura, Y., Hirano, H., Tanaka, F., 2020. Proteomic analysis of exosome-enriched fractions derived from cerebrospinal fluid of amyotrophic lateral sclerosis patients. Neurosci. Res. 160, 43–49. https://doi.org/10.1016/j.neures.2019.10.010

Hayes, M.T., 2019. Parkinson's Disease and Parkinsonism. Am. J. Med. 132, 802–807. https://doi.org/10.1016/j.amjmed.2019.03.001

Henne, W.M., Buchkovich, N.J., Emr, S.D., 2011. The ESCRT pathway. Dev. Cell 21, 77–91. https://doi.org/10.1016/j.devcel.2011.05.015

Hill, A.F., 2019. Extracellular Vesicles and Neurodegenerative Diseases. J. Neurosci. 39, 9269–9273. https://doi.org/10.1523/JNEUROSCI.0147-18.2019

Hipp, M.S., Park, S.-H., Hartl, F.U., 2014. Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends Cell Biol. 24, 506–514. https://doi.org/10.1016/j.tcb.2014.05.003

Hunter, D.J., Losina, E., Guermazi, A., Burstein, D., Lassere, M.N., Kraus, V., 2010. A Pathway and Approach to Biomarker Validation and Qualification for Osteoarthritis Clinical Trials. Curr. Drug Targets 11, 536–545.

Iguchi, Y., Eid, L., Parent, M., Soucy, G., Bareil, C., Riku, Y., Kawai, K., Takagi, S., Yoshida, M., Katsuno, M., Sobue, G., Julien, J.-P., 2016. Exosome secretion is a key pathway for clearance of pathological TDP-43. Brain 139, 3187–3201. https://doi.org/10.1093/brain/aww237

Ingelsson, M., 2016. Alpha-Synuclein Oligomers—Neurotoxic Molecules in Parkinson's Disease and Other Lewy Body Disorders. Front. Neurosci. 10, 408. https://doi.org/10.3389/fnins.2016.00408

Janas, A.M., Sapoń, K., Janas, Teresa, Stowell, M.H.B., Janas, Tadeusz, 2016. Exosomes and other extracellular vesicles in neural cells and neurodegenerative diseases. Biochim. Biophys. Acta BBA - Biomembr. 1858, 1139–1151. https://doi.org/10.1016/j.bbamem.2016.02.011

Janas, Tadeusz, Janas, Teresa, Yarus, M., 2006. Specific RNA binding to ordered phospholipid bilayers. Nucleic Acids Res. 34, 2128–2136. https://doi.org/10.1093/nar/gkl220

Jeon, I., Cicchetti, F., Cisbani, G., Lee, S., Li, E., Bae, J., Lee, N., Li, L., Im, W., Kim, M., Kim, H.S., Oh, S.-H., Kim, T.-A., Ko, J.J., Aubé, B., Oueslati, A., Kim, Y.J., Song, J., 2016. Human-to-mouse prion-like propagation of mutant huntingtin protein. Acta Neuropathol. (Berl.) 132, 577–592. https://doi.org/10.1007/s00401-016-1582-9

Jiang, C., Hopfner, F., Katsikoudi, A., Hein, R., Catli, C., Evetts, S., Huang, Y., Wang, H., Ryder, J.W., Kuhlenbaeumer, G., Deuschl, G., Padovani, A., Berg, D., Borroni, B., Hu, M.T., Davis, J.J., Tofaris, G.K., 2020. Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. J. Neurol. Neurosurg. Psychiatry 91, 720–729. https://doi.org/10.1136/jnnp-2019-322588

Kajimoto, T., Okada, T., Miya, S., Zhang, L., Nakamura, S., 2013. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. Nat. Commun. 4, 2712. https://doi.org/10.1038/ncomms3712

Katsu, M., Hama, Y., Utsumi, J., Takashina, K., Yasumatsu, H., Mori, F., Wakabayashi, K., Shoji, M., Sasaki, H., 2019. MicroRNA expression profiles of neuron-derived extracellular vesicles in plasma from patients with amyotrophic lateral sclerosis. Neurosci. Lett. 708, 134176. https://doi.org/10.1016/j.neulet.2019.03.048

Kim, G., Chen, X., Yang, Y., 2022. Pathogenic Extracellular Vesicle (EV) Signaling in Amyotrophic Lateral Sclerosis (ALS). Neurotherapeutics 19, 1119–1132. https://doi.org/10.1007/s13311-022-01232-9

Kitamura, Y., Kojima, M., Kurosawa, T., Sasaki, R., Ichihara, S., Hiraku, Y., Tomimoto, H., Murata, M., Oikawa, S., 2018. Proteomic Profiling of Exosomal Proteins for Blood-based Biomarkers in Parkinson's Disease. Neuroscience 392, 121–128. https://doi.org/10.1016/j.neuroscience.2018.09.017

Kordower, J.H., Chu, Y., Hauser, R.A., Freeman, T.B., Olanow, C.W., 2008. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat. Med. 14, 504–506. https://doi.org/10.1038/nm1747

Krämer-Albers, E.-M., Bretz, N., Tenzer, S., Winterstein, C., Möbius, W., Berger, H., Nave, K.-A., Schild, H., Trotter, J., 2007. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? Proteomics Clin. Appl. 1, 1446–1461. https://doi.org/10.1002/prca.200700522

Lachenal, G., Pernet-Gallay, K., Chivet, M., Hemming, F.J., Belly, A., Bodon, G., Blot, B., Haase, G., Goldberg, Y., Sadoul, R., 2011. Release of exosomes from differentiated neurons and its regulation by synaptic glutamatergic activity. Mol. Cell. Neurosci. 46, 409–418. https://doi.org/10.1016/j.mcn.2010.11.004

Landles, C., Sathasivam, K., Weiss, A., Woodman, B., Moffitt, H., Finkbeiner, S., Sun, B., Gafni, J., Ellerby, L.M., Trottier, Y., Richards, W.G., Osmand, A., Paganetti, P., Bates, G.P., 2010. Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. J. Biol. Chem. 285, 8808–8823. https://doi.org/10.1074/jbc.M109.075028

Lee, H.-J., Suk, J.-E., Patrick, C., Bae, E.-J., Cho, J.-H., Rho, S., Hwang, D., Masliah, E., Lee, S.-J., 2010. Direct Transfer of α-Synuclein from Neuron to Astroglia Causes Inflammatory Responses in Synucleinopathies. J. Biol. Chem. 285, 9262–9272. https://doi.org/10.1074/jbc.M109.081125

Leidal, A.M., Debnath, J., 2020. LC3-dependent extracellular vesicle loading and secretion (LDELS). Autophagy 16, 1162–1163. https://doi.org/10.1080/15548627.2020.1756557

Leidal, A.M., Huang, H.H., Marsh, T., Solvik, T., Zhang, D., Ye, J., Kai, F., Goldsmith, J., Liu, J.Y., Huang, Y.-H., Monkkonen, T., Vlahakis, A., Huang, E.J., Goodarzi, H., Yu, L., Wiita, A.P., Debnath, J., 2020. The LC3-conjugation machinery specifies the loading of RNA-binding proteins into extracellular vesicles. Nat. Cell Biol. 22, 187–199. https://doi.org/10.1038/s41556-019-0450-y

Leng, B., Sun, H., Zhao, J., Liu, Y., Shen, T., Liu, W., Liu, X., Tan, M., Li, F., Zhang, J., Li, Z., 2020. Plasma exosomal prion protein levels are correlated with cognitive decline in PD patients. Neurosci. Lett. 723, 134866. https://doi.org/10.1016/j.neulet.2020.134866

Liu, S., Hossinger, A., Göbbels, S., Vorberg, I.M., 2017. Prions on the run: How extracellular vesicles serve as delivery vehicles for self-templating protein aggregates. Prion 11, 98–112. https://doi.org/10.1080/19336896.2017.1306162

Lizarraga-Valderrama, L.R., Sheridan, G.K., 2021. Extracellular vesicles and intercellular communication in the central nervous system. FEBS Lett. 595, 1391–1410. https://doi.org/10.1002/1873-3468.14074

Lo Cicero, A., Stahl, P.D., Raposo, G., 2015. Extracellular vesicles shuffling intercellular messages: for good or for bad. Curr. Opin. Cell Biol. 35, 69–77. https://doi.org/10.1016/j.ceb.2015.04.013

Lotankar, S., Prabhavalkar, K.S., Bhatt, L.K., 2017. Biomarkers for Parkinson's Disease: Recent Advancement. Neurosci. Bull. 33, 585–597. https://doi.org/10.1007/s12264-017-0183-5

Ma, L., Li, Y., Peng, J., Wu, D., Zhao, X., Cui, Y., Chen, L., Yan, X., Du, Y., Yu, L., 2015. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. Cell Res. 25, 24–38. https://doi.org/10.1038/cr.2014.135

Moon, H.E., Paek, S.H., 2015. Mitochondrial Dysfunction in Parkinson's Disease. Exp. Neurobiol. 24, 103–116. https://doi.org/10.5607/en.2015.24.2.103

Mor, D.E., Ugras, S.E., Daniels, M.J., Ischiropoulos, H., 2016. Dynamic structural flexibility of α-synuclein. Neurobiol. Dis. 88, 66–74. https://doi.org/10.1016/j.nbd.2015.12.018

Münch, C., O'Brien, J., Bertolotti, A., 2011. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc. Natl. Acad. Sci. 108, 3548–3553. https://doi.org/10.1073/pnas.1017275108

Nabhan, J.F., Hu, R., Oh, R.S., Cohen, S.N., Lu, Q., 2012. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. Proc. Natl. Acad. Sci. U. S. A. 109, 4146–4151. https://doi.org/10.1073/pnas.1200448109

Niu, M., Li, Y., Li, G., Zhou, L., Luo, N., Yao, M., Kang, W., Liu, J., 2020. A longitudinal study on α-synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. Eur. J. Neurol. 27, 967–974. https://doi.org/10.1111/ene.14208

Nonaka, T., Masuda-Suzukake, M., Arai, T., Hasegawa, Y., Akatsu, H., Obi, T., Yoshida, M., Murayama, S., Mann, D.M.A., Akiyama, H., Hasegawa, M., 2013. Prion-like Properties of Pathological TDP-43 Aggregates from Diseased Brains. Cell Rep. 4, 124–134. https://doi.org/10.1016/j.celrep.2013.06.007

Norman, M., Ter-Ovanesyan, D., Trieu, W., Lazarovits, R., Kowal, E.J.K., Lee, J.H., Chen-Plotkin, A.S., Regev, A., Church, G.M., Walt, D.R., 2021. L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma. Nat. Methods 18, 631–634. https://doi.org/10.1038/s41592-021-01174-8

Ohmichi, T., Mitsuhashi, M., Tatebe, H., Kasai, T., Ali El-Agnaf, O.M., Tokuda, T., 2019. Quantification of brain-derived extracellular vesicles in plasma as a biomarker to diagnose Parkinson's and related diseases. Parkinsonism Relat. Disord. 61, 82–87. https://doi.org/10.1016/j.parkreldis.2018.11.021

Otake, K., Kamiguchi, H., Hirozane, Y., 2019. Identification of biomarkers for amyotrophic lateral sclerosis by comprehensive analysis of exosomal mRNAs in human cerebrospinal fluid. BMC Med. Genomics 12, 7. https://doi.org/10.1186/s12920-019-0473-z

Pasetto, L., Callegaro, S., Corbelli, A., Fiordaliso, F., Ferrara, D., Brunelli, L., Sestito, G., Pastorelli, R., Bianchi, E., Cretich, M., Chiari, M., Potrich, C., Moglia, C., Corbo, M., Sorarù, G., Lunetta, C., Calvo, A., Chiò, A., Mora, G., Pennuto, M., Quattrone, A., Rinaldi, F., D'Agostino, V.G., Basso, M., Bonetto, V., 2021. Decoding distinctive features of plasma extracellular vesicles in amyotrophic lateral sclerosis. https://doi.org/10.1101/2020.08.06.20169300

Pecho-Vrieseling, E., Rieker, C., Fuchs, S., Bleckmann, D., Esposito, M.S., Botta, P., Goldstein, C., Bernhard, M., Galimberti, I., Müller, M., Lüthi, A., Arber, S., Bouwmeester, T., van der Putten, H., Di Giorgio, F.P., 2014. Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons. Nat. Neurosci. 17, 1064–1072. https://doi.org/10.1038/nn.3761

Peggion, C., Scalcon, V., Massimino, M.L., Nies, K., Lopreiato, R., Rigobello, M.P., Bertoli, A., 2022. SOD1 in ALS: Taking Stock in Pathogenic Mechanisms and the Role of Glial and Muscle Cells. Antioxidants 11, 614. https://doi.org/10.3390/antiox11040614

Peng, C., Trojanowski, J.Q., Lee, V.M.-Y., 2020. Protein transmission in neurodegenerative disease. Nat. Rev. Neurol. 16, 199–212. https://doi.org/10.1038/s41582-020-0333-7

Perez-Hernandez, D., Gutiérrez-Vázquez, C., Jorge, I., López-Martín, S., Ursa, A., Sánchez-Madrid, F., Vázquez, J., Yáñez-Mó, M., 2013. The Intracellular Interactome of Tetraspanin-enriched Microdomains Reveals Their Function as Sorting Machineries toward Exosomes. J. Biol. Chem. 288, 11649–11661. https://doi.org/10.1074/jbc.M112.445304

Pink, D., Donnelier, J., Lewis, J., Braun, J.E.A., 2021. Cargo-Loading of Misfolded Proteins into Extracellular Vesicles: The CSPα-EV Export Pathway. https://doi.org/10.1101/310219

Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I., Nussbaum, R.L., 1997. Mutation in the alpha-synuclein gene identified in families with

Parkinson's disease. Science 276, 2045–2047. https://doi.org/10.1126/science.276.5321.2045
Przybyl, L., Wozna-Wysocka, M., Kozlowska, E., Fiszer, A., 2021. What, When and How to Measure—Peripheral Biomarkers in Therapy of Huntington's Disease. Int. J. Mol. Sci. 22, 1561. https://doi.org/10.3390/ijms22041561
Raiborg, C., Grønvold Bache, K., Mehlum, A., Stang, E., Stenmark, H., 2001. Hrs recruits clathrin to early endosomes. EMBO J. 20, 5008–5021. https://doi.org/10.1093/emboj/20.17.5008
Raiborg, C., Wesche, J., Malerød, L., Stenmark, H., 2006. Flat clathrin coats on endosomes mediate degradative protein sorting by scaffolding Hrs in dynamic microdomains. J. Cell Sci. 119, 2414–2424. https://doi.org/10.1242/jcs.02978
Reed, E.R., Latourelle, J.C., Bockholt, J.H., Bregu, J., Smock, J., Paulsen, J.S., Myers, R.H., Investigators, P.-H.C. ancillary study, 2018. MicroRNAs in CSF as prodromal biomarkers for Huntington disease in the PREDICT-HD study. Neurology 90, e264. https://doi.org/10.1212/WNL.0000000000004844
Riku, Y., Seilhean, D., Duyckaerts, C., Boluda, S., Iguchi, Y., Ishigaki, S., Iwasaki, Y., Yoshida, M., Sobue, G., Katsuno, M., 2021. Pathway from TDP-43-Related Pathology to Neuronal Dysfunction in Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration. Int. J. Mol. Sci. 22, 3843. https://doi.org/10.3390/ijms22083843
Sala-Valdés, M., Ursa, Á., Charrin, S., Rubinstein, E., Hemler, M.E., Sánchez-Madrid, F., Yáñez-Mó, M., 2006. EWI-2 and EWI-F Link the Tetraspanin Web to the Actin Cytoskeleton through Their Direct Association with Ezrin-Radixin-Moesin Proteins *. J. Biol. Chem. 281, 19665–19675. https://doi.org/10.1074/jbc.M602116200
Sathasivam, K., Neueder, A., Gipson, T.A., Landles, C., Benjamin, A.C., Bondulich, M.K., Smith, D.L., Faull, R.L.M., Roos, R.A.C., Howland, D., Detloff, P.J., Housman, D.E., Bates, G.P., 2013. Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc. Natl. Acad. Sci. 110, 2366–2370. https://doi.org/10.1073/pnas.1221891110
Saucier, D., Wajnberg, G., Roy, J., Beauregard, A.-P., Chacko, S., Crapoulet, N., Fournier, S., Ghosh, A., Lewis, S.M., Marrero, A., O'Connell, C., Ouellette, R.J., Morin, P.J., 2019. Identification of a circulating miRNA signature in extracellular vesicles collected from amyotrophic lateral sclerosis patients. Brain Res. 1708, 100–108. https://doi.org/10.1016/j.brainres.2018.12.016
Shi, M., Kovac, A., Korff, A., Cook, T.J., Ginghina, C., Bullock, K.M., Yang, L., Stewart, T., Zheng, D., Aro, P., Atik, A., Kerr, K.F., Zabetian, C.P., Peskind, E.R., Hu, S.-C., Quinn, J.F., Galasko, D.R., Montine, T.J., Banks, W.A., Zhang, J., 2016. CNS tau efflux via exosomes is likely increased in Parkinson disease but not in Alzheimer disease. Alzheimers Dement. J. Alzheimers Assoc. 12, 1125–1131. https://doi.org/10.1016/j.jalz.2016.04.003
Shi, M., Liu, C., Cook, T.J., Bullock, K.M., Zhao, Y., Ginghina, C., Li, Y., Aro, P., Dator, R., He, C., Hipp, M.J., Zabetian, C.P., Peskind, E.R., Hu, S.-C., Quinn, J.F., Galasko, D.R., Banks, W.A., Zhang, J., 2014. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. Acta Neuropathol. (Berl.) 128, 639–650. https://doi.org/10.1007/s00401-014-1314-y
Si, X., Tian, J., Chen, Y., Yan, Y., Pu, J., Zhang, B., 2019. Central Nervous System-Derived Exosomal Alpha-Synuclein in Serum May Be a Biomarker in Parkinson's Disease. Neuroscience 413, 308–316. https://doi.org/10.1016/j.neuroscience.2019.05.015
Sidhom, K., Obi, P.O., Saleem, A., 2020. A Review of Exosomal Isolation Methods: Is Size Exclusion Chromatography the Best Option? Int. J. Mol. Sci. 21, 6466. https://doi.org/10.3390/ijms21186466
Sproviero, D., Gagliardi, S., Zucca, S., Arigoni, M., Giannini, M., Garofalo, M., Olivero, M., Dell'Orco, M., Pansarasa, O., Bernuzzi, S., Avenali, M., Cotta Ramusino, M., Diamanti, L., Minafra, B., Perini, G., Zangaglia, R., Costa, A., Ceroni, M., Perrone-Bizzozero, N.I., Calogero, R.A., Cereda, C., 2021. Different miRNA Profiles in Plasma Derived Small and Large Extracellular Vesicles from Patients with Neurodegenerative Diseases. Int. J. Mol. Sci. 22, 2737. https://doi.org/10.3390/ijms22052737

Sproviero, D., La Salvia, S., Colombo, F., Zucca, S., Pansarasa, O., Diamanti, L., Costa, A., Lova, L., Giannini, M., Gagliardi, S., Lauranzano, E., Matteoli, M., Ceroni, M., Malaspina, A., Cereda, C., 2019. Leukocyte Derived Microvesicles as Disease Progression Biomarkers in Slow Progressing Amyotrophic Lateral Sclerosis Patients. Front. Neurosci. 13, 344. https://doi.org/10.3389/fnins.2019.00344

Sproviero, D., La Salvia, S., Giannini, M., Crippa, V., Gagliardi, S., Bernuzzi, S., Diamanti, L., Ceroni, M., Pansarasa, O., Poletti, A., Cereda, C., 2018. Pathological Proteins Are Transported by Extracellular Vesicles of Sporadic Amyotrophic Lateral Sclerosis Patients. Front. Neurosci. 12, 487. https://doi.org/10.3389/fnins.2018.00487

Squadrito, M.L., Baer, C., Burdet, F., Maderna, C., Gilfillan, G.D., Lyle, R., Ibberson, M., De Palma, M., 2014. Endogenous RNAs Modulate MicroRNA Sorting to Exosomes and Transfer to Acceptor Cells. Cell Rep. 8, 1432–1446. https://doi.org/10.1016/j.celrep.2014.07.035

Stoker, T.B., Mason, S.L., Greenland, J.C., Holden, S.T., Santini, H., Barker, R.A., 2022. Huntington's disease: diagnosis and management. Pract. Neurol. 22, 32–41. https://doi.org/10.1136/practneurol-2021-003074

Stuendl, A., Kunadt, M., Kruse, N., Bartels, C., Moebius, W., Danzer, K.M., Mollenhauer, B., Schneider, A., 2016. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain 139, 481–494. https://doi.org/10.1093/brain/awv346

Stuffers, S., Sem Wegner, C., Stenmark, H., Brech, A., 2009. Multivesicular Endosome Biogenesis in the Absence of ESCRTs. Traffic 10, 925–937. https://doi.org/10.1111/j.1600-0854.2009.00920.x

Sudhakar, V., Richardson, R.M., 2019. Gene Therapy for Neurodegenerative Diseases. Neurotherapeutics 16, 166–175. https://doi.org/10.1007/s13311-018-00694-0

Takamori, S., 2009. Synaptic Vesicles, in: Squire, L.R. (Ed.), Encyclopedia of Neuroscience. Academic Press, Oxford, pp. 801–808. https://doi.org/10.1016/B978-008045046-9.01392-9

Terwel, D., Dewachter, I., Van Leuven, F., 2002. Axonal transport, tau protein, and neurodegeneration in Alzheimer's disease. Neuromolecular Med. 2, 151–165. https://doi.org/10.1385/NMM:2:2:151

Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G.K., Ayre, D.C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N.N., Baxter, A.A., Bebawy, M., Beckham, C., Bedina Zavec, A., Benmoussa, A., Berardi, A.C., Bergese, P., Bielska, E., Blenkiron, C., Bobis-Wozowicz, S., Boilard, E., Boireau, W., Bongiovanni, A., Borràs, F.E., Bosch, S., Boulanger, C.M., Breakefield, X., Breglio, A.M., Brennan, M.Á., Brigstock, D.R., Brisson, A., Broekman, M.L., Bromberg, J.F., Bryl-Górecka, P., Buch, S., Buck, A.H., Burger, D., Busatto, S., Buschmann, D., Bussolati, B., Buzás, E.I., Byrd, J.B., Camussi, G., Carter, D.R., Caruso, S., Chamley, L.W., Chang, Y.-T., Chen, C., Chen, S., Cheng, L., Chin, A.R., Clayton, A., Clerici, S.P., Cocks, A., Cocucci, E., Coffey, R.J., Cordeiro-da-Silva, A., Couch, Y., Coumans, F.A., Coyle, B., Crescitelli, R., Criado, M.F., D'Souza-Schorey, C., Das, S., Datta Chaudhuri, A., de Candia, P., De Santana, E.F., De Wever, O., del Portillo, H.A., Demaret, T., Deville, S., Devitt, A., Dhondt, B., Di Vizio, D., Dieterich, L.C., Dolo, V., Dominguez Rubio, A.P., Dominici, M., Dourado, M.R., Driedonks, T.A., Duarte, F.V., Duncan, H.M., Eichenberger, R.M., Ekström, K., EL Andaloussi, S., Elie-Caille, C., Erdbrügger, U., Falcón-Pérez, J.M., Fatima, F., Fish, J.E., Flores-Bellver, M., Försönits, A., Frelet-Barrand, A., Fricke, F., Fuhrmann, G., Gabrielsson, S., Gámez-Valero, A., Gardiner, C., Gärtner, K., Gaudin, R., Gho, Y.S., Giebel, B., Gilbert, C., Gimona, M., Giusti, I., Goberdhan, D.C., Görgens, A., Gorski, S.M., Greening, D.W., Gross, J.C., Gualerzi, A., Gupta, G.N., Gustafson, D., Handberg, A., Haraszti, R.A., Harrison, P., Hegyesi, H., Hendrix, A., Hill, A.F., Hochberg, F.H., Hoffmann, K.F., Holder, B., Holthofer, H., Hosseinkhani, B., Hu, G., Huang, Y., Huber, V., Hunt, S., Ibrahim, A.G.-E., Ikezu, T., Inal, J.M., Isin, M., Ivanova, A., Jackson, H.K., Jacobsen, S., Jay, S.M., Jayachandran, M., Jenster, G., Jiang, L., Johnson, S.M., Jones, J.C., Jong, A., Jovanovic-Talisman, T., Jung, S.,

Kalluri, R., Kano, S., Kaur, S., Kawamura, Y., Keller, E.T., Khamari, D., Khomyakova, E., Khvorova, A., Kierulf, P., Kim, K.P., Kislinger, T., Klingeborn, M., Klinke, D.J., Kornek, M., Kosanović, M.M., Kovács, Á.F., Krämer-Albers, E.-M., Krasemann, S., Krause, M., Kurochkin, I.V., Kusuma, G.D., Kuypers, S., Laitinen, S., Langevin, S.M., Languino, L.R., Lannigan, J., Lässer, C., Laurent, L.C., Lavieu, G., Lázaro-Ibáñez, E., Le Lay, S., Lee, M.-S., Lee, Y.X.F., Lemos, D.S., Lenassi, M., Leszczynska, A., Li, I.T., Liao, K., Libregts, S.F., Ligeti, E., Lim, R., Lim, S.K., Linē, A., Linnemannstöns, K., Llorente, A., Lombard, C.A., Lorenowicz, M.J., Lörincz, Á.M., Lötvall, J., Lovett, J., Lowry, M.C., Loyer, X., Lu, Q., Lukomska, B., Lunavat, T.R., Maas, S.L., Malhi, H., Marcilla, A., Mariani, J., Mariscal, J., Martens-Uzunova, E.S., Martin-Jaular, L., Martinez, M.C., Martins, V.R., Mathieu, M., Mathivanan, S., Maugeri, M., McGinnis, L.K., McVey, M.J., Meckes, D.G., Meehan, K.L., Mertens, I., Minciacchi, V.R., Möller, A., Møller Jørgensen, M., Morales-Kastresana, A., Morhayim, J., Mullier, F., Muraca, M., Musante, L., Mussack, V., Muth, D.C., Myburgh, K.H., Najrana, T., Nawaz, M., Nazarenko, I., Nejsum, P., Neri, C., Neri, T., Nieuwland, R., Nimrichter, L., Nolan, J.P., Nolte-'t Hoen, E.N., Noren Hooten, N., O'Driscoll, L., O'Grady, T., O'Loghlen, A., Ochiya, T., Olivier, M., Ortiz, A., Ortiz, L.A., Osteikoetxea, X., Østergaard, O., Ostrowski, M., Park, J., Pegtel, D.M., Peinado, H., Perut, F., Pfaffl, M.W., Phinney, D.G., Pieters, B.C., Pink, R.C., Pisetsky, D.S., Pogge von Strandmann, E., Polakovicova, I., Poon, I.K., Powell, B.H., Prada, I., Pulliam, L., Quesenberry, P., Radeghieri, A., Raffai, R.L., Raimondo, S., Rak, J., Ramirez, M.I., Raposo, G., Rayyan, M.S., Regev-Rudzki, N., Ricklefs, F.L., Robbins, P.D., Roberts, D.D., Rodrigues, S.C., Rohde, E., Rome, S., Rouschop, K.M., Rughetti, A., Russell, A.E., Saá, P., Sahoo, S., Salas-Huenuleo, E., Sánchez, C., Saugstad, J.A., Saul, M.J., Schiffelers, R.M., Schneider, R., Schøyen, T.H., Scott, A., Shahaj, E., Sharma, S., Shatnyeva, O., Shekari, F., Shelke, G.V., Shetty, A.K., Shiba, K., Siljander, P.R.-M., Silva, A.M., Skowronek, A., Snyder, O.L., Soares, R.P., Sódar, B.W., Soekmadji, C., Sotillo, J., Stahl, P.D., Stoorvogel, W., Stott, S.L., Strasser, E.F., Swift, S., Tahara, H., Tewari, M., Timms, K., Tiwari, S., Tixeira, R., Tkach, M., Toh, W.S., Tomasini, R., Torrecilhas, A.C., Tosar, J.P., Toxavidis, V., Urbanelli, L., Vader, P., van Balkom, B.W., van der Grein, S.G., Van Deun, J., van Herwijnen, M.J., Van Keuren-Jensen, K., van Niel, G., van Royen, M.E., van Wijnen, A.J., Vasconcelos, M.H., Vechetti, I.J., Veit, T.D., Vella, L.J., Velot, É., Verweij, F.J., Vestad, B., Viñas, J.L., Visnovitz, T., Vukman, K.V., Wahlgren, J., Watson, D.C., Wauben, M.H., Weaver, A., Webber, J.P., Weber, V., Wehman, A.M., Weiss, D.J., Welsh, J.A., Wendt, S., Wheelock, A.M., Wiener, Z., Witte, L., Wolfram, J., Xagorari, A., Xander, P., Xu, J., Yan, X., Yáñez-Mó, M., Yin, H., Yuana, Y., Zappulli, V., Zarubova, J., Žėkas, V., Zhang, J., Zhao, Z., Zheng, L., Zheutlin, A.R., Zickler, A.M., Zimmermann, P., Zivkovic, A.M., Zocco, D., Zuba-Surma, E.K., 2018. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J. Extracell. Vesicles 7, 1535750. https://doi.org/10.1080/20013078.2018.1535750

Thompson, A.G., Gray, E., Heman-Ackah, S.M., Mäger, I., Talbot, K., Andaloussi, S.E., Wood, M.J., Turner, M.R., 2016. Extracellular vesicles in neurodegenerative disease - pathogenesis to biomarkers. Nat. Rev. Neurol. 12, 346–357. https://doi.org/10.1038/nrneurol.2016.68

Thompson, A.G., Gray, E., Mäger, I., Thézénas, M.-L., Charles, P.D., Talbot, K., Fischer, R., Kessler, B.M., Wood, M., Turner, M.R., 2020. CSF extracellular vesicle proteomics demonstrates altered protein homeostasis in amyotrophic lateral sclerosis. Clin. Proteomics 17, 31. https://doi.org/10.1186/s12014-020-09294-7

Umeda, R., Nishizawa, T., Nureki, O., 2019. Crystallization of the human tetraspanin protein CD9. Acta Crystallogr. Sect. F Struct. Biol. Commun. 75, 254–259. https://doi.org/10.1107/S2053230X1801840X

Umeda, R., Satouh, Y., Takemoto, M., Nakada-Nakura, Y., Liu, K., Yokoyama, T., Shirouzu, M., Iwata, S., Nomura, N., Sato, K., Ikawa, M., Nishizawa, T., Nureki, O., 2020. Structural insights into tetraspanin CD9 function. Nat. Commun. 11, 1606. https://doi.org/10.1038/s41467-020-15459-7

van der Bent, M.L., Evers, M.M., Vallès, A., 2022. Emerging Therapies for Huntington's Disease – Focus on N-Terminal Huntingtin and Huntingtin Exon 1. Biol. Targets Ther. 16, 141–160. https://doi.org/10.2147/BTT.S270657

van Niel, G., D'Angelo, G., Raposo, G., 2018. Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol. 19, 213–228. https://doi.org/10.1038/nrm.2017.125

Vanden Broeck, L., Callaerts, P., Dermaut, B., 2014. TDP-43-mediated neurodegeneration: towards a loss-of-function hypothesis? Trends Mol. Med. 20, 66–71. https://doi.org/10.1016/j.molmed.2013.11.003

Vauleon, S., Schutz, K., Massonnet, B., Gruben, N., Manchester, M., Buehler, A., Schick, E., Boak, L., Hawellek, D.J., 2023. Quantifying mutant huntingtin protein in human cerebrospinal fluid to support the development of huntingtin-lowering therapies. Sci. Rep. 13, 5332. https://doi.org/10.1038/s41598-023-32630-4

Veerman, R.E., Teeuwen, L., Czarnewski, P., Güclüler Akpinar, G., Sandberg, A., Cao, X., Pernemalm, M., Orre, L.M., Gabrielsson, S., Eldh, M., 2021. Molecular evaluation of five different isolation methods for extracellular vesicles reveals different clinical applicability and subcellular origin. J. Extracell. Vesicles 10, e12128. https://doi.org/10.1002/jev2.12128

Vijayakumar, U.G., Milla, V., Cynthia Stafford, M.Y., Bjourson, A.J., Duddy, W., Duguez, S.M.-R., 2019. A Systematic Review of Suggested Molecular Strata, Biomarkers and Their Tissue Sources in ALS. Front. Neurol. 10, 400. https://doi.org/10.3389/fneur.2019.00400

Wang, Q., Lu, Q., 2017. Plasma membrane-derived extracellular microvesicles mediate non-canonical intercellular NOTCH signaling. Nat. Commun. 8, 709. https://doi.org/10.1038/s41467-017-00767-2

Wang, S., Kojima, K., Mobley, J.A., West, A.B., 2019. Proteomic analysis of urinary extracellular vesicles reveal biomarkers for neurologic disease. EBioMedicine 45, 351–361. https://doi.org/10.1016/j.ebiom.2019.06.021

Wild, E.J., Boggio, R., Langbehn, D., Robertson, N., Haider, S., Miller, J.R.C., Zetterberg, H., Leavitt, B.R., Kuhn, R., Tabrizi, S.J., Macdonald, D., Weiss, A., 2015. Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients. J. Clin. Invest. 125, 1979–1986. https://doi.org/10.1172/JCI80743

Willms, E., Johansson, H.J., Mäger, I., Lee, Y., Blomberg, K.E.M., Sadik, M., Alaarg, A., Smith, C.I.E., Lehtiö, J., EL Andaloussi, S., Wood, M.J.A., Vader, P., 2016. Cells release subpopulations of exosomes with distinct molecular and biological properties. Sci. Rep. 6, 22519. https://doi.org/10.1038/srep22519

Yelick, J., Men, Y., Jin, S., Seo, S., Espejo-Porras, F., Yang, Y., 2020. Elevated exosomal secretion of miR-124-3p from spinal neurons positively associates with disease severity in ALS. Exp. Neurol. 333, 113414. https://doi.org/10.1016/j.expneurol.2020.113414

Zachau, A.C., Landén, M., Mobarrez, F., Nybom, R., Wallén, H., Wetterberg, L., 2012. Leukocyte-derived microparticles and scanning electron microscopic structures in two fractions of fresh cerebrospinal fluid in amyotrophic lateral sclerosis: a case report. J. Med. Case Reports 6, 274. https://doi.org/10.1186/1752-1947-6-274

Zbinden, A., Pérez-Berlanga, M., De Rossi, P., Polymenidou, M., 2020. Phase Separation and Neurodegenerative Diseases: A Disturbance in the Force. Dev. Cell 55, 45–68. https://doi.org/10.1016/j.devcel.2020.09.014

Zhang, J., Jin, J., Su, D., Feng, T., Zhao, H., 2023. Tau-PET imaging in Parkinson's disease: a systematic review and meta-analysis. Front. Neurol. 14.

Zhang, X., Abels, E.R., Redzic, J.S., Margulis, J., Finkbeiner, S., Breakefield, X.O., 2016. Potential Transfer of Polyglutamine and CAG-Repeat RNA in Extracellular Vesicles in Huntington's Disease: Background and Evaluation in Cell Culture. Cell. Mol. Neurobiol. 36, 459–470. https://doi.org/10.1007/s10571-016-0350-7

Zhao, Z., Wijerathne, H., Godwin, A.K., Soper, S.A., 2021. Isolation and analysis methods of extracellular vesicles (EVs). Extracell. Vesicles Circ. Nucleic Acids 2, 80–103. https://doi.org/10.20517/evcna.2021.07

Zhou, Q., He, L., Hu, J., Gao, Y., Shen, D., Ni, Y., Qin, Y., Liang, H., Liu, J., Le, W., Chen, S., 2022. Increased expression of coronin-1a in amyotrophic lateral sclerosis: a potential diagnostic biomarker and therapeutic target. Front. Med. 16, 723–735. https://doi.org/10.1007/s11684-021-0905-y

Zimmerman, B., Kelly, B., McMillan, B.J., Seegar, T.C.M., Dror, R.O., Kruse, A.C., Blacklow, S.C., 2016. Crystal Structure of a Full-Length Human Tetraspanin Reveals a Cholesterol-Binding Pocket. Cell 167, 1041-1051.e11. https://doi.org/10.1016/j.cell.2016.09.056