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Review

Alzheimer's and Parkinson's diseases: An environmental proteomic point of view☆

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ABSTRACT

Alzheimer's and Parkinson's diseases are severe neurodegenerative conditions triggered by complex biochemical routes. Many groups are currently pursuing the search for valuable biomarkers to either perform early diagnostic or to follow the disease's progress. Several studies have reported relevant findings regarding environmental issues and the progression of such diseases. Here the etiology and mechanisms of these diseases are briefly reviewed. Approaches that might reveal candidate biomarkers and environmental stressors associated to the diseases were analyzed under a proteomic perspective.

This article is part of a Special Issue entitled: Environmental and structural proteomics.

Contents

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1. Introduction

Proteomic approaches are widely used in biology, medicine, agriculture and many other areas. The main idea, regardless of the biological question behind, is to understand the expression, quantification, compartmentalization, mobilization, or modification of proteins under a specific condition. The types and numbers of these conditions vary extensively: development, biotic and abiotic stress, diseases, medical treatment, and so on. (See Figs. 1 and 2.)

Reports on environmental studies using proteomic approaches have increased in the past years. In these cases, "satellite" organisms aided to monitor different kinds of stresses caused by environmental conditions, such as water, air or soil pollution, intoxication by different poisons, heavy metals, organic solvents, ionizing radiation, and electromagnetic field [1–3].

Although there is a wide range of neurodegenerative diseases (NDs), in this review, the neurodegenerative disorders Parkinson's (PD) and Alzheimer's diseases (AD) were chosen to illustrate proteomic approaches and studies focusing on environmental proteomics.

2. Environmental proteomics

Environmental changes caused by different stressors can be studied applying proteomic approaches. These strategies can reflect the physiological response of living beings to changing conditions or stressful environmental states [4]. Minimal alterations on the environment may lead to important adaptations of organisms to this new condition. As pointed out by González-Fernándes and collaborators, environmental proteomics encompasses studies on toxic and defense mechanisms triggered by different pollutants, without previous knowledge about the biological systems themselves, which is one of the advantages of this approach [5]. Although proteomic studies can compare dynamic responses in several conditions, only in recent years has this strategy gained space in environmental issues, particularly biomarker searches for intoxication/contamination, or environmental risk factors [6].

Examples of studies performed in which the "environmental problem" was addressed using proteomics, include terrestrial ecosystems [2], semimetal intoxication [7], and exposure to tobacco smoke [8]. In a work performed by Montes-Nieto and collaborators using *Mus pretus* as a bioindicator, the protein expression profile of animals from Domingo Rubio stream was compared to that of animals from Doñana Biological Reserve (both in Spain), using 2-DE (two-dimensional electrophoresis) and peptide mass fingerprinting by MALDI-TOF (matrix-assisted laser-desorption ionization-time-of-flight). Relevant differences in the animal's proteome were identified, including proteins with a defensive role against the toxic and polluted environment as well as proteins that could make them more susceptible [9].

In a more recent publication also employing 2-DE as protein fractionation method, Company and co-workers compared subproteomes of the mussel *Bathymodiolus azoricus*. This animal lives in a gradient zone at the bottom of the oceans, in which water from the hydrothermal vents mixes with sea water, characterized by extreme variable conditions of pH, high metals and salt contents, and wide oscillations in temperature. Besides these extreme conditions, several reducing chemical species are present in this environment, which can cause severe oxidative damages through generation of reactive oxygen species (ROS). The authors selected mussels from different locations, and performed an enrichment of thiol-containing proteins, by using an activated thiol Sepharose matrix. Proteomic analysis was performed by 2-DE only, without protein identification by mass spectrometry. The authors found a correlation between thiol direct oxidation by ROS and the site of collection [10].

Dieldrin, a powerful organochloride pesticide which blocks gamma-amino-butyric acid (GABA) receptors in the CNS, was widely used in the 1960–1980s. This pesticide is very lipophilic, and accumulates in fish fat and muscle. In a study by Martyniuk and colleagues, gene expression analysis by microarray and iTRAQ were combined to quantitatively evaluate proteins differentially expressed in largemouth bass fishes fed on subchronic dieldrin-containing diets. The applied proteomic approach revealed decrease in the levels of seven proteins and increase of eleven other proteins in the dieldrin-fed group. Several of the identified proteins are known to be involved in human NDs, such as microtubule-associated tau protein, myelin basic protein, enolase 1, stathmin 1a, apolipoprotein E, and parvalbumin. Martyniuk's study has shown that dieldrin affected "pathological pathways" shared by both AD and PD, overlapping with proteomic signatures known for these neurological diseases [11], which are related to energy production, protection from oxidative damage, and synapse integrity. The authors suggested that "common pathways could be activated by stress or injury of the CNS and may be the result of apoptosis, inflammation, and oxidative damage that may precede neurotoxicity and neural damage" [12].

The effects of another important toxic agent, arsenic, was evaluated using SELDI-TOF (surface-enhanced laser desorption/ionization). This semimetal has high affinity to sulfhydryl groups in keratin, and can be detected in high amounts in the skin, hair and nails of intoxicated individuals [7]. In the study by Harezlak and coauthors, plasma samples from a population in Bangladesh known to be exposed to As were analyzed and an extensive questionnaire was applied to the subjects in order to understand their lifestyle. Authors used a

"unified statistical method that simultaneously takes into account different sources of variation that are present in mass spectrometry measurements". The raw data was decomposed into four different stages: baseline, signal, instrumental noise, and random noise. The authors concluded that the 20 superproteins (protein peaks which fitted into the criteria) detected in the population could be used as an early diagnostic for As exposure, and that the statistical method proposed could be expanded to LC–MS and MALDI-TOF approaches [7].

Considering toxicants related to central nervous system, a proteomic approach identified differential protein expression in the cortex of rats after cocaine exposure [13]. In this case, Guan and Guan studied the medial prefrontal cortex, which is highly activated after cocaine exposure, which can lead to irreversible changes in this brain's area. The authors used a conditioned place preference assay in rats, as a model for addictive drugs. Protein was extracted and analyzed by 2-DE and MALDI-TOF/TOF. There were about 71 differentially expressed spots between control and "addicted" groups, belonging to different functional classes and perhaps these proteins could serve as models or targets to understand cocaine addiction [13].

3. Neurodegenerative diseases and proteomics

Neurodegenerative diseases (NDs) are incurable conditions that result in progressive degeneration or loss of neurons in the affected individuals [14]. The occurrence of NDs is

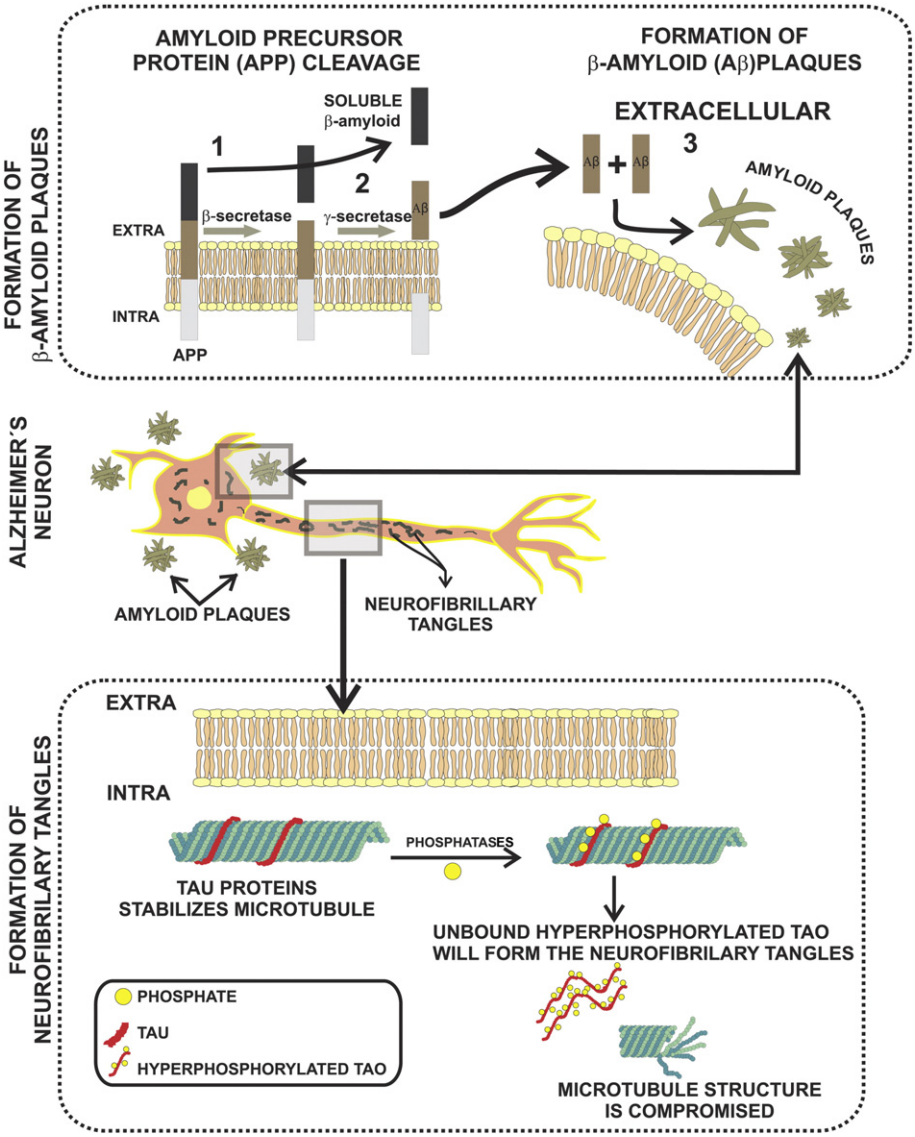

Fig. 1 – In the figure, the formation of β-amyloid plaques is presented in the upper panel. The amyloid precursor protein (APP) is cleaved by β- and γ-secretase and produces one soluble and another insoluble fraction (Aβ). The Aβ aggregation will produce the amyloid plaques. Lower panel: tau protein (TAU) is found in the microtubules and helps the stabilization of this structure. However, hyperphosphorylated TAU causes destabilization of the microtubule because unbound hyperphosphorylated tau aggregates and as result, the microfibrillary tangles are produced. Both (amyloid and tangles) will cause severe damage to the neuron cell.

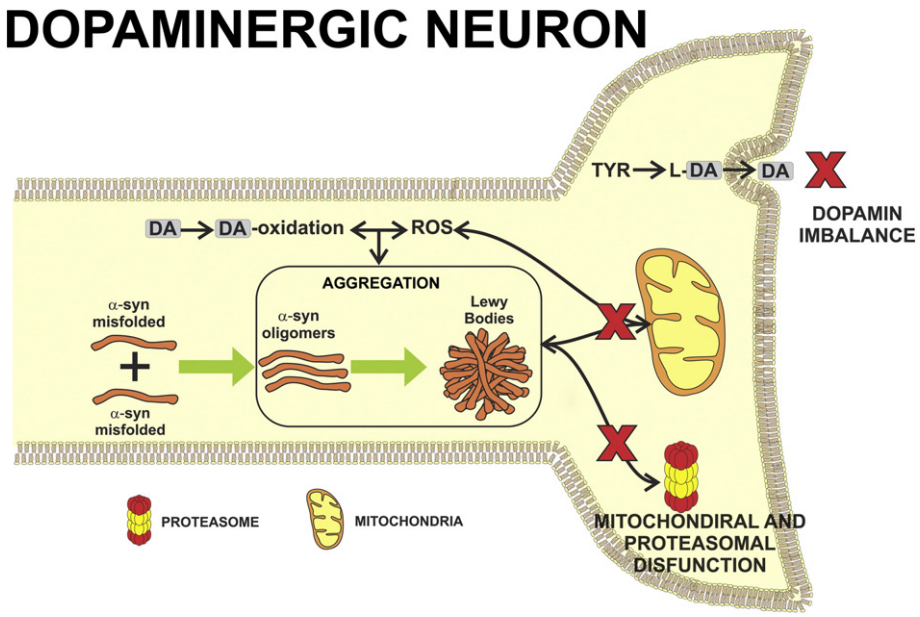

Fig. 2 – In Alzheimer's disease, the pathological process starts at the brain region known as *substantia nigra pars compacta*. It is accepted that alpha-synuclein (α-syn) aggregation will form the Lewy bodies. The α-syn aggregation process will produce severe mitochondrial and proteasomal dysfunction. Reactive oxygen species (ROS) produced in and imbalanced way will contribute to the overall process and at the end. The regular dopamine (DA) metabolism, from tyrosine, levo dopa (L-DOPA) and finally DA will be imbalanced as a result of the entire process and the Parkinson disease will be established. The figure presents a simple representation of a much more complex disease.

significantly increasing over the past decades, especially because of the global increase in life expectancy. Nowadays there is a great interest in understanding the pathogenesis of these diseases, aiming to detect very early signs and symptoms, discover preclinical biomarkers and to develop new therapies for stopping and/or reverting the underlying processes. Some of these diseases constitute a major challenge for health professionals, as very often diagnosis is given only in advanced stages and, despite the different physiopathological processes underlying the diseases, usually these conditions share several clinical symptoms [15–17]. The development of effective diagnostic methods, which could identify patients at risk and the early stages of these illnesses would be of major importance. The early diagnosis of ND diseases ideally should include central nervous system imaging and biomarkers from different sources, such as blood and cerebrospinal fluid (CSF), that could support the clinical diagnosis [18]. A biomarker is defined as a 'characteristic that is objectively measured and evaluated as an indicator of normal biology, pathological process, or pharmacologic responses to a therapeutic intervention' [19]. According to Pal and colleagues, presently no biomarkers exist for reliable diagnosis, tracking of disease progression or monitoring responses to treatment regimes [20]. Therefore, the search for valuable biomarkers for diagnostics and prognostics of NDs are of great interest worldwide [21].

Proteomic approaches have been extensively applied to discover new biomarkers for early diagnostics and prognostics for these diseases. The best "source" to obtain a reliable biomarker for neurodegenerative diseases is the CSF. However, as pointed by Shi and colleagues, the biomarker might be present in the CFS only at later stages of NDs, no longer being useful for early diagnosis or intervention. Hence it is desirable that a biomarker would first be detectable in the blood and subsequently in the CSF, if possible [22].

Proteomic approaches applied in studies of the CNS performed with embryonic and postnatal brain tissue, different brain regions, CSF, neural stem cells, pre and post synaptic proteomes and neurodegenerative diseases were extensively reviewed by Zang [23]. One very interesting and elegant investigation was carried out by Bernay and colleagues, aimed to study the secretome of the CNS; in different words, the contents of the extracellular compartment. A secretome of rat striatum was obtained by performing microdialysis to collect proteins and peptides that participate in the complex network of communication within and between brain regions [24]. In this study, the microdialysis fluid (secretome) was fractionated into proteins of >100 kDa, >10 kDa and <100 kDa and peptides around 20 kDa. Two different mass spectrometers, an Orbitrap (Thermo) and a Q-TOF Ultima (Waters) were used for the analysis. According to the authors, the differential pre-fractionation methods and combination of two mass spectrometers were essential for the success of the study, in which they detected for the rat striatum secretome about 88 proteins and 100 peptides (derived from 29 different protein precursors), potentially involved in signaling of the complex brain network [24].

More than 300 proteins were found altered in brain and CFS of ND patients or other psychiatric condition (the studies comprised Alzheimer's disease, Parkinson's disease, Down's syndrome, Pick's disease, Creutzfeldt–Jakob disease, schizophrenia, bipolar disorder, depression, hypoxia, ischemia and neuropathic pain). This compilation, reviewed in details by Fountoulakis, comprised mainly qualitative studies performed

either by 2-DE followed by MALDI-TOF or LC–MS/MS analyses [25].

Neurodegenerative diseases were also studied under redox proteomic approaches. A comprehensive review by Butterfield about the topic leads to similar results: proteins involved in glucose metabolism, mitochondrial function, structural, and protein degradation are commonly affected in some NDs (Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis), suggesting that there might be a shared mechanism by which neurodegeneration takes place in different diseases [26].

4. Environmental agents and NDs

Many metals can be found in the environment in different forms. Some severe conditions can develop caused by the excessive ingestion or absorption of metals, including acute toxicity, mental retardation, antibiotic resistance and even death. The etiology of several diseases might be related to previous exposure to heavy metals or other intoxicant agents [27–30].

The central nervous system is very sensitive to different agents, and among them, copper can be cited. In the CNS, there is a low level of important antioxidant enzymes, contrasting with a high level of easily oxidized substrates and combined with a high flux of ROS that are generated during neurochemical reactions [31]. Despite its importance in biochemistry, Cu^{+2} ions may disrupt the correct conformation of some peptides and proteins. It is known that Cu^{+2} induces conformational change in the normal prion protein, which modify from a random coil into β-sheet characteristic of the PrP form of the protein, which is associated with Prion diseases [32,33]. Cu^{+2} ions can contribute to the formation of the β-sheet or extended conformation of amyloidal beta peptides, which are associated with AD [33]. On the other hand, α-helical structures are important for the formation of paired helical filaments. It is assumed that in AD, Cu^{+2} participates of the formation of this motif in neurofibril tangles. The stoichiometry of copper binding to peptide R2, one of the four highly conserved regions of tau protein (R1 to R4), was studied by MALDI-TOF and the formation of the R2–Cu^{+2} complex was confirmed although being less strong than the R3–Cu^{+2} complex [32]. Copper is found in several types of wires widely used worldwide and workers exposed to them are susceptible to chronic intoxication.

In another case–control study, Gorell and coworkers analyzed the potential role of occupational exposure to iron, copper, manganese, mercury, zinc, and lead as risk factors for PD, and found a significantly increased association of the disease in patients with more than 20 years of exposure with copper and manganese. The author also reported a greater association of PD with exposure to combinations of lead–copper, iron–copper and lead–iron than with any of these metals alone [34].

High levels of some metals in the brain, including aluminum, zinc and iron levels may also be linked to the development or progression of AD [35]. Zatta and colleagues reviewed the role of these metals in neurodegenerative processes. While aluminum is reported as a very controversial cofactor in AD and other NDs, manganese apparently plays an important role in causing PD, with increased environmental burden of manganese being associated with neurodegeneration in the basal ganglia. The author also reported no evidences associating zinc to ND [36].

Recently reviewed by Bakuslky and colleagues, lead exposure can also be associated with AD. Lead can be absorbed by the lung epithelium and gastrointestinal tract, upon binding to heme groups and consequently can flow around the body through the blood [37]. In short words, early episodes in life and/or continuous exposure to lead can contribute to amyloidogenesis in later life stages [37,38]. In the study performed by Basha and co-workers using rodents, the authors observed that lead exposure induced transient suppression of the β-amyloid precursor protein in neonates, followed by a delayed over expression 20 months after the exposure ceased [38].

The role of environmental factors in the incidence of NDs has been addressed by analyzing mainly pesticide exposure, in special in relation to Parkinson and Alzheimer's disease [39,40]. People who live in a rural area, drinks well water, and works in activities related to farming are more exposed to pesticides from different sources, which may be a risk factor for developing PD [40].

In a prospective cohort in which 1507 French elderly were followed, Baldi and colleagues identified an increased relative risk to develop NDs in subjects who had been occupationally exposed to different pesticides (insecticides, herbicides, and fungicides). However, it was not possible to correlate one specific chemical to the development of any neurological disorder. The focus was given to AD and PD and the "diagnostic" was given based on a simple algorithm based on a standardized questionnaire that classified the subjects as either suspected or not suspected of having dementia [41].

Back in 1997, in a case–control study in Taiwan, Liou and coworkers reported that PD risk was greater among subjects exposed to paraquat and other herbicides/pesticides than those not exposed. However, the author did not find significant differences in occupational exposures to chemicals, heavy metals, and minerals among PD patients and matched control subjects [42].

In case of AD, the contribution of environmental factors is controversial. Some authors report an increased risk to develop AD associated to occupational exposure to pesticides [43,44]. On the other hand, different authors failed to show such risk [45]. The controversy of these studies might be due to the fact that susceptibility to pesticides and other neurotoxins depends on variability in xenobiotic metabolism, possibly generated by genetic polymorphisms, aging and degree of exposure to environmental agents [46].

There are other atypical Parkinson's-like syndromes beyond PD. Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP) are neurodegenerative conditions with clinical features similar to PD, which may be confused considering their clinical aspects. While MSA is a disease associated to α-synuclein accumulation [47] PSP seems to be a tau pathology [15]. MSA incidence has been associated with metal dusts and fumes, plastic monomers and additives, organic solvents, and pesticides [48]. Dexter and colleagues reported an increased concentration of iron patients with MSA and also PSP, suggesting a possible environmental factor in these diseases [49].

Oxidative stress is inherent of several regular physiological processes, in which ROS are generated. Several environmental pollutants, including heavy metals and pesticides potentially exacerbate ROS production [50]. The imbalance on ROS production and physiological antioxidant mechanism, caused by external agents can contribute to the etiology or progress of several diseases, such as AD. Copper, chromium and cadmium are known to cause protein damage through ROS intermediates, and were studied by redox proteomic approaches [50]. Nitrated proteins, specifically enolase, glyceraldehyde-3-phosphate dehydrogenase, ATP synthase, carbonic anhydrase-II and voltage-dependent anion channel were detected by Sultana and colleagues, in frozen hippocampal samples from AD patients analyzed by 2-DE followed by MALDI-TOF [51].

5. Alzheimer's disease

The most common form of age-related neurodegenerative disease in the world is Alzheimer's disease (AD), the leading cause of dementia [52]. In 2006, the worldwide prevalence of AD was 26.6 million and estimates are that by 2050, prevalence will quadruple [53], bringing a very high socioeconomic impact and requiring huge adjustments of governments, social agencies, health insurances and families to deal with these patients.

In 1906 the German neurologist Alois Alzheimer (1864–1915) first described the clinical and pathological features of an unusual brain disease during the Tübingen Assembly of Southwest German Psychiatry [54,55]. His presentation described the case of Auguste Deter, who, at age 51, presented with a rapidly progressive dementia syndrome. Post-mortem examination revealed the presence of amyloid plaques and neurofibrillary tangles. These findings were published by Alzheimer in 1907, in the form of a short report. In 1910, the psychiatrist Emil Kraepelin, a colleague of Alzheimer, introduced the term Alzheimer's disease (AD) in his Handbook of Psychiatry.

The two core pathological hallmarks of AD are amyloid plaques and neurofibrillary tangles. The amyloid cascade hypothesis suggests that the deposition of amyloid β (Aβ) peptide triggers neuronal dysfunction and cell death in the brain. Tau, a microtubule-associated protein, is the major constituent of neurofibrillary tangles. The amyloid cascade hypothesis proposes that changes in tau and consequent neurofibrillary tangle formation are triggered by toxic concentrations of Aβ [56]. Due to the difficulty in the early diagnosis of this disease, in recent years much effort has been made in the discovery of biomarkers for AD, which could allow the disease to be diagnosed at an early stage [57].

Previously, the AD diagnosis included exclusively patients on the dementia stage [58], and the disease was characterized by histology pathological features. Currently, AD is conceptualized as a progressive pathophysiological process in which the accumulation of β-amyloid (Aβ) pathology is thought to set in motion a dynamic sequential cascade of events, including neurodegeneration, inflammatory processes, and neurotransmitter dysfunction. Even the clinical aspects have changed in the last clinical diagnosis consensus, including also preclinical stages and incorporating biomarkers to support the diagnosis [59].

With the current conceptual changes that redefined AD as process, the physiopathological onset, duration and the underlying mechanisms of AD have received great attention of specialized researchers. It is hypothesized that the pathologic process of AD begins around two decades before cognitive decline [60], and may vary among individuals. The most common primary symptom of AD is a decline in cognitive functions, known as mild cognitive impairment (MCI), with deficits minimally interfering in activities of daily life [56,61]. At the MCI stage, considered as an AD prodromal phase [62], biomarkers have a crucial role, in revealing the onset of the pathophysiological process and urging clinical interventions, which are today still on the clinical trial phase. All identified potential biomarkers are still in the testing stage and clinical studies based on large population studies are needed [63].

Diverse approaches searching for AD biomarkers were reported, including plasma proteomics, plasma lipidomics, transcriptome, autoantibodies, microRNA, plasma Aβ species, and plasma tau differential forms [64]. Studies of plasma lipidomics derived from findings that the deregulation of lipid pathways could be implicated in AD [65] and transcriptome profiling-based methods have been used in an attempt to identify a blood-based signature (a serum biomarker) to differentiate AD patients from asymptomatic control subjects [66–68]. Although the presence of autoantibodies in AD has been demonstrated, their role in the pathology of disease is still unclear [69]. There is substantial evidence that alterations in microRNA levels are associated with some parts of AD pathology, however its relevance as a blood-based biomarker requires validation [70]. Some posttranslational modifications have been identified as potential biochemical markers to measure the disease's activity. Increased levels of oxidative modification markers have been demonstrated; recently, mitochondria isolated from lymphocytes of MCI patients were shown to present signs of increased oxidative stress, which may potentially reflect brain damage and serve as a biomarker for AD [71].

Cerebrospinal fluid and positron emission tomography (PET) are the current clinical biomarkers used to confirm AD pathologic changes in patients diagnosed as having dementia. The use of CSF biomarkers is widely discussed. Rosa-Neto et al. recommended considering it at a tertiary care level to improve diagnostic certainty, particularly in those cases presenting atypical clinical features [72].

CSF biomarkers such as amyloid-β1-42 (Aβ42), total tau (t-tau) and phosphorylated tau (p-tau) are 'hallmarks' of the disease, reflecting axonal damage, and phosphorylated tau (p-tau) indicating neurofibrillary tangle pathology [73–75]. CSF tau is considered to be a strong marker of the neuronal injury associated with AD, and the combined detection of Aβ42, t-tau and p-tau levels in CSF are considered to have a high diagnostic accuracy even in the early stages of Alzheimer's disease [76]. Wang and colleagues reported that "decreased cerebrospinal fluid Aβ42 and increased CSF phosphorylated tau_{181} were independently associated with reduced default mode network integrity with the most prominent decreases in functional connectivity observed between the posterior cingulate and medial temporal regions" [77]. The combination of

low CSF Aβ42 and elevated tau in CSF also correlates with higher risks of progression to AD in patients with MCI [78]. CSF biomarkers are thus thought to be useful in the very early diagnosis of AD [75].

Albeit extremely useful, the CSF collection procedure is very invasive, as it requires a lumbar puncture and adequate infrastructure to perform this procedure. Because blood sampling, in contrast to CSF, is less invasive and thus more accepted by patients, biomarkers in blood are highly desirable and helpful in monitoring follow up [14]. Plasma biomarkers combined with baseline demographics have been suggested as a potential screening tool [79]. Urine and saliva have also been tested as possible analytes in AD research.

5.1. *Proteomics in Alzheimer's disease*

Presently most of the AD proteomic data report findings on proteins derived from CSF or blood, either using protein arrays or mass spectrometry-based detection of blood profiles [80–82]. Several promising blood-based biomarkers of AD have been proposed in studies ranging from proteomic analysis in plasma to genetic profiling [64], among which are apolipoprotein E (with controversies), brain natriuretic peptide, pancreatic polypeptide and C-reactive protein [83].

Doecke and coworkers identified a panel of plasma biomarkers that distinguish individuals with AD from cognitively healthy control subjects with high sensitivity and specificity. These include biomarkers with significantly increased levels (cortisol, pancreatic polypeptide, insulin-like growth factor binding protein 2, β2 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide dismutase, and homocysteine) and biomarkers with significantly decreased levels (apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin) in AD [84]. Others studies based on plasma proteome evaluated apolipoprotein A-II, apolipoprotein E (ApoE), serum glutamic oxaloacetic transaminase, α-1-microglobulin and brain natriuretic peptide for AD diagnosis [85]. Apolipoprotein E, immunoglobulin M, eotaxin-3, N-terminal prohormone of brain natriuretic peptide, matrix metalloproteinase 1, pancreatic polypeptide and tenascin-C were evaluated by Soares and colleagues in MCI and AD patients. Their results confirmed studies reporting CSF increased levels of pancreatic polypeptide, eotaxin 3, tenascin C and NT-pro BNP in patients with AD and MCI [79]. Ray and colleagues reported that several serum signaling proteins, as chemokine (C–C motif) ligand-5, -7, -15, and -18; chemokine (C–X–C motif) ligand-8; epidermal growth factor; granulocyte colony stimulating factor; glial-derived neurotrophic factor; intracellular adhesion molecule 1; insulin-like growth factor binding protein 6; interleukin 1α, 3 and 11; macrophage colony-stimulating factor; platelet derived growth factor-BB; tumor necrosis factor α and tumor necrosis related apoptosis-inducing ligand R4 are associated to AD and progression of MCI to AD [86].

As mentioned previously, ApoE is a candidate for an AD biomarker even though some discrepancies have been found when the levels of this protein are related to AD symptoms. Aiming to clarify this point, Simon and colleagues applied the powerful mass spectrometry quantitative approach of selected reaction monitoring (SRM) to quantify ApoE and ApoE4 in AD patients and control subjects. By using 'proteotypic' peptides (cysteine 112–cysteine 158 in ApoE; cysteine 112–arginine 158 in ApoE4) characteristic of each isoform, which differ in only one amino acid, the authors concluded from this target mass spectrometry approach that ApoE and ApoE4 are not clinically significant relevant for AD diagnostics [87].

Adding to the controversy on ApoE and ApoE4 as markers for AD, Wang and colleagues analyzed the same isoforms under a multiple reaction monitoring approach (MRM). In this case, the authors performed protein quantification in the soluble and insoluble cell fractions. Their data have shown that C and N-terminal fragments of ApoE and ApoE4 accumulate in higher amounts in AD tissues, but the full assignment of these fragments identities has not yet be done. The approach used in Wag's study provided "quantitative evidence for a preferable accumulation of apoE C-terminal fragment in the insoluble fraction of AD frontal cortex homogenate" [88].

Zhang and colleagues performed SRM to study histone acetylation in human brain tissue of advanced AD patients, using samples from individuals at different stages of the disease [89]. A considerable lower amount of histone acetylation in AD samples was detected when compared to controls, pointing to the need of further studies to understand the participation of this post-translational protein modification in AD evolution.

Domenico and colleagues performed a quantitative proteomic study, by measuring the levels of phosphorylated proteins in hippocampus of AD patients. Hyperphosphorylation of tau proteins is considered to be a hallmark of AD [90]. According to the authors, several other proteins could also be erroneously phosphorylated and contribute to the evolution of AD [90]. In this work a critical point has been identified, that the PMI period (post modem interval) could influence the outcome of proteomic studies. During the time interval or delay between "death" and "sample" collection, several processes, i.e. proteolysis, unrelated to any pathological process might happen. Although control samples are used for comparison, the delay still inevitably exists. To avoid this situation, the search for biomarkers in alive individuals should continue. The authors reported 17 proteins differentially phosphorylated in AD samples as compared to control samples; nine of them presented increased phosphorylation in AD subjects [90]. In this study, samples were pre-fractionated by 2-DE, and analyzed in an Orbitrap. No phosphopeptide enrichment was performed. Protein phosphorylation was estimated based on ProQ dym staining. The altered phosphorylation patterns in AD patients occurred in proteins involved in energy metabolism and ATP production, signal transduction and neural structure, all key steps for AD development and evolution [90].

The nitration of proteins in tyrosine residues potentially interferes with phosphorylation processes which are vital for many biological functions. Nitrated proteins detected in Sultana's study suggested imbalance of energy metabolism, synaptic loss, and mitochondrial dysfunction, all phenomena nowadays accepted as part of mechanisms leading to AD. Di Domenico and colleagues investigated the role of cellular stress response on AD progression, by evaluating the levels

of HSP 27, 32, 60, 70 and 90 and thioredoxin-1 by Western blot [91]. The main idea of this study was to measure important proteins related to stress response and protein folding. The samples were autopsied from subjects belonging to the aMCI (amnestic) stage at a maximum 3 h PMI, and three brain regions were analyzed: hippocampus, inferior parietal lobule and cerebellum. In general, the HSP levels were higher in AD samples when compared to control samples, except in the cerebellum. HSP 32 was detected at the highest amount, but without significant difference between the groups [91].

Another interesting approach was used by Reed and co-workers in the analysis of proteins that could bind to 4-hydroxy-2-nonenal (HNE) in the human brain. HNE has an important role in lipid peroxidation and can cause oxidative stress in the brain in its free form or bound to proteins [80]. The study compared subjects at two stages of the disease, the mild cognitive impairment (MCI) phase and late-stage Alzheimer's disease. The analysis of HNE-bound proteins was performed by immunohistochemistry and selected proteins were analyzed by MALDI-TOF, after preparation by 2-DE. The authors pointed out that lipid peroxidation seems to have an important role in AD, even in the early stages MCI and EAD (early Alzheimer's disease) [80]. More recently, Hashimoto and colleagues analyzed microdissected hippocampal neurons by O^{18} labeling mass spectrometry. The elegant approach of laser capture microdissection (LCM) allowed the authors to analyze specific neurons population of the brain tissue. LCM was used to extract post mortem neurons of the *cornu ammonis* 1 region from AD and control patients. The *cornu ammonis* 1 is where neurofibrilar tangles are detected even in early stages of AD. The approach allowed detection of up- and down-regulated neuron-specific proteins, when comparing AD to control samples. Through this strategy, one very specific region of the brain could be analyzed in a way that precluded proteins from regions, not implicated in the pathology, to interfere in the proteomics study [92].

6. Parkinson's disease

The English physician James Parkinson in his article "An Essay on the Shaking Palsy" first described the clinical features of this condition in 1817. Parkinson's disease (PD) is the second major neurodegenerative disease in the world, which affects about 0.3% of the general population including all ethnic and socioeconomic groups, with a slight predominance in males. Its incidence and prevalence increase with age, reaching about 1% in people above 60 years and 4% in those above 80 years [93]. Clinically, PD manifests as resting tremor, bradykinesia (difficulty in initiating movements and slow to run them) and muscle rigidity. These changes usually have an asymmetrical onset. Difficulties in gait and postural instability and autonomic dysfunction are symptoms that may be associated with disease progression [17].

The depletion of dopamine in PD is one of the hallmarks of the disease. The postmortem brain pathological evaluation shows the degeneration of *substantia nigra* in the *pars compacta*, leading to dopamine deficiency. Cytoplasmic eosinophilic inclusions termed Lewy bodies (LB), composed mainly of α-synuclein are also found in areas of neuronal degeneration in these patients [94].

Although a definitive diagnosis is only given in the autopsy, the syndromic diagnosis is based on clinical criteria, being the UK Parkinson's Disease Society Brain Bank the most used globally [16]. The PD is not limited to motor disorders as cognitive deficits can also be detected. Studies indicate a 30% prevalence of dementia in individuals with PD, and it is estimated that at least 75% of patients with more than ten years of disease progression develop dementia [95]. PD patients who develop dementia during the first year have been classified as having dementia with Lewy bodies (DLB) [96]. The risk of developing dementia in PD is particularly high in patients older than 70 years [97,98].

In the past years there is an increasing consensus in that exposure to toxicants such as heavy metals, pesticides and other known neurotoxic substances can increase the risk of developing PD [99]. The idea that neurodegeneration, such as that observed in PD, is closely related to oxidative stress is now accepted [99]. Symptoms similar to those of PD patients have been detected in subjects exposed to manganese [99–101]. The accumulation of aluminum in the brain of PD patients was described, and increased incidence of neurological diseases, including PD, correlates to high levels of aluminum in drinking water [102]. Several techniques are available to quantify metals in different tissues. The laser ablation inductively coupled plasma mass spectrometry is one of these strategies. In a work performed by Matush and colleagues, they analyzed MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-exposed mice using this technique to analyze details of Cu, Fe, Zn and Mn mobilization in brain tissues [103].

Exposures to pesticides have been associated with PD in several aspects. The associations of PD with rotenone and paraquat, two worldwide-used pesticides known to easily cross the blood brain barrier, have been reported [104]. The effect of some pesticides, including, paraquat, rotenone and dieldrin over the α-synuclein were studied by Uversky and colleagues (in vitro). The authors used atomic force microscopy to study the α-synuclein conformation and fibril formation, respectively, after treatment with the pesticides. As result, a significant conformational change in the α-synuclein structure and formation of α-synuclein fibrils in a high rate were observed, suggesting strong participation of these pesticides in the development of Parkinson disease [105].

Rotenone inhibits the complex I of the electron transport chain in mitochondria thus disturbing the oxidative phosphorylation process [30,50]. Rotenone intoxication promotes selective degeneration of *nigral* dopaminergic neurons with accumulation of cytoplasmic α-synuclein aggregates, resulting in symptoms seen in PD, such as bradykinesia, rigidity, tremor and nonmotor signs [30,104].

The herbicide paraquat has been pointed as a stronger environmental factor in PD occurrence [106]. Paraquat generates ROS through production of superoxide radicals and induces a parkinsonian syndrome similar in many features to PD. It also increases lipid peroxidation, decreases levels of antioxidants, disturbs mitochondrial function, increases expression and aggregation of α-synuclein and selectively kills nigral dopaminergic neurons [30,107,108].

Under the histopathological point of view, the tau protein, the amyloid protein and Lewy bodies are involved in the development of PD dementia [95,109,110]. The identification of biomarkers that will allow an earlier and more accurate diagnosis in PD with dementia or DLB is urgent as the population ages globally increased [111]. Chang and coworkers suggested that microRNA biomarkers associated with AD could have potentials for other neurodegenerative diseases as well, such as in Parkinson's, Prion and Huntington's diseases [112]. Other studies have proposed that combining clinical findings, biochemical and imaging markers (MRI, PET and SPECT) will be more likely to contribute to early PD diagnosis and follow up [113,114].

6.1. *Proteomics in Parkinson's disease*

Biomarkers for diagnosis and prognosis of PD are not currently available. Actually, some putative candidates for PD biomarkers were proposed but these still have low specificity and sensitivity [18]. Promising findings in the field showed that α-synuclein is a major component of Lewis bodies [115]; that DJ-1 is involved in protection against oxidative stress during ND [116] and that levels of Aβ42 correlate with cognitive impairment.

The current "omic" approaches in PD include transcriptomics, proteomics and metabolomics, aimed at identifying small changes in mRNA, protein or metabolite profiles [114]. Searches for biomarkers in PD were performed in CSF (α-synuclein, tau, β-amyloid peptides and DJ-1) and proteins and urate in the blood [18,113,117]. Recently it has been suggested that tau and β amyloid are critically involved in early PD progression, probably by a mechanism different than that in Alzheimer's disease [118].

The rat ventral mesencephalic tissue gives rise to the dopamine neurons within the *substantia nigra*, which degenerates in Parkinson's disease. Using a quantitative proteomic approach (iTRAQ), Orme and collaborators studied protein expression in tissues in three different stages: immediately before, during and after the dopaminergic neurogenesis. Briefly, extracted total protein was labeled with iTRAQ reagents, submitted to trypsin digestion followed by peptide prefractionation using multidimensional chromatography (strong cation exchange + reverse phase). Using this strategy, the authors identified ca. 3000 proteins by MALDI-TOF/TOF and could explore in details the proteins involved in the dopamine neuron development [119].

Proteomics approaches have been used to understand the role of mitochondria perturbation and oxidative stress in the role of the PD [120]. Van Laar and collaborators analyzed by 2-DIGE (fluorescence difference gel electrophoresis) rat brain mitochondria after exposure to dopamine–quinone (DAQ). In a healthy brain, dopamine (DA) leads to the production of ROS and DA which is not adequately stored in vesicles can be oxidized to form the reactive DAQ [121]. Increased levels of cysteinyl-DA, a covalent modification of DA triggered by DAQ, have been detected post-mortem in *substantia nigra* of PD patients. Furthermore it has been demonstrated that DAQ can cause alteration of respiratory mechanisms in mitochondria [121]. Proteins oxidized in the mitochondria, according to the authors, could also be potential targets for therapeutic agents in AD. The authors performed mitochondrial isolation from brain tissue of rats and the extracted mitochondrial proteins were analyzed by 2-DIGE coupled to MALDI TOF/TOF mass spectrometry. Mitochondrial creatine kinase (MtCK), which is associated with ADP–ATP exchange and the permeability transition pore [122], is highly sensitive to oxidation. Their data showed that DA-induced oxidation of MtCK affected its enzyme activity thus, compromising the integrity and energy metabolism of mitochondria [121].

Parkinsonism mouse models can be obtained by the use of two neurotoxins: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine (METH). MPTP seems to cause death of dopaminergic neurons in the *substantia nigra pars compacta* [123], while METH inhibits oxidative phosphorylation in dopaminergic neurons [124,125]. Analysis of these two systems under trancriptomic and proteomic approaches revealed significant changes on the levels of 86 proteins and of mRNA of 181 genes after toxin(s) treatment(s). The authors concluded that there is a clear mitochondrial dysfunction in PD, with increased oxidative stress, deregulated protein degradation, increased apoptosis and cell death, and a potential activation of the astrocytic response. This study was performed with $^{16}O/^{18}O$ labeling and Cys-peptide fractionation, aiming an accurate quantification and a wide coverage of the proteome [125].

Constantinescu and colleagues analyzed the CSF from patients with different clinical stages of parkinsonian disorders. The authors compared the protein profile of these groups aiming to identify one or more specifics biomarkers by mass spectrometry SELDI-TOF (using three different surface arrays: cation and anion exchange and metal binding). However, no specific biomarker could be detected and used to distinguish the pathologies among the groups. The authors pointed out that SELDI might not be a good strategy, because the detection limit of the approach could be limiting the detection of low abundant proteins [126].

Several studies have pointed to α-synuclein as a biomarker candidate for PD. Even though this small protein can be detected in the plasma, there are still many controversies regarding its use as a biomarker [127]. Chen and colleagues compared the plasma of healthy and PD patient groups, using 2-DE and a Q-TOF mass spectrometer. Abundant proteins were depleted from blood samples and analyzed it separately. A significant difference between the groups for the proteins serum amyloid component P and IgGκL prompted the authors to suggest these two proteins as biomarker candidates. Although ELISA further confirmed these results, the group of PD patients analyzed in the study was small hence more studies are necessary to validate the data [127].

As a matter of fact, a recent systematic review of biomarkers concluded that there is still insufficient evidence to recommend the use of any biomarker for disease progression in PD clinical trials [128].

7. Concluding remarks

This review has briefly highlighted some physiopathological aspects of Parkinson's and Alzheimer's diseases, the most common age-related neurodegenerative diseases in the world. The occurrence of these diseases has significantly increased

over the past decades, in parallel to global increase in life expectancy. Presently no biomarkers exist for reliable diagnosis, tracking of disease progression or monitoring therapeutic outcomes. Here emphasis was given in reviewing proteomic studies aiming at identification of the urgently needed biomarker(s) that will allow an early detection and subsequent therapeutic intervention to deter the progress or at least ameliorate the symptoms of these debilitating pathologies. The role of environmental stressors in the incidence of these neurodegenerative diseases was addressed and proteomic studies dealing with understanding the effects and diagnosing the exposure to different pollutants and heavy metals in relation to Parkinson and Alzheimer's disease were reviewed. The controversial data on candidate biomarkers useful for diagnostic and prognostic of these CNS diseases reveal a field with many gaps yet to be solved, in which proteomic approaches have ensured application.

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