Pathology, Prevention and Therapeutics of Neurodegenerative Disease

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Editors

Springer

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ISBN 978-981-13-0943-4 ISBN 978-981-13-0944-1 (eBook)
https://doi.org/10.1007/978-981-13-0944-1

Library of Congress Control Number: 2018952338

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Neuroscience is a large field founded on the premise that all of behavior and mental abilities have their origin in the structure and function of the nervous system. This book attempts to provide an overview of major neurodegenerative diseases with a special focus on diseases related to the central nervous system (CNS). Neurodegenerative diseases add up to tremendous medical and financial burden due to their non-partisan share for individuals of all ages, with elderly population contributing the largest share. Due to the enigmatic and complex nature of neurodegenerative diseases, therapeutic intervention to address the same is of immense challenge for the researchers. To date, research has suggested the involvement of diverse factors and complex mechanisms in disease etiology, with a bolting approach still lacking to thwart neurodegeneration. Such impuissance of researchers is mainly due to delayed appearance of behavioral symptoms: the only diagnostic marker for most of the neurodegenerative diseases presently. In fact, the visible symptoms manifest at later and peak stage of disease act as barrier for timely intervention.

Brain has postmitotic neurons thereby lacking restoration of damaged neurons. Previous studies have implicated neurogenesis mainly in the hippocampal area of the brain, while the disease pathology may encompass any brain region. Further, restoration of damaged neurons by stem cell therapy failed to achieve the desired effect due to the lack of versatile utilization for treatment and its financial impact. The prime focus of this book is to introduce students to the major CNS-related neurodegenerative diseases. The chapters aim to introduce the readers about disease pathologies, related mechanisms involved, and available therapeutics. As the disease diagnosis is a huge challenge for physicians and researchers alike, specific chapters focusing on the same have been included to assist the reader in getting a comprehensive view of the disease. Further, the book focuses on neurodegenerative diseases involving mental abilities and motor responses, specifically Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Collectively, research to date strongly supports the view that prevention might be a better approach to fight the disease. In line with disease etiology and diagnosis, we have also endeavored to expose the readers to the existing alternative preventive therapeutic approaches. Alternative therapies derived from natural products may outweigh the side effects of the conventional approaches, thereby a potential option for long-term treatment.

We express our gratitude to all the authors for their efforts in bringing out this compilation in the field of neurosciences. We are also thankful to Eti Dinesh at Springer for her constant support throughout the project. N. S. Pandian (Senior Production Manager) and Kumar Athiappan (Project Coordinator) are also acknowledged for their contribution.

Lucknow, India — Sarika Singh
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About the Editors

Sarika Singh completed her postgraduate degree in biochemistry at Lucknow University in 2001 and subsequently received her doctoral degree from the same university for a dissertation on the role of nitric oxide in the pathology of Parkinson's disease. In 2006, she assumed her current position as a Senior Scientist at CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India. She is a life member of the Indian Academy of Neurosciences and a member of the Indian Academy of Sciences. She is a recipient of international Indo-US and CSIR-Raman research fellowships and has worked toward the identification of diagnostic markers for autism and Parkinson's disease. Having published in several international peer-reviewed journals, she also serves as an editorial board member and reviewer for various international and national journals. Her chief research focus is on investigating the neurodegenerative and neuroprotective mechanisms involved in various brain diseases.

Neeraj Joshi received his master's (M.Sc.) degree in biochemistry from Lucknow University, India, in 2001, after which he was recruited to Bhabha Atomic Research Center (BARC), Mumbai, India. After completing the Orientation Course in Nuclear Science and Engineering at BARC, he worked at its Radiation Biology and Health Sciences Division as a Scientific Officer from 2002 to 2006. His research at BARC focused on investigating DNA damage repair and radiation hormesis in the context of cancer biology and neurodegeneration. To further understand the mechanisms of genomic integrity, Neeraj chose to pursue his doctorate at Cleveland State University, USA, where he explored the mechanistic aspects of meiotic chromosome segregation. This resulted in (1) unraveling the role of genome architecture in DNA damage repair (DDR), (2) the discovery of a new, ultrasensitive DNA damage responsive checkpoint system, and (3) the development of a novel molecular assay: "Homolog Pairing Capture."

Collectively, his doctoral studies provided a new perspective on cellular DDR mechanisms and the indirect involvement of proteasome in the DDR process. From 2015 to 2017, his postdoctoral work at the University of California-San Francisco (UCSF), USA, centered on investigating both the selective and comprehensive repertoire of Cullin-RING-like (CRL) ubiquitin ligases under defined stress conditions.

Alpha Synuclein and Parkinson's Disease

1

Arti Parihar, Priyanka Parihar, Isha Solanki, and Mordhwaj S. Parihar

1.1 Introduction

Parkinson's disease (PD) is the age-related neurodegenerative disorder diagnosed by tremor at rest, rigidity, and bradykinesia symptoms. The prevalence of PD increases with the increase in age and about 2–3% population worldwide suffer from the disease ≥65 years [1]. The major neuropathology of PD patients is the deficit of dopaminergic neurons the substantia nigra pars compacta (SNpc) region of the midbrain. The lesions caused in these brain regions cause severe depletion of striatal dopamine. Non-motor symptoms like dementia, depression, anxiety, insomnia, excessive daytime sleepiness, rapid eye movement sleep disorder, constipation, difficulty in swallowing, and dyspepsia may also be involved in PD symptoms and pathology. Histological characteristic of PD includes the occurrence of Lewy bodies (LBs) in existing neurons [2]. However, little is known about the formation of LBs. The rising evidence revealed that LB biogenesis may involve neuroprotective reactions [3]. Numerous studies have been executed to elucidate the role of α-synuclein in the pathogenesis of PD.

Reports have shown the expression of α-synuclein in neurons which abundantly distributed in presynaptic neuronal terminals of synapses [4]. The distribution of α-synuclein in the synaptic terminals indicates that this protein may take an important role in synaptic plasticity, kinetics of vesicle, and in the dopamine synthesis and its release. The role of α-synuclein in the pathogenesis of PD has been extensively analyzed. The observation of fibrillar α-synuclein in LBs and the occurrence of mutations in the α-synuclein gene in familial forms of PD have led to the belief that this protein has a critical role in PD pathology. The relationship of α-synuclein and PD has been identified by a genetic finding of A53T mutation of α-synuclein gene (*SNCA*) in a family with autosomal-dominant familial PD [5]. Furthermore the implication of α-synuclein in PD has been corroborated by the discovery of the other mutations of *SNCA*, involving A30P and E46K in other families with inherited PD [6, 7]. The function of α-synuclein in PD was further strengthened by the investigation in which presence of this protein was found as the primary structural constituent of LBs [8]. Here, we present an overview of existing knowledge on the physiological functions, oligomerization, and aggregation of α-synuclein and its pathological

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_1

role in PD. Considering the nature of the various α-synuclein structures and its mechanism of toxicity may be important in developing attractive treatment options against the pathologic hallmarks of PD and α-synucleinopathies.

1.2 Localization and the Structure of α-Synuclein

The varied forms of synuclein protein, α, β, and γ are expressed at numerous locations in the nervous system [9]. Synuclein α- and β-forms are chiefly present in nerve terminals, near synaptic vesicles in the central nervous system [10], whereas γ-synuclein is present in neuronal cells of the peripheral nervous system [10]. α-Synuclein is mainly located in the cytoplasm but extracellular α-synuclein has also been studied [11]. In PD, the levels of α-synuclein are higher in cerebrospinal fluid (CSF) than age matched controls [12], indicating that α-synuclein is also present in extracellular brain fluids. Most significantly, α-synuclein oligomers have abundantly distributed in the extracellular space in PD. The presence of α-synuclein both at intra- and extracellular spaces could explain that the extracellular α-synuclein oligomers may disperse from one neuron to another, and this movement might channelize the succession of the disease from one brain region to other regions.

α-Synuclein is a 14 kDa protein (140 amino acids; pKa of 4.7) expressed by the *SNCA* gene on human chromosome 4 [13]. It is the cytoplasmic and/or membrane-bound protein found in presynaptic terminals of neurons [14] categorized by an amphipathic lysine-rich amino terminus (Fig. 1.1a–d). α-Synuclein is intrinsically located in the cytoplasm (Fig. 1.1b) but exhibits alpha helical confirmation when bound to cellular membranes [15]. In addition, α-synuclein is also located in other subcellular compartments such as mitochondria (Fig. 1.1c, d) [16] and it can also be secreted and transferred to nearby cells [17, 18]. The normal cellular state of alpha synuclein is the α-helically folded 58 KDa tetrameric complex that primarily exists as an unfolded monomer in the central nervous system [19]. By structure α-synuclein protein consists of three domains like an amino terminus (residues 1–60), a central hydrophobic region (61–95), so-called NAC (non-Aβ component), and a carboxyl terminus which is extremely negatively charged (Fig. 1.2) and is prone to be unstructured [20]. The N-terminal domain is particularly significant for the pathological dysfunction of *α*-synuclein as the rare point mutations like Ala53Thr, Ala30Pro, Glu46Lys, His50Gln, Gly51Asp, and Ala53Glu are present in this region [21]. However, NAC domain is accountable for the aggregation attributes of *α*-synuclein via inhibition of its degradation and promotion of its fibrillation [22]. Although the normal physiological role of *α*-synuclein is not known, still it appears to be involved in compartmentalization, storage, and recycling of neurotransmitters [23]. α-Synuclein has been shown to interrelate directly with the membrane phospholipids, especially vesicles and have a role in the vesicle trafficking during the neurotransmission release. It also appears to be associated with directive of various enzymes and tends to augment the integer of dopamine transporter molecules [24]. In addition, recombinantly α- and β-synucleins inhibit mammalian phosphatidylcholine (PC)-specific phospholipases D2 activity in vitro [25], suggests that inhibition of PLD2 may be a function of synucleins.

In aqueous solution, α-synuclein normally has natively unfolded protein structure but may assume oligomeric and/or fibrillar conformations in definite pathological conditions like mutations in the SNCA gene, overexpression, oxidative stress, and posttranslational amendment (Fig. 1.3a–d). Studies indicate that the pathogenic species of α-synuclein involve the posttranslationally modified, mutant, oligomeric, or aggregated forms that could induce adverse effects by disturbing the physiological function of α-synuclein in release of neurotransmitters [26, 27]. Pathological form of α-synuclein may impair mitochondrial functions and mitophagy [28, 29]. It may also result in endoplasmic reticulum (ER) stress by disrupting ER-Golgi vesicular transport [30, 31] and vitiating the effectiveness of some protein degradation pathways [32]. Thus α-synuclein adversely affects the cellular physiology which consequently causes cellular injury and death.

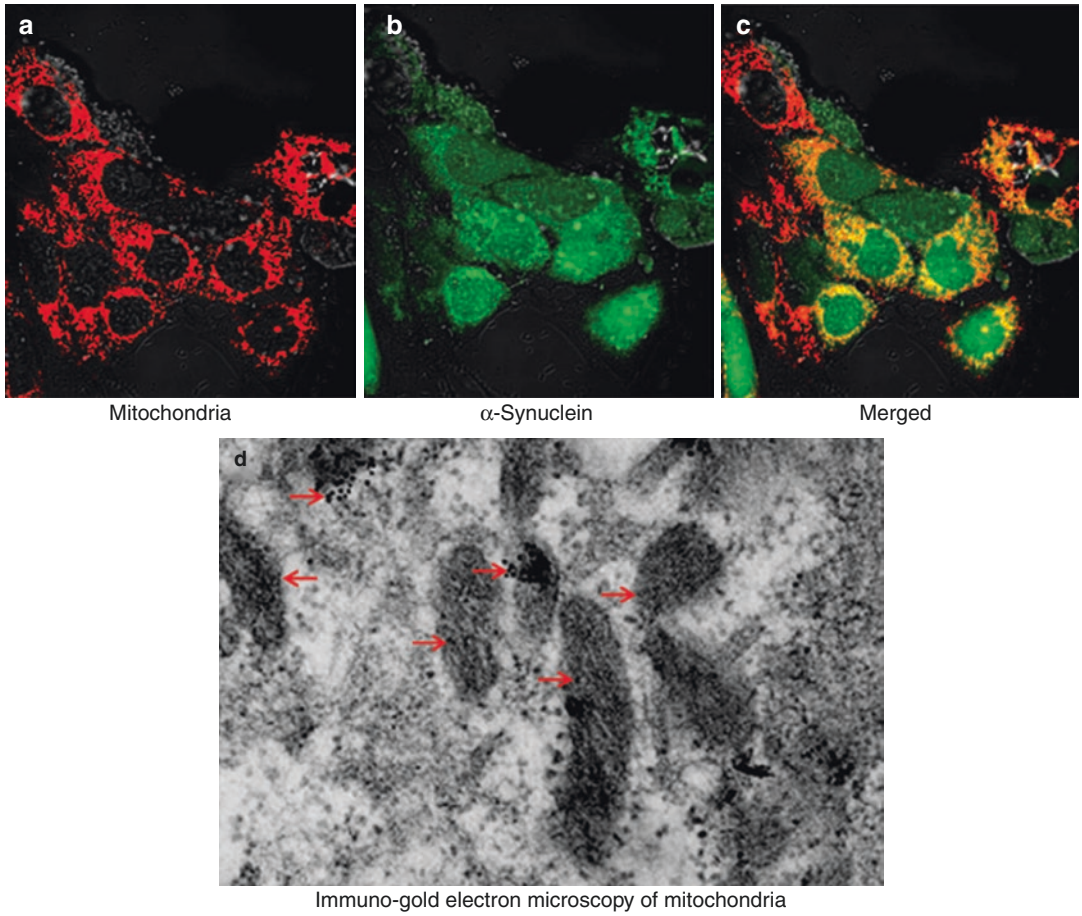

Fig. 1.1 Localization of α-synuclein in the cytoplasm and mitochondria of neurons. (**a**) Human neuroblastoma cells were loaded with mitotracker red (Mitochondria) and (**b**) immunostained for α-synuclein using monoclonal α-synuclein antibody (α-Synuclein). Fluorescence was detected by confocal microscopy. The α-synuclein immunoreactivity is shown in green, mitochondria staining in red, and the merge image (merge) is yellow for overlapping red and green signals (**c**). (**d**) Immuno-gold electron microscopic localization of α-synuclein in the mitochondria of human neuroblastoma cells. Immuno-gold-labeled particles are shown by arrows

1.3 The Transmission and Release of α-Synuclein in Brain Cells

α-Synuclein has self-propagating property, therefore it extends gradually among interconnected brain regions. Different brain regions have the presence of pathological α-synuclein aggregates involving both the peripheral nervous system (PNS) and central nervous system (CNS) [33]. Several observations in human samples revealed the transmission and secretion of α-synuclein in the brain cells. Together monomeric and oligomeric forms of α-synuclein species have been observed in samples of human plasma and cerebrospinal fluid [11, 34], which suggests that α-synuclein can be secreted in brain cells. The exact machinery of α-synuclein release is not entirely understood; however, it is well identified that α-synuclein can be secreted into the culture medium by varied types of neuronal cells [35, 36]. Internalization of α-synuclein has also been demonstrated [37–39], possibly through passive diffusion by enacting with membranes and lipids [40]. Majority of experiments verified that α-synuclein may be spread from one cell to

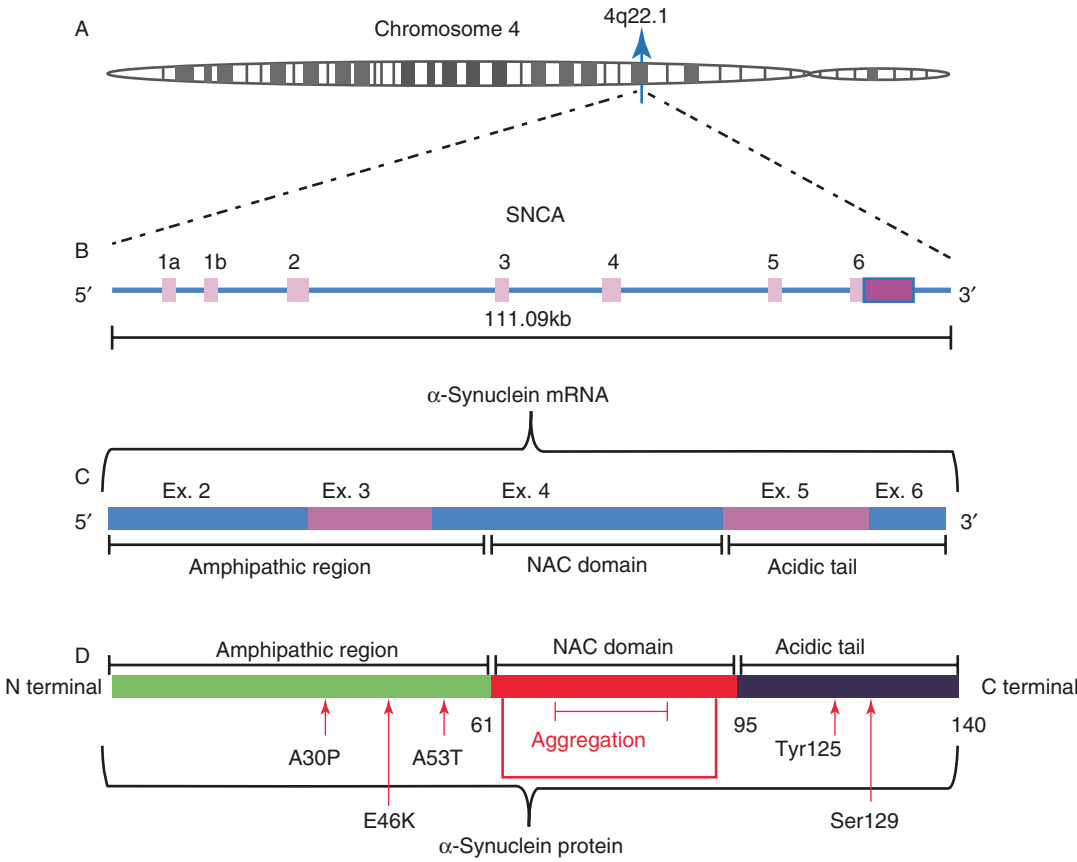

Fig. 1.2 Schematic representation of α-synuclein regions: (**a**) α-Synuclein (SNCA) genomic region on chromosome 4q22.1, (**b**) SNCA gene structure, (**c**) mRNA, and (**d**) protein domains. The amino-terminal from amino acids 1–60 is an amphipathic region. This region is responsible for α-synuclein–membrane interactions. Localized in this region of α-synuclein are three point mutations (A30P, E46K, and A53T). The amino acids 61–95 is termed as central region (NAC), NAC is required for the aggregation process. The C-terminal region from amino acids 96–140 possesses acidic residues and several negative charges. The residue serine 129 in this region is phosphorylated in Lewy bodies. The three missense mutations known to cause familial PD (A30P, E46K, and A53T) lie in the amphipathic region. The non-amyloid-β component or the NAC domain of α-synuclein is associated with an increased tendency of the protein to form fibrils

another by a cell-to-cell transmission machinery [41]. The study confirmed that diverse forms of human α-synuclein, involving monomers, oligomers, and fibrils, might be absorbed by neurons in vivo by endocytosis [42]. In addition, host-to-graft transmission of human α-synuclein has also been demonstrated in rats [43].

1.4 α-Synuclein Physiological Functions

The physiological functions of α-synuclein are the subject most debated in the neuroscience field. However, several researches in the field suggest that α-synuclein enacts at the presynaptic terminal and controls the synaptic transmission. The subcellular localization of α-synuclein at the synapse supports this idea [44, 45]. Evidences suggest that α-synuclein perform many functions at the synapse, i.e., in the rhythm of synaptic vesicles, regulating the vesicle pool size, militarization, and endocytosis [4, 46]. C-terminus region of α-synuclein has been observed to interact with the synaptobrevin-2 (VAMP2) [47], a central player in synaptic exocytosis [48]. Burre et al. [47] reported that the N-terminus of the protein might bind to phospholipids and endorse soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor

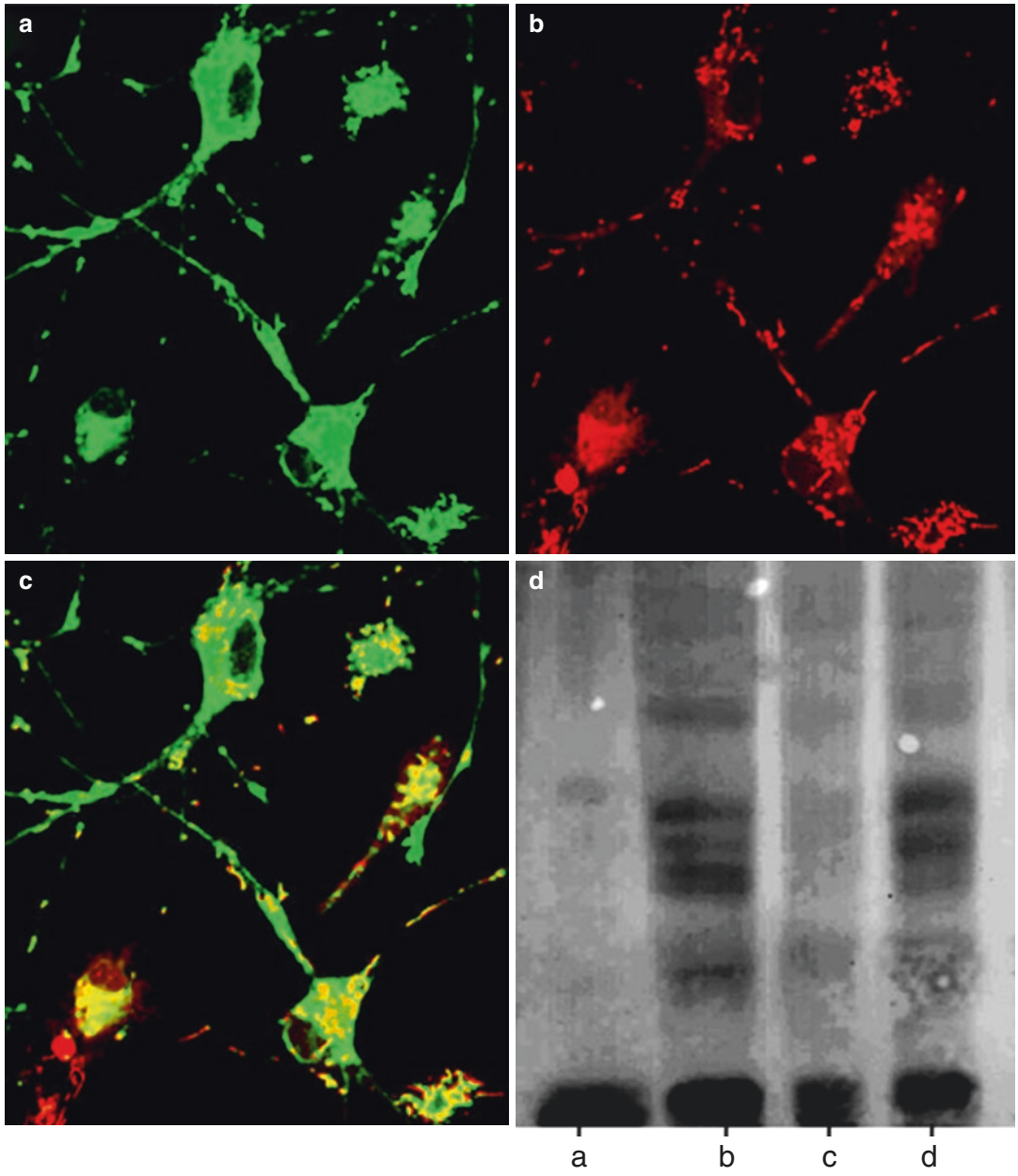

Fig. 1.3 Aggregation of α-synuclein in human neuroblastoma cells. (**a**) Human neuroblastoma cells were overexpressed with wild-type α-synuclein and immunostained for α-synuclein using monoclonal antibody. (**b**) Mitochondria were labeled with mitotracker red. (**c**) Merge shows mitochondria and α-synuclein images overlaid. Aggregates are shown by arrows. (**d**) Silver-stained SDS-PAGE of cell homogenates [lane (a) unaggregated (control) α-synuclein, lane (b) aggregated α-synuclein (mutant A53T), lane (c) aggregated α-synuclein (A30P), and lane (d) aggregated α-synuclein (wild type). Unaggregated α-synuclein migrated at about 19 kDa, consistent with monomeric size. Aggregated α-synuclein showed both low and high molecular mass]

(SNARE) complexes assembly. SNARE proteins encounter important roles in synaptic vesicle exocytosis [49]. Study by Diao et al. [50] revealed that α-synuclein was involved in synaptic transmission by increasing vesicle clustering. These studies suggest that the α-synuclein may delay vesicle trafficking by enhancing vesicle clustering. These studies support the complex multimerization dependent function of α-synuclein, which is vastly reliant on its lipid-binding domains. α-Synuclein can continuously transport between cytosolic monomeric and membrane-bound multimers. α-Synuclein also has an important role in the nucleus. The N- and C-termini of α-synuclein have a signal-like role for its nuclear translocation. Familial mutations and oxidative stress has been found to increase its nuclear localization [51–53]. However, the mechanism of nuclear import of α-synuclein is still not understood. Once α-synuclein enters the nucleus, it may participate in the regulation of transcription. It has been observed that α-synuclein binds to the GC1α promoter, a vital mitochondrial transcription factor, eventually having a negative effect on mitochondria homeostasis [54, 55]. Although several questions are still unclear, currently there is strong evidence for the role of α-synuclein in intracellular trafficking, with particular focus on synaptic vesicle trafficking.

α-Synuclein has been shown to defend dopaminergic cells against apoptosis by signaling pathways involving protein kinase C (PKC). PKC is a serine-threonine kinase involved in phosphorylation of different target proteins and therefore controls many cellular mechanisms, such as apoptosis. PKC is very sensitive to oxidative stress and triggers an apoptotic cascade in dopaminergic cells. α-Synuclein has been shown to be a PKC downregulator that can protect dopaminergic cells against apoptosis. α-Synuclein has been shown to switch off the proteolytic cascade by downregulation of PKCδ expression. Thus in dopaminergic cells, α-synuclein may be considered to be a neuroprotective protein [56]. α-Synuclein regulates different cellular functions via activation of Ras. The activated Ras can activate other signaling molecules including the ERK/MAPK pathway which is involved in sending a signal of growth factor from the cell receptor to transcription factors in the nucleus [57].

α-Synuclein expression has also been recorded in many other cell types, involving cells pertained to secretory processes. α-Synuclein interacts with insulin-containing secretory granules K_{ATP} channels that leads to the inhibition of insulin secretion triggered by glucose stimulation. These observations suggest a function of α-synuclein in diabetes. Moreover, it has been shown that in type 2 diabetes, there is a deposition of amyloidogenic protein in pancreatic β-cells and these patients are most likely to develop PD. However, when α-synuclein combines to amyloid fibrils, an amyloidogenic protein deposits in pancreatic β-cells and forms irreversible damaging complexes in dopaminergic cells [58]. Another important function of α-synuclein has been suggested for modulation of calmodulin (CaM) activity. Calmodulin (CaM) is a messenger protein that can be activated through binding to Ca^{2+} ions and triggers various mechanisms such as those involved in short- and long-term memory. Studies have revealed that both wild-type and mutant α-synuclein can interrelate with CaM both in vitro and in vivo. This interaction of CAM with wild-type and mutant α-synuclein causes α-synuclein fibrillization. α-Synuclein interacts with many cellular proteins and acts as a molecular chaperone, because it comprises regions that are homologous with 14-3-3 proteins which interact with many cellular proteins. Chaperone activity of α-Synuclein is dependent on both its N- and C-terminal regions. The N-terminus is accountable for interfacing of α-synuclein with substrate proteins, leading to the arrangement of a large complex while the C-terminus is responsible for the solubilization of that complex [59].

α-Synuclein may act as an antioxidant in precluding oxidation of unsaturated lipids in synaptic vesicles. Dopaminergic neurons are very sensitive to oxidative damage including the oxidants produced by the metabolism of dopamine. The α-synuclein in its monomeric form can protect lipids from oxidation by interaction with lipid membranes. Fibrillar form of α-synuclein does not have this capability of protecting lipids from oxidation. Thus monomeric form of

α-synuclein could act as an antioxidant which has a significant role in dopaminergic neurons to protect them against oxidative damage [60]. Monomeric α-synuclein can prevent apoptosis by binding to cytochrome c oxidase in mitochondrial membrane and inhibits liberation of cytochrome c from mitochondria to cytosol [61].

One of the key purposes of α-synuclein has been suggested in the determination of dopamine biosynthesis. α-Synuclein acts as the downregulator of tyrosine hydroxylase (TH) activity that may regulate dopamine production and manage its cellular levels. Reduced expression of α-synuclein or its aggregated form may lead to enhanced dopamine synthesis that may lead to oxidative stress caused by dopamine metabolism. Both overexpression of α-synuclein and mutations were demonstrated to upregulate the inhibitory effect of α-synuclein on TH and dopamine levels, leading to downregulation of dopamine synthesis and release [62].

1.5 α-Synuclein Misfolding and Aggregation

Inherently perturbed proteins typically contain primary sequences that preclude aggregation. They are commonly high in charged residues and prolines, and divested of long hydrophobic stretches [63]. The NAC domain of α-synuclein is the main aggregation sensitive region. This region is partially sheltered by the positive and negatives charges of the both N- and C-terminus of the protein. In fact, α-synuclein exhibits vibrant conformations stabilized by retentive interactions which offer considerable degree of compactness [64]. The retentive interactions that happen between the C-terminus and the NAC region, and among the N- and C-termini, may prevent protein aggregation [64]. However, the native compactness of α-synuclein might be disturbed due to the mutations, alterations in environmental conditions, and/or posttranslational modifications, that may lead to misfolding and aggregation of the protein. In an experimental study involving wild type and mutants (A53T, A30P), we showed that α-synuclein aggregates when overexpressed in human neuroblastoma cells (Fig. 1.3) [65]. In another detailed study, we showed that aggregated α-synuclein binds specifically to the membranes including mitochondrial membrane [65]. We showed that overexpressions of wild-type and/or mutants (A53T, A30P) α-synuclein increase the aggregation in cells (Fig. 1.3) and affinity of membrane binding which is exaggerated during oxidative stress [66]. It has been shown that the aggregation tendency of α-synuclein is augmented by the E46K, H50Q, and A53T mutations, whereas the opposite occurs in the G51D and A53E variants. A30P seems to be more susceptible to oligomerization, at the disbursement of fibrillization [67–73]. The oligomeric species detected in patients pretend by synucleinopathies [74–76] has been shown to be the most toxic forms of α-synuclein [77–79]. In addition to the toxicity by oligomeric species, observations sustaining toxicity for fibrillar and mature α-synuclein species are also being described [80–82].

1.6 α-Synuclein and Parkinson's Disease

The role of α-synuclein in PD pathogenesis is controversial. Several data described that the mutations in gene encoding α-synuclein results in familial PD, whereas the *SNCA* polymorphism results in sporadic PD [83]. Transgenic mice overexpressed with α-syn showed reduction in dopamine reuptake, impairments in exocytosis in synaptic vesicles, reduced mass of synaptic vesicle reusing pool, and a reduction in neurotransmitter release [84]. SNCA knockout mice causes disablement in hippocampal synaptic responses [26] that shows that synuclein participate to the extended regulation and preservation of the nerve terminal function [85]. The pathogenic effect of both synthetic and murine disease-associated forms of α-synuclein has been demonstrated to cause PD-like α-synuclein pathology in vivo [80]. Brain homogenates obtained from old α-synuclein transgenic mice when injected intracerebrally into the neocortex and striatum of young asymptomatic transgenic mice, there occur the accruement of the pathological α-synuclein in diverse parts of the brain includ-

ing the spinal cord. The accumulation was connected with the cellular loss in the substantia nigra and caused debilitated motor coordination [86]. In similar experiment synthetic recombinant α-synuclein preformed fibrils when injected to young asymptomatic transgenic mice, the animal produced the α-synuclein pathology in vivo. In an experiment a normal mice were shown to exhibit the α-synuclein pathology after administration of the homogenates from patients with other synucleinopathies, like dementia with LB [81]. Reports have also referenced the probable transmission of α-synuclein pathology from the periphery to the brain. Monomeric and oligomeric α-synuclein are readily taken up by brain cells [87] although to a lesser extent the fibrillar α-synuclein was also taken up by brain cells. Human α-synuclein was also seen in little microglial cells in the olfactory bulb, anterior olfactory nucleus, and frontal cortex. Accumulation of α-synuclein inside microglia signifies that microglia could offer clearing process of the human α-synuclein present into the extracellular space by the neuronal cells.

In cases of autosomal-dominant forms of PD, six different missense mutations have been recognized in the gene encrypting for α-synuclein. These are p.A53T, p.A30P, p.E64K, p.H50Q, p.G51D, and p.A53E [88]. Mutations (A53T, A30P, and E46K) or duplication or triplication of WT α-synuclein have been connected with unusual forms of familial PD [5]. Many α-synuclein transgenic mouse models of the familial forms of PD due to mutations in α-synuclein have been produced [89] which replicate many of the features of α-synucleinopathy-induced neurodegeneration, observed in human PD and diffuse LB disease [90]. Posttranslational alterations of α-synuclein such as nitrosylation, oxidation, and phosphorylation have a role in amending α-synuclein aggregation and toxicity [91].

1.7 Cellular Toxicity of Wild-Type and Mutated α-Synuclein

Numerous ex vivo and in vivo findings showed that in vitro generated α-synuclein species have significant toxic effects on cells [92]. Oligomers were revealed to have different destructive effects on cells in culture conditions. The mechanism of toxicity in inducing cell death was proposed through disturbance of cellular ion homeostasis by a pore-forming mechanism. The increased permeability and influx of ions, as a result of disturbance in pore-forming machinery, from the extracellular space may cause cell death [92]. Oligomers formed by recombinant α-synuclein were exposed to form pores in a synthetic bilayer assay. These protofibril-shaped species when exposed to primary cortical neurons induced a depolarization of the cellular membrane. Another mechanism proposed that α-synuclein could directly penetrate in cells and cause amplified protein aggregation [92]. A significant neurotoxic effect was noted when *C. elegans* and *D. melanogaster* were exposed to in vitro produced α-synuclein oligomers [77]. The transgenic mice exhibiting the artificial α-synuclein variants *E57K* and*E35K* caused oligomer formation and demonstrated an extreme loss of dopaminergic neurons as compared to standard α-synuclein transgenic mice, overexpressing wild-type α-synuclein [78].

Oligomeric α-synuclein may cause a direct synaptotoxic effect [93]. Exogenously added α-synuclein oligomers on hippocampal brain slices from rats cause an impairment on neuronal signaling [94]. Preincubation of tissue with α-synuclein oligomers caused an enhancement in synaptic transmission offering to a suppression of long-term potentiation. In a recent study by Kaufmann et al., [95] two dissimilar types of oligomers were made either by polymerization of monomers or by sonication of fibrils. Despite of variations in the structure of these species, both exhibited similar pessimistic impact on the neuronal excitability. In vivo experiments were also confirmed the outcome of α-synuclein oligomers on the synaptic dysfunctions. In one such experiment, mice expressing the α-synuclein mutants E57K showed widespread synaptic and dendritic pathology in conjunction with the loss of synapsin 1 and reduction in synaptic vesicles [96]. These observations indicate the α-synuclein induced the disruption of presynaptic neurotransmitter release machinery by

the reduction of neuronal synaptic vesicles. The buildup of oligomers chiefly occurs at the synaptic sites and is critical for the neuronal network activity. These oligomeric or fibrillary α-synuclein forms can propagate from one type of neurons to other types and can produce toxic effects in the recipient neurons [97].

The toxicity of α-synuclein depends on its properties of binding to cytoplasmic organelles possibly via N-terminal region. Our previous studies [66] clearly showed the binding of α-synuclein with the mitochondrial membrane when aggregated. The overexpression of either wild-type or mutants (*A53T*, *A30P*) forms of α-synuclein in human neuroblastoma cells increases the accumulation of proteins. The accumulated forms of α-synuclein upon binding to the mitochondria cause decline in the mitochondrial membrane potential and hamper the respiration [66]. α-Synuclein oligomers have been shown to block the proteins import into the mitochondria by communicating with the translocase of the outer membrane 20 (TOM 20) [98]. In addition, the accumulations of α-synuclein oligomers in the endoplasmic reticulum (ER) cause ER stress and perturb its functions including the ER–mitochondria associations [99]. ER possesses interlinked chaperone proteins that guide the correct folding of secreted proteins. These ER chaperones including the grp94, grp78, and PDI have been found to be compromised in the brain stem and spinal cord of an α-*synuclein A53T* transgenic mouse model [100], thus suggesting that α-synuclein may interfere with the process of folding, translocation, or degradation of protein in neurons.

1.8 Mechanisms of α-Synuclein Toxicity

Insufficient is known about the machinery of toxicity innate to cell-to-cell transmitted α-synuclein. The approach of α-synuclein transmission and the variations of the *SNCA* gene manipulate α-synuclein transmission remain to be explored. The cell-to-cell imparted and endogenously expressed α-synuclein both contribute to cytotoxic mechanisms that straightly influence neuronal survival. α-Synuclein aggregation and its cell-to-cell transmission particularly affect neuronal physiological mechanisms like vesicle trafficking including neurotransmitter release and recycling [26, 27]. Its membrane binding affinity with cytoplasmic organelles especially mitochondria and thus consequent dysfunction of mitochondria perturbs not only the metabolic procedures but also degradative mechanisms [101, 102]. The interruption of vesicular transport machinery, specifically those that activate endoplasmic reticulum stress [30] is another important negative effect of α-synuclein. It was reported that extracellular α-synuclein causes activation of astrocytes and microglia in vitro and in vivo executing neuroinflammatory response alike observed in PD pathology [80]. Apparently neurons are extremely susceptible to glial cells-derived proinflammatory factors, consequently representing a substitutional neurotoxic process generated by cells that have attained α-synuclein from the extracellular milieu. α-Synuclein produces both protective and damaging effects. α-Synuclein secreted by neurons could provoke toxicity inside the cytoplasm of neighboring cells and also in the extracellular space. This may cause activation of glial cells in the brain that may induce chronic inflammation, thus participating to the succession of the pathology throughout the brain. Glial cells including both astrocytes and microglia are able to absorb and degrade synthetic recombinant α-synuclein [103]. In fact, α-synuclein can be exchanged among neurons and glial cells in vitro [104]. Neuron-derived α-synuclein exposed to rat primary astrocytes [104] and microglia [105] resulted in induction of an inflammatory reaction. α-Synuclein in aggregated form activates the microglia and thus originates inflammation and damage of exaggerated neurons [105, 106]. α-Synuclein was found to activate the microglia in a primary mesencephalic neuron-glia culture system, which was followed by enhancement of dopaminergic neurodegeneration [105]. In another study, when cultured microglial cells were incubated with protofibrils of α-synuclein, proinflammatory signaling mechanisms involving p38, ERK1/2 MAP

kinases and NF-κB turned out to be activated. Administration of α-synuclein protofibrils into the substantia nigra of adult rats induced the activation of microglia in addition to neuronal cell loss, which could be inhibited by the MAP kinase inhibitor semapimod [106]. These findings suggest that oligomeric/protofibrillar α-synuclein could exert few of its adverse effects by enhancing inflammatory responses in the pretended tissue.

Abnormally high level of α-synuclein may also disturb mitophagy. Postmortem brain tissues obtained from PD patients showed the aggregation of α-synuclein. This aggregation increases oxidative stress and agitates mitochondrial function [47]. Our own work showed that mitochondria are very sensitive for oxidative stress induced by wild-type and mutated α-synuclein [65, 66]. Furthermore, both in vivo and in vitro, expression of α-isoforms of α-synuclein in neuronal cells induces the dysfunction of mitochondria, which will ultimately lead to the declined respiration and neuronal cell death [107]. Overexpression of α-synuclein may be restricted in mitochondria and interrupt the mitochondrial membrane potential by opening the mitochondrial permeability transition pore (mPTP) [66], thus developing mitophagy [108].

1.9 Concluding Remarks

α-Synuclein plays an imperative role in various physiological purposes involving regulation of dopamine neurotransmitter, synaptic transmission, inhibiting oxidation of unsaturated lipids in synaptic vesicles. α-Synuclein accumulates in PD brains, in neuronal cells of the substantia nigra, pons, medulla, and gut leading to inflammation and cellular death and subsequently difficulties in movement, digestion, circulation, and sleep. Experimental studies also support the hypothesis that mutations in *SNCA* gene and α-synuclein oligomers have a vital role in the pathology of PD and other age-related disorders [109]. The mechanisms of toxic and damaging effects of prefibrillar species of α-synuclein have been recognized using molecular and biochemical methods. The main mechanisms of oligomeric α-synuclein cellular toxicity include: mitochondrial impairments, ER stress, synaptic impairment, and affected cell membrane functionality. Furthermore, oligomers of α-synuclein may act as seeds for the arrangement of aggregates and also appear to be prone to transfer among cells. Stopping the α-synuclein from aggregation is the most potential target for treatment of PD. The strong evidence in favor of α-synuclein oligomers indicates that they are predominantly accountable for the dissemination of pathology. Therefore such oligomeric species of α-synuclein should be appropriate targets for early therapeutic intervention in Parkinson's disease and other age-related disorders. Immunotherapy which efficiently interferes with uptake of extracellular α-synuclein has also been recently tried [110].

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2 Molecular Mechanisms of Neurodegeneration: Insights from the Studies of Genetic Model of Parkinson's Disease

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2.1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease with clinical motor syndrome characterized by bradykinesia, resting tremor, muscle rigidity, and postural instability caused by reduced level of dopamine [1]. Although the cause of the disease remains elusive, recent studies suggest that mitochondrial dysfunction, oxidative stress, neuroinflammation, misfolded protein stress, and lysosomal defects lead to the death of dopamine (DA) producing neurons in the SNc (substantia nigra pars compacta) area. Genetic studies over the past 20 years identified several genes mutation which lead to familial forms of the disease. Parkinson's disease may be caused by single gene mutation in autosomal dominant or recessive fashion and these genetic mutations account for about 10–15% of the cases of PD. In the current PD genetics nomenclature, 18 specific chromosomal regions, are termed *PARK* (to denote their putative link to PD), and numbered in chronological order of their identification (PARK1, PARK2, PARK3, etc., where PARK1 and PARK4 are the same gene, *SNCA*) [2]. Mutations in autosomal recessively inherited genes, namely *parkin, PINK1,* and *DJ-1*, typically lead to early onset of PD. The genes *PINK1* and *parkin* appear to work in the same pathway that controls mitochondrial quality and integrity during cellular oxidative stress. Dominantly inherited mutations in leucine-rich repeat kinase 2 (LRRK2) and α-Synuclein (α-SYN) cause late-onset PD and have characteristic Lewy body pathology. Recent GWAS (genome-wide association studies) study suggests that genetic variants of α-SYN and LRRK2 confer an increased risk for late-onset sporadic PD [3].

2.1.1 Neuropathology of PD

The neuropathology of PD is characterized by a specific pattern of DA-producing neuronal loss in substantia nigra pars compacta (SNc) with Lewy bodies (LB) rich in α-SYN in the surviving neurons [4]. It has been suggested that PD may begin in the lower brainstem and olfactory bulb where the substantia nigra only becoming affected during the middle stages of the disease [5]. However, not all the clinical features of PD are attributable to the degeneration of DA neurons [6]. Non-dopaminergic neuron degeneration accounts for other features of the disease like depression, dementia, sleep, olfactory and balance problems [7] that typically occur in advanced stages of PD. The non-dopaminergic features of PD are often the most disabling, and current treatment

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_2

with L-DOPA obviously does not cure these symptoms [8]. Most recently, it has been suggested that connection between spreading of Lewy pathology and development of clinical PD is very weak [9].

2.2 Genetic Models of PD

It is noteworthy that patient-based genetic studies identified the role of genetics in PD which further justify to generate model organisms to elucidate the function of those genes. Animal models are advantageous since it allows manipulation of the condition and yields result in short time. Currently, there are many genetic models of PD, including vertebrate organisms like rat, mice, and zebrafish; invertebrate organisms like *Drosophila melanogaster and Caenorhabditis elegans*. These genetic PD models have been informative in understanding molecular pathways and pathological changes in PD [10].

2.2.1 Vertebrate Models of PD

Mouse is the most preferred model to study neurodegenerative diseases such as PD disorders. This is because mouse possesses human alike neuronal networks and genetic homologs [11]. Since PD is a chronic disorder, promoter should be strong as well as constitutively active throughout the lifetime of mice. Conditional temporal expression of a transgene can also be used to control the expression [12]. A more advanced technique is the tetracycline (Tet)-regulated transgenic switch in which expression of the transgene follows the activity pattern of the promoter in the driver construct. The ability of Tet-transactivator protein (tTA) to change its conformation and affinity for Tet-resistant protein (tetP) by doxycycline allows temporal on/off control of transgene induction [10, 13, 14]. Stereotaxic viral injection of different viruses (e.g., lentivirus, recombinant adeno-associated virus [rAAV], and herpes simplex virus [HSV]) can be used for the expression of desired transgene. Gene knockout (KO) and knock-in (KI) can also be achieved by homologous recombination. In the conventional transgenic system, a gene is overexpressed under the control of a promoter that drives the expression in preferred organ like brain. Usually dominant mutants (i.e., A53T, A30P, and E46K for α-SYN; G2019S and R1441C/G mutants for LRRK2) are preferred to be overexpressed because the mode of inheritance supports a gain of toxicity and hence an exaggeration of its endogenous function. Deletion of important exons or introduction of premature termination should be able to simulate early-onset PD caused by autosomal-recessive gene. Deletion of parkin, PINK1, and DJ-1 has however not yielded mouse with desired phenotype [10]. Even knocking out all three genes together has proved ineffective [15] possibly due to potential compensatory mechanisms elicited in mouse model. The Cre–loxP-mediated conditional KO approach is widely used when embryonic lethality prevents studying deletion of a gene in adult animals [16]. Rat models of PINK1, DJ-1, and Parkin genes have been generated using zinc finger technology. The phenotype of these rats showed progressive neurodegeneration and early behavioral deficits, suggesting that these recessive genes may be essential for the survival of dopaminergic neurons in the SNc area [17]. The neuroanatomy of zebrafish is typical that of vertebrates with forebrain, hindbrain, and spinal cord, and their genes are highly homologous to that of humans and hence it has been used as a PD model organism. For example, transient knockdown of DJ-1 using morpholino antisense oligonucleotides has shown loss of function of DJ-1 in zebrafish.

2.2.2 Invertebrate Models of PD

C. elegans and *D. melanogaster* are small, inexpensive to culture models with short life spans and hence time effective. Although they lack α-SYN homolog and have limited repertoire of cell death effectors, these models offer the advantage of identifying evolutionarily conserved pathways. However, the validity of these studies is a variable on their reproducibility in human system. A large number of mutant strains are available as stocks for the researchers to use them effectively

Table 2.1 Summary of PD genetic model organisms and associated phenotypes

Transgene	C. elegans	Drosophila	Mouse	Rat
α-SYN	Loss of DA neurons but non-progressive, No LB inclusions	Age-dependent DA neuronal loss, DA responsive locomotor deficit, LB inclusions	Functional abnormality with no DA neuronal loss	Age-dependent loss of DA neuron, motor impairment, LB inclusions (viral vector injection)
LRRK-2	Reduced DA levels; DA neuronal degeneration, Increased vulnerability to mitochondrial complex I inhibitors	Decline in age-dependent DA responsive locomotor activity, loss of DA neurons	Minimal or no neurodegeneration except one report. Deficit in DA transmission and DA responsive behavior	Loss of DA neurons in SNc (viral vector injection)
PARKIN	Mitochondrial dysfunction; decreased life span	Muscle degeneration, male infertility, DA neuronal degeneration, mitochondrial defects	Deficit in DA metabolism and behavior without DA neuronal loss, Reduced mitochondrial respiratory chain proteins. Age-dependent motor deficit and neuron loss in BAC mice	Progressive dose-dependent DA neuronal death
PINK-1	Neurite outgrowth defects; mitochondrial cristae defects, sensitive to oxidative stress	Muscle degeneration, male infertility, DA neuronal degeneration with enlarged mitochondrial defect	No neurodegeneration, mild mitochondrial function abnormality. Moderate reduction in striatal DA levels with low motor activity	Motor impairment with robust vocalization deficits, DA cell loss in SNc, LB inclusions. Increased disruption of mitochondrial homeostasis and vulnerability to oxidative stress
DJ-1	Increased vulnerability to mitochondrial complex I inhibitors	Age-dependent loss of DA neurons, no major phenotypic defect	DA neurotransmission defects, mitochondrial dysfunction	Early motor deficit, progressive neurodegeneration

to provide insights on the pathways involved. In reverse genetic approach, RNA interference (RNAi) to knockdown target genes is achieved by simply injecting, soaking, or feeding the *C. elegans* with dsRNA which is complementary to the targeted gene. Similar to human CNS, dopamine plays important functions like locomotion, feeding, sleep/circadian rhythms, and learning in drosophila. The nervous system dysfunction in these models can be studied using the changes in resting and synaptic potentials and linking these changes to behavioral deficits and loss of DA neurons [18, 19]. The Drosophila genome encodes homologs of DJ-1, PINK1, PARKIN, LRRK2, and VPS35. Expression of human SYN in Drosophila results in dopaminergic neuronal loss [20, 21]. Table 2.1 summarizes various genetic models of PD and their observable phenotype.

2.3 Autosomal-Dominant PD

2.3.1 SNCA (PARK1/4)

Missense mutation in the *SNCA* gene identified by Polymeropoulos et al. [22] is the first PD-associated gene identified. Soon, Spillantini and colleagues established that α-SYN protein is the major component of the LB. The three dominant α-SYN mutations identified in separate family studies are A30P, A53T, and E46K [22–24]. Duplication and triplication of the wild-type α-SYN locus are also shown to cause familial PD [25]. Polymorphisms around the *SNCA* locus are also significantly associated in two recent genome-wide association studies of sporadic PD [3, 26]. Thus, α-SYN plays critical role in both familial and sporadic PD [27].

The α-SYN, a presynaptic phosphoprotein, although found to be unstructured in free state, also exists in a variety of structures, including oligomers, protofibrils, fibrils, and filaments. The soluble protofibrils and fibrils seem to be the most toxic forms [28] than the insoluble aggregate present in Lewy bodies [29]. In mouse with pan-neuronal or DA-specific promoters driven expression of α-SYN wild type (WT) or mutants causes the severity and age of onset of disease depended heavily on the promoter and levels of transgene expression. These mouse models however lack the key pathological feature of PD which is DA neuronal loss. However, they show neurodegeneration in other anatomical sites and functional abnormalities in the nigrostriatal system [16, 30]. The mouse prion promoter (mPrP) driven A53T transgenic mice exhibit more pathological phenotype which includes increased phosphorylation, ubiquitination, and aggregation of α-SYN, leading to progressive neurodegeneration [31]. Although the anatomical site is not the one traditionally associated with PD, these systems may however serve to study mechanisms of α-SYN-induced neurodegeneration. Since there is no obvious DA neuronal loss in most α-SYN transgenic mice, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) intoxication is used additionally [32]. Through mitochondrial dysfunction it is sufficient to induce α-SYN aggregation and DA neuronal loss [16]. Interestingly, expression of human WT, A53T, and A30P α-SYN in drosophila produced many pathological hallmarks of PD like the age-dependent DA neuron loss, Lewy body like inclusions containing α-SYN, and other locomotor deficits [20]. Although C. *elegans* overexpressing α-SYN has been shown to cause loss of DA neurons, they lack significant Lewy body like pathology [33, 34].

2.3.1.1 Mitochondrial Dysfunction Causes α-SYN Accumulation

Malfunction in mitochondrial complex I is constantly observed in sporadic PD and this established the link between mitochondrial dysfunction and synucleinopathy [35]. Various studies have established that inhibition of mitochondrial complex I causes selective DA neurodegeneration (Banerjee et al.2009). Impairment of proteasomal and lysosomal systems can lead to α-synucleinopathy, since they play a significant role in catabolizing α-SYN. However, it leads to general neurodegeneration without selectivity for DA neuronal system. But mitochondrial complex I inhibition selectively impairs DA neurons and is thus a causative event while impairment of the proteasomal and lysosomal systems are most probably only the downstream events in the pathogenesis of PD. Further, α-SYN knockout mouse is resistant to the DA neurotoxicity caused by MPTP and other mitochondrial toxins [36]. Transgenic mice overexpressing human A53T α-SYN mutant exhibit mitochondrial abnormalities including mitochondrial DNA damage and degeneration [37]. It has been reported that silencing of SYN prevents dopamine neurons loss when exposed with mitochondrial complex I inhibitor, MPTP [38]. Overexpression of α-SYN, on the other hand, makes mouse more susceptible to mitochondrial toxins like paraquat [39]. In this context, new treatment modalities preventing mitochondrial damage may be more promising for controlling PD.

DA-specific mitochondrial transcription factor A (TFAM) knockout in mouse leads to DA neurodegeneration and Lewy body formation. Moreover, it has been shown that PD patients accumulate α-SYN in the SNc and the striatum has a decreased mitochondrial complex I activity [40]. α-SYN transgenic mice not only show mitochondrial impairment but also enhance DNA damage in which poly (ADP-ribose) polymerase (PARP-1) is overactivated leading to cell death [41]. PARP deletion also protects DA neurons from MPTP-induced toxicity [42]. Thus it may be postulated that α-SYN aggregation may probably impair the regulation of PARP1.

2.3.1.2 SYN Mutation Induces Lysosomal and Autosomal Dysfunction

The autophagy-lysosome pathway plays a critical role in degrading proteins with longer half-lives [43]. Lysosomal pathway inhibition leads to accumulation of α-SYN, suggesting that α-SYN

catabolism is not solely mediated by proteasomal pathway. The chaperone-mediated lysosomal uptake pathway also mediates α-SYN binding to lysosomal membrane receptors. Mutant α-SYN shows deficient translocation blocking uptake of other substrates as well, thus causing misfolded protein stress [44]. Although not directly involved in proteosomal degradation, α-SYN overexpression causes inhibition of proteasome function [44]. A recent study in drosophila showed that expression of Rab11, a regulator of exocytosis could reverse the severe phenotype caused by overexpression of wild-type SYN [18].

2.3.1.3 SYN in Neuronal Synapse

α-SYN was initially identified as a synaptic and nuclear protein. Although their role remains elusive, evidence suggests that the protein plays a role in maintenance of synaptic vesicle pools and activity-dependent dopamine release [45]. The presynaptic cysteine-string protein knockout in mice causes severe phenotype that is rescued by α-SYN overexpression [46], providing evidence that α-SYN might modulate synaptic vesicle function.

2.3.2 LRRK2 (PARK8)

In 2004, the genetic cause of chromosome 12 linked to PD was attributed to mutations in the *LRRK2* (Leucine-rich repeat kinase-2) gene [47]. Mutations in LRRK2 cause autosomal-dominant PD. LRRK2, a large protein with multidomain is ubiquitously expressed in neurons and localizes with membranes and lipid rafts [48]. A deletion mutant for C.elegans *lrk-1* (lrk-1 is similar to human LRRK1, homolog of LRRK2), indicates its significant role in localizing synaptic vesicle proteins to terminals [49]. The most common mutation in LRRK2 is G2019S with a frequency of 1% in sporadic PD and 4% in hereditary PD and the risk increases with age. Autopsy of patients with LRRK2-associated PD shows α-SYN inclusions, and hence LRRK2 and α-SYN might share common pathogenic mechanisms. Unlike α-SYN mutation, dosage effect is not seen in LRRK2 mutations and the disease in homozygotes is clinically identical to heterozygotes carrying the mutation. The toxicity of LRRK2 mutation in vitro is kinase and GTP-binding dependent and this piece of information is invaluable for probable therapeutic interventions [50]. In *C. elegans*, overexpression of either wild-type or G2019S LRRK2 caused DA neuronal loss [51]. LRRK2 G2019S mutation also shows vulnerability to rotenone toxicity compared to wild type. In drosophila, overexpression of human LRRK2 impairs DA-dependent locomotor activity and also causes loss of DA neurons [52]. The *Lrrk* loss-of-function mutant homolog in drosophila also shows deficits in synaptic transmission. In another independent study, using a different clone of *Lrrk* loss-of-function mutant, no difference in EJP (excitatory junction potential) amplitude was found between the mutants and wild type.

The current transgenic mouse models of PD are not very robust in producing PD phenotype compared to cellular and drosophila model systems. Despite several transgenic techniques like conventional, bacterial artificial chromosome (BAC) transgenic, mutant LRRK2 Knock-In, and tet-inducible transgenic, only one of the LRRK2 models reproduced age-dependent DA neuron death in the nigrostriatal system [53]. BAC transgenic mice and conditional expression of LRRK2 WT and LRRK2 G2019S showed no characteristic phenotype and no neuronal loss [54, 55]. R1441C mutation in LRRK2 has been shown to impair stimulated dopamine neurotransmission and D2 receptor function [56]. The R1441G BAC mouse model shows a strong phenotype with akinesia, reversible with L-Dopa and dopamine agonist apomorphine treatment [57]. It is noteworthy that mice that express mutant LRRK2 using varying promoters have different levels of expression. Thus, most LRRK2 transgenic animals although lacking neuronal loss manifest earliest deficits like DA transmission and DA-responsive behavior defects. The aberrant kinase activity of LRRK2 might cause phosphorylation of substrates that misregulates binding partners and other regulators. In HSV-LRRK2 G2019S viral induced DA neurodegeneration models, the aberrant kinase activity of LRRK2 G2019SS was

prevented by inhibitor of LRRK2 kinase activity that abolished its toxicity [58]. Thus identification of phosphosubstrates involved would help in deciphering the pathogenic mechanisms induced by LRRK2 mutations [2, 16].

2.3.2.1 LRRK2 Affects Mitochondrial Function

In *C. elegans*, RNAi-mediated silencing of *lrk-1* increased the toxicity to rotenone treatment and overexpression of wild-type LRRK2 significantly increased resistance against mitochondrial toxins such as rotenone and paraquat [51]. Similar observation was made in drosophila overexpressing human mutant LRRK2. However, *LRRK2* knockout mice are not more sensitive to mitochondrial toxin, MPTP [59].

2.3.2.2 LRRK2 in Neuronal Morphogenesis

LRRK2 knockout mice exhibit normal numbers of dopaminergic neurons in the SNc area without any behavioral deficits. However, in vitro studies strongly suggest a role of LRRK2 in the neurogenesis of dopamine producing neurons by controlling cell cycle. Taken together, it is evident that LRRK2 is required for dopamine neuron genesis or survival in adult animals [49, 59]; however, its substrates, regulators, and binding partners remain elusive. Similar studies in mice suggest no role of LRRK2 in neurogenesis, and drosophila studies show disparate results [49, 55, 60]. LRRK2 plays multiple roles as in neuronal morphogenesis and in other peripheral processes like kidney functions in rats and mice. LRRK2 knockdown in zebrafish causes neuronal loss, developmental abnormalities like axis curvature defects, and ocular abnormalities [61]. Also by its localization to presynaptic vesicles and endosomes, LRRK2 is shown to regulate synaptic vesicle endocytosis by directly interacting with the early endosome marker protein Rab5 [62].

2.4 Autosomal-Recessive PD

The first identified genetic cause of autosomal-recessive juvenile Parkinsonism is the *Parkin* mutation reported in a Japanese family study [63]. With nearly 100 reported mutation (seen in 50% of familial PD cases and in 20% of young-onset sporadic PD), it is the most frequent autosomal-recessive mutation [64]. Mutations in PINK1 gene is the second most common autosomal-recessive mutation (1–7% of early-onset PD) and mutations in DJ-1 are a rare cause of PD [65, 66]. For all the three genes, whole exon deletions cause loss of protein while point mutations destabilize or yield functionally inactive proteins. Various studies in a number of animal and cellular models for parkin, PINK1, and DJ-1 have led to tremendous insight into the role of these proteins in PD. In the recent GWAS, none of the autosomal recessively inherited PD genes (Parkin, PINK1 or DJ-1) have been reported as a risk factor, but such studies might identify only strongly associated genes [3, 26].

2.4.1 PARKIN (PARK2)

Kitada and colleagues [63] first identified mutations in *parkin*, (maps to 6q25–q27), as one of the causes of juvenile Parkinsonism. To date, 100 different *parkin* mutations have been reported both in familial and sporadic PD. This gene extends to about 1.3 Mb of DNA with 12 exons encoding a 465 amino acid protein, with high degree of mutations. Penetrance appears to be complete in individuals with two disease-causing mutations in Parkin [64]. Parkin which is considered as an E3 ubiquitin ligase participates in the ubiquitin-proteasome system [67]. It has an ubiquitin-like (Ubl) domain at the N-terminus followed by two RING finger domains separated by an inbetween RING (IBR) domain, each of which bind two Zn^{2+} atoms. Being vulnerable to oxidative and nitrosative stress, Parkin plays a key role in sporadic PD [68, 69].

In drosophila *Parkin* knock out generates defective flies with reduced climbing ability, life span, and male sterility [70, 71]. Abnormalities in muscle and sperm mitochondria ultimately result in cell death due to activation of autophagy. DA neurodegeneration with reduced TH (tyrosine hydroxylase) level was also observed along with DA-responsive locomotor deficit. To study the role of Parkin in PD, several groups have

generated parkin KO mice [72, 73]. Although these knockouts lack substantial dopaminergic or behavioral abnormalities, they show subtle changes in either the DA nigrostriatal circuit or the locus coeruleus (pons nucleus) noradrenergic system [72–75]. Parkin knockout mice had reduced mitochondrial respiratory chain proteins and stress response proteins. Parkin substrates like AIMP2, FBP1, and PARIS were shown to accumulate in the ventral midbrain of parkin knockout mice [41, 76], [77]. These cellular changes may contribute to deficits in DA metabolism and hence behavior. Parkin mutants might have a dominant negative effect since overexpression of mutant human parkin causes age-dependent progressive DA neurodegeneration in fly and mouse system [78–80].

2.4.1.1 Parkin Mediates Mitochondrial Quality Control

In addition to its role as an E3-ubiquitin ligase, Parkin seems to actively play a role to clear damaged mitochondria. A germ line deletion of Parkin leads to defective mitochondria, suggesting its role in mitochondrial quality control. Overexpression of parkin provides neuroprotection against MPTP toxicity while parkin knockout does not enhance the neuronal susceptibility to MPTP [74]. The mitochondrial defects and upturn wing phenotype due to Pink1 knockout in drosophila are rescued by overexpression of Parkin. This result suggests that Parkin maybe thus essential for clearance or might rescue defective mitochondria to reduce toxicity. However, in vitro mammalian cell culture study shows that Pink1 is essential for recruitment of Parkin to eliminate defective mitochondria [81]. Thus, the protective effect of Parkin during Pink1 knockout suggests that mitochondrial quality control pathways in drosophila can also function independent of Pink1.

2.4.1.2 Parkin Acts as an E3 Ubiquitin Ligase

The ubiquitin-proteasome pathway has been strongly linked to PD pathogenesis, highlighting the significance of the E3 ubiquitin ligase *Parkin* [67]. Parkin catalyzes lysine-48-mediated polyubiquitination, which targets the substrates for proteosomal degradation. Mutations in the parkin gene lead to failure of the ubiquitin-proteasome system due to impaired ligase activity that cause intracellular accumulation of parkin substrates [82]. However, in PARK2 patients, or in parkin knockout mice, the accumulation of substrate is significantly low, thus making the role of Parkin as an E3 ubiquitin ligase insignificant [84], [10]). Parkin is also involved in other forms of ubiquitination, modulating cellular processes like signal transduction, transcriptional regulation, and protein and membrane trafficking [83]. Parkin is capable of modifying proteins with different ubiquitin linkages, including monoubiquitination and polyubiquitination using both lysine-48 (involving receptor turnover, protein degradation) and lysine-63 linkages (involving protein inclusions). However, the exact role of Parkin in the context of these cellular activities is not yet clear [21].

2.4.1.3 Role in Neuronal Synapse

Although a cytoplasmic ubiquitin ligase protein involved in the cellular ubiquitination/protein degradation pathway, *Parkin* can also localize to the synapse and associate with membranes [85]. Interestingly, it is involved in the modulation and metabolism of several presynaptic proteins such as α-SYN and the α-SYN-binding synaptic proteins like synphilin. *Parkin* has been associated with the function of GPR37, aG-protein coupled receptor that interacts with the dopamine transporter DAT [44]. Reduced synaptic transmission is seen in *Parkin* mutant larvae in drosophila and there is reduction of both evoked and spontaneous excitatory junction potential, as well as depolarization of the resting membrane potential in flight muscles. Perturbed synaptic transmission is likely due to reduced glutamate release, because of the changes in synaptic morphology and/or ATP depletion due to mitochondrial deficits [18].

2.4.2 PINK1 (PARK6)

Mutations in the *PINK1* (phosphate and tensin homolog (PTEN)-induced putative kinase 1)

were originally mapped to chromosome 1p35-36 in a Sicilian kindred with autosomal-recessive Parkinsonism by Valente and colleagues in 2004. Analysis in familial and sporadic PD patients identified homozygous and compound heterozygous *PINK1* mutations and is associated with early onset of PD in several families, and in 2–4% of sporadic cases. It is the second most common cause of autosomal-recessive early-onset PD [86], [27]. Inherited in an autosomal recessive fashion, penetrance appears to be complete in individuals with homozygous mutations in PINK1 [65].

In drosophila, PINK1 mutant flies share marked phenotypic similarities with parkin mutant flies [87, 88]. Transgenic expression of *Parkin* was capable of rescuing the PINK1 loss-of-function phenotypes, while overexpression of PINK1 had no effect on parkin mutant phenotypes [87, 88]. Thus parkin and PINK1 may function in a common cellular pathway, where PINK1 acts upstream of parkin. There are evidences supporting that parkin and PINK1 together regulate mitochondrial quality control by clearing defective mitochondria through autophagy [81, 89]. Until today, PINK1 and parkin have no known common substrate that is important for mitochondrial quality control. Thus it was necessary to delineate the pathway in which they act together to protect mitochondrial function [10].

Two independent studies have come out with PINK1-targeted KOs [90, 91] and there are shRNA-mediated knockdown models as well [92]. In contrast to neurodegenerative phenotypes and mitochondrial defects strongly expressed in drosophila PINK1 models [87], PINK1 knockout or knockdown mice failed to replicate these features. Nevertheless, subtle deficits in nigrostriatal DA transmission and mild mitochondrial functional deficits like decreased mitochondrial respiration and electrochemical potential were observed in PINK1 KO mice. In one such study, PINK1 knockout showed significantly less DA content in the striatum and an age-dependent decline in spontaneous voluntary activities [16, 90]. In a novel PINK1KO rat model, motor impairment was documented including significant impairment in ultrasonic vocalizations (USVs) [93].

2.4.2.1 PINK1 Mutation Causes Mitochondrial Dysfunction

PINK1 is a protein kinase localized to the mitochondrial intermembrane and is composed of serine/threonine kinase domain with an N-terminal mitochondrial targeting motif [94]. Topological study suggests that the kinase domain of PINK1 faces the cytosol and hence its substrates should reside in the cytosol [95]. Indeed, it has been shown that cytosolic Pink1 provides neuroprotection against MPTP-mediated neurodegeneration [96]. Although a role of PINK1 to regulate mitochondrial calcium dynamics has been suggested [97], the PINK1 substrates and its site of action need to be elucidated. PINK1 has been linked to the fission and fusion machinery observed in Drosophila and mammalian cell mitochondria. The mitochondrial chaperone TRAP1 (TNF receptor associated protein 1) was the first substrate of PINK1 to be reported. Through phosphorylation of TRAP1, PINK1 suppresses the release of cytochrome c from mitochondrial membrane protecting against cell death due to oxidative stress. Mutations in *PINK1* are known to impair its protective activity [44, 98]. In drosophila lacking PINK-1, mitochondrial degeneration leading to apoptosis in flight muscles and behavioral deficits was seen. Similar to drosophila with parkin deletion, the mitochondria were swollen with reduced mitochondrial DNA, proteins, and ATP levels. There was a small, but significant reduction in number with enlarged mitochondria in DA neurons. PINK-1 mutation may thus lead to mitochondrial dysfunction and cause increased sensitivity to cellular stress leading to apoptosis.

2.4.2.2 PINK1 Mutation Causes Deficient Synaptic Function

The deletion of *Pink1* in drosophila leads to defective synaptic transmission in response to high frequency stimulation at the larval neuromuscular junction (NMJ). However, no perturbation in basal release characteristics like neurotransmitter release, spontaneous release frequency, or response amplitude was observed. Administration of ATP to the synapse rescued

this, supporting a role for *Pink1* in maintaining ATP supply during increased demand [99]. *Pink1* plays a role in the homeostatic regulation of mitochondria through maintaining fusion and fission process and by modulating the activity of the electron transport chain, complex I [100]. *Pink1* mutants were unable to phosphorylate the mitochondrial complex I subunit NdufA10 (NADH dehydrogenase (ubiquinone) 1 alpha subcomplex) at Ser250 in mouse models of study. NdufA10 expression also restored both ATP synthesis and mobilization of the synaptic vesicle reserve pool in drosophila. In drosophila *Pink1* mutant model, the defects in synaptic mitochondrial membrane potential were reversed by feeding *Drosophila* on bacteria synthesizing vitamin K2 suggesting a role for vitamin in PD [18, 101].

2.4.3 DJ-1 (PARK7)

Mutations in the *DJ-1* gene (PARK7) is a rare cause of autosomal-recessive Parkinsonism, clinically similar to the other recessive Parkinson syndromes with early onset and slow progression [44]. A member of the ThiJ/PfpI family of molecular chaperones, DJ-1 is a redox-sensitive protein involved in the oxidative stress response [65, 102, 103]. Individuals with two mutations in DJ-1 [66] exhibit complete penetrance. DJ-1 contains only a single domain with cysteine residue (C106) that can be modified to form sulfinic acid in the presence of reactive oxygen species (ROS). Mutations in DJ-1 result in loss-of-function protein due to defective dimer formation or lack of expression [104]. DJ-1 is expressed widely throughout the body and is primarily in the cytosol and mitochondrial matrix [105]. It has been shown to regulate redox-dependent kinase signaling pathways and antioxidant gene expression in vitro [106]. It may also function as an atypical peroxiredoxin-like peroxidase, where it protects against oxidative stress in mitochondria in vivo [107]. Thus, DJ-1 has pleotropic function such as an antioxidant, oxidation/reduction sensor, chaperone, and/or protease [108].

Although mammalian DJ-1 has a single gene, drosophila possesses two orthologs of DJ-1: DJ-1a and DJ-1b. The DJ-1 double null flies for both DJ-1 homologs are viable, fertile, and have normal number of DA neurons and life span. However, knockdown of DJ-1 by transgenic RNAi in drosophila has been shown to cause neurodegeneration, and age-dependent DA neuronal loss in the dorsomedial cluster [109]. Similar to other autosomal-recessive PD mutations, like parkin and PINK1, the DJ-1 knockout mice do not exhibit any major abnormality and the number of DA neurons and receptors remain unchanged [107, 110, 111]. However, the DJ-1 Knockout mice show defective DA transmission in the nigrostriatal circuit and mitochondrial dysfunction, similar to parkin and PINK1 knockouts [107, 110, 111].

2.4.3.1 Is DJ-1 a Mitochondrial Regulator?

Although predominantly cytoplasmic, DJ-1 is also present in the inner membrane space and matrix of mitochondria. Interestingly, during oxidative stress DJ-1 translocates to the outer mitochondrial membrane suggesting its role in neuroprotection [112]. DJ-1 does not seem to localize with SYN in Lewy bodies; however, oxidized and insoluble forms of the protein accumulate in the brain of sporadic PD patients. Under conditions of oxidative stress in vitro, DJ-1 can interact with parkin [113] and also with α-SYN to prevent formation of fibrils. And this redox-sensitive chaperone activity protects neurons from α-SYN misfolding. DJ-1 knockout mice are more susceptible to aging and the mitochondrial toxin MPTP. The neurons isolated from these mice were shown to be more sensitive to oxidative stress [111]. DJ-1 may be involved in the regulation of protein activity since it is susceptible to protein *S*-nitrosylation similar to *parkin* [114]. DJ-1 may also function as a regulator of apoptosis through interactions with several apoptosis-regulating proteins like PTEN ([8], [115]). DA neurons of DJ-1 KO mice show altered mitochondrial potential and oxidative stress even in the absence of DA neurodegeneration [16].

2.5 Pathogenic Pathways in Parkinsonism

2.5.1 Does α-SYN and LRRK2 Share Common Pathway?

With discoveries of various genes associated with PD, the functional relevance of these molecules in PD pathogenesis suggests a common pathway involving them. The two dominant genes associated with PD, LRRK2, and α-SYN are likely to share common pathogenic mechanisms, as knockout of LRRK2 shows a reduction while overexpression of LRRK2 enhanced the neuropathologic abnormalities in A53T α-SYN transgenic mice [54]. A53T α-SYN failed to show marked pathology in LRRK2 knockout. While expression of LRRK2 mutant alone shows only mild abnormalities, crossing them with A53T α-SYN exacerbated the abnormal accumulation of α-SYN aggregates and caused severe neurodegeneration. LRRK2 PD patients have α-SYN positive Lewy bodies, suggesting that LRRK2 and α-SYN are in the same pathogenic pathway. Overexpression of parkin can limit the toxic effects of both α-SYN [116] and LRRK2 [52] while parkin knockout did not make phenotypes worse [75]. Thus recessive Parkinsonism and dominant PD overlap in their pathology with nigrostriatal degeneration and Lewy body formation, it is possible that they act in multiple pathways that lead to a common outcome [59].

2.5.2 PINK1, Parkin, and DJ-1 Function in a Common Pathway to Regulate Mitochondrial Integrity

Parkin is a cytoplasmic and nuclear E3 ubiquitin protein ligase. PINK1 is a mitochondrial serine/threonine kinase with the kinase domain facing the cytoplasm. DJ-1 is an oxidative stress chaperone protein whose function remains elusive. Since these are recessive mutations, loss of normal function of the proteins probably leads to PD in humans. Links between the function of these proteins have come from modeling these mutations in animals especially Drosophila. Loss of both PINK1 and Parkin results in very similar phenotypes like mitochondrial abnormalities and apoptosis, particularly in spermatid cells and flight muscles [70]. Knockout of parkin can rescue loss of PINK1, the reciprocal is not true and loss of PINK1 does not rescue parkin knockout. This suggests that PINK1 functions upstream of parkin both involved in maintaining the mitochondrial integrity. Mammalian cell culture also supported the concept that PINK1 and Parkin function in a common pathway maintaining the mitochondrial morphology [117]. Although parkin is normally cytosolic, it can be recruited to the mitochondrial surface by PINK1 if the organelle loses membrane potential [81]. The accumulation of markers of autophagy around mitochondria in the absence of PINK1 reveals that cells upregulate autophagy during loss of mitochondrial integrity [118] Since DJ-1 is an oxidative stress response protein that can influence mitochondrial function and morphology, possibly via autophagy it may also function with PINK1 and Parkin in a similar or parallel pathway. DJ-1 overexpression, however, does not rescue PINK1/parkin deficiency phenotypes suggesting it works either upstream of the other two or in parallel [59].

Conclusions

Various genetic animal models of PD have provided valuable information on the molecular mechanisms of neurodegeneration occurring in PD. PD represents broad brain abnormalities of dopamine and non-dopamine dependent circuitry causing Lewy body inclusion. It is true that we currently lack an animal model that simulates all the pathological features of PD. Limitation with most of the available mouse models is that they lack nigrostriatal neuronal loss, although viral models in rats and monkeys have shown more effective phenotype [59]. Mild deficits in DA transmission and behavioral impairments are reproduced in several models, simulating the physiological changes preceding neurodegeneration. Since α-SYN transgenic mice show synucleinopathy with protein aggregation and

neurodegeneration, they can be successfully tested for therapeutics that may reduce the pathology. Genetic PD mouse models that can recapitulate at least the degeneration of DA neuron are highly necessary to provide more light on the PD pathogenesis [16].

Drosophila and *C. elegans* may not stand as perfect models of PD as they are small organisms lacking the complexity of vertebrates, and they do not express α-SYN. More importantly, the traditional clinical features of PD (bradykinesia, rest tremor, rigidity, and postural instability) cannot be exhibited. The reason why mouse LRRK2 transgenic models do not exhibit robust pathology is that penetration of these mutations in humans may not be complete and other genetic or environmental factors play significant role in pathogenesis, which may not be present in the experimental model. Other possibility is that compensatory mechanisms might be present only in the mouse system that prevent loss of DA neurons. The autosomal-recessive models of mice are however useful to provide insights on how parkin, PINK1, or DJ-1 function to induce dysfunction of the nigrostriatal DA system but cannot be used to test therapeutic drugs due to lack of correlates of protection [78–80]. It is surprising that even a triple knock out of PINK1, parkin, and DJ-1 did not cause nigral cell loss. Studies on the lack of a parkinsonian phenotype in these mice might provide useful insight on the protective mechanisms active during the presence of PD gene mutations. The Drosophila loss-of-function models of PINK1 and parkin do have dramatic phenotypes, but unfortunately, these abnormalities reside outside the nervous system [10]. If the PD-related genetic mutations are achieved without inducing any compensatory mechanisms in mouse, DA neuronal loss may be achieved. A detailed study in such a model will provide more light on PD pathogenesis. The development of such PD genetic models will help in testing novel therapeutic approaches like stem cell, viral-mediated therapies, morpholino oligomer (siRNA), and other therapeutic compounds [16]. Also, since PD is an age-related disease, mouse aging models should be combined with current PD genetic models to study the effect of aging on PD pathogenesis. Recent evidences in *C. elegans* suggest that genetically encoded signaling pathways are distinctly compartmentalized to independently control neurodegeneration and the process of aging [119]. Further studies in the direction correlating aging and PD pathology are thus necessary.

Acknowledgments The research grants support from the CMHS, United Arab Emirates University and the National Research foundation, United Arab Emirates to MEH is duly acknowledged.

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3 Pathology and Cell-Based Therapy of Parkinson's Disease

So Young Kim, Sung S. Choi, Dong-Seok Lee, Seung Hoon Lee, Sang Hoon Cha, and Hong J. Lee

3.1 Introduction

The degeneration of the highly specialized central nervous system (CNS) results in serious physical and mental problems. Though it was initially thought that the CNS does not support neurogenesis or regeneration, more recent studies demonstrate renewal of the nervous system using various approaches following neuronal injury. In fact, several studies have revealed that the CNS has moderate regenerative abilities [1]. Therefore, CNS restoration is now a key clinical challenge, the use of stem cell therapy in various neurological disorders considered a very promising therapeutic application.

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease [2, 3]. PD is marked by the progressive loss of pigmented dopaminergic (DA) A9 nigral neurons and the production of α-synuclein-containing Lewy bodies (LB) in the substantia nigra pars compacta (SNpc) [4, 5]. It is characterized clinically by motor symptoms including rigidity, tremor at rest, lateness of voluntary movement, stooped posture, difficulty with balance and shuffling, small-step gait [6], as well as non-motor symptoms such as lack of facial expression, soft voice, olfactory loss, mood disturbances, dementia, sleep disorders, and autonomic dysfunction, including constipation, cardiac arrhythmias, and hypotension [7]. The progressive motor symptoms are caused by a decrease in striatal dopamine levels [8]. Therefore, previous studies have examined PD treatments involving neuromodulation by deep brain stimulation or drug therapies to increase dopamine levels in the brain, such as dopamine precursor L-DOPA, DA agonists, monoamine-oxidase-B (MOA-B)-inhibitors, or the NMDA-receptor antagonist amantadine [9, 10]. However, these medications have limited potential for treatment of patients, due to their adverse-effects caused by the non-physiological delivery of dopamine [11, 12]. Approaches to replace lost DA cells through neural grafting in the striatum of PD patients have shown proof of concept for cell replacement therapy in the human PD brain.

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_3

These trials used intrastriatal transplantation of human fetal mesencephalic tissue containing developing midbrain dopamine neurons and their precursors, resulting increased dopamine levels and restimulation in the striatum [13–17]. These studies showed improved motor symptoms in a number of patients after transplantation [13, 14] as well as increased ^{18}F-DOPA secretion [18, 19], despite the fact that grafted cells showed host-to-graft propagation of LB pathology [20]. However, the clinical utility of human fetal mesencephalic tissue is restricted because of the amount of tissue needed for efficient results within the transplant region [21]. Therefore, cell replacement therapy studies are necessary to better understand the safety and efficacy of transplanting fetal tissue containing DA cells and to assess alternative cell source for stem cell transplantation in PD.

3.2 Pathology of PD

Proposed PD pathologies suggest that the trigger mechanisms of PD are related to genetic predisposition, local neuroinflammation activation, abnormal protein aggregation, aberrant protein folding, and the participation of other neural cells in the degeneration of DA neurons [22]. Recently, oxidative stress, mitochondrial dysfunction, and calcium-induced excitotoxicity, resulting in cell death in the nigrostriatal system, have been generally accepted as trigger mechanisms of PD [23, 24]. Neuronal cell death in PD is associated with the formation of intercellular deposits of protein and lipids. LB and Lewy neurites, with accumulation of α-synuclein (α-SNCA), are strongly expressed in degenerating structures, where they play an important role in neurodegenerative disease progression and neuroinflammation in the brain [25–27]. Histological depositions in tissue are produced in a small number of cells and distributed to distant brain locations by genetic mutation [28, 29]. Therefore, it was first recognized in the autosomal dominant type of PD [30]. Mutations in α-synuclein, a synaptic protein encoded by *SNCA* on 4q22, lead to deposition of α-synuclein, which serves a histopathological marker of PD [30]. In the pathology of PD, LB are large α-synuclein deposits forming round lamellated eosinophilic cytoplasmic inclusions in the neuronal body, and Lewy neuritis are fibers composed of insoluble polymers of α-synuclein, these occur in neuronal processes as well as astrocytes and oligodendroglial at the early onset stage [31]. Consequently, the toxic damage effects of α-synuclein in cells include dysfunction of mitochondria, lysosomes, and the endoplasmic reticulum, as well as interruption of microtubular translocation of ras-related protein (Rab)7 and tropomyosin receptor kinase (TrkB) [31].

Deposition of *α*-synuclein-dependent LB is followed by neurodegeneration, but the lesional pattern and the underlying processes remain unclear. Braak et al. published a detailed description of individual stages of PD pathology, which is only visualized in basal cranial nerve nuclei, such as the glossopharyngeal and vagal nerves, and in the olfactory bulb at stage 1. At stage 2, PD pathology is detected in the pontine areas of the locus coeruleus, the raphe nuclei, and the reticular formation. By stage 4, PD pathology is found at the connection of the substantia nigra and the anterior olfactory nucleus, with equivalent rates of degenerating neurons in the SNpc. Motor symptoms of PD correlate with pathology at stage 4 or later, and this stage is characterized by widespread pathology in the cerebral cortex and limbic system as well as degeneration of a substantial proportion of substantia nigra neurons. Finally, stages 5 and 6 are characterized by pathology of the basal forebrain and cortical regions, including the entorhinal cortex and the Cornu Ammonis (CA) area of the hippocampus [28]. This corresponds to motor symptoms and serious non-motor symptoms of PD, such as dementia, psychosis, and sleep-wake disorders. In advanced PD, dementia with LB is characterized clinically by a predominant dementia syndrome and pathologically by neocortical LB [32–34]. Therefore, PD is a systemic disorder that influences much of the CNS, not only the dopamine system. Future studies are required to totally understand pathology process of PD, thus need to be designed attractive model to treat PD.

Recent studies show that the genes most generally involved in autosomal recessive PD are Parkin,

PTEN-induced putative kinase 1 (PINK1), DJ-1, and leucine-rich repeat kinase 2 (LRRK2) also called as dardarin. Endogenous regulation of PINK1 and Parkin on early-onset PD and characterization of LRRK2 on late-onset sporadic PD are an important factor in understanding the pathophysiology supporting and identical clinical evidence [35–37]. Parkin, an E3 ubiquitin-protein ligase in the ubiquitin proteasome system (UPS), is located in the cytosol and has been implicated in cellular integrity and mitochondrial functions contains mitochondria morphology and mitochondrial turnover [38, 39]. In addition to its role in mitochondrial function, it has been shown that mutation of Parkin leads to a degradation of mitochondria through decreased catalytic activity in PD. Importantly, Parkin is inactivated by nitrosative stress, oxidative stress, and dopaminergic stress in sporadic PD and autosomal recessive PD is non-stimulated by a number of mutations, ultimately breakdown of ubiquitin ligase ability on Parkin leads to deposition of aminoacyl-tRNA synthesis interacting multifunctional protein type (AIMP) 2 and far upstream element binding protein (FBP) 1 as Parkin substrates, which induces neurodegeneration through these pathway [36]. Further, mitochondrial abnormalities have been discovered in 79.5–87.8% of induced pluripotent stem cell (iPSC)-derived neurons with the Parkin mutation [40]. PINK1 is a mitochondrial kinase that reduces mitochondrial stress within cells [41], and deletion or knockdown of PINK1 in mammalian cellular and mouse models leads to various abnormal mitochondrial activities including reduced mitochondrial membrane potential and ATP levels [42]. Moreover, the effect of PINK1 mutation on mitochondria translocation leads to a downregulation in mitochondrial complex I activity and membrane potential [43]. Furthermore, previously established mitophagy mechanisms of PINK1 on Parkin mitochondrial translocation in human DA neurons model using iPSC-derived neurons [44–46]. LRRK2 is a large protein of ROCO family and locate at endolysosomal membrane with functional GTPase and kinase domains [47]. Its role in neuronal function, it has been shown that LRRK2 is important factor in regulating neurite length and outgrowth [48, 49]. LRRK2 missense mutations are commonly known genetic factors of sporadic PD [50]. In PD, neurotoxicity of LRRK2 mutation is characteristically expressed in LB and its regulation is dependent on the presence of α-synuclein [51–53]. Moreover, LRRK2 mutations influence vesicular trafficking, autophagy, protein synthesis, enhancement of oxidative stress, and cytoskeleton function in both in vitro and in vivo models of PD [51, 54–56]. Further, LRRK2-related PD have been discovered in transgenic-induced mouse model with human LRRK2 mutant Gly2019Ser, resulting decrease of neuron proliferation, DA abnormalities, and enhanced levels of phosphorylated tau through strongly express within subventricular zone (SVZ), olfactory bulb (OB), and hippocampus regions [57, 58].

3.3 Cell-Based Therapy for PD

Sources of stem cells include embryonic stem cells (ESCs), iPSCs, mesenchymal stem cells (MSCs), and neural stem cells (NSCs). Stem cell therapy was first studied to treat PD in 1970s. At that time, brain grafts were isolated from developing rat embryo brain and transplanted into a rat PD model. These grafts survived in the host brain and rescued behavioral deficits [59, 60]. However, it is difficult to obtain brain grafts for PD treatment in humans because of ethical issues and problems with immune rejection.

The major goals of stem cell therapy for PD are the regeneration of neurons and glia as well as the improvement of behavioral function. Cell replacement therapy using stem cells is new hope for treatment of PD. This technique has been examined successfully in nonhuman primate preclinical model [61]. Targeting of cell graft and distribution and density of cells is most important component to treat PD [62]. Therefore, stem cells are a promising tool for treatment of several neurodegenerative diseases, and they possess therapeutic effects via neural regeneration and production of neuroprotective molecules [63].

Regeneration of DA neurons is important for treatment of PD. DA neurons are differentiated via gene manipulation of nuclear receptor related-1 (Nurr1), a transcription factor that

assists the differentiation of midbrain precursors from mouse ESCs. DA neurons induced by Nurr1 overexpression show tyrosine hydroxylase (TH)-positive immunoreactivity, a DA neuron-specific marker. Grafted Nurr1 ESCs were integrated into the striatum in a 6-hydroxydopamine (6-OHDA)-mediated rat PD model, and these cells showed electrophysiological properties of neurons and improved motor behaviors [64]. Some studies have reported the beneficial effects of neurons derived from ESCs in enhancing the motor symptoms in rodent PD models [65, 66]. However, ESCs present some challenges, such as difficulties with dopaminergic differentiation, immune rejection, and potential tumorigenic effects [67, 68]. Human ESCs also present ethical problems because they are generated from human embryos, but these cells continue to be used in clinical trials [69].

Various adult stem cells are also excellent sources for PD therapy. NSCs are the most optimal cell type for integration and restoration of nigrostriatal processes. Pitx3-overexpressing NSCs have significant potential for dopaminergic differentiation, and they improve motor function in the 6-OHDA induced rat model after cotransplantation with ventral mesencephalon from E11 rat embryos [70]. In a primate study, undifferentiated human NSCs were transplanted into an MPTP-induced primate model for PD. A small number of engrafted human NSCs differentiated into DA neurons expressing TH and dopamine transporter, and α-synuclein aggregates were reduced [71]. NSCs have the potential to proliferate themselves and differentiate into "wanted" neurons easier than other adult stem cells, whereas it is difficult to isolate the sufficient number of cells for therapy [72].

MSCs also have therapeutic effects through several possible mechanisms, such as secretion of neuroregulatory molecules to assist neural regeneration, activation of endogenous restoration mechanisms to facilitate neurogenesis, immunomodulation, and anti-inflammatory effects [72]. A large number of MSCs can be easily isolated, and they have the ability of differentiation to neurons and glia [73, 74]. Transplantation of bone marrow-derived MSCs in the brains of PD model rats led to markedly increased TH-positive cells and improved behavior [73]. Neurons derived from human umbilical cord-derived MSCs migrate in the substantia nigra and not only integrate in the dopamine pathway but also participate in neurogenesis in the hippocampus and subventricular regions [75]. Transplanted adipose-derived MSCs survive for 4 months and restore normal behavior in MPTP-lesioned rhesus monkeys [76].

Because iPSCs may provide ideal autologous cell transplantation for PD to escape the problem of immune rejection, preclinical studies of iPSCs may have a beneficial potential and lead to clinical application of stem cell. However, iPSCs have risks and are limited by genetic alterations and low efficiency of reprogramming [72]. Transplantation of iPSC-derived DA neurons led to improvement of motor functions in rodent PD models [77]. In a primate study, transplanted iPSC-derived DA neurons survived 2 years and led to behavior improvement without immunosuppression [78].

The therapeutic effects of stem cell treatments are often improved by cell-based gene manipulation and chemical agents. In a study, the administration of estradiol-2-benzoate activated integrin α5β1 in striatal neurons of adult female rats. The activation of integrin α5β1 leads to integrate iPSC-derived dopaminergic neurons into host striatal neuronal circuit [79]. Dual application of estradiol and iPSCs-derived dopaminergic neurons may have the advanced therapeutic potential to treat PD.

H2AX gene is a H2A histone gene to determine DNA repair and cell apoptosis. Mutant gene of H2AX (Y142F) is overexpressed in dopaminergic neuron to improve the survival of transplanted dopaminergic neurons for PD treatment. These cells showed more resistant to DNA damage and apoptosis process under the neurotoxic environment [80]. Another method used viral vectors containing genes for both TH and GTP cyclohydrolase 1 (GTPCH1) infected in human neural stem cells (F3.TH.GTPCH cells). PD model rats transplanted with F3.TH.GTPCH show DA cell regeneration and improvement of behavioral function [81]. These genetic and chemical manipulations provide alternative strategies for improving the beneficial effects of stem cells in PD therapy.

Conclusion

The currently available treatments for PD, such as L-DOPA, DA agonists, MOA-B inhibitors, and amantadine, only delay disease progression and are limited for long-term use because of adverse-effects [9, 10]. Transplanted stem cells can integrate, survive, regenerate, and replace degenerated neurons and glia, restoring their functions in lesions of PD. Stem cell therapy is promising for PD treatment though there are still some obstacles and risks for clinical trials, such as tumorigenesis, immune rejection, and low efficiency. Future research should focus on establishing efficient stem cell lines and improving the survival and integration of engrafted cells in lesions of PD.

Acknowledgement This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011792032017)" Rural Development Administration, Republic of Korea. This research was syupported by the Bio & Medical Technology Development Program of the NRF funded by the Korean gorverment, MSIP (NRF-2016M3A9B6900776).

***Conflicts of Interest*:** The authors declare that they have no competing interests.

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4 The Role of p53 in Alzheimer's Disease: Impact on Tau Pathology

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4.1 Hyperphosphorylated Tau in the Pathogenesis of AD

Alzheimer's disease (AD) is the most prevalent progressive neurodegenerative disorder of the cerebral cortex. It is characterized by personality changes, abnormal emotional and social behaviors, disordered spatiotemporal relationships, and a gradual decline in cognitive functions, of which memory loss for recent events is the most prominent sign [1, 2]. The early involvement of the monoaminergic systems, predominantly the noradrenergic locus coeruleus and serotonergic dorsal raphe nucleus in the brainstem, is thought to be responsible for non-cognitive symptoms of variable severity [3]. In addition to extracellular accumulation of amyloid β (Aβ) peptide in senile plaques, the defining clinico-pathological feature of AD is intraneuronal deposition of hyperphosphorylated tau protein into paired helical filaments (PHF) of neurofibrillary tangles [4–6].

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Tau is the major microtubule-associated protein (MAP) in neuronal cells with an essential role in the stability and dynamics of axonal microtubules. It regulates neurite outgrowth, axonal transport, cytoskeleton maintenance, and neuronal shape [7, 8]. Tau is encoded by a single gene, but several developmentally regulated isoforms with distinct affinities for microtubules are expressed in the human brain by alternative mRNA-splicing [9, 10]. These tau isoforms differ by the presence of two, one, or zero N-terminal inserts (2N, 1N, 0N) and either three or four semi-conserved repeats (3R, 4R) in the microtubule-binding domain at the C-terminus [10, 11].

Tau possesses multiple phosphorylation sites, mainly clustered in the flanking regions of the microtubule-binding domain. Accordingly, the biological activity of tau is principally controlled by phosphorylation as the degree of phosphorylation inversely correlates with binding to microtubules. Hyperphosphorylated tau is unable to efficiently bind microtubules and stabilize the cytoskeletal network. Besides inducing microtubule disruption, hyperphosphorylated tau misfolds and self-assembles into tangles of PHF, sequesters normal tau, and two other neuronal microtubule-associated proteins, MAP1A/MAP1B and MAP2. This ultimately impairs axoplasmic flow and slowly leads to retrograde degeneration [4, 10, 12–14]. Furthermore, tau phosphorylation inhibits tau turnover by proteolysis. The inhibition of tau turnover may be

S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_4

directly responsible for the accumulation of abnormally phosphorylated tau aggregates and the four- to fivefold increment of tau level that is observed in the brains of AD patients [15–17].

The activity of many different proline-directed and non-proline-directed kinases is responsible for the pattern of tau phosphorylation. The major contributors to enhanced tau phosphorylation are glycogen synthase kinase-3β (GSK-3β), cyclin-dependent kinase 5 (cdk5), mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase-1/2 (ERK1/2), JNK and p38, calcium and calmodulin-dependent protein kinase-II, non-receptor tyrosine kinases, casein kinase 1 delta, and cyclic AMP-dependent protein kinase [16, 18–20]. Among them, GSK-3β is identified as a particularly important player in the pathogenesis of sporadic and familial forms of AD. GSK-3β can promote the formation of tangle-like filament morphology in cell-free systems, mediate phosphorylation of tau in neuronal cultures, and induce tau hyperphosphorylation accompanied with the decline of cognitive functions in animals [21–23].

On the longest brain tau isoform, about 80 putative serine (Ser) or threonine (Thr) phosphorylation sites have been identified, and most of them are on Ser-Pro and Thr-Pro motives. Together with five additional Tyr residues, amino acids that can be phosphorylated comprise approximately 20% of the whole tau protein [24, 25]. The number of identified phosphorylated residues in aggregates from AD brain is at least 45 [24, 26]. It is considered that proline-directed kinases, such as GSK-3β, that act on serine/threonine followed by proline are probably more important for the AD pathology than non-proline-directed kinases as they are able to phosphorylate tau at a large number of sites. However, although non-proline-directed kinases (such as protein kinase A (PKA) and calcium/calmodulin kinase II) phosphorylate tau at only a limited number of sites, their importance may be underestimated as phosphorylation of tau by these kinases increases the phosphorylation of tau by GSK-3β and cdk5 [3, 9, 27].

In addition, a decrease in the activity of different phosphatases can result in tau hyperphosphorylation. Indeed, activity and/or expression of protein phosphatases-1, -2A, -2B, and -5 (PP1, PP2A, PP2B, PP5), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), are found altered in the AD brains [3, 10, 19, 28, 29]. Among them, PP2A accounts for approximately 70% of the total tau phosphatase activity in human brain and its activity is significantly inhibited in the AD with concurrent hyperphosphorylation of tau [30].

4.2 The Role of p53 in Neuronal Biology

The transcription factor p53, predominantly known as the key mediator of the DNA damage response, plays a crucial role in cell-cycle regulation, apoptosis, and senescence. p53 is activated in cellular response to various genotoxic challenges, including oxidative stress. The pathogenesis of AD is tightly linked to increased oxidative stress. In the brain, oxidative stress-related injuries are characterized by the accumulation of damaged macromolecules (proteins, lipids, nucleic acids, and carbohydrates), mitochondrial dysfunction and ATP depletion, impaired proteolysis and autophagy, axonal degeneration, pronounced microglia activation, and inflammation accompanied with gliosis that over time ends in neuronal apoptotic death [31–33].

In response to a mild oxidative injury, p53 promotes antioxidant activities to ensure cell survival. However, in response to severe oxidative stress, such as is the case with increased nNOS (the neuronal/constitutive form of nitric oxide synthase) expression in reactive astrocytes and microglia in full-blown AD cases [34], p53 induces prooxidative activities that lead to cell death [35]. In the central nervous system, enhanced levels of p53 are often correlated with neurodegenerative processes and induction of neuronal apoptosis, for example, during brain development, in hereditary brain diseases such as spinal muscular atrophy, in excitotoxic cell death, cerebral ischemia, epilepsy, and traumatic brain injury [36–40]. p53 accomplishes its roles by binding to specific DNA sequences and regu-

lating the expression of a panel of genes that mediate p53-dependent functions. In neurons, target genes induced by p53 include the pro-apoptotic regulators of cell death (Bax, Puma-a p53 up regulated modulator of apoptosis, and Noxa), p21 (regulator of cell cycle), and Mdm2 (a regulator of p53). In addition to transcriptional regulation, p53 may directly trigger nuclear-independent apoptosis acting at the level of mitochondria [38]. Namely, following translocation to the mitochondria, p53 may directly induce permeabilization of outer mitochondrial membrane that results in cytochrome c release and induction of detrimental apoptotic cascade [41, 42]. As synaptic terminals and neurites are enriched in mitochondria, impairment of mitochondrial function may lead to synaptic degeneration [43]. Therefore, p53 deficiency or inhibition may protect neurons from various acute toxic insults. This suggests that p53-inhibiting drugs may be a promising approach in the therapy of neurodegenerative diseases [38, 44].

Stability and transcriptional activity of the normally short-lived p53 are mainly regulated by post-translational modifications and protein/protein interactions. Yet another important mechanism in the control of p53 function is its conformational stability [45]. Under normal physiological conditions, negative regulators murine double minute-2 (Mdm2) or the human homolog (Hdm2) keep p53 inactive and unstable, and at low basal levels by promoting p53 ubiquitination for proteasome-dependent degradation. Consequently, this inhibits transcriptional activity of p53. In response to DNA damage and other stressors, various post-translational mechanisms (such as phosphorylation, acetylation, methylation, and sumoylation, among others) disrupt interactions of p53 with Mdm2, leading to its stabilization and accumulation [46]. Recent findings have revealed that human p53 undergoes a combination of alternative splicing, alternative promoter usage, and alternative initiation of translation that gives rise to at least 12 distinct protein isoforms that differ at C- and N-termini. As major isoforms are considered full-length p53 and N-terminally truncated versions Δ40p53, Δ133p53, and Δ160p53 that lack the first 39, 132, and 159 amino acids, respectively. Isoform Δ40p53 lacks the first transactivation domain (TD1) but retains the second (TD2), while Δ133p53 and Δ160p53 lack both transactivation domains but retain the DNA-binding region almost entirely. Most of the alternative splicing of p53 pre-mRNA occurs at the 3′ end. This creates β and γ isoforms of full-length p53, and α, β, and γ isoforms of Δ40p53, Δ133p53, and Δ160p53 [47, 48]. These diverse and highly variable N- and C-termini allow p53 to mediate a remarkable repertoire of different functional outcomes that are still not completely understood [49].

In addition to its well-documented role in neuronal apoptosis, p53 regulates neuronal terminal differentiation during development, controls proliferation and differentiation of neural progenitor cells towards neuronal phenotype, and promotes axonal outgrowth and regeneration after nerve injury [49]. Thus, p53 is highly expressed in proliferative neuroblasts of the developing brain, but is down-regulated in cells undergoing terminal differentiation [50]. In fact, p53 may regulate axonal growth cones and motility in early developing neurons, presumably via a transcription-independent mechanism. In early developmental stages, high expression of phospho-p53 was preferentially found in axons and axonal growth cones and was co-localized with tau. Later during development, phospho-p53 was mainly found in cell bodies and neurites [51]. Accordingly, deletion of the p53 nuclear export signal blocked axonal distribution of p53 and induced collapse of the growth cone, probably by suppressing Rho kinase activity [51]. In another study, inactivation of endogenous wild-type p53 blocked both apoptosis and differentiation of neurons and oligodendrocytes [52]. Similarly, the suppression of p53 expression in knock-out p53 mice accelerated neuronal differentiation and was associated with rapid cytoskeletal maturity that also included a premature dephosphorylation of tau proteins [50].

Unexpectedly, by studying a *Drosophila* model of tauopathy, it was discovered that p53 may play a neuroprotective role during aging by

regulating expression of synaptic genes. It was confirmed that p53 regulates the expression of the same synaptic-function genes in mammalian cortical neurons. More importantly, genetic manipulation of these genes was able to modify tau neurotoxicity in tau transgenic flies. Hence, the authors suggested that in response to DNA damage, p53 protects postmitotic neurons from degeneration and dysfunction by counteracting synaptic injury and maintaining synaptic function [53]. As previously mentioned, p53 is also required for axonal outgrowth and regeneration after neuronal damage. In particular, p53 is involved in the integration of extracellular signals (neurotrophins and axon guidance cues) and modulation of cytoskeletal response associated with neurite outgrowth [54]. Tedeschi and coauthors [55] demonstrated that the acetylated form of p53 participates in neuronal differentiation and axonal outgrowth by driving expression of growth-associated protein 43 (GAP-43) or neuromodulin, a prototypical regeneration protein and axon growth factor. GAP-43/neuromodulin expression was preferentially regulated by a CREB-binding protein (CBP)/p300-dependent p53 acetylation complex. Namely, p53 and its acetyltransferases CBP and p300 form a transcriptional complex that regulates axonal GAP-43. In an axon regeneration model, both CBP and acetylated p53 were induced following axotomy. Similarly, the p53/GAP-43 transcriptional module was switched on during axon regeneration in vivo. In another study, acetylation of p53 mediated axonal regeneration in mice and primary neurons by targeting the actin-binding protein Coronin-1B and the GTPase Rab13, both of which associate with the cytoskeleton and regulate neuronal outgrowth [56].

4.3 p53 in the Pathogenesis of AD

Elevated p53 immunoreactivity has been found in brain tissue of patients with sporadic AD and transgenic mice carrying mutant familial AD, both in neurons and glial cells. Western blotting revealed that p53 is primarily up-regulated in the superior temporal gyri and frontal cortices of AD brains compared with age-matched controls [46, 57, 58]. Conformational changes in p53 tertiary structure that impaired protein function were seen in peripheral cells derived from AD patients and were associated with a dysfunctional response to stressors [59, 60]. For example, skin fibroblast cultures were more resistant to oxidative damage due to suppressed activation of p53 and p53-target genes and diminished induction of apoptosis. Fibroblasts, as well as mononuclear blood cells from AD patients, specifically expressed an unfolded, point mutations-free form of p53 [61, 62]. Based on these findings, it is assumed that impaired activation of p53 may contribute to the genesis of AD. On the other hand, these results indicate the possibility of using conformational analysis of altered p53 forms in the blood as a potential biomarker to improve the early diagnosis of AD [59, 60].

4.3.1 The Specific Role of p53 in Tau Pathophysiology Is Mediated via GSK-3β

In HEK293a cells, it is shown that p53 induces phosphorylation of human 2N4R tau at the Ser199/Ser202/Thr205 epitopes. The effects of p53 on tau phosphorylation were indirect due to the compartmental segregation of the two proteins and attributed to the transcription of a p53 target genes or a kinase downstream in a p53 signaling [46]. For the AD pathology, it is significant that GSK-3β, the principle tau kinase, interacts synergistically with p53 (Fig. 4.1). Apoptotic stimuli induce accumulation of p53 in the nucleus and the mitochondria that results in association of p53 with GSK-3β [63]. By these nuclear and mitochondrial interactions, the activities of both proteins are increased. While binding of p53 directly stimulates kinase activity of GSK-3β, active GSK-3β promotes transcriptional activity of p53 in the nucleus. GSK-3β also contributes to p53-mediated apoptotic signaling in the mitochondria. Namely, inhibition of GSK-3β prevents cytochrome c release and activation of caspase-3 [63, 64].

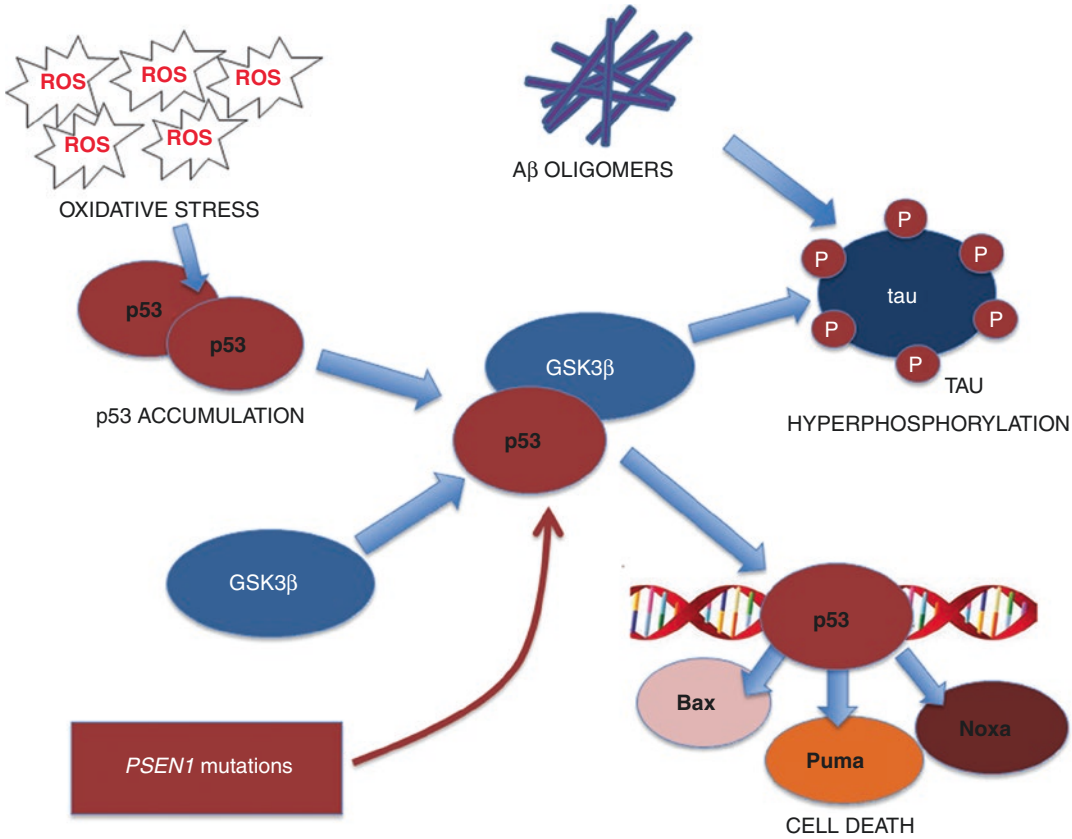

Fig. 4.1 Oxidative stress results in high levels of p53, hyperphosphorylated tau, and cell death. Oxidative stress-induced association of p53 and GSK-3β increases activities of both proteins. p53 directly stimulates kinase activity of GSK-3β, and active GSK-3β promotes transcriptional activity of p53 in the nucleus. In neurons, target genes induced by p53 include the pro-apoptotic regulators of cell death such as Bax, Puma, and Noxa. High levels of Aβ oligomers also trigger the downstream pathways involved in phospho-tau pathology. Increase in intracellular Aβ42 activates the p53 promoter and further exacerbates p53-mediated cell death. Presenilin 1, a part of γ-secretase complex, brings tau and GSK-3β in close proximity. AD-related mutations in presenilin 1 possess higher ability to bind GSK-3β and promotes tau hyperphosphorylation

Neurons bearing neurofibrillary tangles do not preferentially die by classic apoptosis but instead go through chronic (necrotic), long-term degeneration [34]. Results of Wang and co-workers [65] suggest that tau hyperphosphorylation by GSK-3 might be the key factor that renders cells more resistant to apoptosis. In their study, the overexpression of tau prevented apoptotic death in neuroblastoma N2a cells and in a tau transgenic mouse model. The overexpression was associated with lower constitutive expression of p53 and inhibition of transcription-independent, p53-mitochondria-mediated pro-apoptotic program, together with reduced phosphorylation and elevated nuclear translocation of β-catenin, a component of Wnt signaling. According to them, it seems that tau overexpression exerts protective effects on p53 by inhibiting activation (phosphorylation) at Ser33, likely by kinases Cdk-5 and GSK-3 [65].

Besides regulating p53 activity by nuclear and mitochondrial associations, GSK3 also controls p53 abundance via Mdm2 phosphorylation. GSK-3-dependent phosphorylation of Mdm2 is needed for p53 proteosomal degradation to proceed [66]. The model of Proctor and Gray [67] also proposed that GSK-3β might be the kinase responsible for the p53-induced tau hyperphosphorylation.

Namely, when neuronal cells are stressed, p53 accumulates and is free to interact with GSK-3β, leading to increased activity of GSK-3β. Increased GSK-3β activity in turn stimulates p53 activity, thus generating a positive feedback loop. Increased GSK-3β activity directly induces hyperphosphorylation of tau, but also stimulates production of Aβ and formation of Aβ aggregates that further promote tau phosphorylation [23, 33, 68, 69].

The shortest isoform of p53, Δ40p53 or p44, contributes to accelerated aging and reduced life span. Mice with $p44^{+/+}$ genotype display tau hyperphosphorylation, synaptic impairment and premature cognitive decline [70]. In these mice, only Δ40p53 and full-length p53 are able to bind to the promoter of several tau kinases, including GSK-3β, and activate their transcription. The level of Δ40p53 increases with normal aging in the mouse brain, suggesting that an imbalance in the full-length p53/Δ40p53 ratio may be responsible for the altered tau metabolism that characterizes aging [48].

4.3.2 Interplay Between p53, Phosphorylated Tau, and Aβ in AD

The dominant theory of AD pathology, the amyloid cascade hypothesis, emphasizes the crucial role of increased processing of transmembrane amyloid precursor protein (APP). APP is first cleaved by β-secretase (BACE-1), and then by γ-secretase complex, yielding Aβ peptides, a 39- to 43-amino acid fragments. Fragment Aβ42 is considered particularly neurotoxic and prone to aggregation. Although neurodegeneration in AD is historically attributed to extracellular deposition of Aβ in senile plaques, the focus recently shifted to the small diffusible Aβ oligomers. Namely, Aβ42 immunoreactivity is also detected in the cytosol and nuclei of degenerating neurons in Tg mice and AD brains [58]. The accumulation of Aβ oligomers revealed a better correlation with the disease onset and severity in comparison with the insoluble amyloid plaques [71]. It has been shown that high levels of Aβ oligomers trigger the downstream pathways involved in phospho-tau pathology (Fig. 4.1) and facilitate the development of tau-related pathological hallmarks in animal models [72, 73]. Furthermore, intracellular Aβ42 directly activates the p53 promoter and exacerbates p53-mediated apoptosis indicating that Aβ42/p53 pathway may be directly responsible for neuronal loss in AD. Oxidative DNA damage may induce nuclear localization of soluble Aβ42 and p53 mRNA expression in primary neurons. Accordingly, in AD brains, DNA fragmentation was detected in the cells that exhibited increased p53 immunoreactivity [57]. On the other hand, wild-type APP strongly inhibits p53-DNA binding activity and p53-mediated gene transactivation whereas mutant APP does not [74]. This indicates that APP offers protection against neuronal apoptosis by controlling p53 activation at the post-translational level and suggests that disruption of this function may increase neuronal susceptibility to secondary insults and further neurodegeneration.

A connection between soluble Aβ and increased tau protein phosphorylation has been recognized and regarded as an important factor in the progression of AD [75]. As tau hyperphosphorylation results from Aβ accumulation, GSK-3β represents important link between Aβ and tau pathologies [69]. Aβ42 oligomers may induce endoplasmic reticulum stress and Ca^{2+} released from endoplasmic reticulum stores may promote GSK-3β activation and tau phosphorylation [76]. Furthermore, presenilin 1, a part of γ-secretase complex, participates in the regulation of tau phosphorylation as it brings tau and GSK-3β in close proximity. AD-related mutations in presenilin 1 show increased ability to bind GSK-3β and promote tau-directed kinase activity [77].

Evidence also suggests that non-fibrillary Aβ forms bind to membrane receptors and modulate GSK-3β activity. Thus, the ability of Aβ to bind to the Wnt receptor Frizzled permits GSK-3β activity. This activity further inhibits the canonical Wnt signaling involved in the regulation of synaptic function and plasticity [78]. Moreover, GSK-3β is downstream target of protein kinase Akt. Akt phosphorylates GSK-3β and induces its

inactivation in physiological conditions [22]. Accumulation of Aβ42 peptide inhibits Akt pathway, activates GSK-3β, and triggers apoptosis [79]. Recent findings indicate that Puma, an important member of the BH3-only protein family, is up-regulated in neurons upon exposure to Aβ42 both in vitro and in vivo. The activation of p53 and inhibition of PI3K/Akt pathways are required to induce Puma expression. The transcription factor FoxO3a, which is activated when PI3K/Akt signaling is inhibited, directly binds with the Puma gene and induces its expression upon exposure to oligomeric Aβ42 [80].

Interestingly, protein phosphatase-2A inhibitor-2 (I_2^{PP2A}), an endogenous PP2A inhibitor significantly increased in AD brain, regulates p53 and Akt correlatively. The simultaneous activation of Akt induced by I_2^{PP2A} counteracts the p53-induced apoptosis suggesting that an increase in I_2^{PP2A} may trigger apoptosis by p53 up-regulation, but due to simultaneous activation of Akt, the neurons are aborted from the apoptotic cascade. These novel findings also might help in our understanding of why most neurons in AD do not go through classic apoptosis but instead go through prolonged neurodegeneration [81]. The increase of I_2^{PP2A} is associated with tau hyperphosphorylation and is due to inhibition of PP2A. Down-regulation of I_2^{PP2A} by silencing reduces tau hyperphosphorylation and accumulation. Down-regulation also improves memory deficits in 12-month-old human tau transgenic mice by a mechanism that involves restoration of PP2A activity and inhibition of GSK-3β [30]. Interplay between Aβ42, GSK-3β, and Akt signaling is also important for hippocampal long-term potentiation that might contribute to memory deficits characteristic for AD patients. Aβ inhibits hippocampal long-term potentiation and this process requires cleavage of Akt1 by caspase-3, resulting in activation of GSK-3β [82].

Expression of p53 is also partly regulated by the transcriptionally active intracellular domain of the APP (AICD). AICD is released into cytosol after the γ-secretase cleavage step and exerts cytotoxic effects on neuronal cells [83]. AICD fragments may interact with p53 and enhance its transcriptional activity and pro-apoptotic functions [84]. In addition to increase in p53-mediated activities, enhanced APP processing and an increase in AICD generation could lead to an increase in tau hyperphosphorylation. Namely, AICD induces the expression of GSK-3β followed by the induction of tau phosphorylation, reduction of nuclear β-catenin and enhanced neuronal apoptosis [85]. The inhibition of the β-secretase cleavage site reduces levels of p53 and phosphorylated GSK-3β in 3xTg-AD mice [86]. Furthermore, AICD regulates translation of the p44 isoform (Δ40p53) that is expressed in an age-dependent manner and causes premature aging and reduced life span [87]. AICD directly binds to the second internal ribosome entry site (IRES) of the p53 mRNA and affects translation of p44 through a cap-independent mechanism. As p44 controls transcription of several tau kinases, it is possible that enhanced translation of Δ40p53 finally results in enhanced tau phosphorylation, thus providing a potential molecular link between APP, Δ40p53 and tau [88].

Conclusion

Although not completely understood, the role of p53 in the pathogenesis of AD has been documented by human and animal studies. Accumulating evidence supports the involvement of p53 in Aβ and tau pathology: elevated levels of p53 are present in brain tissue from AD patients, p53 synergistically interacts with GSK-3β that promotes activities of both proteins, GSK-3 controls p53 abundance via Mdm2 phosphorylation, p53 is involved in chronic, long-term degeneration of affected neurons, p53 promoter is activated by Aβ, and p53 activity is partially regulated by APP, AICD, and PP2A-inhibitor 2. Hence, intervention in p53-related signaling might be important for maintaining neuronal survival and restoration of vital neuronal functioning, including cognitive abilities. Modulation of signal transduction pathways associated with p53-mediated cell death might be promising therapeutic target in AD and other age-related neurological disorders.

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Pathophysiological Mechanisms of Huntington's Disease

5

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5.1 Introduction

Huntington's disease (HD) is an inherited, monogenic and autosomal dominant, and typically neurodegenerative disease, whose clinical symptoms begin at the age of 40 years on average and the penetrance is almost complete at approximately 65 years of age [1]. With regard to the number of people affected by HD, the prevalence is around 10 cases per 100,000 individuals [2]. The main symptoms that appear initially are motor incoordination, cognitive decline, and psychiatric disorders [3]. Among psychiatric disorders, major depressive disorder (MDD) appears to be the most prevalent [4] and has relevant neuerochemical correlations with HD [5].

Although HD was described before by some physicians, it was given the eponymous name after the physician George Huntington's systematic description in 1872 [6], about three important basic features of the disease, namely: the hereditary nature, the tendency to insanity and suicide, and the severe manifestation of the disease only in adult life [7].

Initially, the disease was described as Huntington's chorea, due to the characteristics of the movements. Korea means dance in Greek and summarizes uncontrollable and shaken movements, which occur in the head, face, trunk, and limbs. Important voluntary activities are seriously impaired, such as speech, swallowing, walk, and writing, among others [8].

The pathophysiological basis comes from a mutation in the huntingtin (HTT) protein, which is one of the proteins whose gene has a polymorphic CAG trinucleotide repeat tract, leading to the formation of polyglutamine tract in the N-terminal region of the protein. The expansion of CAG

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_5

repeat is the basic characteristic of mutated huntingtin (mHTT) [9]. HD is therefore characterized as one of the polyglutamine diseases (PolyQ) [10]. The pathophysiology of the disease involves a wide range of biological mechanisms, whose alterations culminate in gliosis, with loss of astrocytes and oligodendrocytes, and in neuronal death and atrophy of brain tissues, with the most affected regions starting with the striatum, which integrates circuit of the basal ganglia, and the cerebral cortex [3]. Medium-sized spiny projecting neurons which release inhibitory γ-aminobutyric acid (GABA) neurotransmitter are the most affected, but many other neurons and neurotransmitters are involved in circuit dysfunction [11, 12]. Among biological alterations inherent or consequent to the dysfunctions in the neurotransmission system are cellular inclusions of protein aggregates [13], changes in cellular signaling pathways [14], energy metabolism [15], oxidative balance, inflammatory mechanisms [16, 17], and neurotrophic factors changes [18]. This chapter discourse some well-defined basic pathophysiological features and some mechanisms that are the most recent study objects in HD.

5.2 Basic Neuroanatomy of Huntington's Disease

Neurodegeneration can cause about 20–30% of reduction of brain mass, depending on the severity of the disease [19].The main losses and morphofunctional changes occur in the basal nuclei, more specifically in the neostriate, which is constituted by the caudate nucleus and putamen, two structures of cellular mass that integrate the circuit of the basal ganglia [11]. The striatal circuit consists mainly of the group of projection neurons, whose main neurotransmitter is the inhibitory γ-aminobutyric acid (GABA), and the interneurons that modulate the projection neurons. Projection neurons are almost all medium-sized spiny neurons (MSN) [20] which project mainly to the globus pallidus and substantia nigra pars reticulata (SNr) that is located in the midbrain, but is closely connected to the striatal circuit [11, 12]. The striatum receives projections from practically all cortical regions, especially the sensory and motor cortices. Subcortical nuclei, namely: thalamus globus pallidus, substantia nigra pars compacta (SNc), dorsal nucleus of the raphe, and pedunculopontine nucleus of the midbrain also projects to the striatum. The cortex projects information to the striatum through two pathways: a so-called direct pathway, which sends information from the striatum to the internal segment of the globus pallidus (GPi) and SNr; and an indirect pathway, which sends information to the external segment of the globus pallidus (GPe), that in turn projects to the subthalamic nucleus (STN), which sends information to the GPi. By these two pathways, the signals converge to the GPi and, from this follow information to the ventral thalamic nuclei, anterior and lateral. From the thalamus, the signals are projected to the premotor and frontal cortices, which will modulate the output signals of the primary motor cortex, which activate the complex muscular movements (for a more in-depth anatomical description, see: [11, 12]). Also, there is an excitatory pathway that projects directly to the STN and increases the excitability of the STN projection to the GPi, being faster than the cortico-striatal pathways. The pathway was termed the hyperdirect pathway of the basal ganglia [21].

Although the brain regions most affected belong to the connections of the basal nuclei, the brain as a whole suffers losses, which may be reflecting the variability of symptoms among HD patients [22, 23]. Another important factor is that the degree of striatal impairment does not correlate with the severity of the functional impairment and therefore, the variability and severity of the clinical and functional symptoms seem to be related to a variability of affected brain regions, especially the cortical areas [24]. The evolution of studies along more of a century has allowed HD to be considered a multisystem degenerative disease of the human brain [12]. In addition and parallel to the striatal neuronal loss, the HD presents gliosis, with loss of astrocytes and oligodendrocytes, which are happening in the same direction of the neuronal loss [3].

Although earlier studies have suggested that neuronal losses would begin after the onset of motor symptoms, more recent studies have shown that the volume and shape of basal nuclei, cortex,

and other brain regions are undergoing changes before motor symptomatic onset [25, 26]. The reductions in cortical volumes were correlated with cognitive impairment before motor symptoms in HD [26].

5.3 Genetics of Huntington's Disease

HD is a typically autosomal and dominant disease. Extensive research has also noted that homozygosis or the non-mutant allele do not influence the age of onset of the disease and therefore the changes occurring from a mutant allele appear to already exceed the threshold required for the onset of the disease [27]. However, homozygosis seems to induce a greater severity of the disease as verified on the neuronal losses and glioses in the striatum, hippocampus, cortex, and thalamus of transgenic mice [28]. In addition, homozygosis, although rare in HD patients, was related to more severe motor, cognitive, and behavioral phenotypic alterations, which were consistent with neurodegeneration [29]. The disease consists of a mutation in the IT15 gene located on the short arm of chromosome four, which encodes a protein called huntingtin (HTT). The gene contains a variable number of citosine-adenine-guanine (CAG) trinucleotide repeats [9], consisting of a polymorphic region of polyglutamine (PolyQ), both in the non-mutant and mutant genes. The expanded polyQ stretch of HTT is located in the N-terminal region, in the first exon, forming therefore a variable number of N-terminal regions of the protein [10]. However, non-HD individuals present a genotype in which the CAG range from 12 to 36 repeats, while in HD the number is higher, varying from 36 to 120 folds [30]. The expanded polyQ trait of mutant HTT (mHTT) directs the protein to a detrimental conformational flexibility in the N-terminal region, impairing the protein–protein interaction and the functional dynamics of HTT [31]. Losses in the binding activity of the protein from the polyQ expansion can alter biological processes and impair cellular homeostasis, impacting on neuronal survival losses [14]. The highest number of repetitions is positively correlated with the lower age of onset of HD symptoms [9]. The HD phenotype, which is mostly manifested after the age of approximately 40 years, seems to depend on the accumulation of the overexpression of the HTT and ubiquitin fragments in intranuclear inclusions that begin before phenotypic changes, as verified in studies from transgenic mice [32]. However, studies with transgenic mice observed a small percentage of intranuclear inclusions in regions related to motor and behavioral phenotypic changes in HD [33].

HTT protein was observed inside and outside the CNS, in compartments such as the nucleus, Golgi complex, and endoplasmic reticulum. In the synapses, the protein is found in vesicular compartments, such as the endosomes, microtubules, and clathrin-coated vesicles [13].

5.4 Neurotransmission and Cell Signaling Pathways in Huntington's Disease

Among all the neuronal circuits that are impaired in HD, the network of MSNs in the striatumin the one that suffers the greatest degeneration [34]. Striatal GABAergic projections have been well studied. The striatopallidal neurons group whose projections contain enkephalin appear to be the most affected in the early and intermediate stages of HD. However, in the more advanced stages of the disease, practically all GABAergic striatal projections are deteriorated [35].

The neuronal loss in the striatum appears to be a secondary process to alterations in the function of the microcircuitry related to the striatal output neurons. Prior to neuronal loss, the projection neurons are overestimulated, possibly by a reduction of the GABAergic inhibitory current [36]. These latter authors suggest that the reduction of the output inhibitory signal involves alteration in the glutamate astrocytic reuptake, culminating in increase of extracellular glutamate, activation of receptors mGluR5 and thus, endocannabinoids release. Activation of CB1 cannabinoid receptors on GABAergic terminals leads to a reduction in GABA release. Thus, the balance between excitatory glutamatergic and inhibitory GABAaergic activity is dysregulated, leading to an increase in

output striatal excitatory signal. Other researchers have demonstrated that the decrease in the inhibitory current in striatal neurons also arises in the function of astrocytes. The authors verified that the astrocytic release of GABA appears to depend on the uptake of glutamate by astrocytes, an event that depends on the characteristic of the astrocyte membrane potential. The reduction of the astrocytic release of GABA in HD seems to have originated in the reduction of glutamate uptake, possibly from a change in the function of astrocytic glutamatergic transporters [37].

Several receptors were shown to be reduced in brain regions of patients and animals as a model of HD. Reductions of mRNAs and receptors expression, namely D1 and D2 dopaminergic, A2a adenosinergic, CB1 cannabinoids, metabotropic and ionotropic glutamatergic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and NR2a and NR2b subunits of *N*-metil D-aspartato (NMDA) receptors were observed [38]. Studies with PET imaging suggest that the striatal D1 and D2 receptors are the most significant markers of the stages and evolution of HD. D2 receptors seem to suffer a greater reduction in pre-manifestation stages of the disease, suggesting therefore to be more appropriate markers for the identification of HD signals before the most prominent phenotypic manifestations of the disease [39]. The greatest losses in receptors or mRNA expression were in the striatum. However, NMDA receptor losses were higher in the hippocampus than in the striatum [38]. Furthermore, reductions of D1 and D2 receptors were also observed in cortical areas at stages of HD manifestations and studies have observed reductions of D2 in stages of pre-manifestation of HD pathophysiology [39]. With registration to the NMDA receptor-mediated neurotransmission, studies have shown that the mHTT presents a reduction in the interaction with post-synaptic density protein 95 (PSD-95), a member of the family membrane-associated guanylate kinase and which binds to NMDA and kainate receptors at post-synaptic density. Reduced interaction with PSD-95 causes more PSD-95 to be released, leading to over-activation and sensitization of NMDA receptors and, thus, neuronal excitotoxicity. It may also be responsible for the impairment of spatial learning, a condition that is present in HD patients [40].

In cortico-striatal synapses, brain-derived neurotrophic factor (BDNF) controls the release of glutamate, allowing the survival of GABAergic neurons against excitotoxic neurodegeneration [41]. Besides, the function of BDNF as a protector of cellular excitotoxicity induced by glutamatergic activity involved the prevention of the reduction of phosphorylated Akt (p-Akt) and of the antiapoptotic effect of Bcl2 protein, as well as the increase of apoptotic function by the caspase 3 protein [42]. In both animal models and HD patients, transcription of the BDNF gene is impaired [43]. The BDNF is located along with HTT in approximately 99% of pyramidal motor cortical neurons that project to the striatum [44]. It can also be transcribed into striatal neurons, as verified in mouse brain [45], but the larger part is synthesized in the cerebral cortex [18, 45–47]. BDNF is transported into vesicles and released into cortical axon terminals near striatal neurons [48, 49] and seems to be crucial for the maturation of a large part of MSN neurons [18, 20], as well as for the differentiation of GABAergic neurons in the striatum [18, 48, 49]. Changes in the morphology of dendritic spines of MSN neurons appear to result from reductions in the release of cortical BDNF and as one of the consequences, MSN neurons require greater cortical stimuli to reach the activation threshold [47]. HD patients present reduced BDNF transcription in the cortex and, both in the cortex and in the striatum, BDNF protein expression is reduced [43, 50]. In addition, reduced release of cortical BDNF results in reduction of cortical and striatal brain volumes, with loss of MSN neurons as well as motor changes in mice, which resemble the HD phenotype [47]. Transcription from the BDNF exon II promoter, which is responsible for the expression of the BDNF protein in the cerebral cortex is severely affected in HD mice. This suggests that the stimulatory action of wHTT on BDNF transcription is lost from expression of mHTT [43, 51]. A study with HD transgenic mice showed that peripheral administration of recombinant BDNF increased BDNF transcription in the CNS, along with reversal or reduction of various altered motor parameters, as well as neurophysiological parameters in

HD, such as size and morphology of striatal neurons, intranuclear inclusions, microglial reactions, and phosphorylated extracellular signal-regulated kinases (p-ERK), which tends to increase with the progression of HD. In addition, the phosphorylated cAMP response element-binding protein (p-CREB) was also increased, suggesting that the BDNF-TrkB signaling pathway was triggered, involving transcriptional activity [52].

An enzyme that is suggested as a biomarker of both the disease and the progression of HD is phosphodiesterase 10A (PDE10A). The activity of the CREB, BDNF, as well as neurotransmitters and ion channels in the MSN neurons are modulated by PDE10A. PDE10A participates in the hydrolysis of both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), being an important mechanism of cyclic nucleotide intracellular signaling, which is involved in dopaminergic and glutamatergic synaptic transmission, among other neurotransmitters. PDE10A expression appears reduced in brain regions associated with various functional impairments during the course of HD [39].

Another enzyme that participates in the degradation of cAMP, phosphodiesterase 4 (PDE4), seems involved in pathophysiological mechanisms of HD. HTT interacts with the scaffolding protein DISC1 and PDE4, forming a ternary complex, which regulates the function of PDE4. The regulation of PDE4 is important for the balanced function of cAMP and appears to be a mechanism underlying psychiatric behavioral homeostasis. Studies with postmortem brains of HD patients and in HD animal models have shown that mHTT aggregates with DISC1, reducing the interaction and consequently the regulation of PDE4 function. These studies have verified that PDE4 dysregulation is involved in anhedonia behaviors of HD mice, suggesting that it is a pathophysiological pathway involved in mood changes that occur in HD patients [53].

The tyrosine hydroxylase, rate-limiting enzyme for catecholamine synthesis, and dopamine beta-hydroxylase enzyme, responsible for converting dopamine to norepinephrine are reduced in the brain of HD transgenic animals and HD human [40]. Changes in dopaminergic neurotransmission are involved in the motor and cognitive dysfunctions of HD patients [54]. The level of dopamine is elevated and expression of DA receptors is reduced in the early stages of HD when the motor phenotype is characterized by chorea, but dopamine levels are reduced in the late stages, when the phenotype is characteristic of parkinsonian symptoms, such as akinesia [55].

Recent studies have shown that HTT is connected to a large network of molecules and cell signaling pathways. The same authors argue that the great interconnectivity of HTT is due to its wide function in relevant physiological processes, as well as to the degree of pathogenic processes by mHTT in HD [14]. These authors also emphasize that the HTT connection network involves proteins and signaling important for neuronal morphology and synaptic density, whose attributes important for cellular integrity and synaptic transmission may be lost in the interaction with mHTT.

Toxic intracellular aggregates occurring in HD from mHTT may be influenced by the mammalian target of rapamycin (mTOR) signaling pathway [56]. Although mTOR activation is a positive mechanism for transcription, translation, and neurogenesis [57], the interaction of mHTT with molecules of the mTOR pathway is involved with a likely abnormal increase in mTOR activity and with neural, behavioral, and motor phenotypic changes of HD [58]. One of the possible justifications argued by the authors is that increased mTOR activity may impair autophagy, a mechanism that is impaired by mHTT [58] and therefore may underlie the increase of intracellular toxic protein aggregates [56]. A study on mHTT transfected cells showed that mTOR is sequestrated and localized along with HTT aggregates. However, the study also observed that when cell inclusions with mHTT and mTOR increase after accumulation for a prolonged time, inhibition of mTOR has no beneficial effect on increasing autophagy. Additionally, the mTOR-dependent translation is impaired by the polyglutamine expansion in the mHTT [59]. Furthermore, an extensive and recent study observed several molecular parameters related to mTOR as well as phenotypic parameters of HD and found that molecular, morphological, and motor phenotypes related to HD were attenuated in both humans and HD mice models by increasing

mTOR activation [60]. Among several parameters observed in this last study are mechanisms associated with mitochondrial function, epigenetic mechanisms and increased autophagy via the mTOR pathway, striatal plasticity and function, cholesterol homeostasis, and improvement of striatal dopaminergic signaling. Therefore, requirements of the mTOR signaling pathway on HD pathophysiology still require further studies in order to observe which mechanisms may be affected and whether an increase or decrease in mTOR activity underlies pathophysiological mechanisms.

Very recent studies have suggested that another pathway of intracellular signaling, Hippo signaling pathway, from which, cell proliferation, tissue growth, and development are regulated, is involved in the pathophysiology of HD. Through in vitro and in vivo imaging techniques and studies, the researchers observed that the expression of mHTT induces a type of necrotic cell death in primary cortical neurons, through Hippo signaling pathway, which was termed transcriptional repression-induced atypical cell death (TRIAD) [61]. In a later study, it was shown that the activation of effectors of the Hippo pathway is involved in the TRIAD of neurons in postmortem brain of HD patients and in the striatum in HD mouse model [62].

5.5 Oxidative Stress and Neuroinflammation in Huntington's Disease

Neuroinflammation is a common condition in neurodegenerative diseases and may start before some relevant neuronal loss in the course of the disease [16]. In addition, neuroinflammation and oxidative stress are mechanisms that are closely intertwined and can form a vicious cycle under certain physiological conditions, impairing homeostasis [16, 17].

Oxidative stress is one of the major villains involved in various mechanisms of the central nervous system, such as increased cell death, reduced neuronal plasticity and neurogenesis, increased autoimmune responses in neurodegenerative diseases [63, 64]. The brain is especially vulnerable to oxidative and nitrosative stress because it has a high metabolic rate [65] and, consequently, a high rate of oxygen consumption [66], coupled with lower average levels of antioxidants [65]. In addition, the brain is highly vulnerable to lipid peroxidation due to the large amount of polyunsaturated fatty acids present in neuronal membranes [67].

Among many pathophysiological mechanisms, increase of oxidative and nitrosative stress is related to mitochondrial dysfunction. In turn, damage in mitochondrial function are related to increased oxidative stress, creating a vicious cycle that culminates in neurodegeneration [68]. The activity of mitochondrial respiratory chain complexes was impaired, while oxidative damage was also observed in striatal structures of postmortem HD brain [15].

Intracellular aggregates from mHTT appear to increase reactive oxygen species (ROS) levels in neuronal and non-neuronal cells. Studies suggest that increased oxidative damage and inefficiency in damaged DNA repair underlie the somatic expansion of CAG repeats and neuronal loss [69]. On the other hand, ROS levels from high dopaminergic activity inhibits the formation of autophagosomes and causes death of dopaminergic cells from human neuroblastoma expressing transgenic mHTT. Prevention of ROS formation restores the autophagy process and reduces the dopaminergic toxicity of mHTT-expressing neuronal-like cells [70].

Changes in homeostasis of metal ions, such as iron and copper, underlie HD [17, 71]. Iron contributes to relevant mechanisms of oxidative damage [72]. Iron can react with hydrogen peroxide and molecular oxygen to form hydroxyl radicals (Fenton reaction). Exposure of biomolecules to increased levels of iron can cause oxidative damage to proteins, lipids, and nucleic acids, and thus potentiate neurodegeneration [73]. However, if high iron levels are the cause or effect of HD, it is still unclear [71]. It has been found that these metal ions accumulate in the brain tissue of HD patients, as observed in postmortem brain and through magnetic resonance imaging (MRI) [73]. In addition, in animal models of HD has also been shown accumulation and a pro-oxidant copper–protein interaction involved in disease progression [74].

Quinolinic acid (2,3-pyridinedicarboxylic acid) is a tryptophan metabolite, along the kynurenine pathway in glial cells, and is also a potent NMDA agonist. It is typically implicated in excitotoxic damage with increased concentration of cytosolic $Ca^{(2+)}$ and consequent mitochondrial dysfunction and oxidative damage. Toxic damages affect striatal gabaergic neurons leading to motor dysfunctions [75]. The kynurenine pathway is stimulated by cytokines under neuroinflammation conditions, mainly by interferon-γ(IFN-γ)-activated macrophages. In addition, concentrations of induced NO synthase (iNOS) were also elevated from the activated macrophages, with levels of both metabolites of the kynurenine pathway and iNOS exceeding the respective neurotoxicity thresholds for excitotoxicand apoptotic neuronal death as well as neuronal and glial damages from nitric oxide (NO) [76]. A study outlined by Colle et al. [77] showed that striatal mechanism damages are involved with metabolic impairment, ROS formation and oxidative stress, through two toxic models implicated in the quinolinic acid pathway, with activation of NMDA receptor and in the inhibition of succinate dehydrogenase of the mitochondrial respiratory chain (complex II) by 3-nitropropionic acid (3-NP) a mitochondrial toxin. These authors suggest that oxidative stress remains as a key mechanism involved in HD.

Among the parameters related to neuroinflammation, studies have observed an increase in glial activation in the basal ganglia and cortical regions, both in vivo and in postmortem brain tissue of HD patients [78–80]. Some studies also suggest that glial activation is positively correlated with disease severity [79, 80] and progression [81]. It is important to note that glial activation appears to occur before the onset of motor symptoms, as observed in studies with mHTT carriers [81]. A recent study noted that nuclear inclusions are present in all types of glial cells, both in HD animal models and in the postmortem brain of HD patients, although in a smaller size than nuclear neuronal inclusions [82]. Some authors suggest that neuroinflammation is a strong component underlying all stages of HD and highlights that mHTT interferes in important processes directed by microglia and astrocytes, such as cytokine release, cell signaling, and transcription mechanisms [83].

Peripheral immune responses appear to be activated due to the ubiquitous expression of mHTT in a variety of cells. Thus, studies have observed an excessive production of inflammatory cytokines in myeloid cells of the HD patient [84]. Anti-inflammatory cytokines are significantly increased in the plasma of HD patients, both prior to phenotypic motor manifestations and in the early stages of symptoms. On the other hand, inflammatory cytokines are increased in the plasma of patients in later stages of HD [85]. In animal models of HD, it has been observed that pro-inflammatory cytokines are increased in both microglia and serum. While anti-inflammatory cytokines were reduced in microglia and cerebrospinal fluid [86]. Some studies have also observed that inflammatory markers observed in the plasma of HD patients in different stages of the pathology can be considered as markers of HD progression [87]. Interestingly, some researchers suggest that mHTT itself may be considered an inflammatory marker of HD, since mutated protein levels in monocytes and T cells have been positively correlated with the severity and degree of striatal tissue atrophy [88].

5.6 Considerations

Although HD is monogenic, mHTT is widely distributed in tissues and therefore must play important roles from development to the end of life, beyond those that have already been unraveled. Despite numerous researches and findings on a wide variety of mechanisms in which mHTT is involved, a pathway that can link the myriad mechanisms for pursuing a treatment strategy has not yet been signaled by the research. The inclusion of nuclear protein aggregates and the process of autophagy incite the researchers. However, many mechanisms discovered following the studies, such as the involvement of alterations in mitochondrial function and energetic metabolism, neurotrophic factors, cell signaling pathways, transcriptional mechanisms, oxidative balance, and neuroinflammation are highlighted in the researches (See Fig. 5.1). The stages before

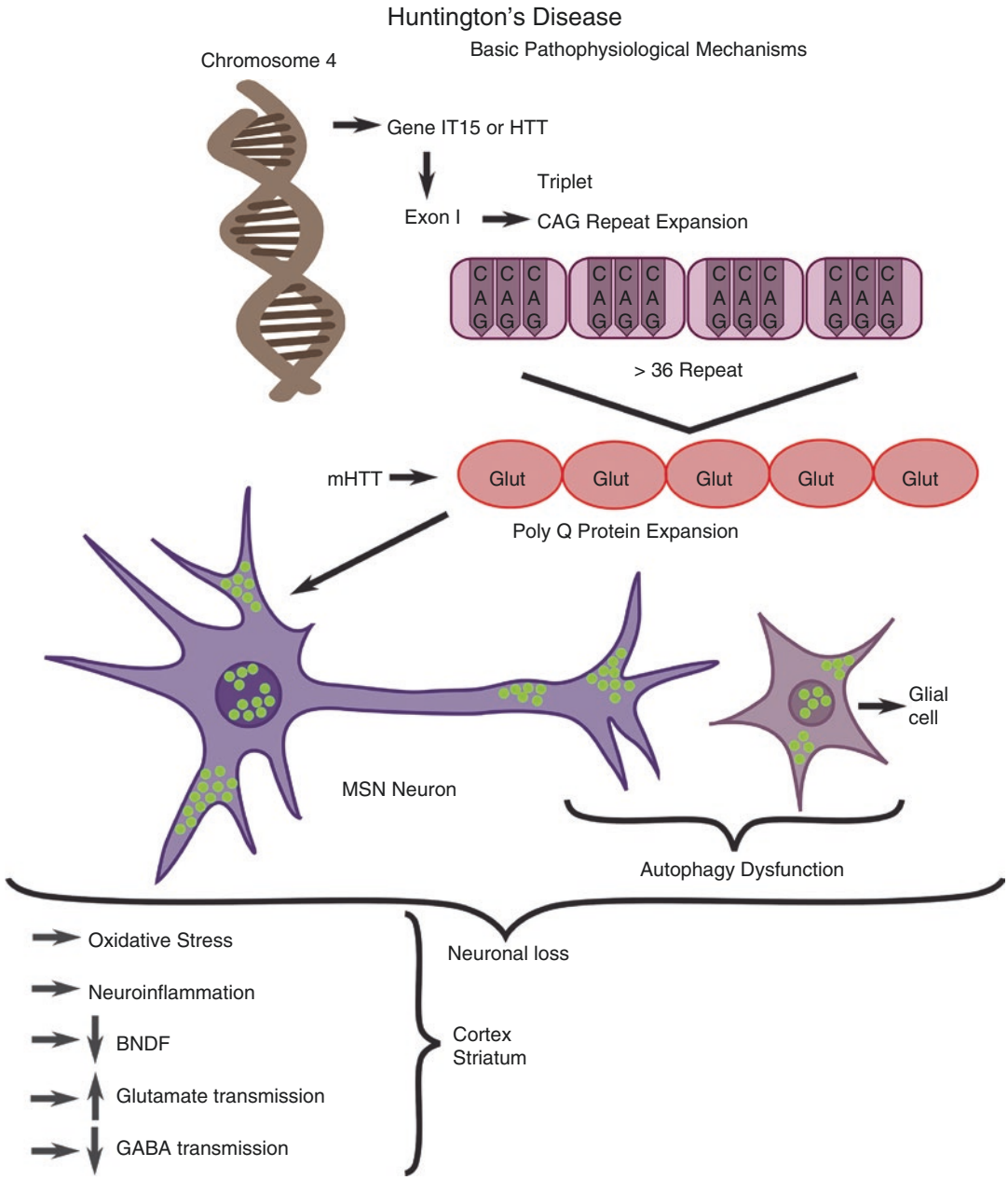

Fig. 5.1 Basic pathophysiological mechanisms involved in the Huntington's disease. The IT15 or HTT gene is located on the short arm of chromosome 4. In exon 1 of the coding region of the huntingtin protein (HTT), there is a polymorphic cytosine-adenine-guanine (CAG) trinucleotide sequence, leading to repeats of the glutamine amino acid in the N-terminal region of the protein. Proteins with the polyglutamine sequence are referred to as PolyQ. In non-HD subjects, CAG trinucleotides can reach up to 35 repeats. From 36 to about 120 repeats is characteristic of the mutant gene (mHTT). mHTT protein aggregates form nuclear and cytoplasmic inclusions in neurons and glial cells, especially in striatal medium-sized spiny neurons (MSN). Dysfunctions of the autophagy process prevent the cell aggregates clearance. Initially and throughout the course of HD, there is a reduction in the release of brain-derived neurotrophic factor (BDNF), an increase in cortical glutamatergic transmission on MSN and a reduction in GABAergic transmission by MSN neurons. There is also an increase in oxidative stress and neuroinflammation, among other detrimental alterations, such as impairments in cell signaling, transcriptional factors, and mitochondrial function. Gradual neurodegeneration of brain tissues occurs, which begins and is more potent in the striatum and cortex

and during phenotypic motor manifestations are crucial points for the investigation of a convergent pathway between all the biological mechanisms involved in HD.

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Glutamate in Amyotrophic Lateral Sclerosis: An Ageless Contestant

6

Alida Spalloni, Michele Nutini, and Patrizia Longone

6.1 Introduction

Glutamate is a crucial amino acid which serves a fundamental function in the central nervous system (CNS) and acts as a signaling substance at many excitatory synapses, ordaining on practically all central neurons. It remains in millimolar concentrations chiefly in the presynaptic terminals of excitatory neurons but obtainable throughout in the brain and spinal cord. As a closely regulated process, glutamate release and uptake are vigilantly regulated. The exposure to this neurotransmitter must be concise to neurons and at its appropriate levels it exhibits proper synaptic neurotransmission and/or neurotrophic effects. Indeed, when the extracellular concentrations of glutamate are increased and remain high for an abnormally long duration, as it happens in certain pathological conditions, glutamate acts as a toxin. In this regard, the notion of glutamatergic excitotoxicity was introduced by Olney and collaborators in the 1960s early 1970s [1–4], and studies in last decades have strongly supported the involvement of this hypothesis in neuronal death [5–7].

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6.2 Glutamate, Astrocytes, and Mitochondria

6.2.1 Glutamatergic Excitotoxicity in ALS

The case for excitotoxicity in Amyotrophic Lateral Sclerosis (ALS) began to surface thanks to the pioneer work of Andreas Plaitakis [8–10]. His work leads the way to the hypothesis that a systemic defect in the metabolism of the excitatory amino acid glutamate may lie behind the ALS-related motor neuron death, directing the attention to the role played by glutamatergic excitotoxicity in the ALS etiology. With his colleagues at the Department of Neurology at Mount Sinai School of Medicine in New York, suggested that the delivery of the glutamate between the intracellular and extracellular pools could be altered, possibly due to the outcome of a defected uptake system or release machinery(s) [11]. In the same years, Rothstein and collaborators, from the Department of Neurology at Johns Hopkins reported irregularities in excitatory amino acids in the CNS of ALS patients [12, 13]. In the 1990 study, they measured significantly higher concentrations of glutamate (by 100–200%) in the cerebrospinal fluid (CSF) from ALS patients. Although, at first, there were conflicting evidences since other groups reported a lack of raise in the glutamate concentrations of CSF and plasma of ALS patients

S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_6

[14–17], these observations set off a line of research looking at the glutamatergic system and excitotoxicity in ALS.

An additional evidence endorsing the involvement of excitotoxicity in the pathology of sporadic ALS (sALS) was provided once more by Rothstein and collaborators [18]. Using synaptosomes preparations from spinal cord or other impacted brain regions of sALS patients, they detected a functional deficiency in the uptake of high-affinity sodium-dependent glutamate, the GLT-1 glial glutamate transporter. Glutamate elimination from the extracellular space, by high-affinity and low-affinity sodium-dependent carriers expressed by astrocytes and neurons, is the primary mechanism for its inactivation [19, 20]. The low-affinity glutamate transporter sub-serves common metabolic performance. The high-affinity carrier is a constituent of the glutamate neurotransmitter scheme and is accountable for the elimination of neurotransmitter glutamate from the synaptic cleft [21]. If the extracellular concentration of glutamate remains elevated at the cleft, it becomes toxic to neurons. Hence, they examined the glutamate-transport system in brain and spinal cord tissue received from the postmortem brain of ALS patients. They found a significant alteration (decrease) in the efficiency of the glutamate transport in spinal cord and brain tissue from ALS patients, identifying excitotoxic injury as one of the noxious processes beneath motor neuron death.

These observations fuelled the interest on glutamate in ALS and encouraged studies to elucidate the underlying mechanisms.

6.2.2 Glutamate Receptors

A considerable line of research has looked at the receptors that are activated by the neurotransmitter. Physiologically when released glutamate binds to its post-synaptic receptors triggering an increase in Na+ and Ca2+ concentrations. Glutamate activates both ionotropic and metabotropic receptors, with distinct pharmacological and molecular profiles. The metabotropic receptors are members of the G-protein coupled receptor superfamily. They arbitrate synaptic neurotransmission through the activity of intracellular second messenger. Hence, they mediate slow responses.

The ionotropic are ion channels associated with the glutamate-mediated rapid responses. These are classified into three different subtypes: the *N*-methyl-D-aspartate (NMDA), the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and the kainate receptors. The NMDA receptors are usually associated with neuronal plasticity. Pathologically, they mediate several acute insults to the CNS, which is consistent with their predominant role during a long exposure to elevated levels of glutamate [22]. Even though slower acting, AMPA or kainate agonists are also compelling neurotoxins and may cause extensive neuronal devastation [23]. Ionotropic glutamate receptor is highly permeable to Ca2+ (with the exception of the AMPA receptors containing the edited GluR2 subunit). Intracellular Ca2+ overload is the key feature of the glutamate-mediated excitotoxicity as demonstrated by Dennis Choi in 1985 [24]. In this landmark study, glutamate excitotoxicity in neuronal cultures was enhanced in a calcium-rich extracellular solution, while a calcium-free extracellular solution noticeably decreased neurodegeneration. Then, following evaluations have established that in the glutamate-mediated injury the intracellular calcium sequestered into mitochondria plays an important role [25]. When over-activated the NMDA receptors allow the entry of excessive amounts of Ca2+ that leads to a mitochondrial calcium overload that in turn triggers mitochondrial dysfunction and activates death signals [26], leading to cell death [27]. The NMDA receptors are considered to be the primarily responsible for the glutamate-mediated Ca2+ entry [28, 29]. In ALS, however, since the 1993 work by Couratier and co-workers [30], AMPA receptors have been considered to be a major player. The authors reported that rat neuronal culture exhibited the poisonous effects when exposed to CSF obtained from ALS patients which was reversed by CNQX, an antagonist to the AMPA/kainate receptor, but not by MK-801 and AP7, two NMDA receptor antagonists. Hence, their data were a strong

indication that AMPA receptors are the main intermediaries in the glutamate-mediated motor neuron death. An additional evidence is offered by the environmental neurotoxins β-*N*-methylamino-L-alanine (BMAA), believed to be correlated to the Amyotrophic Lateral Sclerosis-Parkinsonism Dementia Complex of Guam [31, 32], that again is supposed to be toxic through the commencement of the glutamate receptors [33], mainly of the AMPA subtypes [34]. These evidences have shaped the studies that have looked at the glutamate-mediated excitotoxicity standpoint, focusing essentially on role played by the AMPA subtype of receptor in several "in vitro" [35–38] and "in vivo" [39–43] findings. Although the impact of NMDA receptors in ALS excitotoxicity shouldn't be overlooked [44–47].

These observations while helping define the role of glutamate in motor neuron death have linked glutamatergic-mediated toxicity to two additional frontrunners in the ALS-related pathophysiology: astrocytes and mitochondria.

6.2.3 Spinal Cord Astrocytes in ALS

Astrocytes are the ample populated cells in the CNS (~50% by volume). They have a virtual interaction with neurons, with a metabolism, involving energy generating pathways and amino acid homeostasis firmly coupled to that of neurons [48, 49]. When Rothstein and collaborators reported a dramatic decrease in the GLT-1/EAAT2 immunoreactive protein in motor cortex and spinal cord of patients of ALS [18], they highlighted the glial contribution to the motor neuron demeanor. Then, they punctually demonstrated, by chronically inhibit the synthesis of the glutamate transporter, employing antisense oligonucleotides, that GLT-1/EAAT2 and GLAST/EAAT1 are the main accountable for the amplified glutamate concentration extracellularly and the subsequent glutamate-mediated toxicity [50]. Beyond their precise proposition for perceptive ALS, the studies by Rothstein's group supported the view that astrocytes are directly involved in the pathological process of ALS. This hypothesis was also suggested by a study on postmortem human spinal cords, where the authors concluded that the disease mechanism in sporadic ALS may involve alterations in spinal cord astrocytes [51].

Bruijn et al. [52] reported in a study carried on in the transgenic mice overexpressing human G85R SOD1, a murine model of familial ALS (fALS), the presence of numerous inclusions in astrocytes that preceded the appearance of similar inclusions in neurons. They suggested that besides the glutamate transporter malfunction, molecular targets, present within the astrocytes, and possibly damaged by mutant SOD1, while affecting astrocytes were harming motor neurons. Additional studies confirmed that neuronal Lewy-body-like hyaline inclusion and astrocytic hyaline inclusion were morphological trademark of SOD1-linked familial ALS patients and mice expressing the human SOD1G85R mutation [53, 54]. These findings were further confirmed by Watanabe et al. [55] in two other SOD1 fALS mouse models the SOD1G93A and SOD1G37R. They reported the presence of proteinaceous accumulations in astrocytes and concluded that abnormal astroglial biology could be important in the cell death in ALS.

To clearly understand glial role in ALS genetically engineered mice with a restricted overexpression of mutant SOD1 only in astrocytes or only in neurons have been extremely valuable. Gong et al. [56] generated transgenic mice with mutant SOD1 overexpression restricted to the astrocytes to see whether these mice would extend unplanned motor neuron degeneration and astrocytic pathology. Their experiments demonstrated that when mutant SOD1 expression is limited to astrocytes it causes significant pathological changes within astrocytes but was insufficient to cause motor neuron death or motor dysfunction in vivo. Their conclusion was that astrocytosis in mutant SOD1 is the result of a combined neuronal function impairment as well as prime straight astrocytic dysfunction. Soon after Rouleau and co-workers [57] generated transgenic SOD1G37R mice driven by the neurofilament light chain promoter, to test whether motor neuron restricted expression of mutant SOD1 was adequate for disease

occurrence. They found that the neuronal cell-specific expression of mutant SOD1 does not originate noteworthy motor neuronal cell death and reported that their mice seems healthy at age of more than 18 months. On the contrary, when ubiquitously express the SOD1G37R gene causes the disease as early as 3.5 months and produces clear pathological features in motor neurons (cytoplasmic vacuoles in dendrites, proximal axons, and perikarya, including degenerating and swollen mitochondria) [58]. Another group [59] created a G85R mutant SOD1 deletion, with a confined expression to spinal motor neurons and interneurons. Their transgene generated pathological (loss of motor neurons) and immunohistochemical symbols of motor neuron degeneration (ubiquitin staining) only in the mutant SOD1-immunoreactive cells, without any clear phenotypical signs. They believed that their mice did not build up the clinical disease because the mutant SOD1 expression occur only in a few motor neurons and that a more extensive motor neuron degeneration would be necessary for the disease to become clinically apparent. Hence, they argued that their data diverged, for this reason, from earlier published studies in which mutant SOD1 focused by neuronal promoters abortive to generate either clinical or pathological verification of motor neuron degeneration [57, 60]. Whether or not this is the case, these data clearly assess that mutant SOD1 has to be overexpressed in both neurons and glia to be able to trigger the disease and show its phenotype "in vivo." An interesting manuscript is the one by Hensley et al., [61] showing that primary cultures astrocytes carrying the SOD1G93A mutation hold an altered unstable phenotype prone to produce proinflammatory substances and enter a proinflammatory state.

These observations set the tone to a new view for ALS, as a non-cell autonomous disease [62]. Classically, neurotoxicity in neurodegenerative diseases is viewed as a process where a particular neuronal population is mainly susceptible to a collective toxic load (i.e., toxic mutant proteins). The chronic damage caused by this toxicity, combined with aging, reaches a verge that crushes the neuron's protective machineries leading to its death. Initially, this process was seen as cell autonomous, self-regulating for the damage gathered within other cell types interacting with the neuronal cells. This view has now changed. Cleveland and co-workers using a Cre/loxP SOD1G37R transgenic mice have showed a contribution of diverse cell types to mutant SOD1-induced motor neuron disease. They constructed chimeric mice that incorporated combination of normal and mutant SOD1-expressing cells. Their analyses show that elevated levels of expression of mutant SOD1 in most [63] or all [64] motor neurons are insufficient for early onset of disease, thus linking disease initiation to the synthesis of mutant proteins by non-motor neurons. Then, cell type-dependent excision in mice-expressing transgenes flanked by lox sites has contributed to ascertain the identities of cells whose mutant SOD1 synthesis participates in the disease pathology. The same authors elegantly proved that the selective expression to motor neurons of a ALS-linked SOD1 mutant delayed disease onset, but the degree of disease progression did not alter after the disease onset [62, 65]. Specifically, they demonstrated that a decreased expression of SOD1G37R in microglia and activated macrophages offered slight effect on the initial phase of the disease onset, but their effect could increase with disease progression and could significantly slow down the late phase. In other words, the disease onset between this model and the one that overexpresses mutant SOD1 ubiquitously was similar, while the disease duration after the onset was significantly higher in the selective-expressing mutant.

Apart from the role played in ALS, the dependence of neurons on astrocytes for their energy metabolism and glutamate synthesis [48] is critical. Neurons need astrocytes to maintain the right levels of glutamate, behind its clearance from the cleft. They lack the enzyme pyruvate carboxylase, for this reason they rely on astrocyte cells for de novo glutamate synthesis [66–68]. Moreover, the astrocyte-derived glucose is an essential precursor for the glutamate synthesis [69], and in maintaining its optimum concentration [70].

6.3 Mitochondria and Calcium Loading in Glutamate Excitotoxicity

As discussed above, the excessive activation of the ionotropic glutamatergic receptors leads to the deficit of post-synaptic structures (i.e., dendrites) and neuronal cell bodies. In this context in a neurodegenerative disease as ALS, where neuronal cell death take place over an comprehensive time period, we can envision a condition of chronic glutamate-mediated excitotoxicity. In other words, a long repeated activation of the glutamatergic receptors determined by an increased extracellular glutamate concentration may lead to the nerve cell death.

As a proof of concept, organotypic spinal cord cultures have been utilized to investigate chronic glutamate toxicity [71]. These organotypic cultures may be asserted for up to 3 months. Using two different glutamate uptake inhibitors (threohydroxyaspartate and pyrrolidinedicarboxylic acid), the authors obtained a continual increase of glutamate in the cell culture medium that they linked to a concentration- and duration-dependent motor neuronal cell death. They also reported that the glutamate-mediated neuronal death was neutralized by non-NMDA receptor antagonists and inhibitors of glutamate synthesis or its release. Their experiments revealed that a moderate and prolonged increased of extracellular glutamate can induce toxicity.

Chronic excitotoxicity has also been linked with the Guamanian amyotrophic lateral sclerosis/Parkinson-dementia complex (ALS/PDC), BMAA toxin, from the cycad Cycas circinalis, is considered a possible cause [72]. Although the evidence of its association to the Guanamian ALS/PDC is still controversial [72], the oral dosing of BMAA to macaques causes a motor system impairment affecting both upper and lower motor neurons and also on the extrapyramidal system [73].

Mitochondria are the cellular power plant. They are highly dynamic organelles controlled by an array of physiological stimulus that change their shape through the fission/fusion cycle. Metabolic function during physiological cellular life may contribute dysfunction of mitochondria and their damage. A significant burden for their homeostasis mainly in post-mitotic tissues, such as the brain, is the oxidative damage. With age they accumulate altered proteins in their matrix (i.e., oxidized and glycoxidized), and their ATP-stimulated proteolytic activity decreases considerably [74].

They play complex, interdigitated roles in cellular physiology, have a crucial role in providing the brain with energy (ATP generation), and are central in cell death mechanisms through the activation of cellular suicide programs (i.e., apoptosis) [75]. In addition to providing the ATP necessary to maintain ionic gradients, they can also buffer cytosolic Ca2+ [76], thanks to their large electrochemical potential [77]. Mitochondria shape the Ca2+ responses in neurons by taking up large amounts of the ion [78, 79]. This has been observed in neurons stimulated with glutamate [80, 81]. Hence, there is a direct dependency between the glutamate-mediated Ca2+ response and mitochondrial homeostasis.

Mitochondrial alterations in ALS were suggested by neuropathological studies on postmortem human patients [82]. Then, the mitochondrial involvement in ALS became obvious when, thanks to the mutant SOD1 transgenes, the anatomical analyses of the affected tissues revealed the presence of numerous membrane-bound vacuoles in the G93A and G37R lines. These vacuoles were evident prior to the last phase of the disease and seem to be originated from dilated mitochondria [58, 83] and the endoplasmic reticulum [83]. Subsequent studies have confirmed that mitochondrial abnormalities are an early feature in ALS and that mitochondrial degeneration is an important early event [84, 85].

Weiss and co-workers were within the first to investigate, in spinal neurons, the downstream sequelae of Ca2+ entry by the Ca2+ permeable AMPA/Kainate ionotropic glutamate receptors [35, 86, 87]. They found that motor neurons were extremely susceptible to the chronic Ca2+-dependent mediated injury of those receptors [35, 86]. Then, they extended their analyses and focused on mitochondria and reactive oxygen

species (ROS) generation [87]. They reported that motor neurons are more susceptible, than GABAergic cortical neurons, to AMPA/kainate receptor-mediated damage essentially because their activation triggers substantial mitochondrial Ca2+ excess, mitochondrial depolarization and ROS production. They concluded that the expression of Ca2+ permeable AMPA receptor channels by motor neurons probably contributes to their extreme susceptibility in ALS.

Consistent with these data supporting the role of calcium and oxidative stress in the pathology of ALS is the work by Kruman et al. [88]. The authors further confirmed the augmented vulnerability of MOTOR NEURONs from mutant SOD1 to excitotoxicity and clarify some of the fundamental machineries. They identify elevated basal-oxidative stress and disturbed mitochondrial functions in the mutant spinal cord cultures. Moreover, excitotoxic experiments let them to conclude that mutant motor neurons were extremely vulnerable to the AMPA-mediated glutamate toxicity and that their Ca2+ homeostasis is perturbed. They also reported that antioxidant and Ca2+-reducing agents were protecting against glutamate-mediated toxicity. We have reported a differential expression of the AMPA receptor subunits in mutant SOD1G93A spinal motor neuron in culture [38]. Using the single-cell PCR technology, we were able to demonstrate that the mutant SOD1 alters the AMPA receptor isoforms and subunit composition leading to the expression of a high-gain AMPA receptor that desensitizes more slowly with a longer receptor open time. This provokes an elevated Na+ influx with a resulting extended cell depolarization and opening of voltage-sensitive Ca2+ channels, with an increase in the intracellular Ca2+ and subsequently increased excitotoxicity [38]. The mitochondrial involvement in ALS has also been demonstrated for the cortical motor neurons. Van Westerlaak and co-workers using a rat cortical explant culture model determined that the persistent mitochondrial inhibition ensued in a dose-dependent rise of cortico-spinal motor neuron death. The neuronal death was reverted by the NMDA antagonist MK-801 and the non-NMDA antagonist CNQX clearly showed the role of glutamate through both non-NMDA and NMDA receptors [89, 90].

In the work by Avossa et al. [91] using organotypic slice cultures from wild-type and SOD1G93A spinal cords, early signs of mitochondria vacuolization in the mutant ventral horns were not found. However, other works confirmed the occurrence of altered/malfunctioning mitochondria in spinal and cortical motor neurons, combined with glutamatergic excitotoxicity.

Calderò and collaborators working with an organotypic slice culture from chick embryos spinal cord examined the motor neuron response to various excitotoxins. Their results confirmed the high motor neuron sensitivity to kainate and NMDA. Moreover, their results show that motor neurons are also highly vulnerable to persistent inhibition of mitochondrial functions with malonate and 3-nitropropionic acid (3-NP), which did cause excitotoxic-like lesions. They conclude that their data reveal a positive association among excitotoxicity and mitochondrial dysfunction in MOTOR NEURONs [45]. The protective effects of pyruvate and β-hydroxybutyrate (βHB) as energy substrates in association with the antioxidants glutathione ethyl ester and ascorbate in a chronic AMPA-induced neurodegeneration were also demonstrated by Tapia and co-workers [92]. Again, more recently Tapia's group [93] showed that AMPA perfusion in the lumbar rat spinal cord causes motor neurons death and the permanent paralysis of the ipsilateral hind limb. Interestingly, they reported mitochondrial dysfunction as an early hallmark of neuronal degeneration, prevented when AMPA was perfused together with pyruvate. The authors demonstrated that the progressive motor deficits, massive death of lumbar spinal MOTOR NEURONs, and noteworthy astrogliosis in the ventral horns following "in vivo" AMPA infusion was prevented by the co-infusion of pyruvate or βHB, while the antioxidants co-infusion was ineffective. They concluded that the protection observed with pyruvate and βHB, two

well-recognized mitochondrial energy substrates, is indicative of the importance that the deficit in mitochondrial energy metabolism has in the excitotoxic AMPA-dependent motor neuron death.

Mitochondria are central in the ALS-related pathology as self-governing organelles, and as interconnected organelles in cross talk especially with the endoplasmic reticulum [94]. In this context, the endoplasmic reticulum–mitochondria–Ca2+ cycle (ERMCC) and its link to the disruption of the Ca2+ homeostasis, determined by glutamate-mediated is gaining momentum [95, 96]. Indeed, Ca2+ dysregulation, which is generally triggered by neuronal over-activation, is closely interconnected with the mitochondrial pathology Cozzolino and Carri [97].

It has been reported that glutamatergic excitotoxicity is one of the first pathological pathways related to the motor neuronal death in ALS [98]. However, this event could be submissive by other players in the ALS pathophysiology, which may lead to hyperexcitability (i.e., GABAergic interneurons impairment, Na+ channels malfunction, and altered K+ concentration at the cleft; [99]). Since the central role of glutamate in neuronal function and brain homeostasis is well accepted, it retains a noteworthy role in the disease pathology (Fig. 6.1).

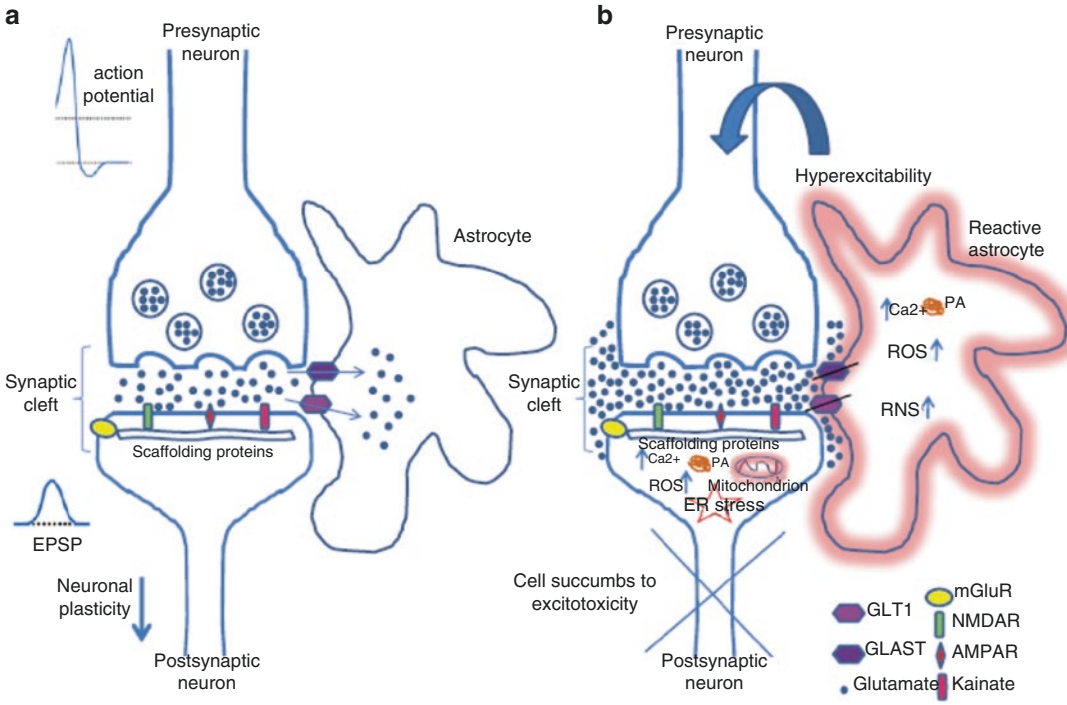

Fig. 6.1 Glutamatergic transmission in ALS pathology. (**a**) During physiological conditions, glutamate released by the presynaptic motor neuron which stimulates its receptors on the post-synaptic neuron to generate excitatory post-synaptic potentials (EPSPs) and contribute to neuronal plasticity. (**b**) In ALS presynaptic motor neuron generates excessive glutamate release. In addition, the concurrent incident of a reduced expression of the glial glutamate transporter GLAST/GLT1 ascertains a pathological rise in the extracellular levels of glutamate in the synaptic cleft. This offers an over-stimulation of the glutamate receptors on the post-synaptic neurons with a resultant cellular excitotoxicity on top of synchronized factors such as mitochondrial failure and endoplasmic reticulum (ER) stress. Moreover, both neuronal and astrocyte cells build up proteinaceous aggregates (PA), increased Ca2+ and reactive oxygen/nitrogen species (ROS/RNS) levels. The incidence of all these measures leads to cellular death. (From Spalloni et al. [47])

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Inherited Neurodegenerative Disorders

7

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7.1 Introduction

The molecular mechanisms of many neurological disorders including Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis are being explored using genetic technologies. Studies regarding genetics of diseases is relevant due to its potential direct clinical effect on the family in terms of disease diagnosis and its care. In addition, it also impacts the future of patient populations through the insight gained in pathogenesis of the disease. Genetic knowledge also offers the development of experimental disease-related animal models which could be utilized to expand ideas about pathogenesis and to test the process of medical care [1].

Though the most common neurodegenerative diseases are sporadic, Mendelian inheritance patterns have been well documented [2]. It is interesting to note that clinical symptoms and neuropathological observations of hereditary neurodegenerative diseases are often identical to sporadic diseases, offering the probability that common pathophysiologic mechanisms lie behind both hereditary and sporadic forms of disease [3].

7.1.1 Mendelian Pattern of Inheritance

Mendelian basis of inheritance of disease that follows a dominant or recessive model of inheritance is the rarer form of disease inheritance seen in neurodegenerative diseases. One example of Mendelian inheritance is Huntington disease, where 90% of the disease is hereditary with a dominant inheritance pattern [2].

7.1.2 Complex Pattern of Inheritance

In complex disease, there are many risk genes for disease and interactions are intricate including interactions with non-genetic factors such as the environment. The universal concept of multifactoral disease is the common disease/common variants hypothesis [4]. In concordance to this hypothesis single nucleotide polymorphisms in a specific gene or genes will increase the risk of disease onset but when acting alone are insufficient to cause the disease.

7.1.3 Mitochondrial Dysfunction

Mitochondria contribute an important role in the neuropathogenesis of neurological disorders [5]. Dysfunction of mitochondrial proteins expressed

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_7

by either mitochondrial or nuclear genomes has been linked with these diseases [6]. Mitochondria are the foremost cellular source of energy. It produces energy through oxidative phosphorylation and mediates biosynthesis of amino acid, steroid metabolism, fatty acid oxidation, calcium homeostasis, and free radical scavenging [7, 8].

In the subsequent sections, we will delineate the genetic knowledge on common neurodegenerative diseases.

7.2 Huntington Disease (HD)

HD is characterized by degeneration of neurons that reside in basal ganglia and cortical regions of brain, causing chorea, dementia, and psychiatric symptoms [9]. One of the pathological descriptions of HD is the manifestation of nuclear and cytoplasmic inclusions [10]. The expanded CAG repeat in the *HTT* gene, which induces the disease occurrence, encodes a polyglutamine strand of inconsistent length. Research indicates that this expansion leads to a toxic gain of function. However, the particular mechanism by which this is brought about is still under investigation [11].

7.2.1 Genetics of HD

HD is caused by a heterozygous expansion of the (CAG)n (encoding glutamine) trinucleotide repeats in the Huntington gene (*HTT*; 613004) on chromosome 4p16. Normal alleles at this site include up to 35 CAG repeats, but when these repeats reach 41 or more, HD disease is completely penetrant. Incomplete penetrance happens with 36–40 repeats. The numeral of CAG repeats accounts for approximately 60% of the discrepancy in age of onset, with the remnants represented by modifier genes and environment [12].

CAG repeats in excess of 28 repeats shows unsteadiness on replication. With each meiosis repeat size may change; mostly leading to expansion (73%), but narrowing can also take place (23%) [13]. Spermatogenesis shows greater repeat expansion than oogenesis. The larger number of repeats with each generation is the basis of "anticipation", in which the onset of Huntington disease occur earlier in consecutive generations. In addition this accounts for the increased probability of paternal inheritance in children with juvenile onset symptoms. Similarly, *de novo* clinical cases of Huntington disease in families that had no family history, which accounts for the 10–30% of all cases appear to take place due to extension of an allele in the borderline or normal range (28–35 CAG repeats) and is regularly paternal in origin [14, 15].

7.2.2 Mitochondrial Dysfunction in HD

HD pathogenesis includes the mitochondrial dysfunction as observed in patients, and functional studies using mutant transgenic mice and cell culture models [16]. Magnetic resonance imaging spectroscopy has also shown augmented levels of lactate in the cerebral cortex and basal ganglia of brain in HD patients [17]. In addition, phosphocreatine levels are decreased in resting muscle [18], and negative alterations in aconitase, complexes II and III of the respiratory chain have also been delineated in the basal ganglia of postmortem brains of HD patients [19]. Mouse striatal knock-in cells carrying a mutant htt fragment showed both flawed energy metabolism and inhibited mitochondrial ATP levels and ADP uptake [20].

7.3 Alzheimer Disease (AD)

Alzheimer Disease (AD) is one of the foremost causes of dementia in the elderly. Clinically, AD can be categorized into early-onset (patients younger than 65 years) and late-onset (those older than 65 years). AD is recognized by the evidences of amyloid β peptides plaques and intraneuronal tangles of hyperphosphorylated forms of microtubule associated protein tau (MAPT) on pathological examination [21].

7.3.1 Genetics of AD

Earlier only one genetic risk factor *APOE ε4* allele was implicated in AD. However with advances in genetic technologies, such as large-scale genome-wide association studies, risk genes for late-onset AD disease were identified. These genes were instrumental in deciphering new disease-related pathological pathways, such as lipid metabolism, the immune system, and synaptic functioning mechanisms [22].

Early-onset AD families with autosomal dominant patterns of inheritance usually harbor highly penetrant mutations in three genes: *APP, PSEN1*, and *PSEN2* [23, 24]. Mutations in *APP* result in modified amyloid β production, alteration in the ratio of amyloid β42 to amyloid β40, or augmented fibril formation. Mutations in *PSEN1* and *PSEN2* weaken the γ-secretase mediated cleavage of APP, and consequentially result in an increased ratio of amyloid β42 to amyloid β40 [25]. These three genes however account for only 13% of early-onset AD patients [26].

The APOE ε4 allele is a well-known risk factor in both late-onset and early-onset forms of AD [27]. Humans with one ε4 allele have an approximately three-times-more risk of AD, and those with two ε4 alleles have an approximately 15-times-more risk, compared with those with the most common genotype, APOE ε3ε3 [28]. In addition, nine novel risk loci were discovered as a result of European and international genome-wide association collaborations. The nine loci genes were single nucleotide polymorphisms in or near the following genes: *CLU*, *CR1*, *PICALM*, *BIN1MS4A* cluster, *CD2AP*, *CD33*, *EPHA1*, and *ABCA7* [29, 30].

7.3.2 Mitochondrial Dysfunction in AD

AD pathogenesis has been linked with mitochondrial dysfunction, with oxidative damage observed to occur before amyloid β plaque aggregation [31]. Impaired activities of the enzymes of tricarboxylic acid cycle including pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase have been found in postmortem brains of patients with AD [32]. Besides this the reduced respiratory chain complex IV activity has also been found in platelets and in postmortem brain tissue [33, 34].

7.4 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurological disorder recognized with rapid and progressive paralysis and death from respiratory failure, typically within 2–3 years after appearance of symptom [35]. A pathogenic feature of ALS is the specific death of motor neurons in the spinal cord and brain, causing paralysis of voluntary muscles [36]. Roughly 10% of ALS cases are classified as familial, while the remaining 90% of cases are considered as sporadic [35].

7.4.1 Genetics of ALS

Approximately 10% of ALS cases are familial following Mendelian pattern of inheritance and 13 pathogenic genes and loci have been identified [37]. Of the known genes, the foremost is *SOD1*, followed by *TARDBP* and *FUS*. *SOD1* mutations account for 12% of familial ALS and 1% of asporadic ALS [38]. Mutations in *TARDBP* account for 5–10% of familial ALS, while *FUS* mutations are for 5%, and mutations in *ANG* for about 1% [39]. Mutations in *SOD1*, *TARDBP*, *FUS*, *ANG*, and *OPTN* cause a typical clinical phenotype [39]. All genes mutated in familial forms of ALS and have also been seen in sporadic forms with no clinical difference, suggesting a similar pathogenic pathway.

People with ALS characterized as having sporadic disease, possibly have mutations in a single gene which in combination with environmental factors trigger disease onset [40]. For these patients, family aggregation studies have recognized an genetic overlap between ALS and other common neurodegenerative disorders, signifying

the existence of susceptibility genes that might amplify the overall risk of neurodegeneration in families [41]. Some ALS-related genes include *FUS*, *C9ORF72*, *TARDBP,* and *VCP* have been shown to sporadically give rise to other disease phenotypes such as cerebellar ataxia, dementia and Parkinsonism [42]. Despite the identification of candidate gene for sporadic ALS, the underlying disease mechanism is not understood in many instances [40].

7.4.2 Mitochondrial Dysfunction in ALS

A subset of familial ALS cases is outcome of mutations in the gene encoding Cu, Zn-superoxide dismutase (*SOD1*) [43]. Mitochondrial impairment has been involved in SOD1-amyotrophic lateral sclerosis pathogenesis as mitochondrial structural irregularities have been observed in motor neurons from mutant SOD1 transgenic mice [44]. It has also been demonstrated that abnormal mitochondria with vacuolization accumilate in the dendrites before disease onset and preceding motor neuron death [45]. It has been suggested that this vacuolization develops from expansion of the mitochondrial intermembrane space and extension of the outer mitochondrial membrane [46].

7.5 Parkinson Disease (PD)

Parkinson disease (PD) is an progressive neurological disorder with features including resting tremor, bradykinesia (slowed movements), rigidity (increased muscular tone), postural instability, and gait impairment [47]. PD motor manifestations are attributed to dopaminergic neuron loss within the substantia nigra, pars compacta, and basal ganglia which contribute to the initiation and execution of movement [48] PD that begins after the age of 50 years is called late-onset disease. If disease-related symptoms appear before 50 years of age, the condition is called early onset while if the cases begin before the age of 20 it is referred to as juvenile-onset PD [49].

7.5.1 Genetics of PD

Roughly 15% of PD patients have a family history of this disorder. These familial cases of PD are mainly due to mutations in the *LRRK2*, *PARK2*, *PARK7*, *PINK1*, and *SNCA* genes, or by alterations in genes which have not been identified yet [50]. Sporadic PD also involves the mutations in some of the above said genes; however, the pattern of inheritance varied based on the gene mutated. If *LRRK2* or *SNCA* genes are mutated, the disease is inherited in an autosomal dominant manner, while if *PARK2*, *PARK7*, or *PINK1* genes are involved, the disease is inherited in an autosomal recessive manner [50].

Majority of cases of PD occur in a sporadic manner and in many instances the cause remains unclear and may possibly outcome from a multifaceted interaction of environmental and genetic factors. Numerous PD-susceptibility genes have been identified, and the recognized genetic variants cause a wide range of disease risk, including both familial and sporadic disease [51]. A few genes, involving *SNCA* and *LRRK2*, carry rare, vastly penetrant Mendelian alleles in addition to common polymorphisms that have a more modest effect on disease susceptibility [1]. When genetic alterations modify the risk of developing PD, the pattern of inheritance is frequently unknown. Alterations in certain genes, including *GBA* and *UCHL1*, do not cause PD but appear to modify the risk of developing the condition in some families [50].

7.5.2 Mitochondrial Dysfunction in PD

In patients with PD the presence of intracytoplasmic inclusions (Lewy bodies) containing α-synuclein is noted in addition to the pathogenic loss of dopaminergic neurons in the substantia nigra and pars compacta. Despite the lack of a direct connection to mitochondria, there is evidence that mutant α-synuclein may cause mitochondrial dysfunction. It has been found that overexpression of mutant α-synuclein in cultured cells impairs mitochondrial function and leads to oxidative damage [52].

Martin et al. (2006) has demonstrated that transgenic mice overexpressing mutant α-synuclein exhibit neuronal degeneration, depleted complex IV activity in the spinal cord, and aggregation of intraneuronal inclusions. These inclusions consist of both α-synuclein and degenerating mitochondria [53]. Together, these findings present an even more convincing link between mutant α-synuclein, mitochondrial degeneration, and cell death.

Conclusion

In this review, we have summarized some of the aspects of a selected group of neurodegenerative disorders. Many of these diseases remain without effective curative therapies. In inherited neurodegenerative disease with high penetrance such as HD, prenatal testing and subsequent abortion of affected fetuses is a preventive option. However, as an adult-onset disease many parents choose not to test and state hope for future treatment options becoming available [54]. In many diseases, survival of affected persons in the areas where medical technology is not easily accessible is alike to that of populations with ready access to treatments [55]. Thus, underscoring the fact that very little progress has been made in combating these diseases. Genetic counselling may offer hopeful effects on patients, their spouses, and individuals at risk. Caregivers groups are priceless sources of information and insight that may help patients and families through the recurring troubles. Lack of such support could lead to increased disease severity and behavioral and psychological symptoms [56]. Therefore, it is essential that health services and social services work hand in hand to support families with patients affected with these conditions.

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8 Astrocytes and the Synucleinopathies

Andrew O. Koob and Paola Sacchetti

8.1 α-Synuclein

8.1.1 Native α-Syn

The synucleins pertain to a family of highly conserved proteins expressed only in vertebrates and encoded by three distinct genes, α-synuclein (α-syn), β-synuclein, and γ-synuclein. The α-syn gene (SNCA; HGNC 11138) is located on human chromosome 4q21.3-q22 [1, 2] and has also been referred previously as non-A4 component of amyloid precursor or NACP [3], PARK1, PARK4, or PD1.

α-Syn is a 140 amino acid protein that was originally detected in the Pacific electric ray *Torpedo californica* and in rat brain at very high levels compared to other cytosolic protein [4, 5]. Several years later, a cDNA encoding for a 140 amino acid protein purified from Alzheimer's disease amyloid plaques and named non-A4 component of amyloid precursor or NACP [3]. NACP mRNA was detected abundantly in the brain [6] and proven to be identical to human α-syn and highly homologous to rat α-syn [7].

Although detected in the cytoplasm soma and axons, the α-syn protein has been localized predominantly in neuronal presynaptic terminals [4, 8]. The primary structure of α-syn is substantially divided into three main domains: an amphipathic N-terminus (aa 1–60) containing several repeats of 11-mer with a consensus KTKEGV sequence, a core hydrophobic NAC (non-amyloid β component) region (aa 61–95) and an acidic C-terminus (aa 96–140) [9]. The 11-mer repeats present in the amino terminal are reminiscent of the consensus class A2 amphipathic α-helix found in the lipid-binding domains of the exchangeable apolipoproteins. These repeats favor an α-helical secondary structure conformation and are responsible for the interaction with lipid bilayers. This structural feature conveys to α-syn the capacity to bind to neuronal vesicles [10] and is essential for protein tetramerization [11]. The NAC domain and its core region (aa 68–78) fold into β-sheets and are at the core of fibrils formation [12, 13]. Ubiquitinated and phosphorylated α-syn are the main constituents of Lewy bodies, the abnormal protein aggregates typically detected in the synucleinopathies [14]. In fibrillar Lewy bodies, Ser129 is phosphorylated in 90% of the α-syn detected, while only 4% is phosphorylated in this position in normal brains [15–17].

8.1.2 α-Syn and the Synapse

Although the physiological role of α-syn has not been fully clarified, its clustered expression in presynaptic terminals and its capacity to associate

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_8

with lipid membranes suggests an involvement in trafficking of neurotransmitter vesicles and neurotransmitter release [18].

A direct link between α-syn and exocytosis is starting to emerge and centers around the SNARE complex formation. α-Syn favors interaction with small unilamellar vesicles (10–110 nm) similar to small secretory vesicles and large unilamellar vesicles (100 nm–1 μm) that contain unsaturated fatty acids and display high membrane curvatures. Recently, a direct link between SNAREs and α-syn has been recognized as it interacts with v-SNARE synaptobrevin [19] via its C-terminal domain [20] and thus potentially participates in the direct assembly of the SNARE complex. A triple α-syn knockout mouse model showed age-dependent SNARE complex assembly deficit and the deficit could be rescued by overexpression of α-syn in cultured cells derived from these same transgenic animals [20]. Interestingly, only the multimerized α-syn bound to membranes modulates the SNARE complex formation, not the monomeric form [19, 21].

Additionally, α-syn is localized within the vesicular lumen and released from neurons through exocytosis. This occurs independent of endoplasmic reticulum and Golgi apparatus vesicular exocytosis and α-syn release via this mechanism is increased in cases of mitochondrial and proteasomal dysfunction. As α-syn in the vesicle also appears to be more likely to aggregate [22], this mechanism may contribute to α-syn inclusions seen in synucleinopathies. Furthermore, misfolded forms of α-syn due to oxidation under stress conditions were more likely to translocate to vesicles and aggregate before release [23].

8.1.3 α-Syn Oligomers, Toxicity, and Clearance

What is quite clear is that further association of monomeric α-syn molecules generates oligomers of different sizes in a step-wise matter resulting first in protofibrils and then in amyloid fibril aggregates [13]. Oligomeric intermediates appear to be cytotoxic by permeabilizing membranes [24] through membrane association via the N-terminal of α-syn. Interaction with membranes initiates the conformational change into helical form and favors α-syn aggregation into protofibrils that cause neurotransmitter leakage from vesicles and loss of mitochondrial membrane polarization. Interestingly, mutant forms of α-syn generated to produce oligomers and amyloid fibril aggregates demonstrated in vivo that the oligomeric forms are most toxic to dopaminergic neurons in the midbrain [25].

Fibrils have also been linked to cytotoxicity and insoluble fibrils aggregates are the main components of the proteinaceous inclusions featured in all synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DBL), and multiple system atrophy (MSA) [18]. α-Syn can also accumulate in human and rat mitochondria in vitro and in vivo [26, 27] and does so more readily in Parkinson's disease (PD)-related brain regions such as the substantia nigra and the striatum [27]**.** The accumulation of α-syn in mitochondria of the substantia nigra and striatum is particularly enhanced in individuals affected by PD [26] and oxidative stress through mitochondrial dysfunction is thought to play a role in dopaminergic cell loss in disease and familial forms of Parkinsonism [28–31].

Removal of α-syn occurs through the ubiquitin proteasome system [32] after phosphorylation at Ser129 [33], with lysosomal degradation via chaperone-mediated autophagy occurring as α-syn levels increase [32, 34]. Macroautophagy pathways through vacuole trapping of misfolded toxic α-syn and damaged organelles can be stimulated under conditions when chaperone-mediated autophagy is impaired [35]. Overexpression of α-syn will likewise impair macroautophagy pathways [36]. Dysfunction to the autophagy system is thought to be one of the possible causes of PD and synucleinopathies, as lysosomal marker depletion has been one of the pathological findings in human PD [37].

8.1.4 α-Syn and Prion Theory

Recent evidence has indicated that α-syn may be able to spread from cell-to-cell in a prion-like fashion [38], whereby amyloid-fibril forms of α-syn can influence other α-syn proteins to aggregate in proteinaceous inclusions as well [39].

Neurons grafted into patients with Parkinson's disease in order to repair brain degeneration accumulated Lewy Body pathology, demonstrating that endogenous α-syn in these brains can seed cells to cause further degeneration [40]. This occurred in long-term embryonic nigral transplants as well, resulting in degeneration of the introduced cells [41]. Inoculation of healthy mice with pathological tissue containing α-syn aggregates led to the spreading of α-syn aggregation in these mice [42, 43]. This transmission also resulted in characteristic Parkinsonism behavior typical of transgenic mice and loss of dopaminergic neurons [43]. This type of host transmission of detrimental forms of α-syn inclusions was also observed in the rat brain [44].

Although the oligomeric form appears most toxic, it seems that the fibrillized form of α-syn is the most capable to induce other forms of α-syn to fibrillize and create inclusions [45]. Also, gastric administration of fibrillized α-syn in rats was shown to migrate to the CNS through retrograde transport in the vagus nerve [46]. Interestingly, in the human adenine 53 to threonine (A53T) amino acid substitution transgenic mouse model of synucleinopathy, which is responsible for a familial synucleinopathy [47], accelerated prion-like spreading of α-syn was seen to progress through the brain after inoculation with amyloid fibrils from brains of older transgenic mice [42, 44]. Median survival times were half as long than A53T mice inoculated with healthy brain, and also demonstrated more widespread pSer129 α-syn as shown by Western blot and immunohistochemistry (Fig. 8.1). Other models using A53T α-syn demonstrated accelerated α-syn phosphorylation on Ser129 and amyloid fibril aggregates after intravenous and intramuscular inoculation with α-syn [45, 48].

However, another study of the inoculation of the brain with fibrillized α-syn resulted in widespread α-syn fibrils and neurodegeneration only in the A53T transgenic model and not in other α-syn mutant models [49]. Likewise, α-syn derived from human MSA patients induced α-syn aggregation in A53T heterozygous transgenic animals, while α-syn from the brains of PD patients could not [50]. Therefore, it seems that the A53T α-syn form is particularly susceptible to prion-like induced misfolding, phosphorylation, and aggregation [49]. Although encouraging, further studies are worthy to determine whether the A53T mechanism of prion-like spreading occurs with native forms of α-syn in human brains, and if so, what the initial cause of propagation is in idiopathic synucleinopathies.

8.2 Astrocytes and α-Synuclein

8.2.1 Astrogliosis and α-Syn

While the mechanisms of α-syn accumulation in laboratory models have been partially understood, the cause of native α-syn accumulation is unknown. Release of α-syn can occur from neurons through exocytosis, and such release is increased in oxidative stress conditions, often associated with neurodegenerative disorders. Since astrocytes are tasked with maintaining brain homeostasis and synaptic oversight, the role of astrocytes in oxidative stress conditions and in the cause of α-syn associated neurodegeneration is of interest.

Although astrocytes are more understood in terms of their response to neurodegeneration in proteinopathies, evidence is beginning to emerge that astrocytes are also involved in disease initiation and progression [51, 52]. The term astrodegeneration [51] refers to the possibility of degenerative diseases of the central nervous system originating from astrocytic dysfunction. Early evidence on the role of astrocytes in injury and neurodegeneration demonstrated an astrocytic response termed astrocyte reactivity or

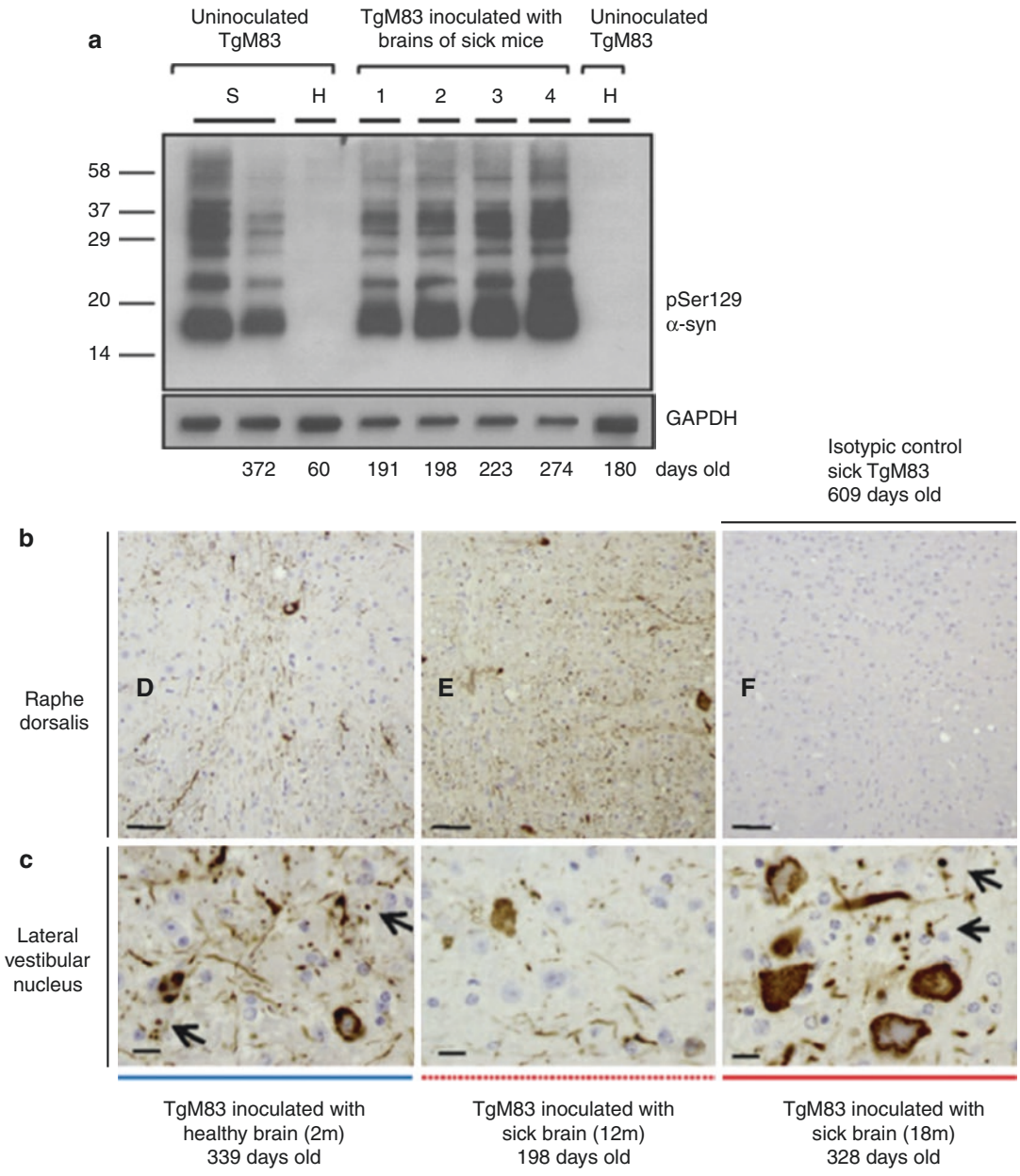

Fig. 8.1 Western blot and immunohistochemical detection of pSer129 α-syn in mice overexpressing human A53T α-syn (TgM83) inoculated with brain homogenates from sick aged TgM83 mice. In (**a**), insoluble pSer129 α-syn Western blot of TgM83 mice inoculated with brains of sick aged (12–18 months) mice (l–4). Days old at time of death is listed at the bottom of the figure. Increased expression of pSer129 was seen in older inoculated TgM83 mice. Uninoculated healthy control TgM83 mice (aged 2–6 months, H) did not exhibit pSer129 expression. Uninoculated sick TgM83 aged mice demonstrated pSer129 expression less robustly at older ages (S). In (**b**), α-syn immunostaining in the raphe nucleus revealed dystrophic neuritis in 198 day TgM83 mouse inoculated with a 12-month sick TgM83 brain compared to a 338–day-old mouse inoculated with a healthy 2-month-old TgM83 brain. Scale bar = 16 μm. Similarly, in (**c**), arrows point to spheroid-like inclusions as well as obvious perikaryal inclusions in TgM83 mice in the lateral vestibular nucleus, with more robust staining in the TgM83 mouse inoculated with a sick 18-month-old TgM83 mouse brain. Scale bar = 64 μm. Reprinted from Mougenot A-L, Nicot S, Bencsik A, Morignat E, Verchere J, Lakhdar L, Legastelois S, Baron T (2012) Prion-like acceleration of a synucleinopathy in a transgenic mouse model. Neurobiol Aging 33:2225–2228, with permission from Elsevier

astrogliosis [53]. Astrogliosis has been observed in response to α-syn in tissue culture and in transgenic mouse models overexpressing α-syn [54–59]. Although astrogliosis was originally thought to be an all-or-none phenomenon in injury and disease, it is now known that astrocytes respond to neurodegeneration by upregulating growth factors, cytokines, chemokines, and anti-oxidant enzymes [60, 61]. A characteristic upregulation of intermediate filaments, glial fibrillary associated protein (GFAP) or vimentin, is typically observed in addition to a hypertrophic morphology [62], and in some cases, proliferation [63].

It appears that the role of astrogliosis is likely neuroprotective to degenerating nervous tissue under normal conditions and occurs along a continuum of injury or disease severity [64, 65].

8.2.2 Astrocytes, α-Syn, Oxidative Stress and Autophagy

A main component of synucleinopathies is neuronal oxidative stress, through increased oxidative damage, mitochondrial dysfunction, and inflammation [66]. Astrocytes treated with oligomeric α-syn increased their rate of reactive oxygen species production, which resulted in lipid peroxidation and cell death [67]. Overall astrocytes are thought to protect neurons from oxidative stress and can promote neuronal survival and neurogenesis though the release of growth factors such as bFGF, BDNF, or GDNF [68].

As α-syn in monomeric and aggregate forms released from neurons are increased in states of oxidative stress, it appears that the astrocytic response induced by α-syn is guided by the Toll-like 4 receptor (TLR4) [69]. Activation of TLR4 by α-syn in astrocytes causes the release of nitric oxide synthetase and cyclooxygenase-2 [70]. Correspondingly, TLR4 KO in astrocyte cultures resulted in decreased reactive oxidative species production and a suppressed response to the c-terminally truncated version of α-syn [69]. However, as $TLR4^{-/-}$ astrocytes are capable of α-syn uptake [70], it seems quite clear that TLR4 does not directly mediate the endocytosis of α-syn in these cells.

Wild type α-syn and its mutated forms induce the release of ICAM-1 and IL-6 from astrocytes via MAP-kinase pathway activation [71]. In fact, mRNA expression profiles of astrocytes in culture treated with α-syn aggregates derived from expression in SH-SY5Y neurons demonstrated increased expression of many other chemokines [72]. Interleukin chemokines (IL-α, IL-β, IL-6, and IL-18) expression was increased within 6 h of treatment, with IL-α and IL-β augmenting 33 and 76 times by 24 h [73]. Colony stimulating factors (CSF-1, CSF-2, and CSF-3) were also increased after neuron-derived α-syn aggregate treatment [73, 74]. Pro-inflammatory cytokines known to be involved in microglial recruitment, cell proliferation, and synaptic regulation were also increased significantly. These included the CC-type (CCL-3, 4, 5, 12, and 20) and the CXC-type (CXCL-1, 2 5, 10, 11, 12, and 16) cytokines [73].

Autophagy is thought to maintain cellular homeostasis and be activated in response to stressed conditions such as oxidative stress. As α-syn is degraded through the lysosomal autophagy pathway, there seems to be a role for astrocytes in the removal of excess α-syn and a cause for dysfunction if such task is disrupted. In lysosomal storage disease, cortical neurodegeneration was caused by astrocyte autophagy dysfunction [75]. Similarly, mitochondrial function in astrocytes is dependent on autophagy pathways, and human primary astrocytes treated with α-syn show increased LDH released as a measurement of mitochondrial dysfunction [56]. Additionally, α-syn colocalization with mitochondria and astrocytes demonstrated reduced cell viability [56]. Interestingly, in the MPTP model of striatal destruction, neurons could be rescued from mitochondrial complex II inhibition if astrocytes overexpressed the cytoprotective transcription factor NF-E2 p45-related factor 2 (Nrf2) which is known to promote antioxidant programs in order to oppose cellular stressing conditions [76].

8.2.3 Astrocytes, the Synapse, and α-Syn

As it is now clear that astrocytes are integral to synaptic communication and synaptogenesis [77–85], and it is known synapse loss correlates with cognitive decline and disease progression in dementias such as Alzheimer's disease and dementia with Lewy bodies [86, 87], α-syn-astrocyte synaptic interactions and mechanisms are of interest. For example, astrocyte cholesterol synthesis, a component in membrane integrity and synaptogenesis [84], was decreased in response to α-syn [55]. Also, cholesterol inhibitors demonstrated reduced α-syn aggregation in mouse models overexpressing α-syn, indicating a possible feedback pathway for α-syn involvement in astrocytic cholesterol regulation [88].

Additionally, it has been shown that cell proliferation factor Wnt1 released from astrocytes is neuroprotective to dopaminergic neurons through the Fzd1/β-catenin pathway [89, 90]. Likewise, recent evidence is emerging on astrocytes involvement in the basal ganglia pathway that degenerates initially in PD. Astrocytes regulate inhibitory GABAergic striatopallidal synapses that are dysregulated by dopamine depletion and exert their effects through release and control of glutamate transmission at synaptic terminals in response to dopamine [91]. In contrast, dopamine transmission alterations affected astrocyte plasticity in the striatum and dopaminergic areas [92].

Astrocytes are much more heterogeneous than previously believed, and two main types have been described, protoplasmic astrocytes in gray matter and fibrous astrocytes in white matter [93]. Release of α-syn from neuronal synapses results in accumulation of α-syn in protoplasmic astrocytes, but not fibrous astrocytes [94]. Protoplasmic astrocytes isolated in tissue culture from human cortex have been shown to accumulate α-syn monomers and dimers, and respond by upregulating GFAP [55] (Fig. 8.2). Additionally, in transgenic mice overexpressing human α-syn, protoplasmic astrocytes in gray matter demonstrated widespread accumulation of α-syn, which was shown to enter the cell through endocytosis and cause cytokine and chemokine release from astrocytes [74]. Inclusions of α-syn in astrocytes have been observed in transgenic mouse models overexpressing neuronal-derived human α-syn and in models of MSA with oligodendroglia-derived α-syn [59, 95].

It is apparent that astrocytes can accumulate the monomeric and oligomeric forms of α-syn and some evidence exists that the fibrillized form will accumulate in astrocytes as well [96]. In vitro studies have demonstrated that α-syn derived from human-derived Lewy bodies is more efficiently taken up through endocytosis in astrocytes compared to neurons, and that further release of α-syn aggregates from astrocytes is toxic to neurons [97]. Recently, it was described that fibrillized α-syn enters into neurons through endocytosis via the LAG-3 receptor. Correspondingly, $LAG3^{-/-}$ mice developed reduced neuronal cell loss [98]. However, no LAG receptor was detected in astrocytes, discarding this mechanism as a possible way of entry. Therefore, like TLR4, this receptor does not appear to be the method of endocytosis of α-syn in astrocytes.

After internalization and accumulation of α-syn, astrocyte functioning is disrupted and synaptic neurotransmission could also be affected. It is known that astrocytes express most transmitter receptors, and upon receptor stimulation they respond with changes in internal Ca^{+} concentration and subsequent transmitter release [99]. Astrocytes communicate at synapses through calcium signaling from their internal organelle calcium stores [100]. Monomeric and oligomeric α-syn was shown to stimulate calcium signaling in astrocytes, effectively contributing to synaptic communication and neurotoxicity through calcium upregulation [101]. Furthermore, the presence of dopamine receptors on astrocytes has been largely supported and a recent study demonstrates the capacity of the neurotransmitter dopamine to directly regulate astrocyte Ca^{+} levels [102].

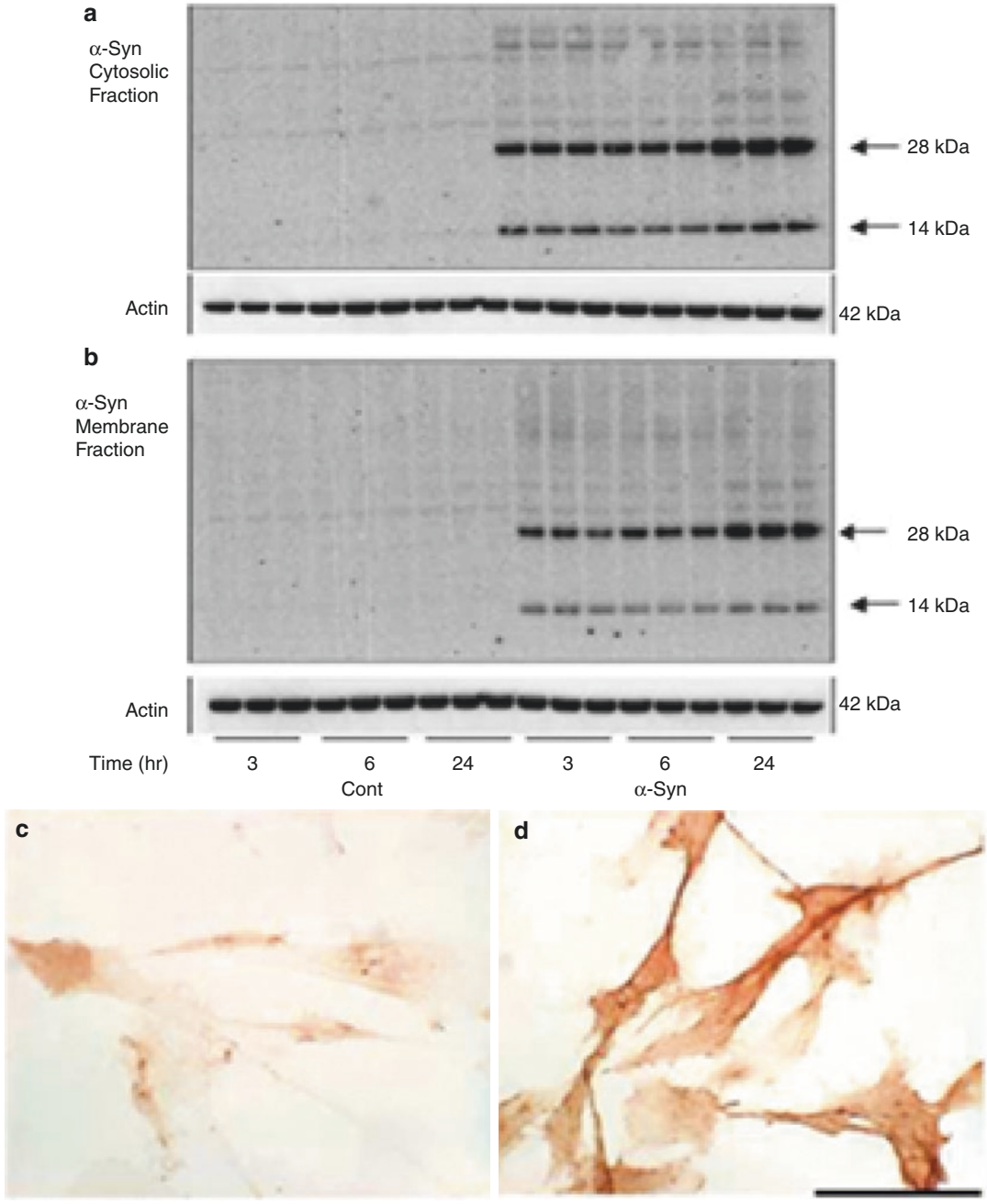

Fig. 8.2 Time course levels of α-syn in human astrocytes treated with α-syn peptide. In (**a**) and (**b**), human primary cortical astrocytes treated with 100 nM of α-syn peptide at 3, 6, and 24 h. Astrocytes accumulated α-syn in monomer and dimer forms in the cytosolic and membrane fractions, which increased at 24 h. Twenty-four hour treatment of human cortical astrocytes with 100 nM α-syn also resulted in increased expression of reactive astrocyte marker glial fibrillary acidic protein (GFAP) in (**d**), compared to control in (**c**). (scale bar—20 μm). Reprinted from Koob AO, Paulino AD, Masliah E (2010) GFAP reactivity, apolipoprotein E redistribution and cholesterol reduction in human astrocytes treated with alpha-synuclein. Neurosci Lett 469:11–14, with permission from Elsevier

8.3 Astrocytes, Familial Synucleinopathies, and Familial Parkinsonism

8.3.1 Astrocytes and Familial Synucleinopathies

Genetic diseases in families have been discovered due to the identification of mutations in the α-syn gene. Synucleinopathy comprised of Lewy body pathology and Parkinsonism occurs with gene duplication and several point mutations in the gene resulting in amino acid substitutions A53T, E46K, A30P, and H50Q, with a late-onset mutant form G51D [103]. Although the familial disease resulting from gene duplication is likely the result of α-syn overproduction in these families as demonstrated by widespread synucleinopathy, the various point mutations cause different types of aggregation in vitro [104]. The A53T, A30P, and E46K mutant forms of α-syn showed an increased rate of fibril amplification and lipid-induced aggregation compared to wild type α-syn, while it was discovered that H50Q and G51D developed at slower rates [104].

Perhaps the most studied mutant is the A53T form of α-syn which has been shown to induce prion-like spread of α-syn. The onset of illness of the A53T mutation is 46 ± 13 years of age and results from a single gene defect [47]. In families affected, a single base pair change occurs from guanine to adenine in position 209 in the fourth exon [47]. The resulting change in the α-syn protein is the amino acid substitution of an alanine to threonine at position 53 (A53T) [47]. This leads to α-syn misfolding by converting segments of the protein from an α-helical tetramers to monomers with a β-sheet shape, with oligomeric accumulation and resulting neurodegeneration [105].

When A53T α-syn was selectively expressed by astrocytes in mice, a degenerative disease was prevalent [106]. Astrocyte toxicity induced by α-syn was shown to be crucial for healthy brain function, as mice developed midbrain dopaminergic neuronal degeneration and motor spinal cord neuronal loss (Fig. 8.3). Likewise, microglial activation was increased, and astrocytosis was seen throughout the brain as demonstrated by increased GFAP immunohistochemistry [106]. Also, aquaporin-4 expression was no longer associated with astrocytic end-feet and instead exhibited a somatic and proximal processes location, indicating compromised blood–brain barrier in these mice [106].

In addition, direct transfer of α-syn A53T to astrocytes resulted in release of TNF-α and pro-inflammatory chemokine CXCL-1 [74]. Interestingly, it appears that overexpression of Nrf2 in astrocytes can rescue neurons from degeneration caused by A53T [107].

The other α-syn variant studied with respect to astrocytes is the A30P mutant, which was shown to decrease neurogenesis in the hippocampus of mice upon overexpression [108]. Neuronal-restricted overexpressed α-syn A30P was detected in neighboring astrocytes in the hippocampus, suggesting uptake of the protein variant by astrocytes as well, and a potential involvement of the astrocytes in neuronal dysfunction [108]. In addition, A30P as A53T and E46K treated astrocytes increased release of ICAM-1 and IL-6 through the MAP-kinase pathway [71, 108].

8.3.2 Astrocytes and PINK1

Homologous mutations on chromosome 1 to the PTEN-induced putative kinase 1 (PINK1) gene (PARK6) result in early-onset Parkinsonism between 32 and 48 years of age. The mutation causes a truncated form of the protein and a G309D amino acid substitution in the kinase domain [109]. Mutations in PINK1 result in Lewy Body pathology in older patients, levodopa responsive Parkinsonism, impaired gate and psychiatric symptoms [110].

PINK1 has been shown to locate to the mitochondria and associate with the E3 ubiquitin ligase protein parkin to target mitochondria for degradation [111]. The PINK1 N-terminus associates with the outer mitochondrial membrane of damaged mitochondria while the kinase region phosphorylates parkin, inducing its recruitment

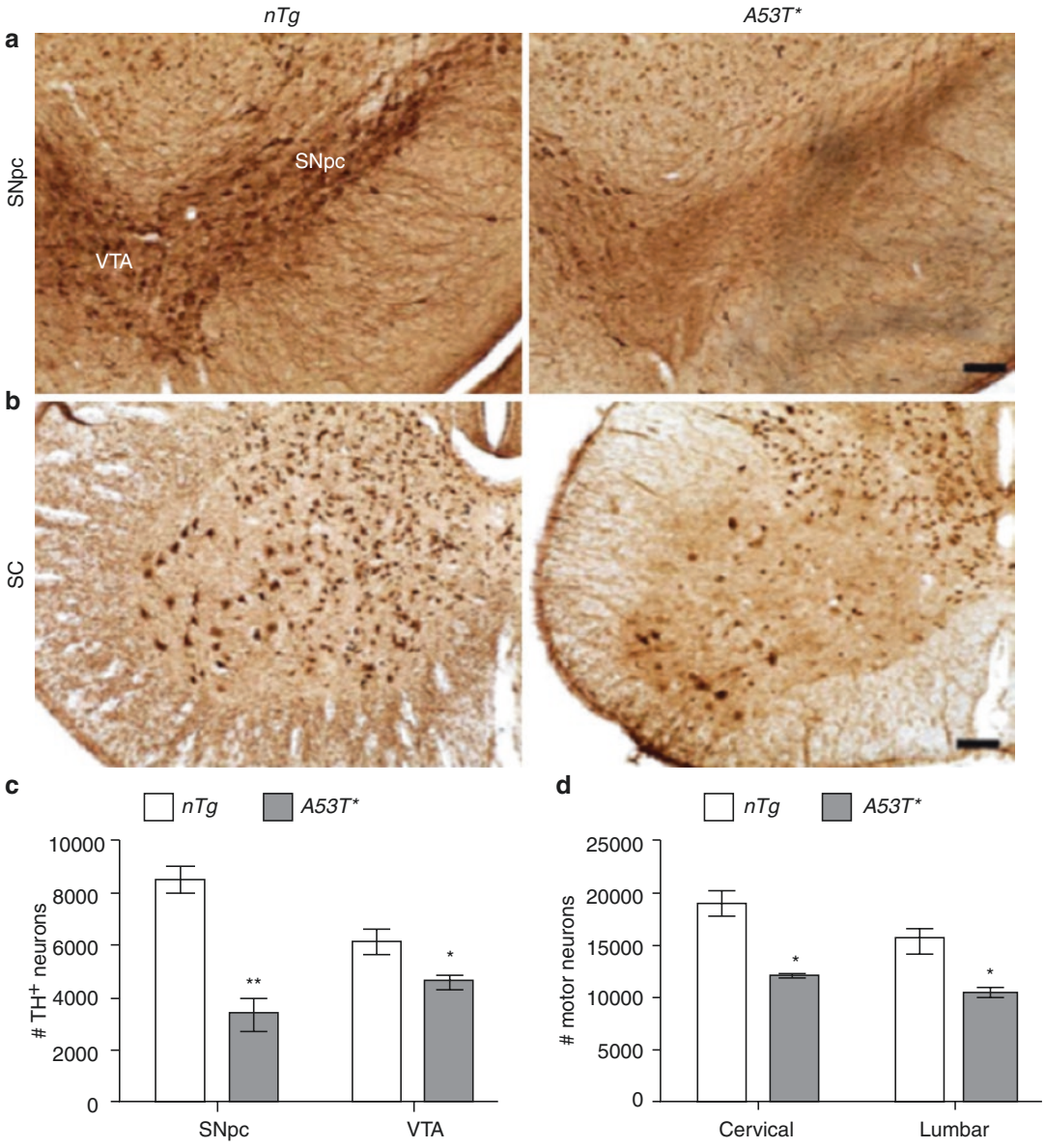

Fig. 8.3 Neuron loss occurred in the midbrain and the spinal cord of symptomatic mice with α-syn A53T expression in astrocytes. (**a**) Overexpression of α-syn A53T in astrocytes resulted in neuronal cell loss compared to age-matched nTg littermates as demonstrated by tyrosine hydroxylase(TH)/NeuN double staining in the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA). NeuN staining in the ventral horn of the lumbar spinal cord in (**b**) also indicated neuronal loss. Scale bars = 100 μm. (**c**) Stereology of TH staining in SNpc and VTA demonstrated significant neuronal loss. $*p < 0.05$, $**p < 0.01$. In (**d**), loss of motor neurons in the spinal cord was also significant in astrocyte expressing A53T mice $*p < 0.05$. Reprinted from Gu XL, Long CX, Sun L, Xie C, Lin X, Cai H (2010) Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. Mol Brain 3:12, Copyright © Gu et al.; licensee BioMed Central Ltd. 2010. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY)

to the dysfunctional mitochondria to target them for autophagy degradation [111].

PINK1 is constitutively expressed by astrocytes, and mitochondrial function in astrocytes is essential for responses to oxidative stress and regulation of brain homeostasis [112]. It was shown that PINK1 expression increased in mice around embryonic day 12 to postnatal day 1 in developing mouse brains [113]. In a related study, astrocytes isolated in culture from PINK1 KO mice displayed defective proliferation [112]. In a scratch wound healing assay, astrocytes also displayed deficient proliferation to "repair" the area compared to cells isolated from WT mice. In this study, it appears that astrocyte mitochondrial function and glucose uptake were impaired which resulted in the proliferative dysfunction [112].

In addition, PINK1 KO mice exhibited downregulated GFAP expression, which was also observed in the midbrain and cortex of adult mice [113]. GFAP associated astrogenesis was affected from development into adulthood, which might factor in Parkinsonism development [113] (Fig. 8.4).

8.3.3 Astrocytes and Parkin

A deletion in the PARK2 gene on chromosome 6 results in early-onset Parkinsonism in patients less than 40 years of age [114, 115]. This familial disease is referred to as autosomal recessive juvenile Parkinsonism and is marked by dysfunction of the parkin protein, a ubiquitin ligase that associates with Lewy bodies in the synucleinopathies [116], and with similar clinical symptoms to diseases related to the PINK1 gene [111]. The disease manifests itself in younger patients and is typically considered a non-Lewy body disease as well, but Lewy body pathology has been observed in aged patients that have the mutation and develop the disease [117].

Parkin is associated with PINK1 in mitochondrial function and degradation. Similarly to PINK1 KO, midbrain astrocytes from parkin KO mice demonstrated less proliferation and more proapoptotic protein expression [118]. Parkin is also expressed by astrocytes and associates with the nucleus, light vesicles, and the Golgi apparatus [119]. Interestingly, increased expression of parkin was noted in response to

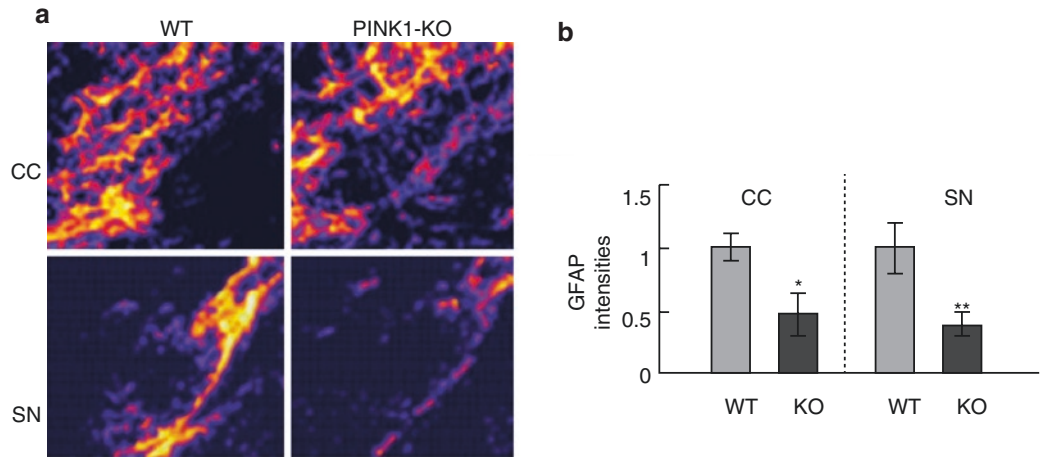

Fig. 8.4 PINK1 deficiency alters GFAP expression in the cortex and midbrain of 8-day-old mice. Pink1-KO mice showed diminished GFAP expression in astrocytes compared to wild type as denoted by GFAP immunohistochemistry in the corpus callosum and substantia nigra in (**a**), and Image J analysis of staining fluorescent intensity in (**b**). Reprinted from Choi I, Choi D-J, Yang H, et al. (2016) PINK1 expression increases during brain development and stem cell differentiation, and affects the development of GFAP-positive astrocytes. Mol Brain 9:5 Copyright © Choi et al.; licensee BioMed Central Ltd. 2010. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY)

unfolded protein stress in astrocytes, and not in neurons [119]. Also, parkin was redistributed in astrocytes as a result of treatment with unfolded protein stress inducers contributing potentially to dysfunction due to non-cleared accumulated proteins [119]. Parkin KO mice-derived astrocytes also exhibited decreased antioxidant glutathione release in response to oxidative stress and were susceptible to oxidative stress [118].

8.3.4 Astrocytes and DJ-1

Deletions in the PARK7 gene result in dysfunction of the DJ-1 protein in families presenting with early-onset Parkinsonism [120]. Patients with this genetic profile do not tend to accumulate widespread synucleinopathy. Recently, however, a novel L172Q PARK7 mutant demonstrated diffuse Lewy bodies and glial pathology [121]. DJ-1 has been hypothesized to protect neurons from oxidative stress, and an increase in DJ-1 is seen in reactive astrocytes, with astrocytes expressing DJ-1 more abundantly in normal as well as synucleinopathy-affected human brain regions than neurons [122].

DJ-1 overexpression in astrocytes protects neurons from pesticide-induced oxidative stress [123]. It appears that this is likely due to interference with complex 1 of the mitochondria [124]. When astrocytes were co-cultured with neurons, impaired mitochondria were located in the soma and proper fusion dynamics was observed in the processes. To the contrary, knockdown of DJ-1 expression in astrocytes resulted in impaired mitochondrial function as noticed by reduced mitochondria fusion in astrocytic processes [125].

DJ-1 deficiency in astrocytes interestingly impaired expression of flotillin-1 and caveolin-1, the two main protein constituents in lipid rafts [126, 127]. This resulted in impaired lipid raft-mediated endocytosis, as well as disruption to membrane fluidity and cholesterol levels [127]. It has been previously shown that α-syn can decrease cholesterol levels in astrocytes [55] indicating a possible mechanism where α-syn and DJ-1 cooperate in astrocytic regulation of synaptogenesis. Also, glutamate reuptake through EAAT1 transporter was impaired in DJ-1 deficient astrocytes, indicating involvement in synaptic communication [127].

Astrocytes are known to protect dopaminergic neurons from 6-hydroxydopamine, and DJ-1 deficient astrocytes demonstrated an impaired ability to protect neurons in mouse models of 6-hydroxydopmaine toxicity [128]. Additionally, pro-inflammatory mediators were decreased in DJ-1 KO astrocytes, with TNF-α and prostaglandin E2 significantly diminished [36].

8.3.5 Astrocytes and LRRK2

Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) result in autosomal dominant late-onset Parkinsonism [129, 130]. LRRK2 is expressed in astrocytes in the brain, including in the substantia nigra, hippocampus, and striatum [131]. LRRK2 is involved in a number of processes linked to α-syn, including vesicular recycling, mitochondrial homeostasis, and macroautophagy [132].

Inhibition of LRRK2 in astrocytes induced autophagy as demonstrated through increased levels of LC3-II in rat primary astrocytes in vitro [133]. Also, expression of the common Parkinsonism LRRK2 mutant G2019S caused enlarged lysosome morphology in mouse primary astrocytes [134]. Additionally, long-lived proteins were not as likely to be degraded through the lysosomal pathway in mouse primary astrocytes overexpressing G2019S mutant LRRK2, which seem to be dependent on LRRK2 kinase activity and autophosphorylation [134].

8.4 Astrocytes and Idiopathic Synucleinopathies

8.4.1 Parkinson's Disease

In idiopathic neurodegeneration, synucleinopathy typically correlates with Alzheimer's disease-type pathology characterized by beta-amyloid plaques

and neurofibrillary tangles expressing hyperphosphorylated tau [135, 136]. Additionally, in normal controls, without the development of neurodegenerative disease symptoms, incidental Lewy Body disease, or synucleinopathy can be present in postmortem tissue [137]. Although accumulation of amyloid-β_{40} and amyloid-β_{42} in plaque formation results in astrogliosis near the site of the plaque, with early studies demonstrating astrogliosis four times greater in cortical areas of patients diagnosed with AD [138–140], in Parkinson's disease (PD) astrogliosis does not seem to correlate with α-syn aggregation [94, 141, 142]. Astrogliosis is also a hallmark of an astrocyte proximal to neurofibrillary tangles expressing hyperphosphorylated tau [143], and in frontal temporal dementia, noted by extensive tauopathy, astrocyte apoptosis, and not neuronal apoptosis, was associated with disease initiation and progression [144]. However, recent studies have demonstrated that astrogliosis is only increased in the frontal lobe in PD, and that it is actually decreased in substantia nigra compared to normal controls [141], despite α-syn itself stimulating astrogliosis in transgenic mice and in vitro, indicating astrocytic dysfunction and inability to respond to α-syn early in disease. [55–58, 95].

Gliosis in the substantia nigra that was previously described in PD was likely microglia in nature [145], and α-syn-induced release of cytokines and chemokines from astrocytes in the midbrain in disease was shown to induce microglial proliferation [74]. However, astrocytes can accumulate α-syn in human synucleinopathies, and in PD and Parkinson's disease dementia, it was demonstrated that astrocytes immunopositive for α-syn correlated with disease progression, without influence of β-amyloid plaques and tauopathy [146, 147].

The role of astrocytes in midbrain degeneration in PD appears to be related to the regulation of oxidative stress, and downregulated astrogliosis may not be able to effectively protect susceptible dopaminergic neurons from these processes. Cytokines shown to be induced by α-syn treatment of astrocytes are increased in the CSF and brain of PD patients, including TNFα, IL-1β, IL-2, IL-4, IL-6, TGFα, TGFβ1, and TGFβ2 [148]. Astrocytes also show increased expression of myeloperoxidase in PD [149], an enzyme related to oxidative stress during inflammation as well as release of brain-derived neurotrophic factor (BDNF) [150] and glutathione peroxidase [151], all neuroprotective factors. Regarding mitochondrial dysfunction in PD, it was shown that decreased Dlp-1, which promotes mitochondrial fission, was observed in astrocytes, and was an early event in disease progression [152].

8.4.2 Dementia with Lewy Bodies

In contrast to Parkinson's disease dementia (PDD), where a progression to cognitive deficits occurs after initial Parkinsonism due to midbrain degeneration, in Dementia with Lewy Bodies (DLB), widespread cortical α-syn aggregation is observed pathologically, with cognitive deficits independent of Parkinsonism [153]. DLB is the second leading cause of dementia, and pathologically representative of 10–15% of patients [153]. Like in PD, α-syn in Lewy body aggregates only accounts for 10% of the pathological formation, and most α-syn is localized to synaptic aggregations and neurites [154] (Fig. 8.5). α-Syn accumulation in postmortem brains was shown in the brainstem, basal ganglia, and cerebral cortices in DLB [155].

In patients with DLB, a significant increase of GFAP was observed in the cerebral spinal fluid [156], indicating possible astrodegeneration or astrocytosis, something that was not observed in MSA or PD [157]. In addition, IL-1 α, TNF- α, and iNOS were all increased in the amygdala, hippocampus, entorhinal, and insular cortices of the brains of patients with DLB [158]. Further analysis revealed that astrocytic processes that were positive for TNF-α and iNOS colocalized with extracellular Lewy Body formation [158].

In patients diagnosed with PD and DLB, neocortical aquaporin 1 and aquaporin 4 were increased compared to subcortical regions, and α-syn was diminished in areas of astrocytes with high aquaporin expression, indicating possible removal through the glymphatic pathway

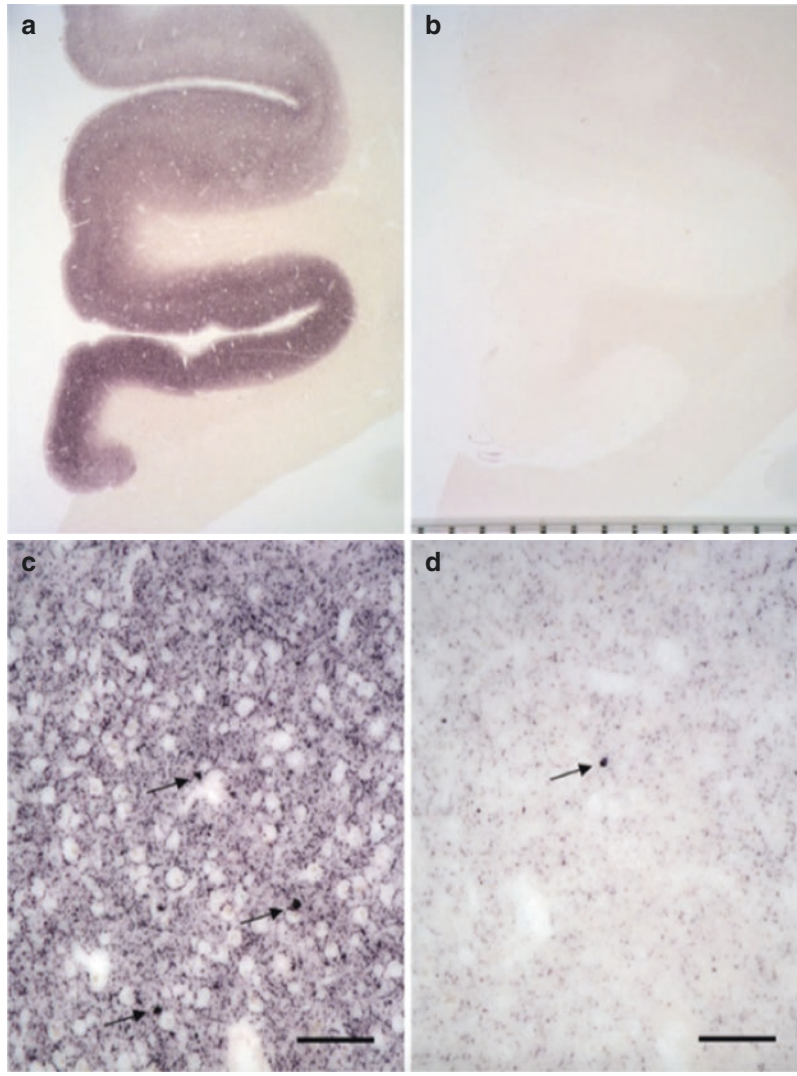

Fig. 8.5 α-Syn aggregates are predominant localized at synapses. In the frontal cortex of DLB patients, more than 90% of α-syn aggregates are located at synapses, and not in Lewy bodies. (**a**) Low magnification of DLB patient frontal cortex α-syn compared with control patient (**b**). Higher magnification indicates α-syn aggregates are mostly not Lewy bodies (arrows) in (**c**): antibody-mAB 4B12, 1:10,000. Using pSer129 1:5000 (**d**), only a fraction of aggregates is detectable demonstrating the sensitivity differences between antibodies and fixation (scale bar = 100 μm). Reprinted from Schulz-Schaeffer WJ (2010). The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. Acta Neuropathol 120:131–143, with permission from Springer

[159]. In the glymphatic system, astrocytes remove waste products from the parenchyma via aquaporin channels located on astrocytic end-feet associated with the vasculature [160]. In patients with PDD and DLB, serum levels of α-syn were increased compared to normal controls [161]. Likewise, in aging brains, disruption to the glymphatic pathway has been observed, demonstrating a possible role of this pathway in the synucleinopathies [160]. In fact, α-syn has been shown to be elevated in the cerebrospinal fluid in Parkinson's disease,

Alzheimer's disease, and Creutzfeldt-Jakob disease [162].

These results are interesting as astrocyte control of vasculature definition and maintenance is well defined [163]. Perivascular astrocytes comprise the integrity of the vascular system [82] and 84% of patients with Alzheimer's disease (AD) have been shown to also have cerebral vascular disease noted by vascular degeneration [164]. Additionally, vascular dementia has been shown to occur in PD with more pronounced degeneration of the vasculature in the substantia nigra, middle frontal cortex and brainstem nuclei [165].

8.4.3 Multiple System Atrophy

In contrast to Lewy Body formation and synaptic α-syn aggregates, multiple system atrophy (MSA) is characterized by increased oligodendroglial inclusions of α-syn and widespread astrogliosis [57, 166] (Fig. 8.6). Astrocyte reactivity, as characterized by GFAP immunopositive astrocytes, increases in cases of MSA in relation to disease progression and coincides with glial cytoplasmic inclusions (GCIs) in oligodendrocytes [167]. GCIs are seen throughout the brains of patients with MSA, but more abundantly in midbrain, brainstem, and spinal cord structures.

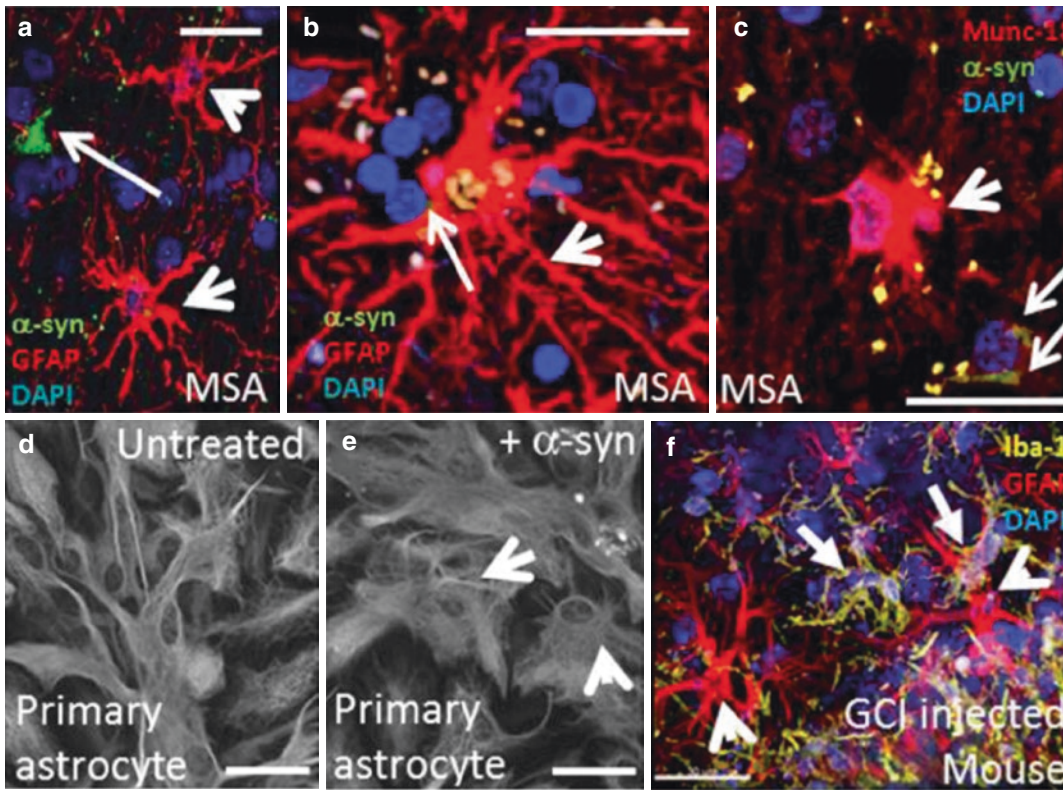

Fig. 8.6 Multiple system atrophy is characterized by widespread oligodendroglial α-syn inclusion bodies, astrogliosis and microgliosis. In (**a**) (putamen) and (**b**) (visual), reactive astrocytes denoted by GFAP immunohistochemistry are in close proximity to α-syn GCIs in MSA. A subset of astrocytes colocalizes with exocytotic vesicular marker munc18 (red) and α-syn (green), ((**c**)—scale bar = 20 μm). In (**d**) and (**e**), rat primary astrocytes treated with α-syn peptide exhibit reactive astrogliotic morphology as demonstrated by hypertrophy (scale bar = 20 μm). Activated microglia (Iba-1) and astrocytes (GFAP) are prevalent near GCI injection of unilateral-lesioned mice in (**f**) (scale bar = 30 μm). Reprinted from Vieira BDM, Radford RA, Chung RS, Guillemin GJ, Pountney DL (2015). Neuroinflammation in Multiple System Atrophy: Response to and Cause of alpha-Synuclein Aggregation. Front Cell Neurosci 9:43 Copyright © 2015 Vieira, Radford, Chung, Guillemin and Pountney. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY)

As MSA coincides with abundant astrogliosis, factors that are associated with astrogliosis are released by astrocytes. For example, the neurotrophic factor midkine, which is more abundantly expressed by astrocytes undergoing astrogliosis [168] is found associated with GCIs and abundantly expressed in MSA brains compared to controls [169]. Also, BDNF increased expression in astrocytes is seen in MSA brains compared to normal controls in the basal ganglia [170]. Similarly, stress-induced molecular chaperone heat shock protein 70 (Hsp70) was upregulated in astrocytes and GCIs in MSA brains [171].

In multiple system atrophy, phosphorylated α-syn aggregates were observed in astrocytes in the subpial and paraventricular regions and increased in later disease stages [172]. Also, extracellular matrix modeling proteins metalloproteinase 2 and 3 were shown to be increased in astrocytes in the striatum in MSA, with metalloproteinase 2 only in GCIs [173]. Using a Giesma stain, increased astrocyte numbers in the caudate and putamen of patients with MSA was observed indicating astrocytic proliferation [174].

8.5 Discussion

Astrocytes respond to and accumulate α-syn, which is increased in the synucleinopathies, and astrocytes are involved in protein degradation and clearance of waste products from the parenchyma through the glymphatic pathway. As an abundantly expressed protein, α-syn could begin to aberrantly aggregate as a by-product of initial astrocyte dysfunction. Therefore, transition of α-syn to phosphorylated amyloid fibrils, may propagate in a prion-like manner as a result from initial astrodegeneration.

Familial diseases that result in Parkinsonism reveal that α-syn pathology is only common and more widespread in aged patients with the disease, with many of the genetic disorders causing mutation in proteins constitutively expressed in astrocytes. This may indicate that initial astrocyte dysfunction can cause disease symptoms, but that synucleinopathy occurs afterwards. Also, in genetic mutations of the α-syn gene, the cause of accumulation may be due to an astrocytic inability to process or respond to the mutated form naturally, which likely could cause α-syn overload and fibrillization.

In idiopathic synucleinopathies, α-syn accumulation appears in aged patients and can occur in patients that don't present with Parkinsonism or dementia, indicating that α-syn itself may not be the cause of disease symptoms. In both familial and idiopathic diseases, oxidative stress is a possible cause of dopaminergic cell loss in the midbrain in Parkinson's disease. The astrocytic ability to protect neurons from oxidative stress, also points to an initial astrocytic dysfunction causing neurodegeneration and α-syn aggregation. Likewise, in disease, mitochondrial function is disrupted in astrocytes as well as neurons, and α-syn oligomer accumulation may develop in the initial disease progression due to mitochondrial dysfunction in astrocytes.

Curiously, astrogliotic responses stimulated in vitro by the α-syn protein seem to be diminished in DLB and PD as revealed by GFAP immunostaining, but dramatically increased in another synucleinopathy, MSA. The different cellular and pathological accumulation of α-syn between these diseases results in different astrocyte morphology, raising additional questions regarding non-neuronal cellular dysfunction at the onset of disease, with α-syn accumulation possibly as a by-product instead of a cause. Likewise, in the Lewy body diseases, PD and DLB, a lack of astrogliosis may indicate astrodegeneration.

Lastly, synapse loss correlates with cognitive decline in dementias, and astrocytic-α-syn interaction may be integral to normal synaptic function. Since 90% of α-syn aggregates occur at the synapse and astrocytes are responsible for synaptic oversight, more evidence on the effect of astrocytic dysfunction on synaptic mechanisms could reveal the cause of the symptoms and pathology that result in the synucleinopathies.

Acknowledgements The authors would like to thank the Biology Department and Neuroscience Program at the University of Hartford for their support. Also, special thanks to Irene Luccia Pearl for help with manuscript preparation.

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9 The Diagnosis of Parkinson's Disease: Current Clinical Practice and Future Trends

Roberto López Blanco and Álvaro Sánchez Ferro

9.1 The Clinical Diagnosis of Parkinson's Disease

9.1.1 Overview

Parkinson's disease (PD) has been traditionally characterized by the progressive occurrence of motor signs. These cardinal motor features are represented by bradykinesia (i.e., slowness manifested by either difficulties in the initiation of movement or by a decrease in the movement amplitude), rest tremor, and rigidity. At more advanced stages of PD, it is common to exhibit postural and gait impairments. The cardinal motor features are still the foundation for PD diagnosis. Based on these features, consensus criteria for clinical diagnosis have been proposed (Table 9.1), and diverse disease phenotypes have been defined (i.e., tremor-dominant, akinetic-rigid, and parkinsonism with postural instability and gait disorder phenotypes) [1, 2].

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However, the motor features are commonly accompanied, or sometimes even preceded, by non-motor symptoms (e.g., olfactory dysfunction, constipation, rapid eye movement behavior disorder, depression, apathy or mild cognitive impairment) [3, 4]. Non-motor symptoms are frequently underdiagnosed and might be as, or even more, disabling than the motor ones [5, 6]. The non-motor symptoms are gaining importance in the diagnosis of PD, as they have recently been proposed to be part of the new clinical criteria [7] and they help define additional disease phenotypes (i.e., a mainly motor form with slow progression, an intermediate phenotype, and a diffuse or malignant form) [8].

9.1.2 Current Diagnostic Criteria for Parkinson's Disease

As stated, the diagnosis of Parkinson's disease is essentially clinical [9, 10]. The prevailing criteria are the Queen's Square UK Parkinson's Disease Society Brain Bank criteria [11, 12]. Hughes et al. published these prospectively proven criteria in 1992. The accuracy of this method ranges from 76 to 90% of patients correctly diagnosed by an expert when compared with the neuropathological "gold-standard" [12–14]. The different steps when applying these criteria are detailed in Table 9.1.

S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_9

Table 9.1 UK Parkinson's disease society brain bank clinical diagnostic criteria [11, 12]

Step 1:	*Diagnosis of parkinsonian syndrome*
	Bradykinesia (i.e., slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions) plus one or more of the following features: • Muscular rigidity • 4–6 Hz rest tremor • Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction
Step 2:	*Exclusion criteria for Parkinson's disease*
	One or more of the following features suggest an alternate diagnosis: • History of repeated strokes with stepwise progression of parkinsonian features • History of repeated head injury • History of definite encephalitis • Neuroleptic treatment at onset of symptoms • 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure • Negative response to large doses of levodopa (when malabsorption is excluded) • More than one affected relative • Sustained remission • Strictly unilateral features after 3 years • Early severe autonomic involvement • Early severe dementia with disturbances of memory, language, and praxis • Oculogyric crises • Supranuclear gaze palsy • Babinski sign • Cerebellar signs • Presence of a cerebral tumor or communicating hydrocephalus on CT scan or MRI
Step 3:	*Supportive prospective positive criteria for Parkinson's disease*
	Three or more of the following features are required for diagnosis of definite Parkinson's disease: • Unilateral onset • Rest tremor present • Progressive disorder • Persistent asymmetry affecting the side of onset most • Excellent response (70–100%) to levodopa • Severe levodopa-induced chorea • Levodopa response for 5 years or more • Clinical course of 10 years or more

Recently, the Movement Disorders Society has proposed a new set of criteria [7]. Their goal is to improve some of the limitations identified for the United Kingdom Brain Bank criteria and adapt the diagnosis to the recent understanding of the disease, where non-motor symptoms and functional neuroimaging are progressively gaining importance. The performance of these criteria has to be validated and confirmed in upcoming prospective studies.

9.1.3 Differential Diagnosis

The main conditions from which Parkinson's disease has to be differentiated are summarized in Table 9.2. They are represented mainly by essential tremor and atypical and secondary parkinsonisms. One of the most important elements for this differential diagnosis is the initial and continued assessment of the patient over the course of the disease. This allows for identifying alarm signs that suggest these alternative conditions [7, 9]. Ancillary tests can help support or discard alternative diagnoses, whereas acute drug challenges are not usually recommended [9, 15]. Nonetheless, in selected cases, the absence of a chronic levodopa response up to 1 g per day after 1 month might suggest an atypical parkinsonism [10].

9.1.3.1 Essential Tremor

Essential tremor represents the main alternative diagnosis to PD due to its high frequency and occasional overlap with disease-related manifestations (e.g., tremor) [16]. The principal differential features are a positional/kinetic tremor in the upper limbs, a compatible family history, a tremor response to alcohol intake, and the absence of parkinsonian signs.

9.1.3.2 Atypical Parkinsonisms

Initially, PD can be identical to these entities. It is only through the existence of accompanying features over the course of the disease evolution when atypical parkinsonisms can be suspected. The main conditions are:

Table 9.2 Main conditions in the differential diagnosis of Parkinson's disease

Atypical parkinsonisms	Secondary parkinsonisms		Tremor and other movement disorders
Multiple system atrophy Progressive supranuclear palsy Corticobasal degeneration Lewy body disease Alzheimer disease	Drugs (antipsychotics, anti-emetics, flunarizine, cinnarizine, valproic acid, chemotherapeutics) Toxics (Mn, methanol, CO, carbon disulfide, CN, organophosphates, MPTP) Vascular Hematoma Post-hypoxia Posttraumatic Hydrocephalus Tumors Post-infectious abscess Prions (CJD,GSS)	Metabolic disorders (Wilson, Fahr, extrapontine myelinolysis, chronic liver and parathyroid disease, mitochondrial disease, Niemann pick-type C, Gaucher disease) HD (Westphal variant) Dystonia-Parkinsonism Spinocerebellar atrophy FTD-parkinsonism Cr.17 NBIA (i.e., PKAN) Neuroacanthocytosis	Essential tremor Drug induced-tremor Dystonia Holmes' tremor Psychogenic tremor

Mn manganese, *CO* carbon monoxide, *CN* cyanide, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *CJD* Creutzfeldt-Jakob disease, *GSS* Gerstmann-Sträussler-Scheinker, *HD* Huntington's disease, *FTD* frontotemporal dementia, *NBIA* Neurodegeneration with Brain Iron Accumulation, *PKAN* Pantothenate-Kinase-Associated Neurodegeneration

- *Multisystem Atrophy (MSA)* can initially be indistinguishable from PD in what has been known as the MSA-P variant [17]. Early dysautonomia (orthostatic hypotension, urinary incontinence), postural instability, the lack of response to levodopa, subtle cerebellar features, irregular postural and action tremor with superimposed jerks, and the presence of orofacial dyskinesia after dopaminergic treatment are all indicative of an MSA diagnosis [18].
- *Progressive Supranuclear Palsy* is differentiated from PD by a progressive bilateral parkinsonism usually from the disease-start, by early postural instability, and by an oculomotor impairment, especially with vertical ocular saccades. Frontalis muscle dystonia and apraxia of eyelid opening with or without early fronto-executive impairment can also be part of this syndrome.
- *Corticobasal Degeneration* typically presents a unilateral parkinsonism with upper limb dystonia, ideomotor apraxia, or even alien limb phenomena. Sometimes, it associates with focal myoclonus of the affected extremity. A fronto-executive cognitive impairment can also be an early feature.
- *Dementia with Lewy Bodies (DLB)* is considered to have a very similar basis as PD, and both are classified as Lewy-body disorders. Within this spectrum, it is difficult to differentiate Parkinson's disease cases from dementia with DLB cases. This difficulty is further amplified by the common coexistence of comorbid conditions, i.e., Alzheimer's disease, that can also influence the presence of cognitive impairment [19]. This diagnostic challenge is controversial and some experts even consider DLB to be part of the PD spectrum [20, 21]. Nonetheless, in DLB, dementia occurs within the first year of motor sign onset, and it is characterized by a prominent executive, visuospatial and attentional impairment. Additional core features of this condition are recurrent visual hallucinations and fluctuating performance in cognitive domains. Rapid eye movement sleep behavior disorder and severe sensitivity to antipsychotics are also clues to its diagnosis.

9.1.3.3 Secondary Parkinsonisms

These parkinsonisms are related to known structural, metabolic, drug-induced or toxic etiologies (Table 9.2). A special emphasis should be made in identifying a drug-induced parkinsonism because of its prognostic and therapeutic implications. This condition is often associated with the chronic use of neuroleptics or even more "conventional" drugs used to treat flatulence, nausea, or vertigo. A symmetrical parkinsonism affecting mainly the

superior half of the body is the typical picture of a drug-induced parkinsonism, and 90% of the affected subjects will improve after ceasing the responsible agent [22]. Vascular parkinsonism, on the other hand, is characterized by a lower limb parkinsonism. This entity is currently under review [23]. Finally, in chronic adult hydrocephalus (formerly known as normal-pressure hydrocephalus) parkinsonism can be accompanied by the triad of cognitive impairment, gait disturbances, and urinary incontinence [24].

9.2 Ancillary Tests and Clinical Scales in Parkinson's Disease

9.2.1 Neuroimaging in Parkinson's Disease

Structural neuroimaging [i.e., computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography] is generally not recommended in the routine management of PD [9, 10]. Nonetheless, MRI can be occasionally used as a supportive tool to differentiate the disease from atypical parkinsonisms and other structural causes. In addition, transcranial ultrasonography has been proven to be useful in the differential diagnosis of movement disorders. Functional neuroimaging is used for the detection of the nigrostriatal degeneration associated with Parkinson's disease [15, 25, 26]. Intense research is currently underway in this area, and it will likely generate new applications of imaging tools in PD [27].

9.2.1.1 Structural Neuroimaging

Magnetic Resonance Imaging and Brain Computed Tomography

Computed tomography is an easy and accessible tool to identify brain structural lesions such as microvascular leukoencephalopathy (representative of a vascular parkinsonism) or large ventricles (characteristic of a chronic adult hydrocephalus). However, a limitation of CT is its resolution to identify subtle white matter and infratentorial lesions. MRI overcame this limitation and can be used for excluding secondary etiologies (Table 9.2) [28]. We have listed in Table 9.3 the most relevant MRI findings in PD and atypical parkinsonisms.

Table 9.3 Main magnetic resonance findings in PD and atypical parkinsonisms [15]

Parkinson's disease	Multisystem atrophy	Progressive supranuclear palsy	Corticobasal degeneration
Iron stores in SNpc loss of signal Loss of volume in SNpc	*Putaminal:* • Atrophy • Slit sign (hyperintensity in dorsolateral zone) • Hypointensity relative to globus pallidus *Brainstem and cerebellum:* • Pontine and/or bulbar atrophy • Cerebellar and/or dentate atrophy • Atrophy of the MCP • Reduced MCP diameter (<8.0 mm) • Dilatation of the fourth ventricle • Signal increase in MCP • Signal increase in cerebellum • Signal increase in inferior olives • Signal increase in pontine fibers (hot cross bun sign)	Midbrain atrophy • Direct • Indirect signs of midbrain atrophy: – Decreased AP < 14 mm – Abnormal superior midbrain profile – "Penguin silhouette" or "hummingbird" sign (Fig. 9.1) – Reduced ratio midbrain and pontine areas – Increased MRPI Signal increase in globus pallidus Signal increase in red nucleus Putaminal atrophy Dilatation of the third ventricle Atrophy of the SCP Signal increase in SCP (on FLAIR images) Frontal and parietal atrophy	Cortical atrophy (mostly frontoparietal and asymmetric) Putaminal hypointensity Hyperintense signal changes in the motor cortex or subcortical white matter

SNpc substantia nigra pars compacta, *MCP* middle cerebellar peduncle, *SCP* superior cerebellar peduncle, *AP* anteroposterior axis, *MRPI* multiplying the ratio of pontine to midbrain area by the ratio of the MCP to SCP width

Additional MRI techniques, such as diffusion-tensor imaging, magnetization transfer, inversion recovery ratio, volume analysis, and spectroscopy of specific brain zones, can help differentiate some characteristics of atypical parkinsonisms (Fig. 9.1), but their role remains to be elucidated. Additional research fields are 7-T-MRI [29] and multimodal approaches that combine functional and structural information such as positron emission tomography-magnetic resonance imaging (PET-MRI), shown in Fig. 9.2.

Ultrasonography

Ultrasound imaging of the midbrain structures can identify the existence of a hyperechogenicity of the substantia nigra in Parkinson's disease [30]. The increased signal reflects an increase in the iron content and an accompanying loss of neuromelanin. Typically, it is unilateral and/or asymmetric. Ultrasonography has also been proposed as a useful tool in the differential diagnosis of PD.

Moreover, it is a relatively inexpensive and harmless technique. It has not been universally implemented because of the need of an expert operator to acquire it. An additional limitation is

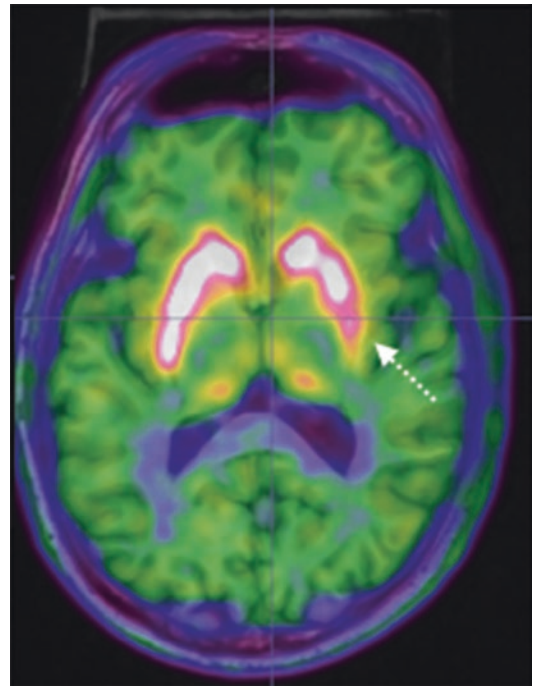
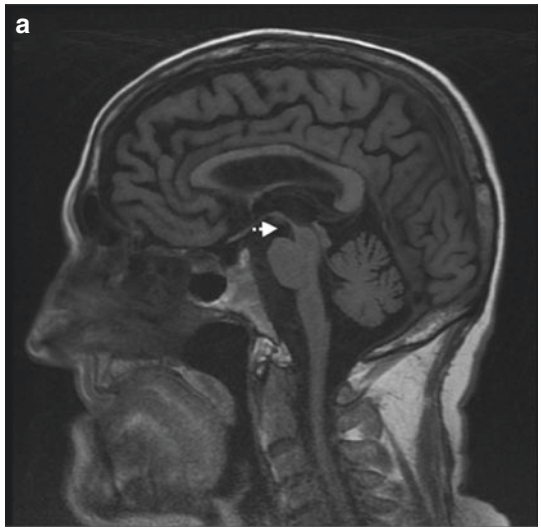


Fig. 9.2 Combined neuroimaging of a PD patient with PET-MRI**.** Axial slides of 18-F-DOPA-PET combined with MRI in a patient with Parkinson's disease. Note left striatum reduced radiotracer uptake (Arrow). Reproduced with permission of José Pineda (PhD)



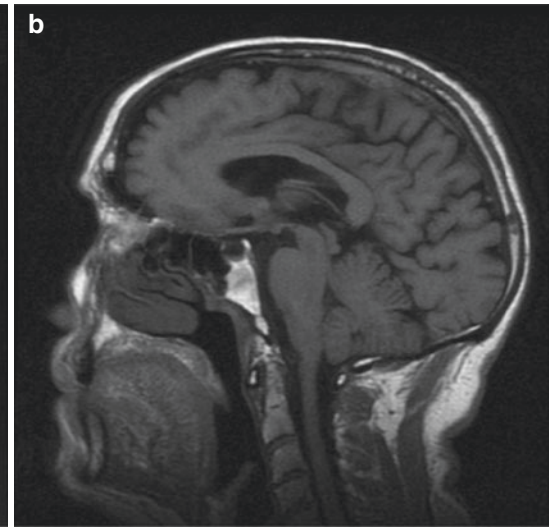


Fig. 9.1 An illustrative example of the use of MRI in the differential diagnosis of PD. Sagittal axis T1-weighted MRI. (**a**) Patient with progressive supranuclear palsy. The arrow indicates the characteristic midbrain atrophy or "hummingbird" sign. (**b**) Parkinson's disease patient with normal midbrain imaging for comparison. Courtesy of Dr. A. Ramos

the existence of inadequate acoustic bone windows in a variable percentage of subjects, ranging from 5 to 40% of the population depending on their ethnicity [15, 31].

9.2.1.2 Functional Neuroimaging

Different radiopharmaceuticals are used in combination with single photon emission (SPECT) or positron (PET) tomography to label the nigrostriatal dopaminergic pathway (Fig. 9.3). The most used is DaTscan, which combines SPECT and an imaging marker that labels the dopamine transporter. Other compounds are used, and new markers are being developed. The impairment of the nigrostriatal pathway can be identified with the tracers described in Table 9.4.

Functional neuroimaging also helps in the differential diagnosis of PD. A progressive nigrostriatal denervation occurs in Parkinson's disease (PD). Hence, the striatal uptake of dopamine markers correlates with the severity of the disease [32, 33]. For disorders such as essential tremor or drug-induced parkinsonism, in which case the synapses are preserved, the presynaptic functional neuroimaging is normal and can hence aid in differentiating them from PD [9, 15, 34]. However, in some drug-induced parkinsonisms, a presynaptic dopamine deficit can also exist when there is an underlying degenerative parkinsonism unmasked by the effect of these drugs [35].

To note, above 4–15% of the studies from patients diagnosed with PD have normal presynaptic imaging in what has been termed as Scans Without Evidence of Dopaminergic Deficit or SWEDDs [36–38]. Follow-up studies of SWEDD

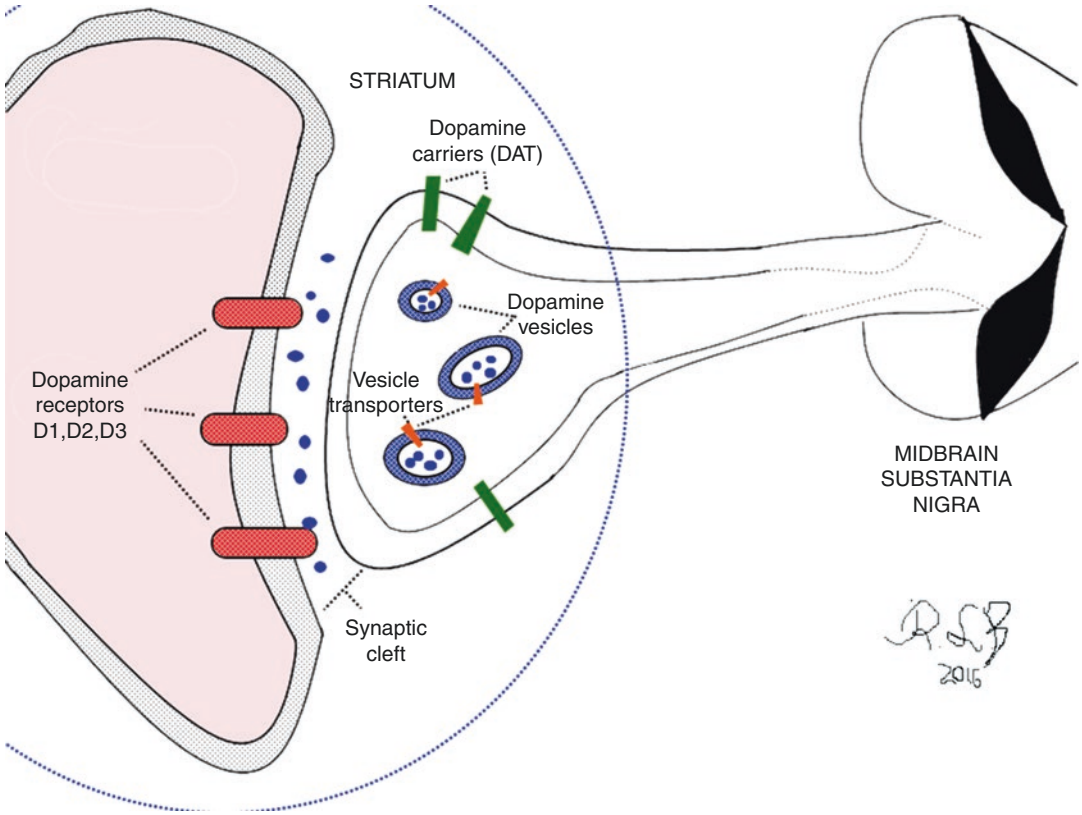


Fig. 9.3 Depiction of the nigrostriatal dopaminergic pathway and the associated imaging targets. Midbrain substantia nigra emits axons that connect with striatum by a synaptic cleft. In synaptic knobs are located vesicles with dopamine storage. Vesicle and dopamine transporters (VMAT and DAT, respectively) contribute to carry and keep dopamine in these terminals. In postsynaptic membrane are located the dopamine receptors

Table 9.4 Functional neuroimaging tracers

	Targets		
Presynaptic striatum imaging	Dopamine transporter (DAT):	Dopamine storage	Vesicular monoamine transporter type 2 (VMAT)
SPECT	– *123 I-FP-CIT (DaTscan™)* – 123 I-β-CIT – 123 I-altropane – 99mTc-TRODAT-1	–	–
PET	– 18F-FPCIT	18F-DOPA	– 11-C-DTBZ – 18F-DTBZ
Postsynaptic striatum imaging	***Dopamine D2-D3 receptors***		
	– IBZM-SPECT	11C-raclopride-PET	
Glucose metabolism imaging	***Cortex and basal ganglia neurons***		
	18F-fluorodeoxyglucose-PET		
Postganglonic sympathetic nervous system	***Cardiac sympathetic neurons***		
	– MIBG-SPECT		

SPECT Single photon emission computerized tomography, *PET* Positron emission tomography, *123I-FP-CIT* Ioflupane-DaTscan™, *123 I-β-CIT* (123)I (2beta-carbomethoxy-3beta-(4-iodophenyl)tropane), *123 I-altropane* (123)I-2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-(3-iodo-E-allyl)nortropane, *99m Tc-TRODAT-1* 99mTc-labeled tropane derivative, *18F-FPCIT N*-[3-[18F] fluoropropyl]-2β-carbomethoxy-3β-(4-iodophenyl) nortropane, *18F-DOPA* 6-[18F] fluoro-L-3,4-dihydroxyphenylalalnene, *11-C-DTBZ* 11C-dihydrotetrabenazine, *18F-DTBZ* 18F-fluoropropyl-dihydrotetrabenazine, *IBZM* (123)I-iodobenzamide, *MIBG* (123)I-metaiodobenzylguanidine (norepinephrine analogue)

patients have revealed that only a few of them evolve to have abnormal scans with dopaminergic deficits indicative of PD [36, 39]. This subgroup might include other underlying conditions different to Parkinson's disease, and there is an ongoing debate on this definition [40].

Characteristically, in atypical parkinsonisms, the decrease of dopaminergic terminals is accompanied by postsynaptic striatal impairment. Then, an impaired postsynaptic imaging exam can confirm the diagnosis of one of these alternative forms because postsynaptic imaging in PD remains normal [41].

In addition to cerebral functional imaging, cardiac 123 I-metaiodobenzylguanidine (MIBG) scintigraphy is used to evaluate the postganglionic presynaptic denervation found in Parkinson's disease [42].

Finally, PD and other neurodegenerative disorders might produce cortical impairment and changes in the cortical metabolism. Tools such as [18F] fluorodeoxyglucose PET have been used in research to detect specific zones of decreased cortical activity, especially in participants with suspected cognitive impairment [43, 44].

9.2.2 Genetic Testing and Parkinson's Disease

Despite not knowing the ultimate etiology of Parkinson's disease, proven monogenic causes explain 5% of all cases [15, 45, 46]. Specific tests to detect the most common mutations are used to confirm a genetic origin when familial aggregation exists (several first degree cases in the family) or there is a young-onset (<45 years). The purpose of genetic analysis is mainly diagnostic, but it is also helpful for genetic counseling when the proband is of childbearing-age.

The pattern of inheritance can be autosomal dominant, recessive, and even X-linked. The most frequent dominant forms are produced by mutations in the *LRRK2* and *SCNA* genes. Regarding the recessive forms, the most representative genes are *Parkin*, *PINK1* and *DJ1* [15].

A recent review proposed a new classification for the different genetic forms, and it is summarized in Table 9.5 [47]. This new classification uses prefixes to define the phenotypes, a suffix related to the gene name, and aims to use a more understandable and pathophysiology-related nomenclature.

Table 9.5 Genetically determined parkinsonism

New designation	Pattern of inheritance	Locus symbol
Classical parkinsonism		
PARK-SNCA	Autosomal dominant	PARK1
PARK-LRRK2	Autosomal dominant	PARK8
PARK-VPS35	Autosomal dominant	PARK17
Early-onset parkinsonism		
PARK-Parkin	Autosomal recessive	PARK2
PARK-PINK1	Autosomal recessive	PARK6
PARK-DJ1	Autosomal recessive	PARK7
Atypical parkinsonism and complex phenotypes		
PARK-ATP13A2	Autosomal recessive	PARK9
NBIA/DYT/ PARK-PLA2G6	Autosomal recessive	NBIA2 PARK14
PARK-FBX07	Autosomal recessive	PARK15
PARK-DNAJC6	Autosomal recessive	PARK19
PARK-SYNJ1	Autosomal recessive	PARK20
DYT/PARK-ATP1A3	Autosomal dominant	DYT12
DYT/PARK-TAF12	X-linked	DYT3
DYT/PARK-GCH1	Autosomal dominant Autosomal recessive	DYT5A No locus symbol
DYT/PARK-TH	Autosomal recessive	DYT5B
DYT/PARK-SPR	Autosomal recessive	No locus symbol
DYT/PARK-QDPR	Autosomal recessive	No locus symbol
DYT/PARK-PTS	Autosomal recessive	No locus symbol
DYT/PARK-SLC6A3	Autosomal recessive	No locus symbol
DYT/ PARK-SLC30A10	Autosomal recessive	No locus symbol
DYT/PARK-GLB1	Autosomal recessive	No locus symbol
NBIA/PARK-WDR45	X-linked	NBIA5
NBIA/DYT/PARK-CP	Autosomal recessive	No locus symbol

Parkinsonism, dystonia, and neurodegeneration with brain iron accumulation genetically determined are traditionally labeled as PARK, DYT, and NBIA, respectively, followed by the gene name [47]

Other genetic conditions that include parkinsonism as a non-predominant part of a more complex phenotype are not listed here (e.g., *POLG*, *SCA-ATXN2, NBIA/DYT-PANK2)* [47]. In spite of being one of the most powerful genetic risk factors for PD, being a carrier of a heterozygous mutation in the glucocerebrosidase gene *(GBA)* is not considered as a monogenic cause of parkinsonism due to its low penetrance [48].

9.2.3 Other Tests

Additional tests are used in the diagnosis of PD to detect-related manifestations (See Table 9.6). In addition, routine blood biochemistry, including thyroid hormones and B12/folate, are commonly requested at the initial visit.

9.2.4 Clinical Scales in Parkinson's Disease

Clinical tools to quantify the manifestations of PD are mainly used in research but some results are practical in the conventional follow-up. The ideal scale has to be accurate, responsive, valid, reproducible and reliable. In addition to these criteria, the ease and rapidity of use are also required for more implementation in practice. Detailed below are some of the most commonly used scales (Table 9.7).

One of the initial scales to measure PD was proposed by Margarete Hoehn and Melvin Yahr in 1967. This scale was useful for assessing the progression of PD [51]. Afterwards, Schwab and England proposed an instrument to evaluate the daily life activities as a proxy for the affected person's functionality and the disease evolution [52]. The ease of use of these scales facilitated their implementation in standard practice. However, they were limited by their inability to capture several relevant aspects of the disease, such as motor and non-motor features and even treatment-related complications. This led to the development of "multidimensional scales." Illustrative examples are the *Unified Parkinson's Disease Rating Scale (UPDRS)* and the *Scales for*

Table 9.6 Non-exhaustive list of other useful tests in differential diagnosis of PD

Neurologic function	Test	Value
Olfaction	University of Pennsylvania Smell Identification Test (UPSIT)	Anosmia identification [49]
Autonomic system	Urodynamic studies	Differential diagnosis of MSA vs PD High post-void residual urine in MSA [50]
	Orthostatism test	Diagnosis of orthostatic hypotension: Decrease of blood pressure after 3 min of standing defined by a ≥ 30 mmHg or 15 mmHg drop in the systolic and diastolic pressures, respectively
Movement neurophysiology	Electromyography and accelerometry	Tremor analysis/polyneuropathy

Table 9.7 Non-exhaustive list of common scales used in Parkinson's disease

Domain evaluated	Instrument	Reference(s)
Motor features		
Motor features and disability	Hoehn and Yahr	[51]
	Schwab and England	[52]
	Intermediate Scale for Assessment of Parkinson's Disease (ISAPD)	[53]
Multidimensional scales	Unified Parkinson's Disease Rating Scale (UPDRS)	[54]
	Scales for Outcomes in Parkinson's Disease-Motor (SCOPA-M)	[55]
	Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS)	[56]
Dyskinesias	Unified Dyskinesia Rating Scale (UDysRS)	[57]
	Abnormal Involuntary Movement Scale (mAIMS)	[58]
Fluctuations	Wearing-off Quick Questionnaire (WOQ-19)	[59]
	Wearing-off Questionnaire Q10	[60]
	Home diary for Motor Fluctuations and Dyskinesia	[61]
Gait	Gait and Balance Scale (GABS)	[62]
	Freezing of Gait Questionnaire (FOGQ)	[63]
Non-motor features		
General	Non-Motor Symptoms Questionnaire (NMS-QUEST)	[64]
	Non-Motor Symptoms Scale (NMSS)	[65]
Cognitive impairment		
Abbreviated assessment or Level I: Screening neuropsychological tests [66]		
	Montreal Cognitive Assessment (Moca)	
	Scales for Outcomes in Parkinson's Disease-Cognitive (**SCOPA-COG**)	
	Parkinson's Disease-Cognitive Rating Scale (PD-CRS)	
	Mattis Dementia Rating Scale (MDRS)	
Comprehensive assessment or Level II: Core neuropsychological battery [66]		
Attention and working memory Executive function Language Memory Visuospatial function	Trail Making Test-A (TMT-A); Symbol Digit Modalities Test [SDMT]; TMT-B Clock Drawing Test Boston Naming Test; Animal naming Free and Cued Selective Reminding Test (FCSRT) Figural Memory Judgment of Line Orientation (JLO) Intersecting pentagons	
Depression	Hamilton Depression Scale	
	Beck Depression Inventory	

(continued)

Table 9.7 (continued)

Domain evaluated	Instrument	Reference(s)
Anxiety	Parkinson Anxiety Scale (PAS)	
Apathy	Lille Apathy Rating Scale (LARS)	
Sleep	Parkinson's Disease Sleep Scale (PDSS).	
	Parkinson's Disease Sleep Scale 2 (PDSS-2)	
Disability		
Quality of life	Parkinson's Disease Questionnaire in 39 version (PDQ-39)	
	Parkinson's Disease Quality of Life Questionnaire (PDQL)	

Outcomes in Parkinson's Disease-Motor (SCOPA-M). Both scales have been commonly used in research, and the UPDRS is the most widely used instrument for PD to date [54]. To overcome some pitfalls of this scale, a new version, the *Movement Disorders Society Unified Parkinson's Disease Rating Scale* (*MDS-UPDRS),* has been proposed [56].

An important dimension in PD is the assessment of cognitive function. Cognitive assessments are typically performed in two steps. In the first step, a neuropsychological screening test can be performed during the practice (i.e., Montreal Cognitive Assessment test). In the case of a positive screening suggesting a cognitive impairment or when there is a clinical suspicion, a comprehensive neuropsychological assessment can help determine the grade of cognitive impairment. Cognitive assessments are also commonly used in the research setting (Table 9.7) [66].

Additionally, home diaries have been developed to monitor the participant's symptomatology at home. These have shown to be simple and feasible for patients and are commonly used to evaluate motor fluctuations in the context of advanced therapies. Sometimes, information acquisition can be challenging, and it has been shown that their reliability increases with the number of days that the diary is used. An additional limitation is that there is a lower compliance over time [61].

Despite their utility, most of the scales and methods described here are time-consuming, prone to bias, the Hawthorne effect, the subjectivity of the rater, and can only be performed in specialized settings by trained personnel. This is the basis for the increasing and exponential development of new methods for the assessment of PD [67, 68].

9.3 New Insights into Diagnosis

9.3.1 Overview

Despite the fact that Parkinson's disease is still diagnosed clinically, upcoming biological and neuroimaging markers targeting misfolded proteins implied in neurodegeneration, such as α-synuclein or β-amyloid [69], as well as the use of new technologies to measure the disease, are expected to revolutionize the disease management.

Diverse biological specimens, e.g., serum samples, skin or mucosal biopsies, are being investigated as potential biomarkers in PD. They are not yet formally established in the routine clinical practice, but will likely be part of the diagnostic pipeline in the near future [70–74]. One of the main studied proteins is α-synuclein that has been found in cerebrospinal fluid, blood, saliva and mucosa samples [70, 72, 75]. Beyond the known involvement of α-synuclein in the pathophysiology of Parkinson's disease, new mechanisms are being researched. The striking immune dysregulation described recently in Parkinson's disease is opening a new way to seek potential peripheral blood biomarkers [76].

These research efforts are focused on diagnosing the disease earlier, in what has been named as the prodromal phase of Parkinson's disease [77], and on developing more objective assessment methods.

For the latter, the miniaturization of inertial measurement units (IMUs) in microelectromechanical systems (MEMS) has opened the possibility to translate these measurement tools to the participant's natural environment (what has been termed as ecologically valid measures). This is part of the wider field referred to as "mHealth" where industrial partners are actively developing new solutions or using existing off-the-shelf smart systems (i.e., *smartwatches*, *smartphones,* or *smart bracelets)* to measure PD. For now, these systems are restricted to research applications and mainly for an in-the-clinic or specialized movement disorders center's use. It is expected that these systems will soon be introduced as an aid in common clinical practice and be translated also to the patient environment.

9.3.2 Wearables, an Example of New Technologies

The use of miniaturized IMUs is integrated into almost every smart device that most of the population owns (i.e., 75% of the UK population owns a smartphone) [78, 79]. These systems can contribute to gathering objective knowledge about the yet unexplored quotidian *movement states* of Parkinson's disease patients. The raw data obtained from these devices can be easily sent to a remote server by wireless connection and, thanks to the increasing development of artificial intelligence, they can be properly processed to generate relevant information related to the disease. The developed methods analyze a diversity of data measuring aspects of PD, such as tremor, bradykinesia, dyskinesia, fluctuations, gait impairment, and postural instability. Furthermore, some methods are measuring other dimensions, such as sleep, falls, or physical activity [79–82]. Hence, this approach will enable a fast source of relevant, ecologically valid and clinically objective information [83]. Nonetheless, there are issues that will need to be resolved before these new methods of assessment are fully implemented. An example is the protection of the patient's privacy to avoid unsolicited third-party uses [78]. A new era is opening, and we are certain that these upcoming technologies will soon change how PD is diagnosed.

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Clinical Symptomatology of Huntington's Disease

10

Jan Roth

10.1 Definition

Huntington's disease (HD) is a dominantly inherited autosomal neuropsychiatric degenerative disease with a fatal prognosis. The main clinical features are motor impairment (especially choreatic dyskinesias and the impairment of voluntary movements) and behavioral changes (especially cognitive deterioration and personality changes). The mutation is the expansion of the C-A-G (cytosine-adenine-guanine) triplet repeats 40 and more repetitions on the short arm of fourth chromosome. The prevalence of HD is approx. 1:10–15,000 [1]. The typical onset of HD is in the fourth decade, though there also occur relatively rare cases of juvenile or late onset HD forms.

10.2 Introduction

In 1872, a 22-year-old doctor, James Huntington, published a description of a disease that occurred in the region of East Hampton, Long Island, where he was born and where he lived [2]. In his publication, he summarized all the basic features of the disease: its hereditary character, fatal prognosis, adult onset and, of course, its characteristic symptoms: movement disorder, behavioral changes, and dementia. His patients came from the immigrant families of East Anglia, presumably from the town of Bures in Suffolk, who had settled in New England. These families were probably the original source for spreading the disease in the USA.

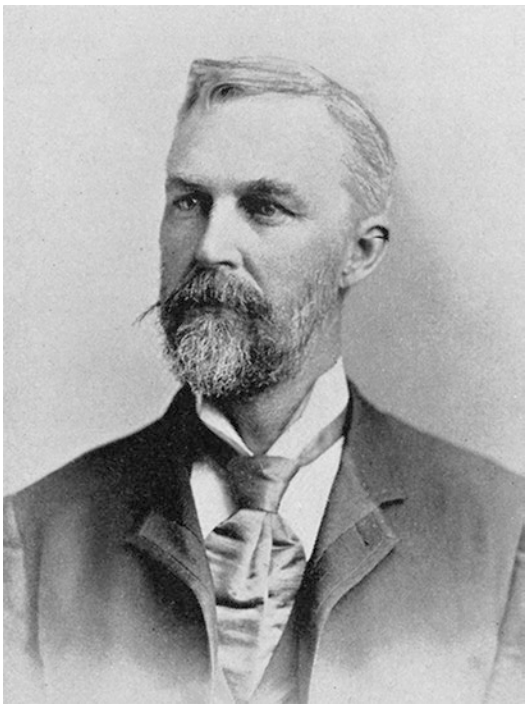

Photo 10.1 George Huntington

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_10

Of great importance for HD research was a discovery by a Venezuelan physician named Americo Negrette, who in the 1950s detected the source of HD. It was found within an isolated community by Lake Maracaibo with about 10,000 inhabitants of whom more than 100 suffered from the disease [3]. Blood samples of the affected individuals of this region enabled the identification in 1983 of a genetic marker on the short arm of the fourth chromosome, and also the mutation itself: an unstable C-A-G (cytosine-adenine-guanine) triplet on the short arm of fourth chromosome [4].

The "healthy" gene produces huntingtin protein. Not all the functions of this protein have been examined in detail, but it is known for its essential role in embryonic brain development and for hematopoiesis [5].

Mutation produces huntingtin with abnormally expanded polyglutamine chains. Pathological huntingtin differs from the physiological one structurally and functionally. Physiological and mutated huntingtin both intervene in a number of cellular processes: apoptosis, axonal transport, structural and functional changes of the cell membrane, production of neurotrophic factors, etc. [6, 7]. Most significant is that the organism is unable to remove the mutated protein from the nerve cell. The expanded polyglutamine chain prevents the protein from entering the ubiquitin proteasome system, degrading intracellular proteins and likely causing a great number of pathological processes through the accumulation of protein in aggregates [8].

Recent estimates of HD prevalence in Europe and the Americas have been approximately 5.70 per 100,000 [1]. HD is relatively more frequent in host countries of historical European migration (USA, Canada, or Australia).

The typical age of onset of the first HD symptoms is between 35 and 50 years. Both sexes are affected to the same extent. The average survival time ranges from 15 to 20 years. The disease manifests itself considerably more rarely (about 5% out of all cases) in the premature age (juvenile HD) or by the age of 20 (HD with an early onset). This form of the disease usually has other clinical signs than the so-called classical form of HD. Late onset of HD with the first signs after the age of 60 is also very rare (about 5%, see below).

As mentioned above, the substance of the mutation which gives rise to HD is an expansion of C-A-G triplet repetition. 40 and more triplets means the full penetrance and the individual is sure to develop the disease [9, 10]. In cases of individuals with 35–39 triplets, the prognosis is uncertain (so-called "grey diagnostic zone" or "incomplete penetrance") [11]. A number of triplets between 27 and 34 will not cause HD to manifest, but it is considered "unstable" as in up to 10% of all cases the number of triplets may increase through intergenerational transfer above the critical level necessary for HD occurrence [12, 13]. However, intergenerational contractions (reductions of the number) of triplets have also been rarely recorded. The higher the number of triplets, the more unstable the condition and the more likely to affect onset of HD—see Fig. 10.1 [14, 15].

The inverse relationship correlation between the number of CAG repeats and the age at onset

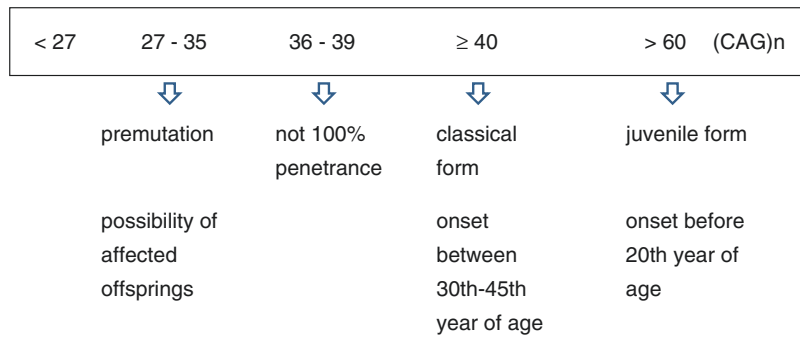

Fig. 10.1 The relation between the number of CAG triplets (CAG)n, clinical form of Huntington's disease and age at the onset

of the clinical signs of HD (the more CAG triplets present, the sooner the disease develops) has been demonstrated in many studies (e.g., [16, 17]). However, this relationship is obvious only in individuals with big (over 60) and marginal (36–39) numbers of triplets. The number of CAG triplets is a crucial but not sufficient factor that determines the age of onset of clinical HD, it determines it only partially [18, 19]. We can presume the existence of a range of other influencing factors, so-called gene modifiers, that are currently objects of further investigation and research [20]. It is interesting here that homozygous composition does not appear to influence the age of onset of HD, but does predict more serious clinical process [21].

Paternal transmission is a significant factor in the number of CAG triplets as the expansion of CAG triplets takes place most frequently during spermatogenesis [12, 22] or through some as yet unknown mechanism depending on the sex of the ancestor and the embryo of descendant [13, 23, 24].

10.3 Clinical Manifestation

10.3.1 Preclinical Findings in the Mutation Carriers and Phenoconversion

The onset of "soft" signs preceding the full HD manifestation (or in other words the transition from health to the disease phenotype) is called "phenoconversion". Phenoconversion in HD is traditionally defined as the onset of chorea. However, it is inadequate to use just one motor sign to characterize the disease onset. Many nonspecific symptoms (motor, cognitive, psychiatric, functional) could precede the definite clinical manifestation, sometimes by as long as 10 years [25–30].

MRI neuroimaging can also capture significant changes, such as atrophy of the caput nuclei caudate [31], cortex, or white matter, many years before the full clinical manifestation itself [32–34]. In studies with functional magnetic resonance of individuals at risk of HD, some changes were spotted before the clinical manifestation of HD [35].

There are also interesting findings regarding a decline in ability to decode facial expressions not only in cases of HD sufferers but also in carriers of the HD mutation [36–38].

A few large sample observational studies have focused on capturing the complex nature of abnormalities in the "preclinical" period: Cohort, Pharos, Predict-HD, or Track-HD.

10.3.2 Clinical Forms

10.3.2.1 Classical HD Form

The classical HD form is most frequent (app. 90% out of all cases), with the first signs appearing between the ages of 35 and 50, though the character and combination of symptoms may vary significantly (see Tables 10.1 and 10.2).

Movement Impairments

Choreatic dyskinesias are abrupt, involuntary, irregular, and non-stereotypical movements in random distribution of both proximal and acral muscle groups.

Dyskinesia is accentuated by physical and mental effort. It could be partially inhibited by psychic relaxation and disappears during sleep.

At low intensity, chorea may be overlooked or mistaken for signs of psychomotor restlessness.

Table 10.1 Neurological symptoms of Huntington's disease

Common symptoms	Less common symptoms (except juvenile form)
Chorea	Epileptic paroxysms
Dystonia	Cerebellar symptoms
Rigidity	Lesions of pyramidal tract
Bradykinesis, hypokinesis, akinesis	Myoclonus
Motor impairment	
Eye movements impairment	
Dysarthria	
Dysphagia, hyperphagia	
Cachexia	
Incontinence	
Sleeping disorders	

Table 10.2 Psychiatric symptoms of Huntington's disease

Personality changes; behavioral disorders – Alcohol abuse, changes in sexual behavior, lack of sexual restraint, aggression, criminality, apathy
Depression
Irritability
Anxiety
Psychomotor restlessness
Hallucinations and delusions
Isolated cognitive deficits
Dementia

While chorea is the classic symptom of HD, and often indicative of the diagnosis, considering its relatively late presentation in the course of the disease, its diagnostic function may be overestimated. As chorea typically manifests only after years of subtle psychopathological symptoms, it is difficult to accurately determine the actual onset of the disease.

Despite its crippling effects, chorea may be overestimated from the therapeutic point of view as well as the impairment of voluntary movement and dystonia usually are more significant. Sometimes it really accomplishes an invaliding impact though.

During incipient stages of the choreatic syndrome, involuntary movements are present especially in the perioral area of the face and may be mistaken for voluntary grimacing or expressions associated with excitement, stress, and anxiety. Also, mild choreatic movements of the upper limbs could be misinterpreted as simply expressive gesticulations.

Random movements of the limbs may be described as aimless or purposeless, even though some patients are very good at camouflaging them by shifting the involuntary movement into one that seems "purposeful," e.g., scratching one's cheek, playing with small objects, crossing one's legs, etc. (so-called "parakinesia" phenomenon).

During the progression, these movements become more pervasive and striking (though with variable intensity) in various muscle groups, with facial expressions ranging from surprise to anger, amazement, fear, etc. Grimacing, tongue protrusions, and moving lips become more frequent, as well as chaotic, random eye movements with diminished ability to focus. Rapid and brief eyelid contraction similar to blepharospasm accompanied with elevation of the eyebrows is also characteristic. Sound phenomena could be present, such as phonation of sighing or grunting as a consequence of involuntary movements of the respiratory muscles and vocal cords.

The neck muscles execute irregular swinging movements of the head, subtle elevations of the limbs at the shoulder and elbow joints, and abduction—adduction or flexion—extension of individual fingers.

Besides proximal and acral movements (e.g., typical shifting of feet on the floor—shuffling with the sole when sitting) of the lower limbs, choreatic dyskinesia may be manifested by hyperextension of the big toe ("PseudoBabinski" syndrome, "PseudoSiccard"), similarly to dystonia, in which case hyperextension of the big toe is fixed for longer period.

Some symptoms are very characteristic of chorea. They result from so-called global motor impersistence, i.e., the inability to maintain the sustained position.

Tongue Protrusion Test

The patient is not able to keep the protruded tongue still—involuntarily keeps putting it back into the mouth. Also, lateral movements of the tongue are not smooth and coordinated.

Grasp Sign

When pressing hands of the examiner, the patient involuntarily loosens and clutches hands as if he or she was "rubbing" the doctor's fingers.

Dance-Like Gait

Those afflicted by HD very often perform a "dance-like gait," they waddle from side to side. The gait can sometimes resemble a "gluteal, myopathic type" of gait, but in this case there are also dyskinesias of the limbs present. Waddling gait in hips which gives impression of a dancing act gave name to this choreatic phenomena (in Latin "chorea" and in Greek "choros" means "a dance").

Due to dyskinesias, the affected persons may not be capable of performing appropriate daily activities; their movements are inadequate; their aim, intention, and coordination get stuck. During such intensive involuntary movements, the affected may be at risk of injury. Later in the disease progression, speech abilities degrade considerably, with characteristic explosive—sometimes even saccadic—dysarthria and altered phonation. Dyskinesia also interferes with swallowing. Intensive choreatic dyskinesias may restrict one's capacity for self-care, worsen stability and lead to falls. More often, however, chorea is rather a source of social difficulties.

In the progression of the disease, chorea worsens significantly, but after some time the intensity spontaneously diminishes and transforms into dystonia and then finally akinesia.

Dystonia (sustained muscle contractions that result in twisting and repetitive movements or abnormal postures of the affected parts of the body) usually occurs during the middle stages of the classical form of HD, for example, trunk dystonia often becomes a source of significant gait disorder.

Besides chorea and dystonia, some patients also show signs of myoclonus (an involuntary, brief, and short twitching of muscles), or rarely as tics.

Severe, generalized **parkinsonian syndrome** does not manifest itself until the late stages of HD (so-called secondary Westphal variant—i.e., immobility without dyskinesias). The affected persons are then completely immobile and subject to secondary complications, such as decubiti and/or infections. Rigidity is often present in middle stages of HD, though this may be a consequence of antipsychotic therapy or other causes.

In contrast to the symptoms outlined above, **the impairment of voluntary movement** is underestimated in clinical practice. However it contributes significantly to the patient's invalidity as it is responsible for motor failures at many basic daily activities. Clumsiness, slowness of movements, and a lack of coordination both concerning the upper limbs during a focused, aimed activity and the whole body during coordination of the gait stereotype are not just the consequence of dyskinesias but an independent symptom.

Unlike dyskinesia, impairment of voluntary movement correlates to the progression and length of the disease and also with cognitive deterioration [39].

Another strange and characteristic sign of HD is a specific facial expression of emotional blunting with a hint of slight annoyance to disgust (facies Huntingtonica).

Gait disorders, usually developing during the middle stages of the disease significantly contribute to worsening quality of life. There occurs a distinctive "dancelike" gait interrupted by sudden involuntary movements, though falls are relatively rare. In later stages with worsening dystonia, bradykinesia, rigidity and postural instability, falls occur more frequently.

Dysarthria is a very frequent symptom of HD. Speech disorder develops in the middle to late stages of the disease, but may also appear in earlier years. In the course of the disease, speech degrades and eventually becomes totally inarticulate. An explosive or saccadic speech pattern—called hyperkinetic dysarthria—is characteristic. Sometimes speech may also be interrupted by involuntary sounds of grumbling and sighing.

Dysphagia is a serious symptom of HD and may even have fatal consequences especially in patients at later stages. It is necessary to monitor carefully problems with swallowing liquid or solid food. For the aspiration of food is typical coughing; wet or gargling vocalization immediately after eating; vomiting within several minutes of a meal; recurrent respiratory tract infections. Physicians/caregivers should be aware of so-called quiet aspiration which may even remain clinically mute for long periods and have to be examined specifically, e.g., by videofluoroscopy. There may also be rare instances of so-called hyperphagia, i.e., swallowing large unchewed bites, probably under the influence of an uncontrollable feeling of hunger, when the patient may be at risk of suffocating.

Gradual **cachectization** in most patients is a typical feature of the late stages of the disease. It need not be related to a striking loss of appetite or problems with standard food intake. The weight

loss cannot be satisfyingly explained and does not correspond with the impact of dyskinesias. Degeneration in the lateral nuclei of the hypothalamus is presumed to affect the process [40–42]. Early cachexia indicates a worse prognosis.

With individual variation, after 10–15 years of development of the abovementioned symptoms, patients with the classical form of HD become fully dependent on caregivers, and die after 15–20 years of the HD development in a marantic state, usually from complications, such as infection, decubiti, etc.

Neuropsychiatric Disorders

The first symptoms of HD are usually subtle **changes in behavior and personality** [43–45]. There are two typical scenarios of development.

In the first model, the patient manifests and develops a gradual loss of interest in one's surroundings, children and their needs, one's partner, appearance. An early development of feelings of **apathy**, distinct from the potential presence of depression, and emotional numbness are characteristic [46–50].

The affected individuals suffer a decline in work performance as a consequence of the development of executive dysfunction. This scenario may result in the chronic loss of employment, poverty, and a decline in social and economic status.

This situation may come about years before the onset of even minor choreatic dyskinesias which is typically the trigger for the clinical diagnosing and genetic testing.

The second clinical scenario also begins with the development of behavioral and personality disorders, but of a different, productive type. Instead of apathy, patients manifest increased **irritability** and states of **anxiety** [46, 47, 51–54]. Due to this anxiety, patients are very often incapable of handling formerly trivial tasks. Family members may notice dramatic and anxious reactions of the sufferer to casual and inconsequential events, such as the late arrival of a family member; deciding what clothes to wear; and whether to accept an invitation. In many cases of HD patients, the anxiety is often and vehemently manifested through somatoform symptoms, such as headaches, backaches, and digestive problems, which the patients describe as dominant problems.

Depression develops in 40% of all cases [43, 46, 47] and bipolar affective disorder could be present. Suicidal tendencies are a serious problem for this group of patients, with suicide rates of 4–6 times higher than the general population [46, 47]. Some data suggest a prevalence of suicide attempts as high as 13% within this population. It is necessary to emphasize that suicidal behavior is a threat at any stage of HD, even prior to the diagnosis [55, 56].

While **psychotic manifestations** are relatively rare in the initial phases of the disease [53, 54], common neuropsychiatric features of HD include paranoid tendencies together with irritability and aggressive behavior. Early symptoms may include feelings or acts of jealousy, and suspicion. Hallucinations are relatively rare.

Obsessive thoughts, compulsive behavior, and perseverance are also common [57, 58].

Interestingly, many HD patients are unaware of their symptoms [59] with almost half the cases of HD diagnosed patients [60] reporting no symptoms. Such a **lack of self-awareness** (anosognosia or denial) can cause problems, especially when the sufferer wants to pursue activities he is not capable of managing (e.g., driving, dealing with finances).

While problems associated with hypersexuality—sexual aggressivity, promiscuity, and sexually provocative behavior—are sometimes observed in early phases of HD, impotence is a more frequent phenomena [61]. Verbal and brachial aggression [25, 43, 46, 47] very often directed only at their closest family members is noted particularly in cases of patients who have had these tendencies throughout their entire life. Anxiety, depressive and psychotic symptoms, obsessive-compulsive disorder, and delirium may predispose sufferers of HD to aggressive behavior. Very often the triggering factor is psychosocial stress brought about by a change of environment, being assigned multiple tasks within a short period, feelings of inadequacy resulting from imperfect speech comprehension, troubles with routine tasks, or conflicts with authorities.

Manifestations of minor criminality (e.g., petty thefts) or problems with alcohol [62–64], while often perceived as simply "unprincipled" demonstrations of asocial behavior may be early indications of pathological changes characteristic of HD. Pathological changes in affects and behavior may also result in divorce, the loss of custody of children, and alienation. Not until years later, when other clinical manifestations of HD surface, the aforementioned signs can be attributed to this diagnosis.

Cognitive Disorders

Minor cognitive changes very often precede the typical clinical picture with dyskinesias [50, 65–67], though the decline of cognitive function in HD is neither universal nor progresses evenly, as the rate of development of dementia varies among individuals.

In early stages of HD, isolated cognitive deficits dominate, especially disorders of executive functions, attention, learning, memory, and changes in psychomotor speed [68]. Sometimes a long-term stationary character can be observed. The extent of cognitive deterioration need not correspond proportionately with other behavioral or neurological symptoms.

As far as memory is concerned, working and short-term memories are affected most, while long-term memory is relatively stable and well preserved. For basal ganglia affection generally (thus including HD) disorders of procedural memory are typical (motor skills—driving, walking, etc.). Memory storage appears to be unaffected by HD. Impairments in retrieval may be remediated through recognition, cues, or associations.

Memory disorders are also impacted by the executive dysfunction: the inability to conceptualize action, or plan for the future; a lack or disruption of control over certain performance procedure and time structures; and at the same time a diminished capacity to accommodate disruptions or unexpected shifts in the activity.

Executive dysfunction also inevitably impacts the capacity for selection, storage, and voluntary recollection of substantive information from memory. Such executive function disorders, typical of the early stages of HD, are mainly responsible for HD sufferers' incapacity first for professional work/activity and later for normal everyday activities [69].

The progression of the disease ultimately leads to full-blown dementia, with a global loss of cognitive function incommensurate with age which interferes with daily activities.

Dementia of the so-called subcortical type is typical for HD, with executive dysfunction typified by changes in psychomotor pacing, behavior disorders (irritability, apathy, obsessive-compulsive manifestations, etc.), mood, and anxiety. Unlike dementia of cortical type (e.g., Alzheimer's disease), the fatic, practic, and gnostic functions are relatively preserved in case of HD, though with further cognitive deterioration, cortical functions also become substantially affected.

10.3.2.2 Juvenile Form of HD (JHD)

JHD starts before the age of 20 and occurs in approximately 5% of all HD cases [70]. In about 1–2% of all cases, symptoms manifest before the age of 10 and very rarely even in the preschool age.

The manifestation of HD in the affected children or adolescents usually differs significantly from the classical form of HD. A child and parent both suffering from HD would present very different symptoms, such that one would hardly identify the same disease in progress.

The onset of JHD varies considerably, due largely to the fact that the disease is manifesting in a developing brain. For this reason the diagnosis is often very difficult, especially in the absence of a positive family history.

The transition from subclinical manifestations detected only by specific methods (e.g., neuropsychological testing) to the stage of the obvious clinical manifestation detectable by objective examination, observation, and interview (phenoconversion—see above) is very vague, making it difficult to assess the chronology of the disease.

The first manifestations may be either motor, cognitive, or behavioral. Siesling [71] found behavioral disorders in JHD as the initial manifestation in 70% of all cases, compared to motor in 48% and cognitive in 27%. Similar results have also been detected by the Ribaï study [72].

So far there have been very few clinical studies dealing with JHD symptoms and its dynamics and there is a lack of systematic data. The life expectancy in JHD is shorter than in the classical form, approximately 10 years from the first manifestations [70, 72, 73], though some studies do not indicate a distinctively shortened life span in comparison with the classical form of the disease [74].

Psychopathology of JHD

The first indications of a JHD disorder are that of intellect. A typical initial manifestation is failure to cope with school demands particularly due to a combination of some aspect of cognitive disorder (at a very early age there occurs mental retardation, in older children already indications of some cognitive deterioration) with a slowing of motor function, lack of coordination of movement, and voluntary movement disorder.

Manifestations as temper tantrums, aggressivity, antisocial behavior, and obsessive-compulsive features are frequent. Depression is also a very frequent symptom. Psychotic manifestations in JHD occur more often than in the adult form.

Motor Symptoms of JHD

The character of motor symptoms is what distinguishes the clinical image of JHD from its adult form. The most characteristic symptoms of JHD are hypokinesia, rigidity, and dystonia, accompanied by rapidly progressive stability and gait disorders. In the middle stages, there is a distinctive shaking of the head and the upper part of the trunk, kinetic tremor of upper limbs with occasional trunk myoclonus. In JHD, there may also occur compulsive spasmodic movements. Neurological examination often reveals pyramidal tracts lesions as increased reflexes and the presence of plantar response, etc. Severe involvement does not occur.

There is a relatively early onset of dysarthria, which in later stages may progress to mutism and dysphagia sometimes accompanied with hypersalivation. Dysphagia can cause choking, coughing, postprandial vomiting, and aspiration pneumonia.

The most common manifestation of JHD is a rapidly progressive and invalidizing atypical parkinsonian syndrome in combination with diverse psychopathologies. This form is sometimes referred to as primary Westphal variant of HD, unlike secondary Westphal variant which is the late stage of the adult form of HD (when akinesia replaces dyskinesia), and the diseased is not able to carry out voluntary motor activities.

Supranuclear gaze palsy is another commonly associated symptom, though it does not usually appear until the middle stages of JHD. In such cases, both vertical and horizontal movements get stuck, and in very serious cases the eyes are fixed forward and the whole head moves when trying to look aside.

Rarely, typical choreatic dyskinesias may also occur, but normally it is either absent or not evident to clinical observation. If present, they tend to occur in cases of individuals whose JHD onset started in adolescence. With cases at the onset by 10 years of age, it is totally rare.

Other manifestations are epileptic seizures—which occur in as many as 40% of all cases of JHD [70, 71]. They can be both generalized (very often of tonic-clonic character) and focal and there are cases of the clinical image of progressive myoclonic epilepsy.

From the middle stages of the disease, cachectization appears constantly. Cachexia is a very serious sign. It need not be linked with a lack of appetite or with problems of ordinary food intake. Weight loss cannot be explained satisfactorily and does not correspond with the impact of dyskinesia.

Patients require hypercaloric intake; approximately 4000–6000 calories daily is recommended. Weight loss (or the "onset of weight loss") is always considered an alarming sign which is necessary to try to address.

The advanced stages of JHD are characterized by repeated falls with injuries, which, together with a gradual loss of active mobility and dystonic postures, ultimately leads to total immobilization and mutism. Serious cachexia with dysphagia may require a percutaneous endoscopic gastrostomy (PEG). Infectious complications increase (pneumonia, decubiti) and patients died in a marantic state.

10.3.2.3 Late Onset Huntington Disease

This form of the disease manifests in persons over 60. It comprises approximately 5% of all cases of HD. Onset after the age above 70 is exceptional. It may be presumed that minor symptoms had been present but undetected a long time beforehand.

The clinical features of late onset HD resemble the symptoms of the classical form, but the progression is slower and less functionally debilitating. The hallmarks are mild to moderate chorea and cognitive impairment, gait disorder, and dysarthria. Behavioral symptoms such as apathy or irritability, depression or even psychosis may be present, though only infrequently.

Due to its relatively "benign character" (though not in all cases!), the patients are often able to maintain an active lifestyle for many years, only occasionally requiring nursing support.

Late onset HD is generally underdiagnosed, with major consequences for descendants, who remain unaware of this potentially serious hereditary disease [75–77] (Table 10.3).

Table 10.3 Typical features of late onset Huntington disease [77]

• Dominant motor symptoms (chorea, gait disorders)
• Slow progression of cognitive deficits to dementia
• Slow progression of functional disability
• Frequent negative family history
• Borderline or low pathologic expansion of CAG triplets

10.4 Diagnosis

It is relatively easy to diagnose HD particularly in situations when we are aware of any family history of severe neuropsychiatric disease (e.g., an affected ancestor died in a psychiatric hospital) and when the patient exhibits symptoms of dyskinesia together with a behavioral disorder and cognitive deficit (Table 10.4).

Accurate diagnosis may not be so simple in situations, where family history is missing (unknown paternity or no information about one side of the family in the case of parents' divorce, new mutation, etc.) or the patient and family are in denial about the condition and refuse to share relevant information. The clinical picture may also vary from one patient to another, depending on the stage of the disease and the prevalence of

Table 10.4 The manifestation of main symptoms in particular forms of Huntington's disease

Symptom	Juvenile form (onset by the age of 20)	Classical form (onset between 35 and 50)	Late form (onset over the age of 60)
Chorea	Usually not present	From the early stage	From the early stage
Dystonia	Present from the early stages	From the middle stage	Not present
Parkinson's syndrome	Present from the early stages	From the late stage	Not present
Epileptic paroxysms	Present	Atypical symptom	Not present
Lesions of pyramid pathway	Present	From middle stage	Not present
Cerebellar symptom	May be present	Atypical symptom	Not present
Affective disorders	Present from the early stage, later it disappears	Present from the early stage, later it disappears	Not present
Dementia	Present, fast progression	From the early or middle stage	Not present
Psychotic states	Present from the early stage	Any stage	Atypical symptom
Disease progression	Very fast (death within 10 years of the first signs)	Medium (death within 15–20 years from the first signs)	Slow
Heredity	Usually paternal	Both paternal and maternal	Both paternal and maternal, however often not present

symptoms (dominant psychiatric or neurologic symptoms may dominate in some families).

Nonetheless, the possibility of HD should be considered whenever the adult slowly develops behavioral changes or cognitive deficits in combination with motor handicaps of a choreatic or dystonic character, even in the absence of similar problems in the family medical history. If the family history is indicative, even minor behavioral changes, personality disorders, or just discrete memory disorders should be taken into consideration.

Genetic test confirms only the presence of the mutation! A positive test for the HD mutation in a person who does not otherwise show any HD symptoms does not confirm a diagnosis of the disease but just a genetic predisposition.

Genetic testing can be theoretically carried out in a few model situations:

Diagnostic testing is carried out in the case of a reasonable clinical suspicion for HD. The testing either confirms or disproves the clinical diagnosis with 100% certainty. The patient must always be informed that his or her blood is being taken for genetic testing which will either confirm or disprove the HD diagnosis. The patient has to agree to the testing procedure and confirm compliance in writing. Exceptions to this written protocol can only be made in cases where the patient so severely affected that he or she is physically incapable of giving consent.

Predictive (presymptomatic and prenatal) testing may be carried out in heretofore healthy individuals at risk of HD. A presymptomatic test may be carried out on descendants of an affected person who wish to know whether or not they have inherited the HD mutation.

The prenatal test is carried out through the analysis of amniotic fluid (amniocentesis, biopsy choria) in the course of pregnancy of a diseased or positively tested woman, or the wife of a diseased or positively tested partner, who wishes to know the genetic status of her unborn child.

Presymptomatic testing of HD has serious ethical considerations [78–81]. The disease is fatal, incurable, and causes progressive devastation of the motor and mental faculties. Known carriers are burdened with high stress not only regarding their own future quality of life but their posterity, as a positive result entails 50% hereditary risk for the next generation. Last but not least, a positive test for the HD also has multiple negative impacts on "healthy" family members and loved ones.

An international protocol-based presymptomatic testing procedure has been designed with the aim of minimizing negative, catastrophic consequences [82]. This includes several consultations: genetic, neurological, psychological, and psychiatric. Individual sessions are aimed at providing a detailed overview of the disease and the testing procedure to the applicant; verifying the patient's insight on the issue; clarifying his/her motivation and the benefit of the test; and last but not least, at determining the applicant's adaptive capacity to handle burdensome situations.

The entire process of the predictive protocol reduces—though does not eliminate—the risk of suicide considerably [79]. Short- and long-term consequences of predictive testing have been examined in many studies [78, 81, 83, 84].

According to the protocol, physicians may not refuse to do the predictive test but they may recommend postponing it. Aside from clear contraindications such as suicidality and depression, a number of other situations may arise whose importance is subjectively determined depending on the personal judgement of the given psychiatrist (for example, their willingness to accept risk and individualize treatment versus an entirely formal approach with no personal engagement of the examiner). As a rule, the recommendation to continue or postpone the test stems usually from the clinical experience of the psychiatrist. The most important variable in this respect is probably played by the presence or lack of motivation and self-advocacy in the patient; furthermore by their personality structure, maturity, adaptation mechanisms, duration and depth of HD awareness and knowledge of the quality of their background and other factors.

Many years of experience with the predictive protocol at many centers worldwide show that the implementation rate of the genetic test is relatively low, ranging between 5 and 25% in various countries [85].

With current preimplantation genetic diagnosis technique it is possible that a person at risk of

HD can give birth to a child who is not a carrier of the HD mutation, without the necessity of confronting the people at risk with their genetic status [86, 87].

10.4.1 Huntington's Disease Phenocopies

Approximately 1% of patients with typical classic HD-like manifestations lack the causative mutation [88]. Such cases are considered "Huntington's disease-like syndromes" or "Huntington's disease phenocopies" (HDP). HDP are clinically and genetically heterogeneous. The etiological diagnosis of the respective HDP is usually difficult to establish (see Table 10.5).

10.4.2 The Problems of Caregivers

Due to its complexity, HD is a typical example of a disease that affects not only its carriers but also the whole family and extended care group. Physicians and scientists have been focused mainly on the biological principles of the disease and on a search for possible therapy. A great deal of attention is given to patients and carriers; however, the problems and needs of caregivers (who bear the burden of psychological stress) nursing the patients are not in the center of the attention [89, 90].

The partner of the HD patient, for example has to face a lot of problems, such as a lower economic status, the loss of free time, and the loss of independence.

Other serious problems include the risk of HD transmission on the descendants (feelings of guilt for passing on the mutation, the inability to convey to the children the nature and level of the risk) and the character of the disease itself which often severely disrupts the partner's psyche.

The afflicted sometimes alter their behavior and personality, even committing violence on their partners or children through pathological jealousy, sexually motivated aggression, phychotic symptoms, or rage.

Children in families with HD often have difficulty comprehending situation with all its ramifications (why the parent is aggressive, understanding the change in behavior, etc.) It is impossible to generalize the consequences of such long-term problems for the development of the children, but they definitely have a considerable negative impact on the whole family.

Under the stress of the manifestation of HD symptoms, not to mention the broader social, economic, and emotional costs, in most cases partners in long-term relationships with HD-affected persons develop severe depression, anxiety and panic disorders, negative behavior and/or aggressive outbursts towards the diseased. Even talking about HD often becomes a family taboo and is not allowed to be discussed in public or even within the wider family circle. The partners, as well as the persons at risk, describe their state as one of "permanent sadness," often requiring medical intervention.

Table 10.5 Huntington's disease phenocopies (HDP) of adulthood

Clinical entities with a typical picture of HDP
Dentato-rubro-pallido-luysian atrophy (DRPLA)
Spinocerebellar ataxia type 17 (SCA 17) or Huntington disease-like 4 (HDL4)
Choreoacanthocytosis
McLeod syndrome
Neuroferritinopathy
Huntington disease-like 1[a]
Huntington disease-like 2[a]
Huntington disease-like 3[a] (onset at preschool age)
Disorders only rarely fulfilling the clinical picture of HDP
Spinocerebellar ataxia type 2, type 3
Friedreich ataxia
Pantothenate kinase-associated neurodegeneration (onset in childhood and adolescence)
Wilson disease

[a]Extremely rare in Europe

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11 Diagnosis of Amyotrophic Lateral Sclerosis/Frontotemporal Dementia Spectrum

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11.1 Introduction

Frontotemporal dementia (FTD) constitutes approximately 15% of all primary degenerative dementias. Its prevalence is approximately 150–220 cases/million inhabitants [1]. FTD is currently recognized as the second most common early-onset dementia under 65 years old, but there is also evidence that FTD occurs in elderly patients. Clinical symptoms usually begin in patients between 45 and 65 years of age, with no significant gender difference, and previous work has reported family history in 30–45% of all cases [2]. The most common clinical features of this disease are altered behaviour with change in personality, altered social behaviour and language impairment. Additionally, a percentage of patients with FTD may present with parkinsonism or associated motor neuron disease.

Approximately 15% of FTD patients develop clinical symptoms of motor neuron dysfunction and different studies have suggested that roughly 50% of patients with ALS have some cognitive impairment and 15% reach criteria for diagnosis of FTD, allowing experts to suggest a link between ALS and FTD [3–5].

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ALS was generally considered a pure motor neuron disorder with no cognitive impairment; but pathological studies have shown that ALS affects multiple areas and structures in the brain, causing not only motor neuron degeneration but also a wide range of alterations in extra-motor areas.

The idea that FTD and motor neuron disease had their own distinctive neuropathology began 20 years ago with the first report of ubiquitin-positive inmunoreactive inclusions in the cytoplasm of motor neurons [6, 7]. Later, the evidence of ubiquitin-positive inclusions in the extra-motor cortex was shown in both pure ALS patients and ALS patients with dementia. These ubiquitin-positive inclusions became the pathological hallmark of the combined FTD-MND syndrome.

In some cases FTD precedes ALS by many years, but in others, ALS precedes FTD. Nevertheless, in some ALS patients with no FTD diagnosis, early behavioural changes were reported even preceding the onset of the ALS symptoms. Prognostic factors like older age, male, bulbar onset, low education, family history of dementia, low forced vital capacity and pseudobulbar palsy have been associated with cognitive involvement in ALS. Therefore, cognitive impairment in ALS has been associated with shorter survival.

In this regard, cognitive impairment in ALS patients is key not only for therapeutic trials of

S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_11

this incurable disease, but also for care planning, compliance to interventions and ultimately end-of-life decisions.

11.2 Pathological Findings

Neurodegenerative diseases can be classified from a histopathological point of view according to the finding of protein inclusions or by the filamentous protein. Frontotemporal lobar degeneration (FTLD) encompasses a heterogeneous group of pathologies that can be classified according to different protein deposits in the central nervous system.

Thus, we can classify them into four subgroups: FTLD with tau positive inclusions (FTLD-tau); FTLD associated with DNA-binding protein TDP-43 inclusions (FTL-TDP43); FTLD associated with fused in sarcoma protein (FTLD-FUS) and a small percentage of cases that cannot be classified within these three major subgroups and constitute FTLD-others [8–10].

At the macroscopic level, atrophy can be observed at frontal and temporal cortex, globus pallidus, amygdala, hippocampus and hypothalamus [11], and in the orbital and cingulate cortex. Besides, the microscopic analysis shows mainly loss of neurons and frontal and temporal astrocytosis.

In the group of FTLD-tau, the tau deposits can be found in neurons, astrocytes and oligodendrocytes, whereas the FTLD groups without tau deposits are characterized by the presence of TDP-43 or FUS deposition [12].

Interestingly, motor syndromes associated with FTD are different according to the presence or absence of tau deposition. In this regard, the presence of parkinsonism (corticobasal syndrome and progressive supranuclear palsy) is suggestive of FTD-tau+, while motor neuron disease is tau-. Also, inclusions of ubiquitinated misfolded proteins are a common finding in the pathology of ALS and FTD with TDP 43 (Transactivation response—DNA-binding protein) being the main component of these inclusions (SLIs, RHIs, LBHIs), both in FTD and ALS cases [13]. Inclusions of FUS have also been observed in cytoplasm of the motoneurons in the anterior horn and in glial cells of patients with ALS and FTD. The study of the deposits of these two RNA-binding proteins has raised the possible implication of RNA processing in the pathophysiology of these diseases [9].

11.3 Genetics

The study of the genetic mutations in ALS and FTD has experienced an exponential growth in recent years. Despite these important advances, many aspects remain to be clarified. In FTD, between 10 and 27% of cases present a family history [14], while in ALS approximately 5–10% of patients have a family history of motor neuron disease [15].

To date, genes associated to FTD are the Microtubule Associated Protein Tau (*MAPT*), progranulin (*PGRN*), *C9ORF72*, valosin containing protein-1 (*VCP-1*), charged multivesicular body protein 2B (*CHMP2B*), TAR DNA-binding protein (*TARDBP*) and fusion protein in sarcoma (*FUS*), being *C9ORF72* and *PGRN* the most important ones in terms of frequency.

In ALS the first gene reported was *SOD1*, giving a great boost to research in this disease since it allowed the development of the first animal model. Later, mutations were observed in the *TARDBP* gene—the gene encoding TDP43—and at the same time mutations in *FUS*. In recent years, a large number of genes related to motor neuron disease have been reported, being the expansion of *C9ORF72* the most important one, considered as one of the main causes of familial ALS (30%) and even sporadic cases.

ALS and FTD are now considered to be a continuum of two overlapping diseases. This concept of a spectrum is supported by the identification of new genes and the description of common pathophysiological pathways and factors. The main genetic causes related to both diseases (ALS-DFT) are mutations that produce alterations in RNA processing binding proteins (*TDP43, FUS, ANG*), proteomic proteins (*UBQLN2, OPTN,*

SQSTM1, VCP, CHMP2B) and the most frequent of all, the expansion *C9ORF72* [16–18]. More recently, significant variants in some genes has recently been found in ALS/FTD: *TBK1*, *CCNF* and *NEK1* [19–22]. In C9ORF72, inheritance is autosomal dominant with a high penetrance (nearly 100% at 80s). Clinical features include bvFTD (with disinhibition as a prominent symptom) and/or motor neuron disease, a variable age of onset, and generally bilateral frontal-predominant damage in neuroimaging (Fig. 11.1).

11.4 Clinical Findings

11.4.1 Frontotemporal Dementia

Early diagnosis of FTD may be difficult because the initial symptoms of the disease may be similar to those presented in other neurodegenerative diseases and even to some psychiatric disorders. Therefore, a comprehensive neurological and neuropsychological assessment is necessary.

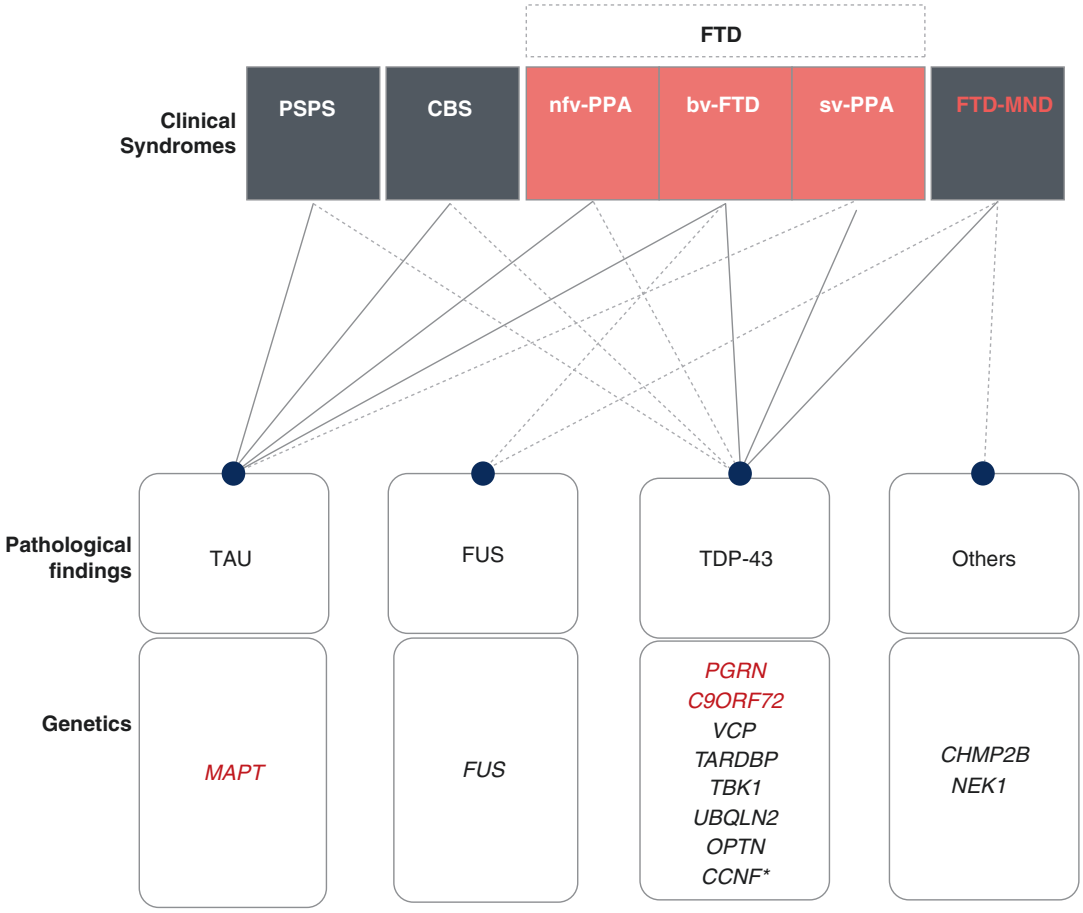

Fig. 11.1 Clinical syndromes, pathological findings and genetics of the frontotemporal lobar degeneration (FTLD). *FTD* frontotemporal dementia, *PSPS* corticobasal degeneration Syndrome, *PSPS* progressive supranuclear palsy syndrome, *svPPA* semantic variant primary progressive aphasia, *nfvPPA* non-fluent variant PPA, *bvFTD* Behavioural Variant of FTD, *FTD-MND* frontotemporal dementia and motor neuron disease, *MAPT* microtubule associated protein tau, *FUS* proteina de fusion en sarcoma, *PGRN* progranulin, *VCP* valosin containing protein, *TARDBP* TAR-43 DNA-binding protein, *TBK1* TANK-binding kinase 1, *CCNF* cyclin F (*whether cyclin F-linked ALS and FTD is a TDP-43 proteinopathy in vivo requires further investigation), *CHMP2B* charged multivesicular body protein 2 B, *UBQLN2* Ubiquilin-2, *NEK1* NIMA related kinase 1

FTD groups three main clinical syndromes, including behavioural variant FTD (bvFTD)—a disorder characterized by behavioural abnormalities—and two language variants—semantic variant primary progressive aphasia (svPPA or semantic dementia) and non-fluent variant PPA (nfvPPA) [23].

The bvFTD is the most frequent subtype of FTD. This subtype is characterized by behavioural alterations and disinhibition, compulsive and persevering behaviour, emotional disturbance, poor planning ability and mental flexibility, overreaction, apathy and personality changes.

On the other hand, semantic dementia may present predominantly left atrophy (associated with progressive loss of conceptual word content) or predominantly right temporal atrophy (associated with difficulty in recognizing faces and individuals). Finally, nfvPPA is characterized by agrammatism, non-fluent language and apraxia of speech [24–26].

Interestingly, some authors have argued to subclassify FTD in several types, according to some clinical, topographical and even genetic findings. In this regard, frontal-dominant, fronto-temporal, temporal-dominant and temporo-fronto-parietal subtypes have been suggested and associated to some clinical and genetic differences. For instance, *C9ORF72* expansion has been associated to bilateral frontal atrophy, tau mutations to anteromedial temporal atrophy, and progranulin mutations to temporo-parietal asymmetric atrophy [27, 28].

The clinical diagnosis of FTD and its main variants was based mainly on the consensus by Neary et al. [29], but due to its restrictive nature, its use has been limited for the diagnosis in early stages of the disease and mainly in its behavioural variant. For this reason, in order to improve its accuracy and to accelerate the diagnosis in this last variant, more flexible and sensitive clinical criteria were recently established [30]. Regarding language variants, specific criteria for PPA and its subtypes have been published [31].

However, it is important to emphasize that the use of clinical criteria constitute a tool to support the diagnosis and for research purposes, but it should not be considered as an absolute method. Today there are complementary tools to make the diagnostic process more accurate.

Regarding cognitive impairment, it most frequently involves attention and executive functioning. Language, verbal fluency, and social cognition are also impaired, while visuospatial and visuoperceptive functions are relatively spared. Thus, a comprehensive cognitive assessment is usually needed (Table 11.1). Motor and/or speech disorders must be taken into account adapting or correcting the cognitive instruments. Behavioural assessment is essential, especially because cognitive examination may be within normal limits in early stages. This is explained by the early impairment of orbitofrontal cortex

Table 11.1 Some examples of cognitive tests for each domain

Domain	Cognitive tests
Screening/global cognitive functioning	– Edinburg Cognitive and Behavioural ALS Screen (ECAS) – Addenbrooke's Cognitive Examination Revised/III
Attention/executive functioning	– Digit span forward and backward – Corsi's blocks – Stroop-Color Word Interference test – Trail making test – Hayling test – Wisconsin Card Sorting test – Tower of London/Hanoi
Constructive praxis, visuospatial functioning	– Rey–Osterrieth Complex Figure – Judgement Line Orientation – Visual Object and Space Perception Battery
Language	– Boston Naming test – Pyramids and Palm Trees test – Verbal fluency
Memory	– Free and Cued Selective Reminding test – Rey-Osterrieth Complex Figure
Social cognition	– Mini-SEA – Faux-pas Recognition test

(mainly associated to behaviour), while dorsolateral cortex (mainly associated to executive tests performance) is damaged later.

Two aspects deserve a commentary. Firstly, in patients with FTD, episodic memory was considered to be preserved in the early stages of the disease, and even its alteration constituted an exclusion criteria in the diagnosis. However, several studies have confirmed that up to 15% of cases of FTD had major alterations in memory, questioning the aspect of "relative preservation of episodic memory compared with executive functions" considered in the clinical criteria we use nowadays.

And secondly, another important aspect is about social cognition. Because this cognitive domain may be very early in the course of the disease (with symptoms such as absence of empathy, emotional coldness, inability to understand the point of view of others, intuit intentionality or difficulties of moral judgement), it is important to include in the protocols of cognitive assessment in patients with suspicion of the ALS/FTD spectrum.

11.4.2 Frontotemporal Dementia and ALS

The ALS-FDT association is generally characterized by a rapid progression of behavioural and cognitive symptoms that continue with motor symptoms within 1–2 years [32]. The behavioural symptoms present in this association resemble those previously described for bvFTD, and psychotic symptoms, delusional ideas and hallucinations can also be observed. In addition, these patients may develop language disorders, fundamentally characterized by non-fluent language, sometimes mixed, progressive aphasia, reaching even mutism [4, 33–35]. Spastic dysarthria in the setting of nfPPA is a specific sign of motor neuron disease, while apraxia of speech is highly suggestive of FTD associated to parkinsonism. Both bvFTD and nfPPA (but exceptionally svPPA) may be associated to motor neuron disease (Fig. 11.2).

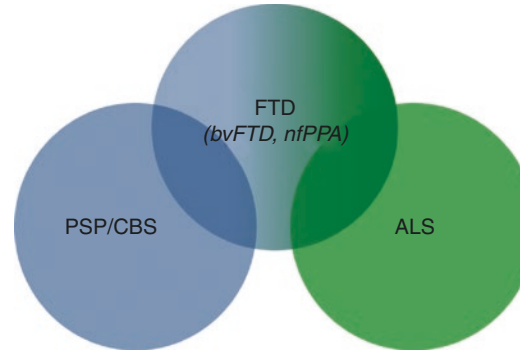

Fig. 11.2 Overlap between FTD and atypical parkinsonisms (PSP, CBS) on the one hand (tauopathies, in blue) and between FTD and motor neuron disease (TDP-43 proteinopathies, in green)

11.5 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is a neuroimaging technique that allows measuring and quantifying the degree of brain atrophy in different neurodegenerative pathologies. It is a useful tool both for the diagnosis and for the monitoring of various diseases, while it is a great help in establishing differential diagnoses [36].

In FTD, the pattern of atrophy evidenced by MRI seems to correlate very well with the different clinical subtypes. Thus, bvFTD is characterized by atrophy of the frontal lobe, the insula, the anterior cingulate and anterior temporal lobe; whereas svPPA is associated with asymmetric atrophy affecting the anterior region of the temporal lobe and nfvPPA with asymmetric atrophy of the dominant hemisphere in the anterior perisylvian cortex.

It has also been shown that some features of these patterns of brain atrophy, both grey matter and white matter, can be used to discriminate between the different clinical variants of FTD with high sensitivity and specificity. For example, temporal versus frontal atrophy can differentiate svPPA from other variants, and laterality of atrophy is an important feature in differentiating svPPA from other FTD subtypes [26, 37–39]. Interestingly, specific types of TDP-43 pathology

(type 1, 2 and 3) have been associated to more atrophy in specific regions of frontotemporal and parietal cortex [27, 40].

Regarding patients with ALS-FTD, MRI is characterized by evidence of the typical findings described for FTD associated in some cases with areas of increased signal in the white matter and pyramidal tract as well as rarely in the globus pallidus and thalamus—characteristic of motoneuron disease—although we must take into account that these findings are not obligatory nor pathognomonic. Some changes in grey and white matter have found with a certain overlap between ALS, ALS-FTD and bvFTD. In ALS-FTD, a temporal lobe impairment was observed in comparison to ALS with no FTD [41].

In early stages of the disease alterations evidenced by structural neuroimaging techniques may be absent. Thus, functional neuroimaging techniques such as positron emission tomography, which we describe in the next section, may be very useful.

11.6 Positron Emission Tomography (PET)

Functional nuclear medicine techniques such as PET have made a great contribution to the study and knowledge of the pathophysiology of several neurodegenerative diseases.

In neurology, the use of 2-deoxy-2-[^{18}F] fluoro-D-glucose (FDG) is able to determine cerebral energetic metabolism, improving the diagnostic sensitivity of dementias and other neurodegenerative diseases [42].

The pattern of brain metabolism is different in different neurodegenerative disorders, and FTD typically shows a predominantly medial frontal, lateral frontal, and temporal lobe hypometabolism. Several studies reported in the literature have shown the usefulness of this technique to differentiate FTD from Alzheimer's disease with high sensitivity and specificity, demonstrating a sensitivity of 90% and a specificity of 82% in a study with histological confirmation [43, 44].

In ALS, a typical pattern of frontal hypometabolism has been described. However, impairment in other areas have been reported, including hypometabolism in the dorsolateral prefrontal cortex, lateral and medial premotor cortex, insula, occipital cortex, anterior temporal lobe, parietal; and hypermetabolism in midbrain, corticospinal tract, superior temporal gyrus, hippocampus and cerebellum [45–51].

Interestingly, the degree of frontal hypometabolism and its extension has been associated to cognitive impairment in patients with ALS [52]. In a study, ALS patients with *C9ORF72* expansion showed a more generalized hypometabolism in comparison with patients without the expansion [53]. Furthermore, in recent years, other tracers have been released or are under development, such as amyloid [52] or tau tracers. These tracers will enable us to more accurately know the histopathological findings in vivo.

11.7 Other Techniques

New techniques are being studied to obtain an early diagnosis of these diseases. For instance, some studies mention the usefulness of motor-evoked potential gain for the assessment of corticospinal dysfunction, as well as the utility of the fasciculation ultrasound score as a simple and noninvasive technique to diagnosis of ALS [54, 55]. Recent studies highlight the suitability of transcranial magnetic stimulation (TMS) as a potential diagnostic biomarker, in order of identifying upper motor neuronal dysfunction, at earlier stages of the ALS disease process. Furthermore, this technique could even have a role in treatment, as has been suggested in pilot studies [56, 57].

Conclusions

The development of clinical symptoms of both ALS and FTD in some patients confirms a TDP-43 proteinopathy continuum. The assessment of the ALS-DFT complex from an integrative point of view (considering clinical, molecular, genetic and neuroimaging techniques) allows a more accurate approximation. Probably, in the coming years, some

subtypes of patients associated to specific histopathological and genetic findings could be identified taking into account clinical characteristics, cognitive profiles and neuroimaging findings.

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12 Nanocarriers for Diagnosis and Imaging of Neurodegenerative Diseases

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12.1 Introduction

12.1.1 Neurodegenerative Diseases

Neurodegenerative diseases gain an accelerating importance depending on the increase in the life span of mankind. Due to the increase in the life time of human, the probablity of observing neurodegenerative diseases increases remarkably. As very well known, neurodegenerative diseases primarily affect neurons resulting in progressive degeneration and/or death of nerve cells, nerve structure which may cause movement problems and mental disfunctioning such as loss in memory and desicion-making. Alzheimer's Disease (AD), dementias, Parkinson's Disease (PD), PD-related disorders, prion disease, Motor Neuron Diseases (MND), Huntington's Disease (HD), Spinocerebellar Ataxia (SCA), and Spinal Muscular Atrophy (SMA) constitute neurodegenerative diseases. However, AD, dementia, PD, and HD are the most widely encountered neurodegenerative diseases. AD is the most common neurological disorder in people older than 65. It has been estimated that the number of AD dementia patients will be 13.8 million in 2050 [1, 2].

PD is the degeneration or loss of dopaminergic neurons in striatum and substantia nigra of brain. Additionally, there is an abnormal accumulation of alpha-synuclein protein bound to ubiquitin in nerve cells in PD which accumulates proteins from spherical inclusions called Lewy bodies. Diskinezia, rigidity, and tremors are the common symptoms of PD. AD is the commonly observed form of dementia and it is the progressive loss of memory by depositing of tiny protein plaques and misfolding of beta-amyloid and tau proteins that damage different parts of the brain. Although PD and AD have different characteristics, they have a similar pathology mechanism involving the accumulation of abnormally folded proteins that leads to fibril formation and amyloidosis. HD is a progressive genetic disorder. It affects major muscles of the body leading to severe motor restriction and may cause death eventually [3–5].

Current treatment strategies of neurodegenerative diseases can only comprise alleviation of symptoms and helping to improve patients' life quality. For example, memantine and donepezil can be used to slow the progression of dementia symptoms in some people with Alzheimer's disease. Levodopa as gold standard in PD treatment can increase the brain's dopamine level to relieve some PD's symptoms; however, its long-term usage can cause some side effects such as on-off periods and diskynesia.

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_12

Due to the lack of effective therapy strategies in neurodegenerative diseases, researchers focus on enlightening the mechanisms of disease and searching on new imaging strategies by using a variety of noninvasive imaging modalities to perform early diagnosis with molecular imaging. Therefore, effective therapy strategies of neurodegenerative diseases can be achieved [4].

12.1.2 Noninvasive Imaging Modalities

A variety of imaging modalities with higher sensitivity and specificity have been developed benefiting from recent developments in the computer engineering and radiation physics in order to diagnose the diseases more accurately by getting better images.

The history of imaging modalities initiates with gamma camera at 1950s which was very similar to photoscanners called scintillation camera [6]. Gamma camera was then functionalized with a rotating detection system with reconstruction algorithms and computers in 1976 called single-photon emission computed tomography (SPECT) [7].

A new age in the diagnosis in Nuclear Medicine and Radiology clinics was performed by invention of PET and other recently used imaging modalities to obtain more sensitive and accurate images. For both clinical and preclinical studies, different imaging modalities are used for nanomedicine researches that have different applications with various pros and cons for the diagnosis and imaging of various diseases. Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT) have some advantages such as higher sensitivity, proper for biodistribution studies, giving quantitative results and unlimited penetration. Limited spatial resolution, use of radioactive probes and lack of anatomical resolution are some of their drawbacks. Computed Tomography (CT) gives anatomical information with high spatial resolution, dynamic imaging and quantitative results. It has poor soft tissue contrast, low contrast agent sensitivity and causes radiation exposure. Magnetic Resonance Imaging (MRI) has high spatial resolution and high soft tissue contrast. Its diasadvantages include low contrast agent sensitivity and difficult quantification. Optical Imaging (OI) is mostly used for preclinical research purposes with high sensitivity. However, it has poor penetration depth, difficulty in quantification, and poor penetration depth. Ultrasound (US) imaging is a dynamic imaging modality with a high sensitivity. It has higher user dependency and low probe versatility, and as another disadvantage whole body imaging can not be performed by US [8].

The launch of hybrid imaging modalities such as PET/CT, SPECT/CT, PET/MR in preclinical and clinical studies are relatively novel. Hybrid imaging modalities have the ability to fuse anatomical and functional images in molecular imaging to obtain more accurate images and better diagnosis by compensating the weaknesses of each individual imaging modalities [9, 10]. Hybrid imaging modalities provide imaging of patient with different imaging modalities at the same time, at the same patient but without causing any disturbance to patient and within a very short time to obtain more accurate images [11].

As a relatively new field, molecular imaging is the diagnosis of different diseases through characterization and quantification of biological processes at cellular and sub-cellular levels before initiation of any symptomatic changes. It is essential to detect a specific target molecule such as hormone, enzyme, lipid, etc., which may occur or increase during the disease process. The quantification of molecular changes related with the initiation, maintenance, and finalization of pathologic processes is significant in providing early diagnosis, prognosis, and early therapy of several diseases [12]. These molecular targeted, nanosized nanocarriers can be used for either diagnosis and/or imaging of different neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and stroke [13, 14].

Although variety of imaging modalities have different working principles, different pros and cons, using different radiocontrast/contrast

agents, all of them are needed higher signal intensity coming from the diseased tissue or organ in order to detect and diagnose the disease accurately. Depending on the launch of novel imaging modalities in Nuclear Medicine and Radiology clinics, novel, more specific, targeted and modified radiopharmaceuticals, radioligands, and radiocontrast agents are needed to specifically image the diseased area within the body for early diagnosis and by this way early therapy. This can be achieved by designing molecular, target specific, nanosized and functionalized radiocontrast/contrast nanocarriers.

12.1.3 Nanocarriers as Promising Approach for Both Diagnosis and Imaging

Benefiting from drug delivery systems as nanocarriers depends on decreased volume of distribution of drug molecules, enhanced accumulation and localisation of drug molecules in desired tissues, increasing bioavailability, organs or even cells, improving undesired properties such as bad taste and odor, and by this way decreasing adverse effects and side effects of drugs [15, 16]. Needing of drug company facilities with developed technology, high costs, and developed drug research and development facilities leading by qualified personnel and longer process times are some of drawbacks and challenging parts. However, these drug delivery systems are very beneficial in decreasing dose and by this way increasing safety and efficacy and decreasing adverse reactions and toxicity of drug molecules [14, 17–19]. These passive and active targeted drug delivery systems as nanocarriers can also be used for diagnosis and imaging of several diaseases such as neurodegenerative diseases by modification of radionuclides and contrast agents. Therapy monitoring can also be achieved by using nanosized radioligands.

Liposomes, niosomes, micelles nanoparticles, nanocapsules, microparticles, dendrimers, colloidal gold, gold nanoshells, cyclodextrins, superparamagnetic particles, and carbon nanotubes are some of drug delivery systems and nanocarriers for not only therapy but also diagnosis and imaging of several diseases such as neurodegenerative diseases [14, 19–29].

The advantages and limitations of some of these drug delivery sytems as nanomedicines for either imaging or therapy have been given in Table 12.1.

Drug delivery systems as nanocarriers have proceeded a long way from first-generation delivery systems which were removed from blood circulation by RES organs such as liver and spleen [32]. They were mostly used for the diagnosis or therapy of RES organs. Second-generation drug delivery systems were modified with amphiphilic polymers such as PEG, chitosan, monosialoganglioside GM1, and glucuronide derivatives, and used for the diagnosis and therapy of other organs and diseases [33]. These small particle sized and hydrophilic polymer coated, passive targeted systems are called "*stealth*" systems [33]. Third-generation nanocarriers comprise modification of target specific ligands such as antibodies, antibody fragments, or peptides for active targeting [19, 34, 35]. Surface modification and architecture for specific targeting and proper radionuclide or contrast agent modification by optimum formulation are essential for designing target specific molecular imaging nanocarriers [14, 36–38].

These nanocarriers can be used for the diagnosis and imaging by modification of either a single radioligand/contrast agent or two or more diagnostic radiocontrast/contrast agents in order to increase signal intensity to obtain more accurate images of neurodegenerative diseases as dual modality or multimodality nanocarriers. Additionally, both the diagnosis and therapy can be achieved by using single nanosized sytems called theranostics. Theranostics give the chance of molecular imaging and therapy monitoring [39]. Early diagnosis and imaging of different neurodegenerative diseases and by this way early therapy and therapy monitoring can be achieved with a great success by designing novel molecular, target specific, nanosized and functionalized radiocontrast/contrast nanocarriers.

Table 12.1 Nanocarriers and application platforms [30, 31]

Nanocarrier type	Composition/structure	Properties	Applications
Lipid vesicles	Liposomes, micelles	Can carry hydrophobic cargo, biocompatible, typically 50–500 nm	Drug delivery and imaging
Dendrimer	PAMAM, etc.	Low polydispersity, cargo, biocompatible	Drug delivery and imaging
Polymeric particles	PLGA, glycerol, chitosan, DNA; monomers, copolymers, hydrogels, etc.	Some biodegradable	Drug delivery; passive release (diffusion), controlled release (triggered) and imaging
Quantum dots	CdSe, CulnSe, CdTe, etc.	Broad excitation, tunable emission, typically 5–100 nm	Optical imaging
Gold particles	Spheres, rods, or shells	Biocompatibility, typically 5–100 nm	Hyperthermia therapy, drug delivery, and imaging
Microbubbles	Microbubbles	Low circulation residence time, typically 100–700 nm	Ability to target, drug delivery, diagnosis in combination with ultrasound, biocompatible
Magnetic particles	Iron oxide or cobalt-based; spheres, aggregates in dextran or silica	Superparamagnetic, ferromagnetic (small remanence to minimize aggregation), superferromagnetic (~10 nm), paramagnetic	Contrast agents (MRI), hyperthermia therapy
Silica particles	Spheres, shells, mesoporous	Biocompatibility	Contrast agents, drug delivery (encapsulation)
Carbon-based particles	Carbon nanotubes, bucky balls, graphene	Biocompatible	Drug delivery and imaging

12.1.3.1 Nanocarriers for Imaging of Neurodegenerative Diseases

A variety of nanocarriers can be used for the diagnosis and molecular imaging of different diseases like neurodegenerative diseases. The diagnosis and imaging of neurodegenerative diseases can be performed by different imaging modalities by using different modified, targeted, nanosized single/multimodal and single/multifunctional radiocontrast or contrast nanocarriers [40]. A schematic representation of multifunctional nanocarriers was given in Fig. 12.1.

Human neural progenitor cells (hNPCs) have the ability to differentiate into cells of the neural lineage, and it is essential in neurodegenerative diseases. Bernau et al. [42] formulated superparamagnetic iron oxide (SPIO) nanoparticles labeled with hNPCs to increase iron content for MRI imaging. This study is essential in enlightening alternative methods for cell detection [42]. Indole-3-carbinol (I3C)-loaded poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles were formulated for antioxidant and neuroprotective effect which is essential in neurodegenerative diseases [43]. BBB impermeability of I3C was improved by preparation of Tween 80 comprising nanoparticles in in vitro cell culture studies [43]. Apoptosis is related to many diseases including incurable neurodegenerative diseases. Roy et al. [44] used a novel method to prepare annexin V antibody (AbA5)-modified graphene quantum dots (GQDs) including photoluminescence properties in order to label and in vivo imaging of apoptotic cells in live zebrafish (Danio rerio) by which found very effective [44]. Translocator protein (TSPO) functionalized luminescent silica coated QD nanoparticles (QD@SiO2 NPs) were prepared for molecular fluorescent imaging of mitochondria. TSPO is generally overexpressed at outer mitochondrial membrane in some pathological conditions such as neurodegenerative diseases and cancers like glioma. These target specific, nanosized silica coated QD nanoparticles were observed as effective imaging agents in vitro [45].

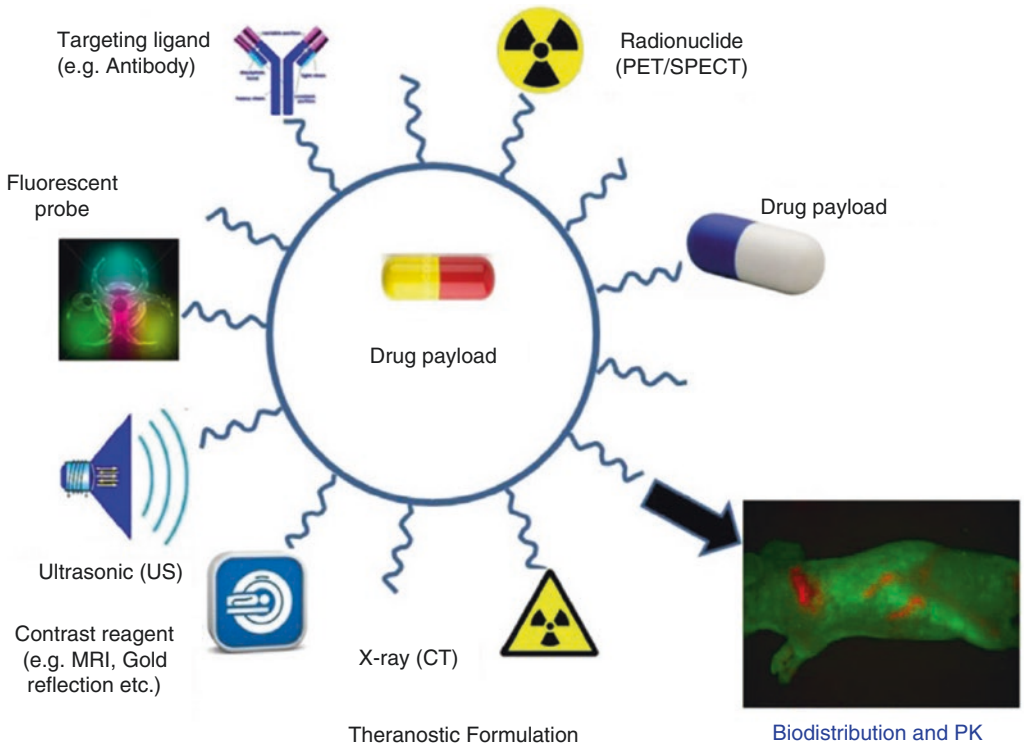

Fig. 12.1 Multifunctionalized nanocarriers for different purposes (Taken and modified from [41])

Amyloid targeted nanoliposomes were formulated for the therapy of AD. These immunoliposomes were prepared by modification of a mAb against Aβ-peptides (Aβ-mAb) and two different curcumin-lipid derivatives on the surface of liposomes. It was observed that these liposomes strongly delayed amyloid peptide aggregation and found potential [46]. Superparamagnetic iron oxide nanoparticles can be used for the diagnosis and imaging of BBB dysfunction related to tumors and other neuroinflammatory pathologies, cerebral ischemia or stroke, multiple sclerosis, traumatic brain injury, and epilepsy. AD is also related with neuroinflammation comprising the BBB so ultrasmall superparamagnetic iron oxide nanoparticles (USPIONs) can be used for imaging and diagnosis of AD by penetrating BBB to the some extent [47]. Yang et al. [48] formulated Aβ1–42 peptide loaded into ultrasmall USPIOs to diagnose and image AD by MRI. Amyloid plaques were successfully identified in mice. Nanosized, Congo Red (ability to specifically bind to amyloid plaques that have extensive β-sheet structures), and Rutin (a phenolic antioxidant) loaded magnetic nanoparticles were formulated as theranostics for both detecting amyloid plaques by MRI and for therapy of AD by controlled release of Rutin by H_2O_2 response to prevent oxidative stress. These theranostic agents found effective in AD diagnosis and therapy in in vivo and in vitro studies [49].

Similar to AD, early diagnosis of PD is also crucial for early and effective therapy of PD. The diagnosis and imaging of PD before the initiation of disease symptoms such as slowness in the movements, rigidity in the muscles, and malfunction in the posture at the level of molecular alterations is crucial [50, 51]. Rhodamine-B conjugated multimodal iron oxide nanoparticles were prepared to image PD. It was observed that about 5×10^5 labeled mesenchymal stem cells were

efficiently imaged with MRI in short term after infusion in the brain striatum of rats developed PD [52]. A fluorescent nanoparticle was prepared for both the diagnosis and therapy of neurodegenerative diseases such as PD. These nanoparticles include fluorescent core/shell CdSe/CdS quantum rods for specific targeting and they were aimed to controlled release of dopamine. These multifunctional nanoparticles showed potential efficacy [53].

Gold (Au) nanoparticles (colloidal gold) can be used for both bio-imaging and as photonics due to their unique optical properties which arise after interaction of light with electrons on the surface of Au nanoparticles [54]. Au nanoparticles can also be used for dissolving amyloid aggregates in weak microwave fields [55]. The dissolving of Aβ aggregates and prevention of Aβ aggregations were performed by local thermal energy formation at a molecular level when surrounded by a weak microwave field [55]. Au-doped TiO_2 nanotube arrays were formulated as a photoelectrochemical immunosensor for the diagnosis of α-synuclein specific in PD [56]. Nanosized, PEG-coated, ^{99m}Tc-labeled and pramipexole encapsulated, neutral and positive charged liposomes and niosomes were formulated for both diagnosis and therapy of PD. The formulation and characterization studies of both teranostic formulations were found proper and potential for further in vivo studies in 6-OHDA lesioned rats [57, 58].

Conclusion

The incidence of the observation of neurodegenerative diseases is on the rise depending on the increament in the life span of human. Therefore, early diagnosis and imaging of different neurodegenerative diseases is very essential especially at molecular level before initiation of symptomatic alterations in order to enhance life quality and standard of patients and to provide early and more effective therapy.

Depending on the development of novel, more sensitive imaging modalities, more specific and active targeted, single/multifunctional contrast/radiocontrast nanocarriers are getting under research increasingly and investigated by combination of modification, chemical and biological design. Apart from many pros of nanocarriers, by eliminating a few cons such as lower stability and toxicity, highly effective, target specific, next generation contrast agent/imaging probes can be developed. The design of novel, effective, BBB permeable, multifunctional contrast/radiocontrast agents is not only crucial for early diagnosis and imaging of neurodegenerative diseases, but also therapy monitoring, identification of molecular targets in the brain and understanding of tissue properties and targeting mechanisms, tracing of biodistribution and pharmacokinetics of drug molecules.

Due to the multidisciplinary studies of medicine, pharmacy, chemistry, and computer engineering, preclinical and clinical studies of these novel, target specific, BBB permeable, multifunctional contrast/radiocontrast agents may take market authorization and enter to the market in the near future. Therefore, its reflection to Nuclear Medicine and Radiology clinics will help to obtain more accurate and early diagnosis by molecular imaging of neurodegenerative diseases such as AD, PD, and stroke.

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13 Parkinson Disease Therapies and Drugs

Rodolphe Hajj

13.1 Introduction

Parkinson disease (PD) is the second most common neurodegenerative and progressive disorder characterized by dopaminergic deficiency due to the degeneration of dopamine-producing neurons that control motor movements [1]. It is characterized by α-synuclein aggregation and deposition as well as by a multitude of molecular disturbances in different areas of the brain [2]. Main characteristic PD symptoms include rigidity, tremor, bradykinesia and postural instability [3]. Other neurotransmitter systems together with the loss of non-adrenergic, serotoninergic and cholinergic neurons are also responsible for non-motor symptoms [4], such as cognitive decline, depression, sleep abnormalities and gastrointestinal disturbances.

Two hundred years after the first description of PD, all currently approved drugs are symptomatic with low to fair benefit, and do not affect the progression of the disease [5]. This could be mainly explained by the poor understanding of the pathogenesis and the pathophysiological mechanisms involved in the aetiology of PD that limit the discovery of more effective symptomatic and disease-modifying agents.

Therapies of PD involve drug treatment, neurosurgery and supportive therapies. In this chapter, I will focus on currently established therapies of PD with the most common drugs and give some examples of drugs still ongoing research and development. Cell and gene therapies will be discussed later in this book.

13.2 Pharmacotherapy for Parkinson Disease

Following the establishment of the clinical diagnosis and after a deep discussion with the patient and relatives about the disease and its implication on daily life, an appropriate treatment should be decided. Chosen pharmacotherapy must take into account the age, the physical impairment by the time of diagnosis, as well as motor complications that can be related to the treatment. The therapy must target first motor symptoms, then ideally non-motor complications that are out of the scope of this chapter.

The currently used pharmacotherapy for PD consists mainly in dopamine replacing therapies due the deficit of production and action of dopamine in the nigrostriatal region of the brain. Other non-dopaminergic therapies are also available (Fig. 13.1).

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_13

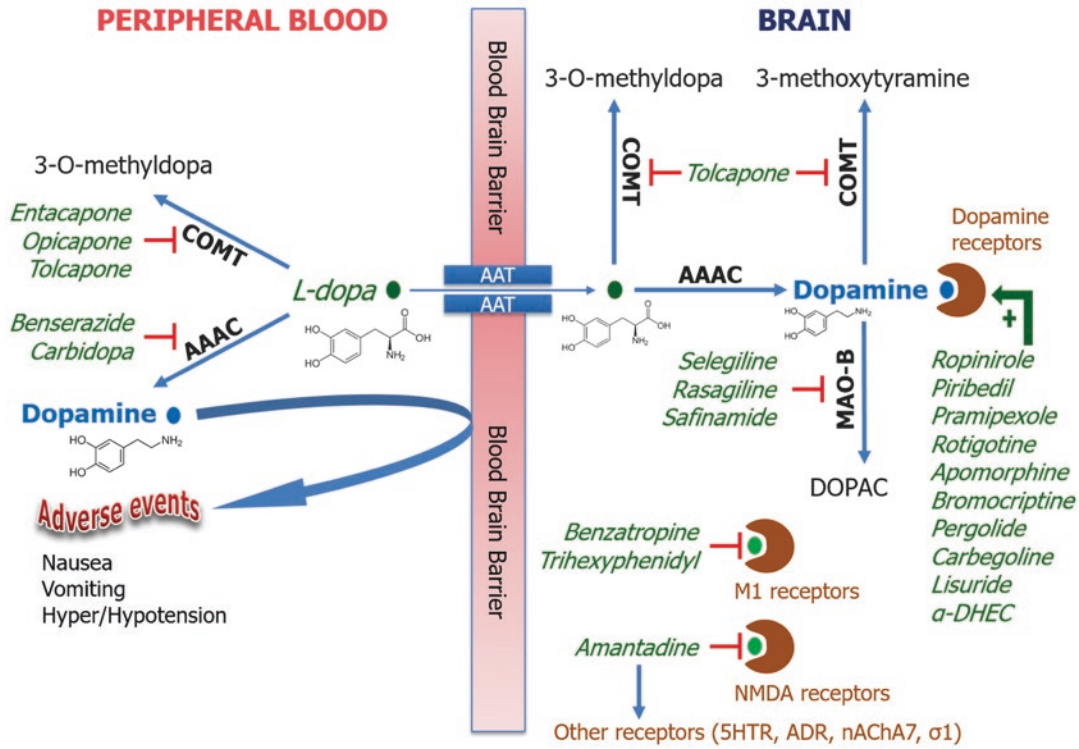

Fig. 13.1 Pharmacotherapy for the treatment of motor symptoms in Parkinson disease. Approved drugs are presented in green. L-dopa is administered mainly orally with an AAAC-I that does not cross the brain barrier to make it more available for the brain. COMT-I may also prolong its half-life and its availability to cross the blood–brain barrier via the amino acid transporter (AAT). Once in the brain, COMT-I (if a safe drug is available) will similarly prevent partly the degradation of L-dopa leading to its major conversion to dopamine that is responsible for the positive clinical effect by binding dopamine receptors. To increase the level of dopamine, MAO-B inhibitors are used to prevent its partial degradation into 3,4-Dihydroxyphenylacetic acid (DOPAC). COMT-I can also contribute the same way. To mimic the effect of dopamine, dopamine agonists are also used alone or in combination to L-dopa. Other secondary therapies act on the muscarinic receptors type 1 (M1), or on *N*-methyl-D-aspartate (NMDA) receptors to moderately alleviate motor deficits in patients. Red lines: inhibition. *α-DHEC* dihydroergocryptine, *5HTR* 5-hydroxytryptamine receptors, *ADR* adrenergic receptors, *nAChA7* nicotinic acetylcholine alpha 7 receptors, *σ1* sigma-1 receptors

13.2.1 L-Dopa

Currently, the gold standard for the treatment of PD is L-dopa (L-dihydroxyphenylalanine), used as a precursor for the replacement of the loss of dopamine in the brain of affected patients. It is always combined with a decarboxylase inhibitor to avoid its conversion to dopamine in the blood stream and increases thus its original half-life. L-dopa is today the most efficacious and efficient symptomatic treatment for PD, devoid of any neuroprotective activity. However, its chronic long-term use is often associated with the development of motor complications such as dyskinesias [6], thought to be due to the repetitive stimulation of dopamine receptors. It was even shown to induce oxidative stress, in PD patients [7], that might have a negative impact under long-term treatment.

L-dopa therapy is recommended in all stages of PD whether patients present non-motor complications or not. Its clinical efficacy was shown to work better or at least equivalently to other dopaminergic drugs [8]. However, the need of an adjunction therapy after manifestation of L-dopa side effects seems to be unavoidable.

One way to decrease the appearance of L-dopa-induced side effects was to formulate it as an infusion in the jejunum (Duodopa) in order to stabilize its concentration in the plasma and thus stimulate dopamine receptors in a constant fashion. This treatment can be prescribed later when the disease is in an advanced stage where dyskinesias are highly manifested [9]. Infusion therapy still needs to prove its use and efficacy since the access to this therapy is not of general use for the time being.

13.2.2 Decarboxylase Inhibitors

Also known as aromatic amino acid decarboxylase inhibitors (AAAC-I), these drugs were introduced shortly after L-dopa in order to increase its efficiency in producing dopamine in the brain, and also to allow the use of L-dopa at lower dosages to decrease its subsequent side effects. Two AAAC-I are extensively used clinically, always in combination to L-dopa: carbidopa and benserazide. These drugs were primarily designed to act in the peripheral blood only, devoid of the capacity to cross the blood–brain barrier.

13.2.3 Catechol-*O*-Methyltransferase Inhibitors (COMT-I)

COMT-I possess a more simple and straightforward mechanism of action than monoamine oxidase inhibitors (MAO-I). Metabolism of L-dopa by COMT is the second important degradation pathway leading to the conversion of L-dopa to 3-*O*-methyldopa (3OMD). Entacapone is the most used COMT-I clinically that prevents peripheral degradation of L-dopa, thus improving the penetrating level of L-dopa into the brain and its conversion to dopamine. Recently in June 2016, opicapone was approved in Europe for the same use, while tolcapone is no longer used due to its toxic effect on the liver. COMT is implicated in the degradation of dopamine in the brain as well, which emphasizes the clinical meaningfulness of COMT-I by not only acting peripherally to increase the amount and half-life of L-dopa in the blood, but also improving the level of dopamine in the brain of PD patients.

13.2.4 Monoamine Oxidase Inhibitors (MAO-I)

MAOs are a class of inhibitors that slow the degradation of dopamine by inhibiting its conversion to DOPAC. Thus, MAO-I will increase dopamine in the brain of PD patients leading to the symptomatic improvement of motor functions. However, this was not the reason for which MAO-I were tested initially in the past. Indeed, it was thought that the metabolism of dopamine is generating oxidative stress responsible of nigrostriatal cell death and associated PD symptoms. Hence, the idea came to inhibit the observed oxidative stress by inhibiting the metabolism of dopamine, particularly by inhibiting the activity of MAO-B that was initially regarded as a major source of free radicals [10]. The first clinical trial with the MAO-I selegiline was based on this idea [11]. Later in subsequent clinical trials, selegiline began to be considered as a disease modifier when patients continued to show improvement after washing out the drug, but this concept was judge insufficient by experts [12]. Other MAO-I such as rasagiline with a wider mechanism of action was considered as promising for disease modification, but its modifying effect turned out to be disappointing clinically [13]. Another recently approved MAO-I, safinamide, proved its moderate efficacy clinically by a mechanism of action that is thought to be beyond a simple action on MAOs [14].

Therefore to date, MAO-I can support therapeutically L-dopa treatment as add-on by increasing dopamine in the brain and by adding other mechanistic components that could help in alleviating PD symptoms.

13.2.5 Dopamine Agonists

Dopamine agonists act by replacement or support to endogenous dopamine that is severely decreased in the striatum of PD patients. These

work similarly by binding and stimulating post-synaptic dopamine receptors. Ten dopamine agonists are currently approved for the treatment of PD and are administered to (1) treatment-naïve patients or as (2) add-on to L-dopa. As stated above, dopamine agonists do not work better than L-dopa and it was initially thought that these do not lead to the occurrence of frequent dyskinesias observed in patients treated initially with L-dopa [15]. However, this idea is not clear yet since long-term treatment and follow-up studies with dopamine agonists followed by the addition of L-dopa have still to be undertaken.

On the other hand, dopamine agonists are known to induce side effects of which profile and intensity depend on the type of the drug: ergot or non-ergot derived drug. Indeed, non-ergot dopamine agonists have been described to have a better safety profile than ergot drugs [16], leading to the idea of using non-ergot dopamine agonists before using ergot ones, depending on the efficacy and tolerability of the first administered drugs to patients.

13.2.6 Non-dopaminergic Drugs

This class of drugs is less used than therapies cited above and considered as secondary for PD treatment. Anticholinergic drugs and amantadine are among this class (Fig. 13.1). Amantadine is still used at advanced stages of PD to manage L-dopa associated dyskinesias and as a mild early anti-Parkinsonian drug.

13.3 Which Treatment Comes First for the Management of Parkinson's?

There is no definite choice in the initial pharmacotherapy to treat PD patients. Generally, patient's age (over or below 60 years old) and lifestyle should be taken into account. More particularly, the patient should participate in the selection of the first medication(s) after these have been well explained by the physician, regarding their short- and long-term benefits and side effects.

The most widely used initial medication in patients over 60 years old is L-dopa associated to an AAAC-I. However, the dose of this combination per day should be kept as low as possible in order to reduce the occurrence of motor complications such as dyskinesias.

On the other hand, the physician in collaboration with the patient may choose to begin the therapy with a dopamine agonist. As a general recommendation, non-ergot dopamine agonists are preferred first. Then, if these have moderate efficacy on motor symptoms, ergot-derived dopamine agonists may be tested with a close monitoring of adverse events. There is no general rule for the use of a given dopamine agonist or another. Each drug should be titrated in each patient to achieve a clinical efficacious response.

Last, MAO-I might also be used as initial treatment, but this is not usually the case since these are almost always used as add-on to L-dopa to manage complications. The same issue is for amantadine that can be used in early PD after other medications having been tested before. COMT-I are always associated at later stages to L-dopa for the management of motor fluctuations. Similarly, apomorphine is used in adjunction to L-dopa when severe motor complications occur. MAO-I and COMT-I can also be associated early to L-dopa to prolong its effect and thus reduce the possibility of occurrence of motor complications, but there is no general rule of using this scheme. All recommendations for the use of different approved drugs for PD were summarized by Ferreira and colleagues [17].

13.4 Drugs in Research and Development for Parkinson's

The therapeutic pipeline for the treatment of PD was growing in the last decades due to the absence of an effective safe, tolerable symptomatic and disease-modifying treatment. Many molecules are in their early phase of discovery and preclinical

research, with diversified mechanisms of action. Overall at the time of writing this chapter end 2016, the total number of therapeutics in all R&D stages for the treatment of PD and its complications is estimated to be higher than 250 (source GlobalData, https://healthcare.globaldata.com). Many non-dopaminergic compounds that reached clinical testing were summarized for their activity in animal models of PD by Stayte and colleagues [18].

New symptomatic therapies are currently ongoing clinical trials to treat motor symptoms in PD. Some still focus on the dopaminergic pathway, more precisely on dopamine receptors such as CLR-4001 (Phase 2, Clera Inc.) and KDT-3594 (Phase 1, Kissei Pharmaceutical) that agonises dopamine receptors type 2, and others focus on non-dopaminergic pathways such as those driven by adenosine A2A receptors that are selectively expressed in the striatum. The blockade of these receptors expressed on the striatopallidal neurons induces the inhibition of their release of GABA in the globus pallidus, potentially enhancing motor function in PD [19]. Istradefylline and tozadenant are examples of such molecules antagonising these receptors. Istradefylline was approved in 2013 in Japan but failed however recently in December 2016 to demonstrate its efficacy in an international Phase 3 trial for the assessment of PD motor symptoms, therefore questioning the successful future of other adenosine A2A-targeting drugs. Other mechanisms of action involve compounds acting on metabotropic glutamate receptors, such as PXT-2331 (Phase 1, Prexton Therapeutics), an agonist of mGluR4. These receptors are localized presynaptically on the striatopallidal neurons and on the subthalamo-nigral projections. mGluR4 activation is expected to decrease GABAergic and glutamatergic transmission to restore motor behaviours in PD.

On the other hand, disease-modifying therapies are also actively sought in order to slow the progression of PD. Caffeine (Phase 3, McGill University) for example is among the molecules tested to modify the course of the disease. Its mechanism of action is identical to molecules antagonising adenosine A2A receptors. Inosine (Phase 3, Massachusetts General Hospital) is another example of molecule tested to raise urate levels believed to be neuroprotective in PD. Isradipine (Phase 3, University of Rochester), a calcium channel blocker originally used for the treatment of hypertension, was repurposed in PD as a potential disease modifier. Simvastatin (Phase 2, Plymouth Hospitals NHS Trust), a lowering cholesterol drug, is also currently repositioned and tested for neuroprotection in PD. Since PD is related to α-synuclein, PRX002 (Phase 1, Prothena Corporation) is now testing as a humanized IgG1 monoclonal antibody directed against aggregated α-synuclein. Initial results showed that it decreased the level of α-synuclein in the blood of PD patients.

13.5 Why Drug Combinations Are the Key for the Treatment of Parkinson's?

Combination therapy is to date used routinely in the management of PD. Dopaminergic therapies are those mainly combined together to provide effective symptom relief and limited side effects associated with the higher doses of each agent [20, 21]. Dopamine agonists can be added on L-dopa and vice versa. When combined with other drugs from the pharmacotherapy of PD, L-dopa efficiency is enhanced at importantly lower dosages, but for a short time period of treatment. At later stages of the disease, L-dopa/carbidopa is also combined to entacapone to prolong its effect on motor symptoms.

Current combinational strategies for the treatment of PD involve up to 4 drugs together (without taking into account AAAC-I) and are commonly used to improve the symptoms and reduce the occurrence of side effects and motor complications of dopaminergic drugs, but still not efficiently and satisfactory. This comes from the fact that the sole mechanism of action of all dopaminergic drugs is the activation of dopamine receptors. Their combinations will hence act on the same receptors without any new brought mechanistic component, therefore mimicking a mono-therapeutic intervention. Since Parkinson's

is a complex multifactorial disease characterized by the disturbance of several molecular pathways, targeting one mechanism will most likely not achieve a satisfactory clinical response.

In the last few years, it became largely admitted that drug combinations would be the most reasonable solution to tackle any disease, especially neurodegenerative ones that are etiologically very complex [22–24]. Drug combinations are of particular relevance when they multi-target different molecular pathways and mechanisms of the disease. Very recently, Hajj and colleagues applied this very promising combinational strategy to treat PD by assuming that two drugs with different mechanisms of action could be more efficient when combined together [25]. More importantly, these drugs in combination were used at very low doses allowing to anticipate future concerns of side effects. They repurposed two drugs, acamprosate and baclofen, and showed that their combination named PXT864 provided neuroprotective activity in Alzheimer disease in vitro and in vivo models while restoring several altered hallmarks in AD [24]. Based on the fact that Alzheimer and Parkinson share genetic, molecular and cellular features, they explored the possibility that PXT864 could also be used for the treatment of PD as well. They demonstrated that PXT864 synergistically protected dopaminergic neurons in vitro, and then confirmed the efficacy of the combination on motor dysfunctions in the 6-OHDA rat model, relevant to PD [25]. The effectiveness of the combination did not only consist in a neuroprotective activity in PD rats, but also in a symptomatic one. They argued that PXT864 is capable of protecting dopaminergic neurons and normalizing the activity of the glutamatergic and GABAergic system reinstating the functioning of brain motor systems [26]. This, if not unique, is an example of how should drug combinations be designed for the therapeutical management of PD. Repurposed or not, drugs should be combined on the basis of targeting different disease mechanisms, and ideally should be synergistic in order to use them at low doses to overcome problems of adverse events.

Conclusions

L-dopa discovery half a century ago [27] was the most significant finding since it is still the main used therapy for PD. However, as discussed above, its symptomatic action is always accompanied by the discomfort of side effect motor fluctuations. Moreover, L-dopa does not possess the ability to protect neuronal cells in patients and thus devoid of a disease-modifying activity. Several therapeutic approaches have been developed to treat patients, but these are still lacking effectiveness and safety. Extensive research efforts have been carried out over years for the discovery of effective and robust treatments for replacement or combination to L-dopa, but most compounds that have proven their efficacy in animal models of PD [28] have failed in human clinical trials [29].

Presently, there is an urgent need for a disease-modifying neuroprotective therapy that should also possess a symptomatic action for the immediate restoration of motor dysfunctions that are already elicited at the moment of diagnosis. It would not be exaggerated to predict the success of a new symptomatic or disease-modifying therapy in the next two decades in replacement or support to existing therapies. This could only occur if the way of treating Parkinson disease is rethinked by designing new polytherapeutic treatments based on targeting multiple disease molecular pathways from the mechanistic standpoint. One obstacle should also be overcome for the success of such therapies: the development of a predictive animal model of PD capable of mimicking patients' neurodegeneration and symptoms. In addition, all developed therapeutics were only tested in these animal models focusing on the nigrostriatal pathway that do not model or take into account the disturbance of other neurotransmitter systems in the brain. Next generation drugs should consider other brain regions together with the current targeted ones, ideally by combining synergistic compounds.

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14 Therapeutic Application of Stem Cell and Gene Therapy in Parkinson's Disease

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14.1 Introduction

As advances in medicine continue to be made, important public health implications are now arising in an aging population with increased life expectancy. These implications are especially noticed in diseases of aging, of which PD is no exception. The prevalence and occurrence of PD amplified almost exponentially with age, and the number of people with the disease is projected to increase by more than 50% by 2030 [1].

PD is considered the majorly frequent movement disorder. It is well-known for its characteristic primary motor symptoms, which include rigidity, resting tremor, hypokinesia, and postural instability [2]. These symptoms are due to degeneration of dopaminergic neurons (DAn) in the *substantia nigra pars compacta (SNpc)*, which thereby causes a severe deficit of the neurotransmitter dopamine in the two target nuclei, the putamen and the caudate nucleus [3].

PD diagnosis is based on the presence of α-synuclein aggregates (a protein abundant in presynaptic terminals) called Lewy bodies [4]. Furthermore, other complex cellular disturbances such as synaptic damage, apoptosis, loss of trophic support, mitochondrial dysfunction, oxidative stress, and neuro-inflammation are also believed to be involved, contributing to disorder progression [5]. These latter disturbances may explain the lesser known secondary motor and non-motor symptoms that also affect patients with PD, and often manifest themselves years before the recognizable primary motor symptoms [1, 6].

The etiology of PD is still unknown, though different theories have been suggested. One theory, known as Braak's hypothesis, emphasizes the role of α-synuclein protein aggregates in disease progression. The theory proposes that these protein aggregates target specific induction sites in the peripheral nervous system which gradually spread to less vulnerable areas in the central nervous system, suggesting that PD may be a type of prion disease [4]. A more recent hypothesis called the "Threshold Theory" suggests that symptoms begin appearing when the functional store of neurons and their corresponding regions of brain can no longer compensate for neuronal loss. According to this theory, the first signs become apparent in less compensated systems of the peripheral nervous system, and later appear in the central nervous system [7]. Overall, it is becoming more clear that PD is a slow, complex, heterogeneous neurodegenerative disorder, affecting several different neuroanatomical areas, and could be influenced by a compounding of environmental risk factors (including pesticide

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_14

exposure, prior head injury, and rural living) and genetic factors (including mutations in genes such as *α-synuclein* (*SNCA*), *leucine-rich repeat kinase 2* (*LRRK2*), *PARKIN*, *PTEN-induced putative kinase 1* (*PINK1*), and *DJ-1 (Daisuke-Junko-1)* [1, 5].

Current treatment options are available, ranging from pharmacological therapy to invasive surgery, and some can have profound effects on increasing the excellence of life for some patients. The current gold standard treatment for PD is levodopa (L-dopa), a dopamine precursor that can cross the blood–brain barrier (BBB) and increases local levels of dopamine [2, 3]. Additional pharmacological treatments include other dopamine agonists, inhibitors of dopamine breakdown (inhibitors of the enzymes catechol-*O*-methyl transferase and monoamine oxidase), and anticholinergics, all of which aim to increase local levels of dopamine. However, these treatment options are often associated with side effects, and eventually cease to be effective [8]. In these cases, some patients qualify for deep brain stimulation (DBS), which has been developed to treat motor fluctuations and tremors resistant to pharmacotherapy [8].DBS is a reversible and adjustable system that involves the implantation of microelectrodes in specific areas of the brain (such as the globus pallidus or subthalamic nucleus), which are activated electrically by a neuro-stimulator, usually placed on the patient's chest [9].

Although these treatments can be highly effective at improving motor symptoms and, thereby, the quality of life of patients at first, they are purely symptomatic and none of them slow down or prevent the disease progression. In addition, few of treatments may develop undesired side effects of their own, like involuntary muscle movements, called dyskinesias. Therefore, alternative methods are currently being investigated, especially in the form of cell replacement and gene therapy.

Here we will review the most recent work being done with cell replacement therapy (CRT) derived from different types of stem cells, as well as advancements being made in gene therapy (GT). We will also review the rewards and disadvantages of each type of therapy and provide an update on ongoing clinical trials working towards the common goal of finding a cure for PD.

14.2 Stem Cell-Based Therapies for PD

Transplants of cellular suspensions obtained from fetal midbrain tissue (ventral mesencephalon, VM), containing DAn and its precursor in developing midbrain, have proven to be effective and safe for PD patients. Fruitful open-label trials have reported improved motor symptoms in a number of PD patients [10, 11], increased ^{18}F-DOPA uptake [12], and sturdy long-term graft survival lasting over more than one decade as shown by postmortem investigation, despite some grafted cells showing Lewy body formation [13–15]. Additionally, the grafted tissue re-innervated the host striatum and became functionally incorporated into the recipient circuitry [16].

However, practical issue in obtaining the enough tissue for a successful transplant in relation to ethical clearance and concerns make this strategy unpractical. Therefore, it is necessary to search for new cellular sources, and stem cells are the most widely used.

Stem cells are undifferentiated self-renewing cells keeping potential to differentiate to particular type of cells in the body. Due to these properties, these are currently measured as the excellent option for developing a uniform source of DAn to be employed in PD therapies.

However, in order to successfully apply stem cells to CRT for PD, they must first qualify for clinical use. To qualify, it is generally accepted that stem cells originated DAn need to be corresponding to those of human VM tissue, and upon transplantation, they must have capability to survive, re-innervate the striatum, and assimilate into the host's neural circuitry. Furthermore, they need to appreciably improve motor symptoms,

not cause undesired effects, and gather numerous safety requirements, like eradicate the risks of tumor formation, immunological responses and the development of involuntary movements such as dyskinesias [17–20].

Precursors of human DAn have been efficiently derived from different sources of stem cells including human Embryonic Stem Cells (hESCs) [21, 22], human induced Pluripotent Stem Cells (hiPSCs) [23, 24], human Neural Stem Cells (hNSCs) obtained from fetal [25, 26] or adult brains [27], and human Mesenchymal Stem Cells (hMSCs) [28].

14.2.1 Human Multipotent Stem Cells

14.2.1.1 Human Neural Stem Cells

These are multipotent stem cells with the limited capacity to differentiate to cells of ectodermal origin like neurons and glia. These stem cells can be acquired from fetal, neonatal, and adult brains or through designated differentiation of pluripotent stem cells. Neural precursor cells obtained from the human VM are measured as the best alternative for cell therapies in PD, however as discussed above their utilization is limited. Apart from high ethical concerns, these cells have unstable phenotypes, present reduced growth potential, and survive poorly after grafting [26, 29, 30].

Several methods have been formulated to optimize the expansion of these cells. These techniques include the development of neurospheres with the availability of growth factors such as basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF) [31] or with the addition of soluble factors like Wnt5a [32]; other strategies consisting transduction with an immortalizing gene such as v-Myc, c-Myc, or TERT [26, 33]. In spite of all these attempts, an efficient method to obtain sufficient mesencephalic dopaminergic neurons from NCSs for large-scale clinical application is still lacking.

14.2.1.2 Human Mesenchymal Stem Cells (hMSCs)

hMSCs are multipotent stem cells that may be found in varied adult tissues including bone marrow, adipose tissue, placenta, and dental pulp [34–37].These cells are quickly emerging as an encouraging new approach to regenerative medicine due to their universal availability in the body, as well as their extensive proliferative potential. These are stromal cells which showed multi-lineage differentiation potential to cells of mesodermal origin [38]. Recently, some authors have reported that hMSCs also contain trans-differentiation potential to cells of a neural lineage (ectodermal origin) [39]. However, this remains somewhat controversial. Results from these reports were contradictory and thus unable to verify the ability of these cells to properly integrate into the host-neural circuitry to form the synaptic connections [40].

Another property of MSCs is their ability to promote protection and repair by secreting a number of neurotrophic factors, growth factors, and cytokines (including VEGF, BDNF, HGF, IGF-1, TGF-β, β-NGF, FGF2, and GDNF). They can inhibit the release of inflammatory cytokines, thus contributing to immunosuppression and other immunomodulatory effects in the brain [41]. Furthermore, it has been shown that following systemic infusion in animals, these cells were found in injury sites, suggesting migratory capability towards damaged areas where these can then enhance repair processes. Finally, one of the greatest advantages of these cells is the possibility of isolating them from autologous sources, which would avoid the requirement for immunological regimes and also eradicate any ethical concerns [42].

Despite these facts, the use of hMSC in clinical trials has been confined, mainly due to the difficulty in obtaining homogenous populations of cells isolated from diverse tissues. However, some clinical trials have been approved using hMSCs isolated from bone marrow or adipose tissue. These clinical trials are investigating the

Table 14.1 Human stem cells used in clinical trials (www.clinicaltrials.gov) for treatment of Parkinson's disease

Stem cell type	Source	Identifier	Status	Transplant type	Method and target	Endpoint classification	Sponsor
hNSCs	Fetal ventral mesencephalic tissue	NCT01898390	Phase 1	Allogenic	Intracerebral implantation	Safety and efficacy study	University of Cambridge
		NCT02538315	Phase 0	Allogenic	Intracerebral implantation	Observational	University of Saskatchewan
		NCT01860794	Phase 1/2	Allogenic	Not provided	Safety and tolerability study	Bundang CHA Hospital
	Fetal brain	NCT02780895	Phase 1	Allogenic	Intraputaminal implantation	Safety study	Celavie Biosciences LLC
	Adult cerebral cortex	NCT01329926	Phase 0	Not provided	Not provided	Observational	NeuroGeneration
hMSCs	Adipose tissue	NCT01453803	Phase 1/2	Autologous	Intravenous and intranasal administration	Safety and efficacy study	Ageless Regenerative Institute
		NCT02184546	Phase 0	Autologous	Not provided	Observational	StemGenex
	Bone marrow	NCT02611167	Phase 1/2	Allogenic	Intravenous administration	Safety, feasibility, and efficacy study	The University of Texas Health Science Center
		NCT01446614	Phase 1/2	Autologous	Intravenous administration	Safety and efficacy study	Guangzhou General Hospital
hpESCs	Non-fertilized oocytes	NCT02452723	Phase 1	Allogenic	Implantation into striatum and substantia nigra	Safety and tolerability study	Cyto Therapeutics Pty Limited

Abbreviations: *hNSCs* human Neural Stem Cells, *hMSCs* human Mesenchymal Stem Cells, *hpESCs* human parthenogenetic Embryonic Stem Cells

efficiency of treatment of autologous and allogenic hMSCs in PD patients, focusing on their immunomodulatory actions and trophic properties (Table 14.1 and Fig. 14.1) [43].

14.2.2 Human Pluripotent Stem Cells

Pluripotent stem cells are specified by their unlimited self-renewal and the ability to differentiate into specialized cells from all three germ layers (endoderm, mesoderm, and ectoderm). However, risks associated with their use (ethical concerns, possible tumor formation, host immune reactions, and issues associated to appropriate differentiation) have led most countries to highly regulate their use, leading to a limited integer of clinical trials approved for the investigation of their therapeutic potential.

14.2.2.1 Human Embryonic Stem Cells

Human Embryonic Stem Cells (hESCs) are considered the most fundamental stem cells, as they are pluripotent by nature. They are obtained from the cell mass of the blastocyst, which derived in about 1 week after fertilization of the embryo [44]. These cells are therefore believed to be the best source for cell replacement therapies.

Recently, a derivative of hESCs, called parthenogenetic embryonic stem cells (phESC), can be obtained via the chemical or electrical activation of unfertilized oocytes (a process known as parthenogenesis) [45]. However, these cells lack the paternal contribution present during normal oocyte fertilization. This could make their inaccurate clinical use, as normal cell cycle progression and their differentiation could be affected. The very first clinical trial in 2015 with pluripotent

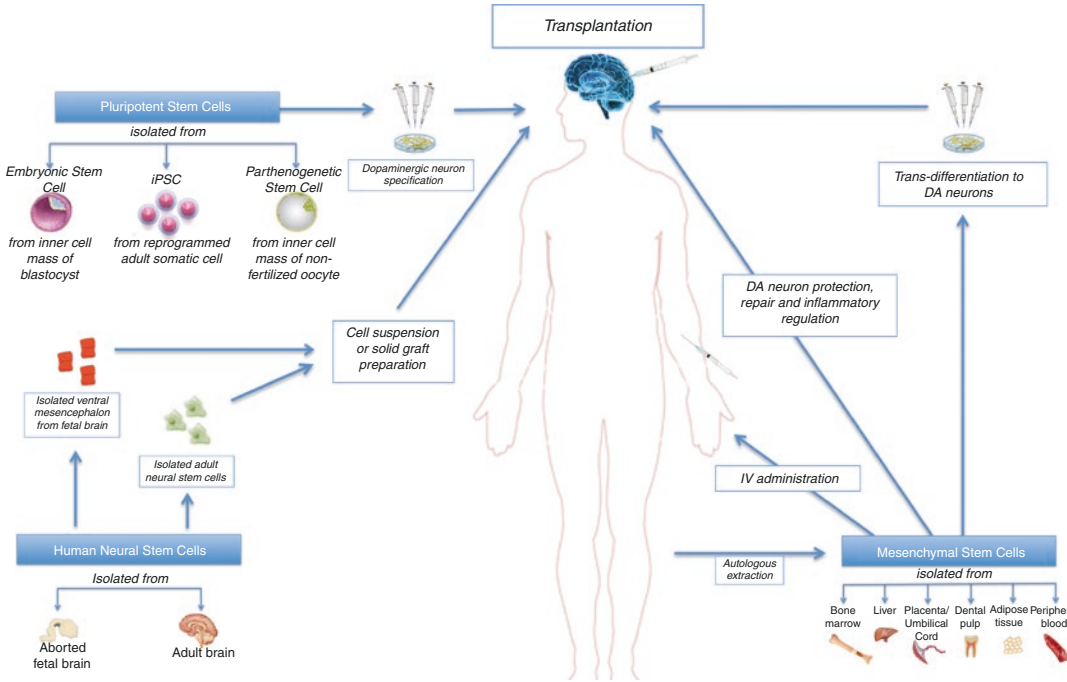

Fig. 14.1 Schematic representation of the different types of human stem cells available for treatment of PD. Several types of human pluripotent stem cells and human neural stem cells can be used for Cell Replacement Therapy (CRT) in PD after differentiation into dopaminergic neurons. Mesenchymal stem cells can be isolated from different tissues and infused intracerebrally or systemically for treating PD patients

stem cells for treating PD was approved, and employed the use of phESCs (Table 14.1 and Fig. 14.1) [46].

Although hESCs are considered the optimal source of cells for clinical use, they must meet several strict requirements to qualify for the treatment of PD. These cells must show safe and efficient differentiation to cells with the correct DAn phenotype. To attain this, numerous varied protocols have been developed. The most effective protocols include the formation of embryoid bodies, dual SMAD inhibition, and the use of Shh and Wnt to help renovate floor plate precursors into DA neurons [47, 48].

However, as optimal as hESCs may seem, several problems persist. These problems include extensive ethical concerns, tumor formation probability, phenotypic instability, and risks of host-graft rejection due to the HLA typing [49, 50]. Besides, it is critical to follow GLP/GMP (Good Laboratory and Manufacture Procedures) to eliminate issues of contamination and optimize the clinical application of these cells (Fig. 14.1).

14.2.2.2 Human Induced Pluripotent Stem Cells

Human induced pluripotent stem cells (hiPSCs) are cells which have been reprogrammed from adult somatic cells to reenforce a state of pluripotency. They share several similarities with hESCs, including the presence of pluripotency markers, cell morphology, epigenetic alterations, the capability to differentiate into cells of all three germ layers in vitro and in vivo (by forming teratomas), and the capability to create viable chimeras [51].

Takahashi and Yamanaka (2006) made a notable breakthrough in stem cell research with the discovery of these cells, which they achieved by

the addition of the four major factors Oct3/4, Sox2, Klf4, and c-myc to reinforce a state of pluripotency [51]. These cells can be utilized as in vitro cellular models of PD and also could be a foundation of autologous cells that would abolish ethical concerns.

Despite their assurance, several issues still bordered the use of hiPSCs on a large-scale clinical level. hiPSCs are anticipated to create the same challenges as hESCs, in addition to adversity of epigenetic memory of autologous tissue, genomic instability, the risk of teratoma formation, and issues with the strategy employed to reprogram the cells, especially when using integrating viral vectors [52]. Despite these limitations, the prospects placed on hiPSCs are still large. Several clinical trials including their use to treat PD are already intended and currently waiting for government approval. One is expected to start in Japan later this year led by the group of Jun Takahashi (Table 14.1 and Fig. 14.1) [53].

Alternative to the use of hiPSCs, some investigations are focused on the straight conversion of fibroblasts to neural stem cells (iNSCs) or to dopaminergic neurons (iDA), which could offer new opportunities for transplantation and modeling of PD [54–56].

14.3 Gene Therapy Strategies for Treatment of PD

Gene therapy is an approach for treating diseases based on the transfer of genetic material, involving DNA and RNA, to cells of an individual in order to modify cellular and/or biological function. Conventionally, gene therapy has been mainly thought of as a means of correcting a genetic defect. However, various other gene-based therapeutic schemes for disorders that are not primarily genetic in origin, such as PD, have been well thought-out and tested [57]. Depending on whether genetic information is directly introduced into the patient's own cells or if the cells are genetically customized in culture and transplanted into the patient, clinical gene therapy is classified as in vivo or ex vivo gene therapy, respectively [58]. Currently human clinical trials related to gene-based therapy in PD have utilized an in vivo approach, using viral vectors to innovate specific genes into the patient's own cells (Table 14.2) [59].

The most frequently used viral vectors are adeno-associated virus (AAV) and lentivirus, which have both revealed promise in experimental animal models, and are now considered potential tools for the implication of neuroprotective therapies in PD patients and other neurodegenerative disorders [60, 61]. All AAV-based gene therapies have shown to be secure and well supported in clinical assessments. More recently, lentiviral vectors have begun being evaluated in clinical trials of central nervous system (CNS) diseases as well [62]. These vectors have repeatedly been shown to transduce neuronal cells with high effectiveness, have low immunogenicity and are able to hold a larger therapeutic cargo than AAV vectors [63]. However, regardless of the vector used, there is a common limitation to both strategies: neither AAV nor lentivirus vectors can cross the BBB. Consequently, all gene therapy clinical trials currently approved for PD included the direct infusion of the viral vector via craniotomy into specified areas in the brain [64].

Several gene therapies have been evaluated in clinical trials of PD, and mostly utilized AAV and/or lentivirus vectors for gene transfer. Such strategies are aimed at modulating basal ganglia activity by transducing genes that alter neuronal phenotypes [glutamic acid decarboxylase (GAD)], increase dopamine production by transducing genes exhibiting neurotransmitter synthesis [aromatic L-amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), guanosine triphosphate cyclohydrolase 1 (GCH1)] and increase the survival of dopaminergic nerve terminals by transducing genes for neurotrophic factors like neurturin and glial cell-derived neurotrophic factor (GDNF) (Fig. 14.2, Table 14.2) [65, 66].

Table 14.2 Gene therapy used in clinical trials (www.clinicaltrials.gov) for treatment of Parkinson's disease

Gene	Viral vector	Identifier	Status	Method and target	Endpoint classification	Sponsor
GAD	AAV2	NCT00643890	Phase 2	Bilateral surgical infusion in subthalamic nucleus	Safety and efficacy study	Neurologix Inc.
	AAV	NCT00195143	Phase 1	Surgical infusion in subthalamic nucleus	Safety and efficacy study	Neurologix Inc.
	AAV	NCT01301573	Phase 0	Surgical infusion in subthalamic nucleus	Observational	Neurologix Inc.
AADC	AAV2	NCT01973543	Phase 1	MRI guided infusion to putamen	Safety and efficacy study	Voyager Therapeutics
	AAV	NCT02418598	Phase 1/2	Stereotactic infusion to putamen	Safety study	Jichi Medical University
AADC + TH + GCH1 (ProSavin)	Lentivirus	NCT00627588	Phase 1/2	Stereotactic injection to striatum	Safety and efficacy study	Oxford Biomedical
	Lentivirus	NCT01856439	Phase 1/2	Bilateral injection to putamen	Safety and tolerability study	Oxford Biomedical
Neurturin	AAV2	NCT00985517	Phase 1/2	Direct surgical injection to putamen and substantia nigra	Safety and efficacy study	Ceregene
	AAV2	NCT00252850	Phase 1	Bilateral stereotactic injection to putamen	Safety, tolerability, and biologic activity	Ceregene
GDNF	AAV2	NCT01621581	Phase 1	Bilateral convection-enhanced delivery to putamen	Safety and efficacy study	NINDS

Abbreviations: *GAD* glutamic acid decarboxylase, *AADC* aromatic L-amino acid decarboxylase, *TH* tyrosine hydroxylase, *GCH1* guanosine triphosphate cyclohydrolase 1, *GDNF* glial cell line-derived neurotrophic factor, *AAV* adeno-associated virus

14.3.1 Types of Gene Therapy in Clinical Trials for PD

14.3.1.1 Inhibition of Subthalamic Nucleus (STN)

The deficit of dopaminergic neurons in PD causes downstream alterations in circuits of the basal ganglia, involving depleted gamma-aminobutyric acid (GABA) input to the subthalamic nucleus (STN). Since GABA is the main inhibitory neurotransmitter in the brain, the reduction of GABAergic afferent fibers from the *globus pallidus* (GPe) causes excessive activity in the STN. In turn, the hyperactive glutamatergic efferent fibers cause alterations in thalamic and cortical motor neuron activity [64, 67]. Consequently, treatments that modulate activity of the STN can help relieve some parkinsonian symptoms [68]. Gene therapy containing inserted gene for glutamic acid decarboxylase (GAD) into the STN works by reducing glutamatergic neurotransmission and increases the GABAergic tone in downstream targets. GAD is the rate-limiting enzyme for GABA production pathway, and the activity of both GABA efferents to the STN and its targets within the basal ganglia circuitry are impacted in PD [69, 70].

After several findings of improvement obtained from AAV2-GAD injection in a standard rodent model of PD, there are various clinical trials using this strategy [71, 72]. Over the last decade, early phase clinical trials have utilized AAV2 vectors for GAD delivery to the STN in order to regulate STN firing rates and alleviate the foremost motor symptoms caused by nigrostriatal degeneration. To date, published results

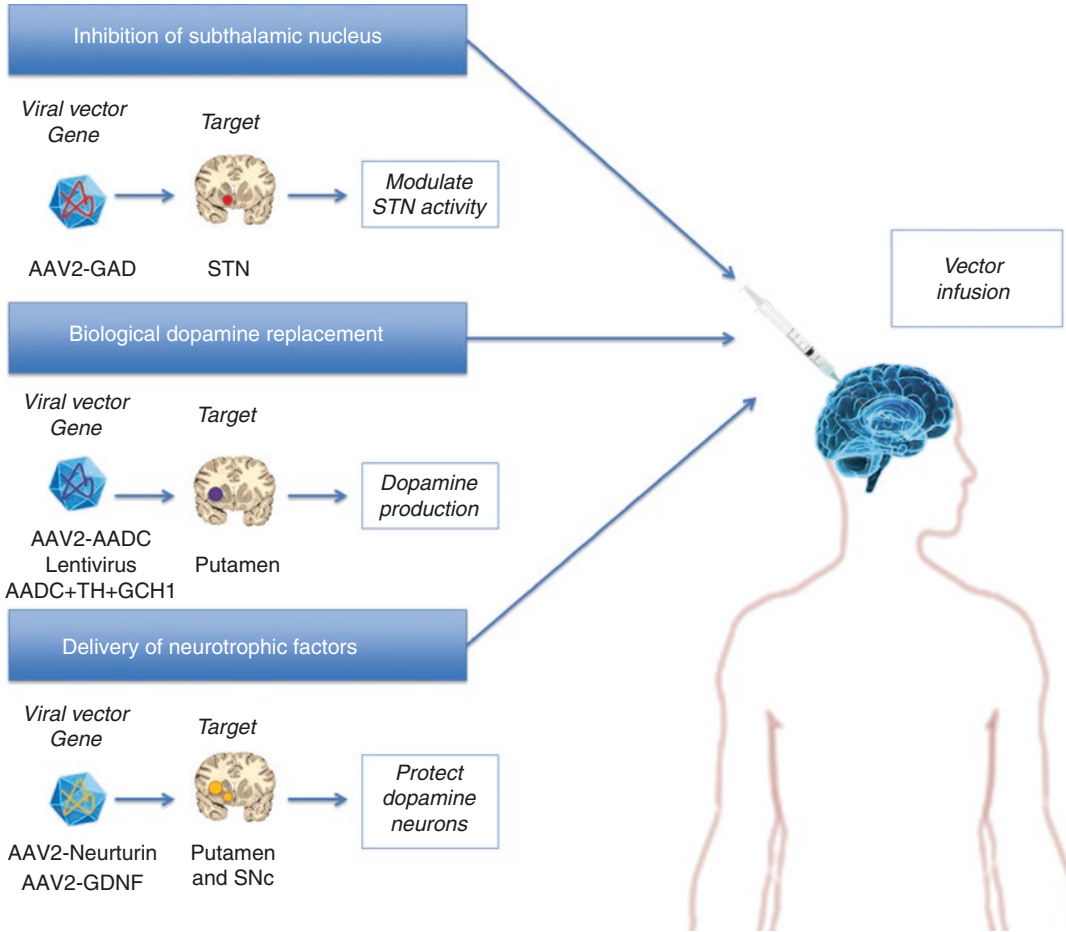

Fig. 14.2 Schematic representation of the main gene therapy strategies used for treatment of PD. (1) Modulation of subthalamic nucleus activity. (2) Biological dopamine replacement. (3) Delivery of neurotrophic factors

from clinical trials show significant improvements in both regional and network-related metabolic activity after unilateral insertion of AAV2-GAD in the STN, as well as good results in efficacy and prophylactic after bilateral infusion of AAV2-GAD in the STN [67, 70]. This shows guarantee for gene therapy to treat neurological disorders and justifies continued development of AAV2-GAD for treating PD.

14.3.1.2 Biological Dopamine Replacement

Dopamine replacement has been the standard pharmacotherapy to treat motor impairment in PD. At initial stages of the disease, this type of treatment can provide great benefit and symptom relief to patients, but as the disease progresses, severe loss of nigrostriatal nerve terminals leads to decreased activity of dopamine-synthesizing enzymes [73]. One approach of gene therapy for treating motor symptoms of PD focuses on enhancing the efficiency of the conversion of levodopa to dopamine. The therapy utilizes aromatic L-amino acid decarboxylase (AADC), the rate-limiting enzyme that converts endogenous or pharmacologically administered levodopa to dopamine, and its activity decreases with the progressive loss of nigrostriatal neurons [74].

Gene therapy consisting of insertion of the AADC gene inside the caudate putamen works to reinstate dopamine synthesis in situ within the striatum, where dopamine is most required.

Along with AADC, tyrosine hydroxylase (TH), an enzyme to synthesize L-DOPA from L-tyrosine, and guanosine triphosphate cyclohydrolase 1 (GCH1), the first enzyme in the biosynthesis of an essential TH cofactor tetrahydrobiopterin, are essential for proficient levels of dopamine [74].

Positive results of recovery obtained from AAV2-AADC injection in in vivo models of PD have led to several clinical trials using this type of gene therapy [75, 76]. Early phase clinical trials have utilized the AAV2 vector for insertion of AADC into the putamen in order to continue dopamine production in PD patients. Until now, good results have been obtained in efficacy, safety, and tolerability [73, 77]. Similarly, other clinical trials have used a lentiviral vector to incorporate the genes namely AADC, TH, and GCH1 into the caudate putamen. In addition to good safety and tolerability obtained, these studies showed improved motor behavior in patients with advanced PD and suggest that this strategy has the capacity to treat neurological disorders (Fig. 14.2, Table 14.2) [62].

14.3.1.3 Delivery of Neurotrophic Factors

The two previous gene therapy strategies are aimed at treating symptomatic effects of PD through normalizing basal ganglia circuits or by increasing dopamine production. However, gene therapy has also been employed to execute therapies aimed at modifying effects of the disease. These perspectives have utilized applied genes of neurotrophic factors to restore function of dopaminergic neurons and to slow disease progression [64].

Gene therapy containing insertion of the glial cell-derived neurotrophic factor (GDNF) gene or the neurturin gene into the putamen and *SNpc* has been tried, in order to hold the purpose, survival, and neurite outgrowth of nigral dopaminergic neurons.

It has been shown that GDNF and neurturin improve function and protection of dopaminergic neurons in in vivo models of PD [78, 79]. However, improvements have not been obtained in double-blind clinical trials with PD patients infused with GDNF directly to the putamen, probably because of inappropriate distribution of trophic factor throughout the target region [80]. This has led to the exploration of viral vectors for delivery of GDNF or neurturin genes as a substitute to GDNF infusion. After repeated findings of recovery obtained by AAV2-GDNF or AAV-neurturin injection in PD animal models, clinical trials using this type of gene therapy have been permitted and are currently in use. Early phase clinical trials have utilized AAV2 vectors for distribution of the neurturin gene into the putamen and *SNpc* to restore function of dopaminergic neurons in patients with PD [81]. Good results have been obtained showing efficacy and safety, and justify continued development of AAV2-neurturin for treating patients with advanced PD (Table 14.2, Fig. 14.2) [65, 81, 82].

14.3.2 Risks of Gene Therapy

Treatment of human disease by utilization of gene therapy has shown varying results over the last two decades. With a committed effort, various challenges are being overcome and there have been many hopeful clinical trials showing efficacy [83]. However, gene therapy contains several risks which need to be measured before conducting clinical trials.

One potential risk of in vivo gene therapy is insertional mutagenesis, where the administered gene could potentially be inserted into the host genome, inducing neoplastic transformation of the host cells. Another potential risk is unregulated abandoned production of the targeted protein, provoking several undesired effects. In addition, gene therapy might induce autoimmune and inflammatory responses, mainly from the use of certain viral vectors [64]. There are strategies to mitigate these risks, and tracking them should be carried out regularly in clinical trials.

14.4 Conclusions and Future Directions

The existing treatment alternatives available for most neurodegenerative disorders, including PD, only offer temporary symptomatic relief

and could not prevent the disease progression. Therefore, the expansion of cell replacement and gene therapies may provide considerable benefits for PD patients. To achieve this, however, a number of requirements must be met. First, in terms of cell therapy, better standardization of surgical methods and tissue sources needs to be established. Second, the immunological response to tissue grafts needs to be better understood to avoid rejection. Third, clinical trial design needs to be improved and fourth, the best source of cells for DAn differentiation needs to be found. However, immense progress has been made in the development of hiPSC technology that allows improved development of viable DAn, and mounting work with hMSCs is also increasing their clinical potential, especially in terms of their trophic and neuroprotective properties.

Furthermore, in the last few years, various gene therapy strategies with mixed responses have been introduced in clinical trials for the treatment of PD. However, compiling data suggest that gene therapy may be safe and well-stood in PD patients. That said, several risks need to be corrected and certain problems must be solved, such as determination of the optimal dose and target, modulation of gene expression, and finding the appropriate patient population to study to help advance gene therapy as a therapeutic alternative for PD.

Though more work needs to be done to confirm the efficacy and safety of such cell and gene therapy approaches, numerous clinical trials are currently being conducted, utilizing different strategies of CRT and GT for treating PD (Tables 14.1 and 14.2). These clinical trials are an exciting start towards improving current treatment options, and encourage more work to be done to eventually find a cure for PD.

Acknowledgements The authors wish to thank members of their laboratory for their research work and fruitful discussions. Research at the authors' laboratory was funded by the MICINN-ISCIII (PI-10/00291 and MPY1412/09), MINECO (SAF2015-71140-R), and Comunidad de Madrid (NEUROSTEMCM consortium; S2010/BMD-2336).

Author Disclosure Statement: The authors declare that they have no competing interests.

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15 Targeting Glucocorticoid Receptors: A New Avenue for Alzheimer's Disease Therapy

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15.1 Introduction

Alzheimer's disease (AD) is age-related devastating neurodegenerative disease specified by a progressive impairment in cognition associated with synaptic and neuronal deficit, and the evidences of neurofibrillary tangles (NFTs) and senile plaques in brain [1, 2]. Senile plaques are made up of amyloid-β (Aβ) peptides and remain insoluble and extracellular, while NFTs are consisting of abnormal hyperphosphorylation of the microtubule-stabilizing tau protein [1, 2]. There are several forms of AD. Familial forms with known mutations of specific genes, representing less than 5% of AD cases, and sporadic forms representing more than 95% of patients, with unknown mechanisms, but with identified risk factors. The principal risk factor for sporadic AD is aging and could be double after every 5 years after the age of 65. Environmental stress also plays considerable role in pathology and could enhance the probability to develop AD [3–5]. Such perspective is supported by the fact that the symptoms of AD patients involving the cognitive impairment and psychiatric problems are associated with an early dysregulated hypothalamic-pituitary-adrenal (HPA) axis, with increased levels of glucocorticoids in cerebrospinal fluid in addition to plasma [6–9].

The HPA axis is implicated in the stress response, and could trigger the adrenal cortex to release the glucocorticoids (corticosterone and cortisol in rodents and humans). Such steroid hormones readily pass the blood–brain barrier and bind to glucocorticoid receptors (GR) and also to receptors of mineralocorticoid (MR) [10]. These two receptors are essential for physiological cellular activity and many functions of central nervous system like memory and learning [11]. While MR are essentially localized in the hippocampus, GR are more ubiquitous and are particularly found in several structures of the limbic system, which is vastly involved in psychological and cognitive functions, but also are imperative machinery of the neural circuitry arbitrating HPA axis activity [12]. Therefore, while structural plasticity in the prefrontal cortex and hippocampus may mediate the impaired cognitive functions, alterations in amygdala probably contribute to the affective facet of stress disorders [13]. In limbic structures also the dysregulated HPA axis activity and modification of GR functioning could be extremely toxic [14] and therefore,

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_15

contribute to the cognitive deficit and psychiatric problem that occur in AD patients. It has also been reported that such structures of the limbic system get affected in AD patients [15]. Moreover, the dysregulation described above is the largely common and well-authenticated neuroendocrine irregularity in stress-related disorders and also in depression [16], a prodromal stage and a component of AD pathology, and may be also an initiator event for incipient AD [17].

15.2 Studies in Human

This association between AD, stress, HPA axis, and glucocorticoids first came from observations in humans. Reports available showed the augmented basal level of circulating cortisol and inability to show cortisol suppression following a dexamethasone challenge, substantiating HPA axis deregulation in patients of AD [6–9, 18–21]. Estimation of cortisol levels in blood serum of AD patients appears to be relevant prognostic marker of disease occurrence. Previous study by Davis et al. evidenced the augmented levels of cortisol in the most severely demented patients [22]. Comparatively more recently, Csernansky et al. also showed that higher cortisol level of serum in the pre-dementia clinical stages of AD predicts a more rapid cognitive impairment [6]. In disparity, an increased level of serum cortisol was observed in the cerebrospinal fluid of AD patients, whereas in patients of mild cognitive impairment the levels were not significantly altered, suggesting that the increased level of cortisol in the cerebrospinal fluid is related to the progression of AD [20]. Chronic stress like loss of life partner or sleep deprivation in addition to memory impairment enhances the vulnerability to develop AD [23, 24]. AD patients, treated with prednisone (a glucocorticoid classically used for its anti-inflammatory properties), showed the increased behavioral decline in comparison to placebo-treated cohort. Another report evidenced a halotype in the gene of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD), an enzyme involved in the activation of glucocorticoids, which increases the risk to develop AD by six times [25]. Likewise, it appears that lots of molecular neurodegenerative mechanisms and cascades identified in humans that trigger the pathogenesis of major depression are also involved in the AD etiology (in particular, chronic inflammation, neurotrophin deficits, and HPA axis dysregulation) [17, 26]. Involvement of environmental factors has been observed for about 25–40% risk for AD development [27].

All these observations in human evidenced that lifetime events, chronic stress, stress-related disorders, and glucocorticoids are involved in the AD etiology and could be thought of as an important risk factor for AD development [17, 26–31].

15.3 Studies in Animal Models

In addition to the observations in humans, many proofs implicating HPA axis dysfunction, glucocorticoids, stress, and stress-related disorders in AD came from studies conducted in rodent models. In chronic animal models of AD (transgenic models), glucocorticoid levels and stress affect the grade of the pathology. For instance, chronic behavioral stress augmented the senile plaque pathology and hastened the initiated cognitive impairment in transgenic Tg2576 and APP-CT100 mice [32, 33]. Socially isolated APP/PS1 mice showed the increased impairment of spatial working memory in relation to increased Aβ in the hippocampus region [34]. Chronic mild social stress or chronic stress-level glucocorticoid administration in 3xTg AD mice triggered the amyloid precursor protein (APP) misprocessing and increased Aβ levels in addition to neuronal damage in hippocampus and cognitive decline, further stimulated the Tau hyperphosphorylation and its accumulation [35, 36]. The corticotropin-releasing factor receptor (CRF1) in Tg2576 mice was also linked with the progression of Tau pathology, suggesting an additional link between stress, HPA axis, and AD [37].

In acute models of AD, in which an oligomeric solution of Aβ-peptides is injected in cerebral ventricles or directly in brain structures, amyloid toxicity modified the HPA axis activity. Indeed, we showed a clear non-functional HPA

axis in one of these acute models induced through single intracerebroventricular (icv) administration of an oligomeric solution of an Aβ fragment ($oA\beta_{25-35}$) [38, 39]. This Aβ fragment found in AD patients and originated from proteolysis of senile plaques [40–42] induced a broad pattern of central modifications suggestive of the human physiopathology [38, 39]. Previously we reported that activation of the HPA axis remains associated with an alteration of the balance between expression of MR and GR. An alteration of the adaptive response to acute stress and an interruption in the GR nucleocytoplasmic shuttling suggested a progressive sequestration of this receptor in the cellular nucleolus [43]. In addition, chronic treatment with corticosterone before the icv injection of $oA\beta_{25-35}$ exacerbated amyloid toxicity (*unpublished results*), suggesting a potential positive synergy between glucocorticoids and amyloid-β peptides.

15.4 Glucocorticoids and AD

Glucocorticoids and GR are particularly involved in the regulation of a few parameters engaged in the etiology of AD [28, 29]. Since glucocorticoids act synergistically with glutamate, dysfunction of the HPA axis could be enormously harmful, especially at the hippocampus level through causing the excitotoxicity, neuronal death, neuroinflammation, oxidative stress, and cognitive impairment [14, 44–50]. In humans, the deregulation of HPA axis with chronic glucocorticoids hypersecretion appears to exhibit the harmful effects in aging, and also in several diseases like AD, Cushing's syndrome, and depression [51, 52]. In fact, it appears that plasma levels of cortisol correlate with the severity of hippocampal atrophy and therefore may contribute to the impaired cognition and occurrence of psychological symptoms that take place in neurodegenerative pathologies and particularly in AD [51].

The impact of glucocorticoids on misprocessing of APP and Aβ pathway could be due to various harmonizing events. A glucocorticoid response element (GRE) in the promoter regions of APP and β-APP cleaving enzyme (BACE1) genes was reported in few studies [53, 54] which proposed that glucocorticoids and GR may directly mediate transcription of APP and BACE1 genes, offering augmented Aβ levels. Reports are scarce regarding the role of insulin degrading enzyme (IDE) which offer clearance of Aβ. However, it was shown that glucocorticoids alleviate Aβ degradation and clearance by astrocytes through increased expression of APP and decreased levels of Aβ degrading proteases [55]. The IDE mediated regulation of glucocorticoids was also reported in aged macaques [56] and in the $A\beta_{25-35}$ experimental model [57].

In the same line of evidence, glucocorticoids and stress seem to provoke hyperphosphorylation of Tau and its accumulation in neurons [35, 58]. It seems that exogenous glucocorticoids also potentiate the capability of chronically infused $A\beta_{1-42}$ which further increased the Tau hyperphosphorylation and thus their cytoplasmic accumulation associated with AD pathology [58]. Thus, as recently reviewed by Vyas et al., information on the mechanisms underlying the relationships between stress, glucocorticoids, and Tau is only emerging [31]. Indeed, in vitro studies show that the effects of glucocorticoids and stress are mediated through enzyme glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5 (CDK5) which are known for their involvement in Tau phosphorylation and the subsequent disruption of microtubules related to AD pathophysiology [31, 59]. Glucocorticoids exposure increase Tau aggregation by affecting its turnover through a reduced level of molecular chaperones (Hsp90 and Hsp70), responsible for its degradation [60]. Interestingly, both of these heat shock proteins also serve to uphold GR in high affinity state, suggesting that these proteins could be the key point at which glucocorticoids and GR signaling interconnect with the cellular machinery and regulating degradation of Tau [31]. In addition, it seems that chronic stress enhances caspase-3-mediated truncation of Tau at c-terminus and causes its anomalous conformation [31]. Such level of abnormal tau facilitates the nucleation and employment of other molecules of Tau into formation of neuropathogenic

aggregates [61, 62] before formation of NFT [61, 63, 64]. Such effect of stress at the hippocampus region seems to be mediated in part by the CRF1 receptor [65].

The interruption of the GR nucleocytoplasmic shuttling as previously evidenced [43] seems to be dependent on the phosphorylation condition of GR [28, 29, 66], underlying another link between AD and glucocorticoid regulations. GR get phosphorylated on serine and threonine residues by kinases involving mitogen-activated protein kinases (MAPK), cyclin-dependent kinases (CDK), and GSK-3β. Such phosphorylation of GR modulates its transcriptional activity, as well as affects its nuclear and/or cytoplasmic shuttling within cells [67]. For instance, it was reported that Jun amino-terminal kinases (JNK) inhibited the transcriptional activity of GR [68–70]. Since previously, the role of these kinases is reported in AD [71], it would be obvious to hypothesize that along with other predicted mechanisms, the inactivation of central GR could be under the control of these kinases. This hypothesis could explain, at least in part, the interruption of the GR nucleocytoplasmic shuttling as reported by us previously [43], but also the resistance observed in AD patients [9, 18, 29].

Apolipoprotein E (ApoE), a chaperone protein particularly implicated in the metabolism of lipoproteins and transport of cholesterol [72]. It has been reported that ApoE in the brain remains involved in regeneration, neuroprotection, and also in development [73]. ApoE is also a known amyloid-β peptide-binding protein highly involved in its clearance. It also facilitates microglial phagocytosis and proteolytic degradation by enzymes like neprilysin and insulin degrading enzyme [74–76]. ApoE is a sturdy genetic hazard for AD particularly ApoE-ε4 allele [77–79]. ApoE seems to be also implicated in the maintenance of activity of HPA axis and particularly in the synthesis of adrenal steroids. Indeed, Raber et al. evidenced in a rodent model of ApoE-deficient mice (ApoE−/−), an age-dependent deregulation of the HPA axis by affected functions of the adrenal gland. In fact, in addition to develop neurodegenerative, behavioral, and metabolic alterations, these mice present an age-related amplified basal adrenal corticosterone level and peculiarly augmented plasma corticosterone levels after restraint stress [80]. Accordingly, alterations in the level or activity of ApoE, by the presence of a specific allele like ApoE-ε4 for instance [81, 82], could be involved in the deregulation of the HPA axis as evidenced in AD pathology, and thus contribute to the connection of HPA axis in the AD pathophysiology.

15.5 A Vicious Circle

All these observations evidenced that dysregulations of the HPA axis would possibly increase the levels of Aβ and Tau, hyperphosphorylation of Tau, resulting inevitably in a vicious circle whereby the pathological events increase the secretion of glucocorticoids. Such release of glucocorticoid further worsens the disease pathology [35, 83–86], In addition, it appears that aging, genetic susceptibility, pathologies associated with high levels of circulating glucocorticoids (chronic stress, depression or Cushing's syndrome for instance) or a stressful lifestyle could also be involved in the etiology of AD, by reinforcing this vicious circle [23, 30, 35] (cf. Fig. 15.1).

In view of above evidences, maintenance of glucocorticoid levels could be sufficient to counteract the harmful effects related to deregulated HPA axis. In fact, the inhibition of glucocorticoid activation with inhibitors like 11β-hydroxysteroid dehydrogenase type 1 may provide improvement in the verbal memory [87]. The inhibition of this enzyme may also offer improvement in memory related to social recognition, amnesia, and spatial memory performance [88–90].

15.6 The Anti-GR Strategy

Based on the different observations previously described in this chapter in humans and in experimental animal models, suggesting a dysregulation of GR functioning, several strategies targeting directly GR were tested and seem to have an

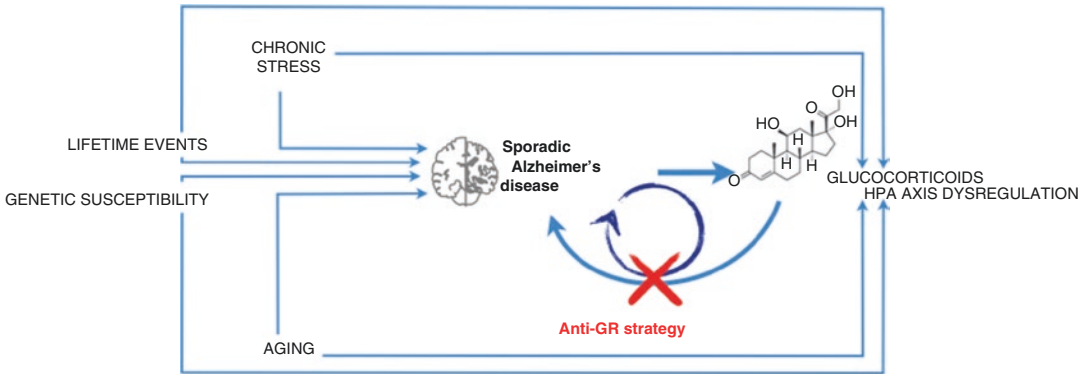

Fig. 15.1 The vicious circle and the anti-GR strategy in Alzheimer's disease

important remedial potential [91]. However, given the ubiquitous expression of GR receptors [92], their antagonists may implicate various undesired side effects and should be used with caution.

Studies tested an anti-GR strategy, with the nonselective antagonist of GR mifepristone (RU486) provided very hopeful results. In fact, chronic treatment with mifepristone in 3xTg-AD mice rescued the impaired cognition, markedly reduced the Aβ levels, as well as accumulated phosphorylated Tau [93]. Another study showed that Tg2576 mice treated with mifepristone rescued early episodic memory and synaptic plasticity deficits [47]. In the oAβ_{25-35} model, mifepristone [94] restored the basal circulating CORT levels, significantly reversed the synaptic deficits and hippocampal apoptosis [57]. Nevertheless, the partial reversal of impaired cognition, APP misprocessing in hippocampus, clearance of Aβ and neuroinflammatory processes are suggesting limits in its efficacy [57]. This limitation was also observed in human as treatment with mifepristone slows the progression of cognitive decline in AD patients [95] and increases morning levels of circulating glucocorticoids [96], suggesting its potential side effects and thus a limited therapeutic potential.

Several potent and selective GR ligand series were recently developed and seem to have an interesting therapeutic potential. The first nonsteroidal selective GR molecules come from anti-inflammatory studies. Indeed, glucocorticoids are normally used to take care of inflammatory diseases; however, the extended use of these steroids leads to a number of deleterious undesired effects that might be due to both activated and repressed GR target genes and are not directly involved in the inflammatory process. The objective of Abbott Laboratories (Abbott Park, IL, USA) was to create a series of molecules capable of preventing its detrimental side effects from anti-inflammatory activity [97]. The Abbott-Ligand 438 (AL-438) was derived by modification in synthetic progestin scaffold ensuing in the discovery of a new series of high affinity, GR-selective ligands. In comparison with prednisone, the steroidal anti-inflammatory molecule of reference, AL-438 shares the similar intracellular receptor binding profile as prednisone in that both ligands have less affinity for MR while high affinity for GR. Though, with a major difference, in MR-dependent reporter gene assays, AL-438 was a very weak antagonist, whereas prednisone is a full agonist at nanomolar concentrations. When tested in vivo, AL-438 retained significant anti-inflammatory effectiveness and potency analogous to steroids with its reduced negative side effects [97]. Unfortunately, this compound and its derivatives were never tested, to our knowledge, in AD studies.

Another series of selective nonsteroidal molecules (1H-pyrazolo[3,4-g]hexahydro-isoquinoline sulfonamides) derived by Corcept Therapeutics (Menlo Park, CA, USA). These GR ligands and in particular CORT108297 and CORT113176 exhibited high affinity for GR while for other nuclear hormone receptors like

PR (progesterone receptor), AR (androgen receptor), MR, and ER (estrogen receptor), no quantifiable affinity was observed [98–100]. Dexamethasone (DEX), the GR agonist augments the activity and expression level of tyrosine amino transferase (TAT) in liver cells. Both CORT108297 and CORT113176 act as full antagonists in the human liver cell line HepG2, as reported by their ability to inhibit the DEX-induced increased TAT activity. Contradictorily Beaudry et al. [98] showed that when these were tested in a similar assay by using rat hepatocytes, both compounds showed incomplete antagonism and partial agonist activity. An additional cell-based functional assay in the A549 cell line showed the effect of CORT108297 and CORT113176 on IL-1β-induced IL-6 production. Both compounds in this cell line exhibited the partial agonism and also acted as partial antagonists when tested in presence of DEX (Review in [57]). Thus, the underlying principle for testing this family of molecules in AD patients comes from their particular modulator properties. These molecules represent a class of ligands which could selectively abrogate the pathogenic GR-dependent processes in the brain, while also keeping the beneficial aspects of GR signaling. Onno Meijer's team reported that CORT108297 induced antagonizing effects were companied with a lack of negative-feedback inhibition of the HPA axis, which further proposed the probability of antagonizing various GR effects without affecting basal systemic glucocorticoid levels [94]. In fact, it appears that this family of molecules acts as "*selective GR modulators*" rather than pure antagonists [94].

Tested in triple transgenic mice, CORT108297 was able to reduce APP-C-terminal fragment (C83) and the Tau hyperphosphorylation via reductions in p25 levels [93]. In the acute experimental model, we showed that treatments with CORT108297 and CORT113176 reverse the hippocampal amyloidogenic pathway accelerated through the inhibition of BACE1 and the increase of IDE. Besides this, the selective GR modulators reinstate the hippocampal levels of synaptic markers, repeal the hippocampal neuroinflammation, apoptotic processes, restore the glucocorticoid levels in plasma, and thus consequently inhibit the cognitive impairment [57].

The diverse efficacy between all of these compounds (mifepristone, AL-438, CORT108297, and CORT113176) could be due to the their unlike selectivity and affinity for GR [97–100]. Such varied effect may also include the fundamental properties of GR nuclear receptors and their capability to differentially employ nuclear receptor coregulators after binding of ligands [94, 97, 101]. These coregulators form a varied group of transcriptionally functional proteins which could arbitrate the transcriptional effects of nuclear receptors which have tissue/ligand/cell-specific expression patterns, and display gene and receptor specific interactions [94, 102, 103]. Onno Meijer's team showed that each GR compound could induce a specific profile of interaction with these coregulators. They suggested, as previously envisaged by Coghlan et al. in 2003 and detailed by Onno Meijer studies more recently, that these particular profiles could explain the distinct efficacy and functionality of these GR ligands [97, 101].

Conclusions

All of the above discussed preclinical investigations designate the potential therapeutic effect of selective GR modulators as an effective treatment for AD patients and further highlight the impact of the glucocorticoid system as a regulator of Aβ accumulation, aggregation, and clearance, as well as of Tau hyperphosphorylation. Thus, this class of compounds, alone or in association, becomes captivating and relevant candidates in the treatment of AD.

This review also evidences that modulator molecules selectively targeting GR could abrogate pathogenic GR-dependent processes induced by a dysregulation of the HPA axis and retain beneficial and primordial aspects of GR signaling. Accordingly, it appears that a better knowledge of the specific molecular interaction profiles of GR compounds, in combination of their regional distribution in brain, may provide insights for dissecting the molecular degenerative signaling pathways underlying pathologies specifically related to

high levels of glucocorticoids. This strategy will participate to create new avenues of investigation on glucocorticoids and GR, and to exploit these avenues to develop novel preventive and/or therapeutic strategies to tackle disorders (neurodegenerative or not), associated with a dysregulation of the HPA axis.

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Multifarious Therapeutic Avenues for Alzheimer's Disease

16

Magisetty Obulays

16.1 Introduction

The etiology of AD pathology involves oxidative stress, deposition of extracellular amyloid beta (Aβ) plaques, formation of intracellular neurofibrillary tangles (NFTs), metal-mediated neurotoxicity, mutations in genes, neuroinflammation, hyperphosphorylation of tau, and apoptosis [1, 2]. In addition, hyperphosphorylation of tau and Aβ accumulation are associated with augmented levels of inflammatory cytokines such as interleukin1β and Tumor Necrosis Factorα (TNFα), perturbed calcium homeostasis, synaptic disintegration, and neuronal dysfunction eventually resulting in neuronal death [3, 4]. Since AD pathogenesis is more than a decade long process before diagnosis, there is a growing need to develop substantial theranostic agents. In line with this, metal-free nitroxide based MRI contrast agent has been developed for Aβ detection. It showed adequate brain penetration in turn demonstrating better affinity for Aβ [5].

Although manifold animal models were employed to study tau pathology yet there is a burgeoning need to develop newer models and novel imaging techniques to tease out appropriate therapeutic avenues [6, 7]. Transgenic AD models which have been extensively used in AD research replicate multiple etiological factors. However, they fail to show clinicopathological events of human AD, thus impeding the progress of therapeutic intervention significantly [8–16]. Growing lines of evidence suggest that human based models such as studies on pluripotent stem cells, neuronal and glial cultures yield robust outcome compared to animal models [16].

Microfluidics or organ on chip systems with multiple cell types are used for AD research and also open new avenues to study therapeutic compounds currently. However, staggering array of research approaches are required to unravel the molecular understanding thoroughly [16]. Growing lines of evidence suggest that nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing-3 (NLRP3) inflammasome provokes inflammation in neurodegeneration more specifically in AD and is the robust therapeutic target currently [17].

Multifactorial etiology with incomplete understanding and inability of therapeutic molecules to span BBB are the major lacunae in AD research currently [4]. Electroacupuncture curtailed Aβ levels significantly by provoking peroxisome proliferator activated receptor γ (PPAR-γ) which ameliorated AD symptoms in Sprague-Dawley rats [18]. Recent reports corroborated the amelioration of AD symptoms by modulating glucocorticoid metabolism in brain via inhibition of 11β hydroxycorticosteroid [19]. Cerebrospinal fluid neurogranin, an appropriate biomarker was found to be correlated with AD

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_16

pathological events such as reduced glucose metabolism and hippocampal volume [20]. Identification of appropriate biomarkers provides substantial impetus in the development of AD therapeutics [7, 21]. Although adequate research has already been done and currently in progress yet the number of studies escalated to clinical trials is significantly low.

16.2 Blood Brain Barrier

BBB impairment plays a pivotal role in neurodegenerative diseases such as AD [22]. BBB dysfunction being the primary event in aging human brain starts in hippocampus, thus resulting in cognitive dysfunction [22–25]. Tight junction architecture of BBB plays a pivotal role in limiting the entry of multifarious molecules into brain [22]. BBB considerably limits the entry of blood circulating therapeutic compounds into brain [26]. A few strategies employed to make brain entry of the therapeutic molecules such as antibodies directed against transferrin receptor or the insulin receptor showed safety concerns [26].

16.3 Astrocytes

Astrocytes play a pivotal role in the neurophysiology by maintaining blood flow, extracellular ionic balance, BBB and providing metabolites to neurons [27–29]. However, a few studies corroborated their role in neuropathological events [29, 30]. In line with this, they are one of the best therapeutic targets to overcome neurodegeneration currently [29]. It has also been reported that the meticulous therapeutic strategy requires a deeper understanding although the astrocytes are the mainstay [29].

16.4 Drugs

After extensive research for almost a century, only a few drugs rivastigmine (Exelon), galantamine (Razadyne and Reminyl), tacrine (Cognex), donepezil (Aricept), and memantine (Namenda) were approved by food and drug administration (FDA). In addition, these drugs merely ameliorate AD symptoms and slow the disease process in a few patients but in many patients they show poor compliance [31–33].

Despite the inadequate acetylcholinesterase (AchE) inhibition by galanthamine, it plays a vital role in strengthening the sensitivity of acetylcholine [34–36]. Furthermore, it attenuates Aβ aggregation by regulating the activity of beta site Aβ cleaving enzyme [36–38]. Non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen and celecoxib showed amelioration of cognitive decline [39].

16.5 Bioactive Compounds

Gypenoside (GP), a vital bioactive component of Gynostemma pentaphyllum, a traditional Chinese herb medicine showed reduction in M1 markers and augmentation of M2 markers leading to the deterioration of Aβ-induced microglial activation [40]. In another study, Vitamin D also has been found to play a pivotal role in the treatment of AD. Its potential neuroprotective properties have been explored in both human and animal studies [41]. Accumulating evidence emphasized that Vitamin D succeeds in reaching memory centers due to the existence of corresponding receptors in the brain [41–44].

It has been reported that the selective reuptake inhibitors such as fluoxetine provoke paracrine signaling regulated by transforming growth factor-β1, thus attenuating Aβ-42 induced toxicity [45]. Furthermore, Puerarin, an isoflavone glycoside and a popular traditional Chinese medicine showed substantial therapeutic effect against AD by attenuating the Aβ-instigated toxicity by provocation of estrogen receptor β [46, 47]. Its structural similarity with mammalian estrogen and ability to instigate estrogen receptors confer it the substantial protective efficacy [47–49].

16.6 Nanotechnology

Wealth of studies showed that 100s of therapeutic compounds failed in clinical trials due to low bioavailability and BBB constraints [33, 50]. A

few FDA-approved AD drugs being oral formulations repose a few gastrointestinal adverse effects, thus resulting in poor patient compliance [51]. In addition, the drugs fail to stop the progression of disease [16]. Almost for a decade, no new drugs have been designed to treat AD with a significantly high clinical failure rate of 99.6% [16]. To overcome these challenges and achieve better patient compliance, nanotechnology based DDS have been extensively used currently [50].

Redox nanoparticles employed to cure AD by improving the bioavailability of piperine yielded substantial therapeutic effects [2, 52]. Despite the difficulty in spanning BBB, recently designed Camelid single domain antibodies with variable heavy chain succeeded in diagnosing the extracellular amyloid plaques and intraneuronal NFTs [26]. They span BBB through receptor-mediated transcytosis.

16.6.1 Curcumin Nanoparticles

Recently designed curcumin-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles showed substantial neuroprotective efficacy against AD [53]. In addition, a curcumin-loaded low density lipoprotein (LDL)-mimetic nanostructured lipid carrier (NLC) modified with lactoferrin (Lf) showed enhanced BBB permeability with robust neuroprotective efficacy [54]. Transferrin functionalized polymerosomes succeeded in delivering curcumin to the brain and ameliorating cognitive dysfunction in intrahippocampal Aβ1-42-injected mice [55].

16.6.2 Liposomes

Liposomes employed for various diseases such as cancer showed significant therapeutic effects [56]. In line with this, rivastigmine-loaded liposomes showed enhanced therapeutic efficacy by augmenting the concentration and half-life of the drug in the brain of mice [57] and rats [33, 58]. Moreover, liposomal vaccines were also extensively used to treat AD. It has been reported that 15 aminoacid sequence of Aβ peptide coupled to fatty acid residues or phospholipid/PEG spacers showed enhanced neuroprotective efficacy against AD [33].

In summary, it is essential to develop appropriate nanoparticles which enhance the therapeutic efficacy of natural compounds such as curcumin. Since both natural compounds and nanoformulations with encapsulated natural compounds showed promising therapeutic efficacy in several studies, further studies on improving these biomaterials may reverse AD symptoms significantly. In addition, nanoparticles with multiple therapeutic effects such as drug-loaded redox-active nanoparticles which can scavenge reactive oxygen species and also show therapeutic effect of loaded drug can open novel therapeutic avenues.

16.7 Viral Vector Therapeutics

Multiple lines of evidence suggested that viral vectors are substantial therapeutics compared to nanotechnology based DDS due to their efficacy and safety [4]. With a view to achieve potential neuroprotection against AD, viral vector expression of Aβ cleaving enzymes, such as neprilysin [59, 60], or anti-Aβ single-chain antibodies to accomplish passive immunization [61] were extensively employed [4]. Furthermore, regulation of amyloid precursor protein degradation was accomplished by genetic transfer of siRNA for β-secretase [4, 62] or shRNA to curtail G protein coupled receptor for γ-secretase [4, 63]. Wide range of other viral vector therapeutics include anti-Apo E antibodies [64], inhibition of acylCoA-cholesterol acyltransferase [65], delivery of growth factors such as cerebral dopamine neurotrophic factor [66], insulin growth factor [67], brain derived neurotrophic factor [68], and nerve growth factor [69] to evade toxic injury to the brain. Despite the merits of viral vector therapeutics, a few demerits such as immune intervention, absence of appropriate entry receptors, and inappropriate cellular uptake of vectors impede their success [4].

Conclusion

Although etiology of AD has extensively been studied yet the understanding of panoply of molecular underpinnings is elusive. The primary molecular players involved in neurodegeneration at BBB level play a pivotal role in AD [22]. Considerable importance to the negative data and employment of human models are strongly recommended by AD researchers [16]. Novel nanomedicine such as Camelid antibodies with robust imaging ability becomes a corner stone to develop appropriate armamentarium for severe neurodegenerative diseases. However, wealth of studies showed that there is a burgeoning need to improve DDS for brain diseases since they are not as appropriate as they are for other diseases such as cancer [50]. Multifactorial etiology of AD needs a robust theranostic arsenal that entails the expertise from several disciplines such as medicine, pharmacy, and biology [2].

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Treatment Paradigms in Huntington's Disease

17

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17.1 Introduction

Huntington's disease (HD) is a neurodegenerative genetic disorder with a mutation in huntingtin (Htt) gene. This mutation is caused by an expanded cytosine-adenosine-guanine repeat in the gene. This mutation results in an abnormally long polyglutamine repeat in the huntingtin protein which is a major driver of this disease. The resultant phenotypic manifestations are uncontrolled as well as excessive motor movements in combination with cognitive and emotional defects [1]. The molecular pathology of this disease has been understood in the past few years which offer the potential of designing new treatment paradigms [2].

Drug discovery is a long (it takes about 12–15 years for an idea to be developed into a marketed drug) and expensive process (it costs US $500 million on an average to discover, develop, and market a drug). Within the disease therapy areas, discovery and development of drugs for treatment of central nervous system (CNS) diseases is a big stumbling block for the pharmaceutical industry. Not only discovery but especially Huntington's drug discovery and development is affected by some of these challenges [3]. The challenges are listed below:

1. There is a lack of good animal models that mimic human CNS diseases and therefore when a drug that appears to be efficacious in an animal model is tested in humans, the probability of success is low.
2. In most cases, understanding of CNS disease is still a work in progress and the utility of a target protein or gene is not completely understood because of poor disease models.
3. The blood brain barrier, i.e., brain endothelium, represents an often impregnable wall that does not allow drugs to get to the brain at sufficient concentrations to be efficacious.
4. The time frame for seeing significant efficacy of a drug candidate in clinical trials for CNS diseases is often long and therefore the trials are expensive.
5. The safety considerations of a drug are critical and the safety requirement bar is high because most CNS diseases afflict the elderly.

Despite these challenges, much progress has been made in treatment of CNS diseases although vast majority of these drugs are "symptomatic drugs." Drugs can be classified in two categories, those that are symptomatic and act only temporarily because they do not treat root cause of disease and those which are curative and may permanently cure or reverse

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_17

the disease. A major treatment gap in Huntington's is that the currently marketed drugs are mainly providing symptomatic relief but offer no benefit towards cure of the disease to the patient [4]. However, there is a strong possibility based on the current drugs in development pipeline that there will be better drugs specifically for this disease in the near future. When this happens, it will be a paradigm shift in the treatment from use of symptomatic to curative drugs.

17.2 Huntington's Clinical Disease Symptom Presentation

The symptoms are obvious and overt in the advance disease. Clinically they are segregated into motor, cognitive, and psychiatric disorders. The presentation of different symptoms is based on the stage of the disease. A brief and simplified summary of various symptoms at different stages of disease is as follows [5]:

I. Early Stage: uncoordinated movements, impairment of gait (locomotor), impairment in thought process, mood changes (cognitive), and sadness/unhappiness (psychiatric).
II. Mid Stage: speech/swallowing difficulty, impaired voluntary movements (locomotor), tendency of getting stuck on thoughts and behavior (cognitive), and insomnia, irritability, anxiety, indecisiveness, etc. (psychiatric).
III. Late Stage: chorea, dystonia, bradykinesia (locomotor), lack of awareness, behavioral disabilities (cognitive), and social withdrawal or suicidal tendencies (psychiatric).

Clinical assessment of the disease is a critical aspect of determining the stage of disease. The Huntington Study Group (HSG) has developed a uniform assessment tool to describe the clinical features of HD, which has been designated as Unified Huntington's Disease Rating Scale (UHDRS). The UHDRS is considered a reliable and validated method to measure outcomes of Huntington's clinical trials. The components of the UHDRS are: (1) Motor Assessment, (2) Cognitive Assessment, (3) Behavioral Assessment, (4) Independence Scale, (5) Functional Assessment, and (6) Total Functional Capacity (TFC) [5].

17.3 Pathogenesis of the Disease

Huntington's disease is caused by CAG repeats, leading to an abnormally long polyglutamine (polyQ) expansion in the Htt protein. The mutated Huntingtin protein (mHtt) has abnormal properties and is neurotoxic. mHtt triggers a cascade of pathogenic events that ultimately results in neurodegeneration. As shown in Fig. 17.1, the mHtt protein is misfolded and it aggregates into clumps [6, 7]. Essentially, mHtt engages in multiple pathogenic pathways leading to neuronal

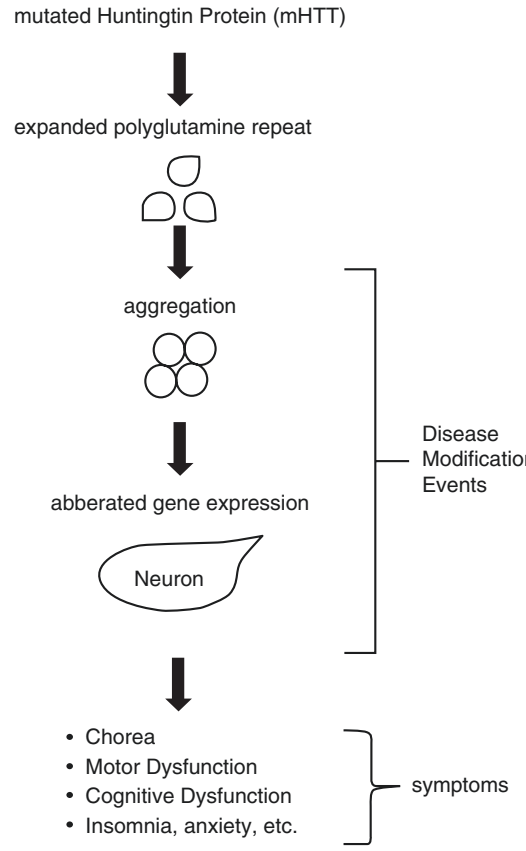

Fig. 17.1 This figure shows a simplified process by which mHtt may play a role in the disease process

Table 17.1 List of investigational drugs and details pertaining to their current clinical phase for Huntington's, mechanism of action and impact

Pipeline drug	Clinical phase	Mechanism of action	Impact in Huntington's
Deutetrabenazine (SD-809)	NDA approved	Vesicular monoamine transporter Type 2 (VMAT-2) inhibitor, thus decreasing available dopamine and reducing chorea	Reducing chorea
Pridopidine (ACR16)	III	D2 dopamine receptor antagonist, thus inhibiting excitatory hyperglutamatergic symptoms	Motor impairment
Selisistat (EX-527)	II	Sirtuin 1 (Sirt1) inhibitor, acts by restoring global acetylation, thus restoring mutant Htt (mHtt)	Restoration of the Htt mutation

dysfunction and ultimately neuronal death. A particularly attractive hypothesis for this dysfunction is that mHtt may cause increased oxidative stress within the neuron. This may induce aberrant gene expression followed by activation of caspase enzymes and apoptosis of neuronal cells [8–12]. Finally, there is neurotransmitter imbalance which produces symptomatic motor and cognitive abnormalities.

17.4 Current Treatment Strategies

Currently the drugs that are used for treatment of Huntington's mainly provide symptomatic relief to patients. Various symptomatic drugs that are used for treating different symptoms of Huntington's disease are presented below [13]:

(a) Suppression of Chorea: Tetrabenazine, Haloperidol, Clozapine, Clonazepam, Diazepam.
(b) Antidepressant/Anxiolytic: Escitalopram, Fluoxetine, Sertraline
(c) Mood stabilizer: Lithium
(d) Anticonvulsants: Valproic Acid, Divalproex, Lamotrigine

Symptomatic drugs are those that only treat the symptoms but not the underlying cause of the disease. Not only these drugs do not treat the disease-causing steps but since the symptoms are treated but not the disease, the disease may continue to worsen. In contrast to this, disease-modifying drugs intervene in a disease-causing step (s) to retard disease progression and/or stop disease process or reverse it.

17.5 Drugs in Clinical Development

Here we have focused on three investigational drugs that are currently active in clinical trials or have been recently approved. The profile of these investigational drugs, their current clinical phase for Huntington's and mechanism of action are shown in Table 17.1. Each of them is briefly discussed below:

17.5.1 Deutetrabenazine (SD-809)

Deutetrabenazine (SD-809) was approved by USFDA in 2016 to treat chorea associated with Huntington's after successful completion of Phase III clinical trials [14]. Deutetrabenazine is a deuterated analogue of TBZ, and deuterium (D)-substitution reduces the metabolism of the active metabolites, resulting in improved pharmacokinetic properties, and increased half-life [15].

Deutetrabenazine is an inhibitor of vesicular monoamine transporter Type 2 (VMAT-2), which leads to the early metabolic degradation of monoamines. This in turn leads to prevention of uptake of serotonin, norepinephrine, and dopamine into pre-synaptic neurons. This leads to reduction in hyperkinetic disorders like chorea [16].

17.5.2 Pridopidine (ACR16)

Pridopidine (4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine hydrochloride) acts similar to a dopamine D2 antagonist as well as through

N-methyl-D-aspartate (NMDA) receptors [17, 18]. Multiple rodent experiments have been conducted using pridopidine that confirmed the preclinical potential of pridopidine as a dopaminergic stabilizer [19, 20]. Recently, Sigma 1 Receptor (S1R) agonism has been proposed to be an additional mechanism of action for pridopidine [21].

In clinical trials, pridopidine has been efficacious on motor symptoms but has not shown improvement in cognitive function. In the trial conducted by Huntington Study Group HART Investigators (ClinicalTrials.gov Identifier: NCT00724048), this drug candidate showed that after 12 weeks of treatment the only dose that showed improvement on the modified motor score was 90 mg/day. Pridopidine was well tolerated at this dose [22]. However, in a recently concluded Phase II clinical trial (ClinicalTrials.gov Identifier: NCT02006472) the drug candidate showed a statistically significant reduction in disease progression from 26 to 52 weeks after treatment with pridopidine at certain doses as compared to placebo control. The efficacy was measured using Total Functional Capacity (TFC) [23].

17.5.3 Selisistat (EX-527)

Selisistat (EX-527, SEN0014196, 6-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide) is a selective Sirtuin 1 (Sirt1) inhibitor. It acts on NAD^+-dependent deacetylation mechanism, thus inhibiting histone deacetylases (HDACs) which in turn restores the gene transcription repression caused by mHtt [24, 25]. It has been demonstrated in genetic as well as phenotypic animal models that selisistat suppresses pathological effects of Huntington's [26].

Selisistat has been studied in a couple of clinical trials (ClinicalTrials.gov Identifiers: NCT01521832, NCT01521585), wherein it has been claimed to have satisfied the criteria of pharmacokinetics parameters, pharmacodynamic efficacy and tolerability up to 12 weeks of treatment [27]. This is one of the few investigational drugs that may act as disease-modifying agent based on the disease-modifying events as described in Fig. 17.1.

Conclusion

Discovering new and better drugs for rare diseases such as Huntington's is fraught with high risk both scientifically as well as from a business perspective. Firstly, the number of patients available for a clinical trial is less than optimal and therefore demonstrating a meaningful and statistically significant therapeutic effect is tough. Secondly, because the number of patients is less the profitability for the innovator has to be carefully thought thru. To this end, the USFDA provides incentives for developing drugs for rare diseases for pharma companies.

Despite such challenges, we believe that in the near future the likelihood of producing a disease-modifying drug is realistic [28]. As shown in the pipeline drugs described here, there are many "shots on goal" and therefore one should be hopeful of success. We should expect a paradigm change in the way this disease could be treated by the entry of disease-modifying drugs that can work together with the symptomatic drugs. Ultimately this could lead to improvement to the patient's health and longevity.

Acknowledgements Dr. Uday Saxena would like to dedicate this chapter to the memories of Dr. Eshwar Raj Saxena and Dr. K. Anji Reddy, his inspiration for being a scientist and an entrepreneur.

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18 Management of HD: Insight into Molecular Mechanisms and Potential Neuroprotective Drug Strategies

Puneet Kumar, Sumit Jamwal, and Anil Kumar

18.1 Introduction

Huntington's disease (HD) is a hyperkinetic movement disorder and dominant inherited neurodegenerative disorder caused by genetic mutation, resulting in an unstable expansion in polyglutamine stretch (CAG repeat) in IT-15 gene. IT-15 gene encodes for a protein known as huntingtin protein [1–3]. HD is characterized by mutant huntingtin protein (mHTT) induced selective degeneration of GABAergic medium spiny neurons in the striatum and is associated with motor (hyperkinetic movements and gait abnormalities) and non-motor symptoms (depression, memory impairment, and anxiety). HD has a worldwide prevalence of 5–8 per 100,000 people. A substantial progress has been made in last three decades about understanding the pathophysiology of HD and several theories have been put forward like mutant huntingtin protein (mHTT) protein aggregation, transcriptional dysregulation, mitochondrial dysfunctions, oxidative stress, apoptosis, and neuroinflammation but the exact mechanisms by which mHTT induces early GABAergic MSN degeneration are still unclear [1, 4]. Numerous studies have indicated that HD pathophysiology may begin both from autonomous processes within defenseless neurons and from alteration in neuron–neuron interactions, most exclusively at the level of the cortico-striatal neurons.

Clinically reported symptoms of HD in early stages include progressive weight loss, sexual behavior dysfunction, and alteration in the sleep cycle, whereas in the later stages HD is characterized by motor signs, progressive cognitive dysfunction, and impairment of the intellectual processes involved in thinking, decision, and memory. Due to progressive motor dysfunction, patients with advanced HD are unable to walk and have very less food intake. Life-threatening conditions in advanced HD result from injuries associated with serious accidents, and most of advanced HD patients ultimately surrender their life due to aspiration pneumonia [4].

During past two decades, significant advancement has been made to develop drugs for HD but still there is no drug available that can arrest the neurodegenerative processes involved in HD. A drug, i.e., Tetrabenazine, belonging to the class of dopamine transporter inhibitors has been

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_18

approved by FDA for treating dyskinetic symptoms in HD patients. This drug provides restricted relief from clinical symptoms and induces various ill effects like suicidal thoughts, depression, and sedation. Accordingly, innovative approaches are required to develop new drugs or agents that may halt neurodegeneration in HD. Other fruitful approaches like suppressing the expression of huntingtin gene and clearance of mHTT aggregates in the neurons might prove to be advantageous in the treatment of HD. In this regard, we have composed and conversed the data of recently assessed drugs in HD animal models on HTT gene expression, mHTT protein metabolism, mitochondrial dysfunction, BDNF level, apoptosis, neuroinflammation, and various other neurodegenerative processes in HD.

18.2 Huntingtin Protein: Structure and Function

The HTT gene encodes for huntingtin protein having a molecular weight of 348-kDa. HTT gene contains a specific DNA segment known as a CAG trinucleotide repeat and is composed of series of three DNA building blocks (cytosine, adenine, and guanine) that appear multiple times in a row. The normal repetitions of CAG segment appear 10–35 times within the gene [4]. Three different forms of huntingtin have been identified matching to human huntingtin protein amino acids, i.e., 1–386 (htt1), 683–1586 (htt2), and 2437–3078 (htt3). Recently, it has been found that the NH_2-terminus of htt1 is the most evolved part of huntingtin, while the COOH-terminus is most conserved portion among all animal.

Physiological HTT is universally expressed cytoplasmic protein and is found in all mammalian cells with uppermost expression reported in the brain. HTT is incriminated in numerous functions including neurogenesis, transcription, cell trafficking, axonal transport and in upregulation of the expression of neurotrophic factors, such as BDNF and NGF [5, 6]. In the brain, the lack of HTT protein gives explanation for some of the clinically observed symptoms of HD. HTT plays a pivotal role in the embryonic brain development, as relentless impairment of neurogenesis has been seen in HTT knockout mice. Even though the exact function of this protein is not understood, it appears to play multiple roles in the brain and is essential for normal brain development before birth.

mHTT is considered as key contestant in arbitrating the HD pathophysiology. mHTT consists of enlarged polyglutamine stretch (polyQ) with more than 40 CAG repetitions. The utmost expression of mHTT has been found in the striatum nuclei of brain. The unusual folding of mHTT protein leads to striatal neurotoxicity as mHTT is not degraded by ubiquitin-proteasomal system but its toxic protofibrils and fibrils are defused in aggregates and inclusions. These mHTT aggregates and inclusions get assembled in the cytoplasm and nucleus of neurons but the mechanism by which mHTT initiates neuronal degeneration is not fully understood. However, the loss of activity of ubiquitin-proteasomal system, defective autophagy-lysosomal system, transcriptional dysregulation, oxido-nitrosative stress, activation of apoptosis, mitochondrial dysfunction, neuroinflammation, and abnormal protein–protein interaction are considered to be key factors in interceding HD pathogenesis [1].

18.3 Mechanism Underlying Huntington Disease Pathogenesis (Fig. 18.1)

18.3.1 Oxidative Stress

Oxidative stress results from imbalance between pro-oxidants and antioxidants. Reactive oxygen species (ROS) production is unavoidable consequence of cellular metabolism. Elevated ROS levels are thought to play a significant role in HD pathogenesis [7]. In particular, high levels of ROS in HD are linked to proteasomal pathway dysfunction, which stimulates mHTT aggregate formation and ultimately leads to mitochondrial inhibition. In addition, mHTT aggregates are the production foundation of ROS because they are

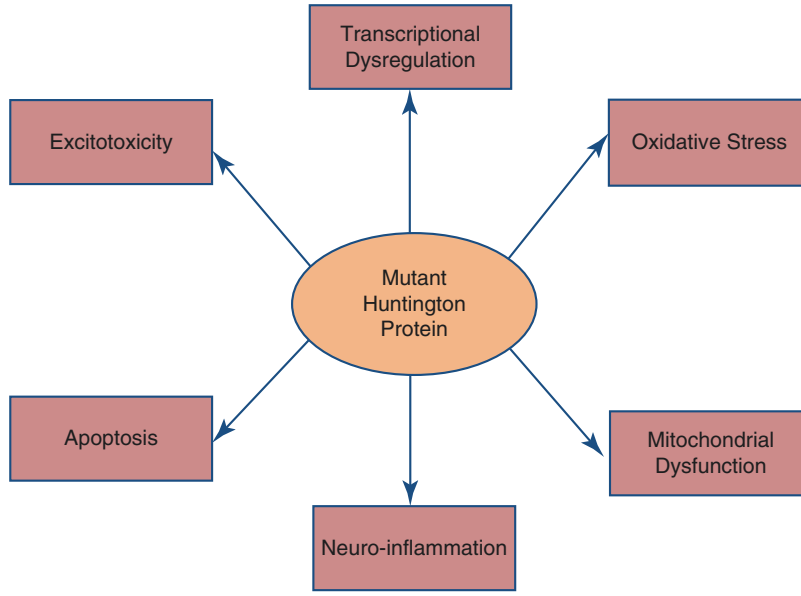

Fig. 18.1 Mutant Huntington protein induced pathogenic mechanism underlying HD pathogenesis

rich source of oxidized proteins. Moreover, HD patients showed decline in mitochondrial aconitase activity in caudate and putamen nuclei of striatum by 90% and 70%, respectively, which is indicator of high levels of ROS production. 3-nitropropionic acid (3-NP) is mitochondrial complex II inhibitor, enhances excessive ROS production, and produces degeneration of GABAergic MSNs in the striatum. 3-NP produces similar brain lesions in rats and mice as seen in HD patients and is widely used as animal model to study HD pathogenesis. Quinolinic acid (QA), an endogenous metabolite of kynurenine pathway produces overactivation of *N*-methyl-D-aspartate (NMDA) receptor, which leads to mitochondrial dysfunction, excessive ROS production, and oxidative damage. Acute intrastriatal administration of QA in rodents reproduces the symptoms of HD. Previous studies have revealed that the use of antioxidants like green tea, lycopene, polyamines, curcumin, resveratrol, quercetin, and epigallocathechin-3-gallate (EGCG) was successful in ameliorating the deleterious effects of 3-NP and QA-induced HD in Wistar rats [1, 8, 9]. Therefore, it is not speculate to conclude that HD onset and progression is well correlated with the elevated levels of ROS in HD subjects.

18.3.2 Mitochondrial Dysfunction

Mitochondrial dysfunction is a key feature in the pathogenesis of neurodegenerative disorders like HD and considered as hallmark for neurodegeneration, occurring as a result of defective mitochondrial calcium handling, ATP production, transcription abnormalities, and electron transport chain (ETC) impairment. Animal models of HD exhibit mitochondrial impairment and metabolic deficits similar to those found in HD patients. mHTT causes mitochondrial dysfunction by directly interfering with mitochondrial dynamics, and organelle trafficking, which in turn result in bioenergetic failure. Loss of the activity of enzymes involved in mitochondrial oxidative phosphorylation further supports the hypothesis of mitochondrial dysfunction in HD. Postmortem HD brain tissue studies have revealed decreased activity of the enzyme succinate dehydrogenase (complex II) in striatum nuclei. In addition, histological examination of the striatum in 3-NP treated animals showed pattern of similar MSNs degeneration without any loss of the NADPH diaphorase interneurons. Severe deficiency in the activities of the mitochondrial

respiratory chain enzymes, specifically complex II/III and aconitase has been reported in caudate and putamen nuclei of HD patients. Aconitase enzyme is highly susceptible to free radicals such as NO• and ONOO–, and the loss of its activity is considered more important than that of complex II/III activity. Moreover, using a proteomics approach decreased expression of aconitase enzyme is also found in the striatum of R6/2 HD mice model. Some studies have shown that mechanism by which mitochondrial dysfunction leads to neuronal death is coupled to excitotoxicity. Loss of mitochondrial energy metabolism is well established in HD subjects but it is still unclear whether these defects are the source or end result of the disease. In contrast, some studies have revealed that there is no direct evidence of impairment in mitochondrial electron transport in the postmortem HD brains and in HD transgenic mice expressing full-length mHTT. Therefore, precise role of mitochondrial dysfunction in HD pathogenesis remains unclear [1, 4, 9].

18.3.3 Excitotoxicity

Excitotoxicity refers to the deleterious neuronal death resulting from prolonged activity of glutamate receptor. The excitatory amino acid glutamate is most abundant neurotransmitters in CNS and plays a crucial role in the pathogenesis of HD by increasing mitochondrial calcium levels, opening of the mitochondrial permeability transition pore followed by neuronal loss. Excitotoxicity hypothesis has been given considerable recognition in the past few decades, because of the fact that direct administration of QA and kainic acid into the striatum nuclei produces similar loss of GABAergic MSN as seen in HD. Numerous strides have revealed that excitotoxic cell death totally depends on the expression of NR1/NR2B subunit of NMDA receptor. Reports are available for enhanced activity of NMDA receptor and decline in GLT-1 (glial glutamate transporter) in HD transgenic models.

Yeast artificial chromosome (YAC46 and YAC72) transgenic mice express mHTT similar to that expressed in HD patients. YAC72 mice showed GABAergic MSNs loss in the striatum at 12 month of age. Mitochondrial analysis of YAC72 HD mice (over-expressed with a full-length mHTT) has revealed Ca^{2+} overload. The Ca^{2+} overload produces NMDA receptor overactivation, which ultimately results in increased mitochondrial depolarization, and induction of mitochondrial permeability transition pore-dependent apoptosis. mHTT has been shown to interact with NR1/NR2B subunits and increases Ca^{2+} through NMDA receptors [10, 11]. Increased level of glutamate has been found in striatum isolated from 3-NP and QA treated rats indicating hyperfunctional glutamatergic system in HD. Studies have demonstrated that calcium channel blocker significantly ameliorated excitotoxic events associated with oxidative damage in animal models of HD [1, 4, 8, 9, 12].

18.3.4 Neuroinflammation

Increased activity of astrocytes and microglial cell has been clearly observed in the HD subjects but exact relationship between HD pathogenesis and neuroinflammation has not been established till date. The inflammation is in general a defensive mechanism to prevent damage to body organs from foreign substances. Postmortem HD brain studies have revealed high activity of microglia and macrophages in the striatal neuron. The level of pro-inflammatory markers, i.e., IL-6, IL-1β, and TNF-α, has been found to be increased in striatum of HD patients as well as in 3-NP, QA, and R6/2 animal models of HD. It has been reported that NH_2-terminus of mHTT directly binds to and activates IKK-NFκB pathway. The activation of IKK-NFκB pathway leads to activation of iNOS and subsequent production of NO^-. Some of studies have suggested hyperfunctional IKK-NFκB pathway in the striatum of HD subjects. The mHTT aggregates are not cleared by ubiquitin-proteasomal system and therefore

predicted as foreign particles by microglial cells, thereby ensuing neuroinflammation. Numerous strides have documented that many antioxidants drugs like green tea, spermidine, curcumin, quercetin, licofelone, and EGCG significantly decreased the level of pro-inflammatory cytokines in 3-NP and QA-induced HD models. In addition, some of pro-inflammatory markers like IL-6, IL-8, and MMP-9 were shown to be increased in cortex and cerebellum. There is a number of studies which stand in support that neuroinflammation plays a substantial role of in the disease progression of HD. Yet, the accurate mechanism underlying increased activity of neuroinflammatory markers in HD pathophysiology remains questionable [1, 4, 9, 13].

18.3.5 Apoptosis

Apoptosis is generally referred to a programmed cell death and is well-established pathogenic mechanism contributing to HD progression. Caspases belong to family of cysteine-aspartate proteases and play a pivotal role in mediating apoptotic cell death. Huntington protein is natural substrate for caspases. During early stages of HD, increase in caspase activity results in cleavage of huntingtin, formation of toxic fragments followed by nuclear translocation of N-terminus fragments of mHTT. This translocation of N-terminus fragments of mHTT to nucleus causes transcriptional upregulation of caspase 1 gene in early stages whereas in middle and late stages, transcriptional upregulation of caspase 9 gene has been reported in HD. Increased expression and transcriptional dysregulation of caspase-1, caspase-3, and caspase-9 have been reported in HD patients. During disease progression, caspase-3, caspase-8, and caspase-9 get activated and followed by release of cytochrome c, which is considered as a key event for triggering apoptosis. Cytochrome c is critical enzyme of the mitochondrial electron transport chain which upon stimulation unites to Apaf-1 for apoptosome formation. Apoptosome (a complex composed of Apaf-1, cytochrome c, and caspase-9) triggers the activation of caspase-9, an upstream architect for the induction of apoptosis, and ultimately stimulates caspase-3 to induce apoptosis in HD [1, 9]. Till now, the search for potential caspase inhibitors is on-going and HD animal models have proved to be instrumental in evaluating the neuroprotective potential of caspase inhibitors.

18.3.6 Misfolding, Aggregation, and Clearance of Mutant Huntingtin

mHTT plays a central role in mimicking HD pathogenesis. mHTT fragments are produced as a result of catalytic activity of calpain and aspartic endopeptidases. Calpains are cysteine proteases and belong to family group of calcium-dependent enzymes which gets activated in response to numerous apoptotic and necrotic stimuli, specifically those altering calcium homeostasis. In this regard, in HD, mHTT induces significant impairment in mitochondrial calcium homeostasis due to excitotoxic events, thereby resulting in activation of calpains. Calpain activation has been well demonstrated in human HD tissue, and in the brain of HD patients. The cleavage of huntingtin protein by calpains I, II, and III depends on the length of polyglutamine segment. The length of huntingtin protein fragments as well as polyglutamine repeat is considered as decisive factors in aggregation process. There are substantial evidences which revealed that huntingtin aggregates get accumulated in the nucleus and cause sequestration of number of transcriptional regulators and impair axonal transport process. Numerous pieces of evidences have implicated the failure of ubiquitin-proteasome system to dissolve mHTT, leading to mHTT aggregates and inclusion formation. Using various transgenic animal models of HD, number of drugs like congo red, thioflavin S, curcumin, gossypol, green tea, and trehalose have been found to prevent huntingtin aggregation. Therefore, therapeutic strategies intended at decreasing aggregate formation might prove to be fruitful in HD [4, 14].

18.3.7 Transcriptional Dysregulation

Using in-situ hybridization techniques, analysis of postmortem HD brains revealed that some of mRNA species which encodes for various neuropeptides and neurotransmitter receptors found to be decreased in striatal neurons, indicating transcriptional dysregulation in HD. Decreased mRNA levels of the dopaminergic receptors D1 and D2 have been shown in R6/2 transgenic mouse model of HD. Numerous studies using different HD transgenic mice (expressing the longer or full-length transgenes, expressing shorter NH2-terminus) have differential impact on gene transcription. Reports are available that HD mice expressing a short NH2-terminal fragment of mHTT showed most robust effects on gene expression. mHTT has been reported to obstruct with the activity of transcription factors or coactivator on susceptible gene promoter region. Numerous transcriptional factors binds to polyglutamine segment of huntingtin protein and some of them are Nuclear receptor corepressor (N-Cor), TATA-binding protein (TBP), transcription regulator (mSin3a), p53, mammalian SIN3 homolog A CBP, coactivator CA150, transcriptional corepressor COOH terminal binding protein (CtBP), and Sp1. Increase in the size of polyglutamine segment in HD gene results in decreased activity of several transcriptional systems in HD. The best examples of transcriptional abnormalities in HD are gene suppression by the transcription factor REST/NRSF and mHTT induced inhibition of regulator of mitochondrial biogenesis (PGC-1α).

Modification of chromatin structure by transcriptional factors and enzymes is key mechanism involved in gene regulation. Histones represent themselves as principal target of modifications that include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation of histones. Acetylation and deacetylation of histones are thought to play pivotal role in gene expression with the help of histone acetyltransferases (HATs) and histone deacetyltransferases (HDACs). HAT enhances the gene transcription process by opening of chromatin architecture and subsequent addition of acetyl groups. In contrast, HDACs inhibit gene transcription process by removing acetyl groups and subsequently resulting in chromatin condensation. The inhibition of HDAC activity results in increased acetylation of histones, followed by the transcriptional activation through relaxation of the DNA conformation [4, 15, 16]. Therefore, it can be concluded that HDAC inhibitors like sodium butyrate may prove to be effective in HD.

18.4 Therapeutic Strategies to Combat Pathogenic Mechanisms

Various therapeutics strategies used to target HD are premeditated to improve HD symptoms. These drugs met with limited success and do not halt the inevitable disease progression. Here are some of potential therapeutic strategies for HD (Table 18.1):

Table 18.1 List of potential drug/drug therapy for Huntington disease with specific targets

Targets	Drugs/drug therapy
Excitotoxicity	Riluzole, memantine, lamotrigine, remacemide, verapamil, diltiazem
Oxidative stress	Green tea, curcumin, quercetin, polyamines, EGCG, lycopene
Mitochondrial dysfunction	Creatine, coenzyme Q10, eicosapentaenoic acid, green tea, polyamines, lycopene, curcumin
Neuroinflammation	Green tea, curcumin, EGCG, quercetin
Apoptosis	Caspase inhibitors
BDNF therapy	BDNF, diet restricted regimen, irregular fasting, environmental enrichment
Autophagy inductors	Rapamycin, trehalose, polyamines
Aggregation inhibitors	Minocycline, congo red, trehalose, green tea
HDAC inhibitors	Sodium butyrate, SAHA, pimelic diphenylamide

18.4.1 Targeting Excitotoxicity

Excitotoxicity call death in HD results from increased NMDAR activity eventually resulting in impaired calcium homeostasis followed by degeneration of striatal neurons. Studies have confirmed that relatively high expression of NMDA receptor containing NR1A/NR2B subunits is accountable for the striatal neuronal death. To neutralize excitotoxic events by targeting extreme glutamate release from corticostriatal terminals, riluzole (inhibitor of glutamate release) was first drug tested and found to be defensive in counteracting glutamate hyperactivity in toxin and transgenic animal models of HD. In this series, memantine (NMDAR antagonist) become second drug after riluzole and showed more promising result than riluzole. Memantine has been shown to decrease striatal neuronal loss and improvement in cognition to a great extent in toxin based animal models of HD. Nevertheless, an exploratory studies on larger population is urgently required to authenticate the neuroprotective role of memantine in HD. In addition to memantine, lamotrigine, (NMDA antagonist) and remacemide (a noncompetitive inhibitor of the NMDAR) have shown promising results in HD rodents but their effectiveness cannot be translated in clinical trials. Another drug ifenprodil (NR2B subunit specific inhibitor) reduced excitotoxic loss of MSNs in HD transgenic mice and wild-type mice exposed to NMDA. Thus, targeting excitotoxicity by using NMDA blockers has brought some hope for their therapeutic efficacy in HD subjects. Dopamine is key neurotransmitter in striatum and required for variety of functions in CNS. Dopamine is released by neurons of substantia nigra *pars compacta* into striatum. Experimental evidences have proved that dopamine is itself neurotoxic at higher concentration in the striatum. It has been reported that hyperactivity of dopaminergic system might contribute to HD like symptoms. Tetrabenazine (a dopamine depletor) has been reported to improve the motor impairment and lessen striatal neuronal loss in HD mice, and confirms the neurotoxic role of hyperactive dopaminergic system in HD pathogenesis. In 2007, tetrabenazine become first drug to be approved by US-FDA for the treatment of choreiform movements in HD. However, several adverse effects have been reported to be associated with the use of tetrabenazine such as sedation, depression and parkinsonism, and sedation [1, 4, 17].

18.4.2 Maintenance of BDNF Levels

Reduced level as well as expression of BDNF and its receptors, i.e., TrkB receptors, have been found in HD subjects and this finding has led to a hypothesis that maintenance of BDNF level could be a promising drug therapy for HD. It has been reported that number and activity of TrkB receptors get decreased in the striatum of HD animals and patients. There are number of hurdles to use BDNF therapy in the HD patients. The major challenge is regarding monitoring of BDNF produced by the local neurons because excessive levels of BDNF may possibly have a lethal effect on neurons and memory. One of the fascinating approach is to increase BDNF levels by use of diet restricted schedule, irregular fasting, physical exercise, use of natural products, and environmental enrichment. Preclinical study using transgenic HD N171-82Q mice have reported that irregular fasting and use of diet restricted regimen normalized the BDNF levels in brain and postponed the onset of motor dysfunction. In addition, environmental enrichment with the help of physical exercise and use of natural foods has been documented to delay motor symptoms in R6/1 and R6/2 transgenic animal models of HD and prevented BDNF shortage in the brain [1, 4, 5, 18].

18.4.3 Targeting Caspase Activities, mHTT Aggregation, and mHTT Clearance

Caspases and calpains play key role in the production of toxic fragments of mHTT. The neuroprotective efficacy of caspase inhibitors in HD pathology has not been elucidated but numerous efforts are

continuing in this direction. It has been reported that minocycline (second-generation tetracycline) inhibited caspase 1 and 3 in R6/2 transgenic mice, thereby improving the HD phenotype. The efficacy of compounds that stand in as aggregation inhibitors has been evaluated in the animal models of HD. In this regard, congo red was evaluated for its efficacy as aggregation inhibitors and was successful in decreasing mHTT aggregates in a mouse model of HD. In vitro and in vivo studies have confirmed that congo red inhibits oligomerization of polyglutamine (CAG) segment, improves mitochondrial performance, and enhances the clearance of expanded CAG repeats. Emerging evidence has indicated autophagy induction as a neuroprotective strategy in HD and polyglutamine like diseases. Rapamycin (mTOR inhibitor) has been shown to induce autophagy and thereby produce substantial decrease in mHTT aggregates, enhancing neuronal endurance in HD Drosophila model system. Similar results were shown with induction of autophagy by rapamycin in HD mice on motor deficits and improved striatal neuropathology. Alas, the use of rapamycin is associated with some severe side effects like immunosuppression, limiting its use in clinical settings. The thrust of finding novel drugs for HD has come up with combined use of rapamycin (autophagy enhancer) and lithium (inositol monophosphatase inhibitor) and this yielded additive clearance of mHTT aggregates in vitro and in vivo. Similarly, trehalose has been reported to induce autophagy thereby clearing mHTT aggregates and protect neurons from apoptotic death. It is therefore speculate to conclude that trehalose acts on multiple targets, i.e., by inducing autophagy, reducing mHTT aggregates, and promoting the clearance of toxic fragments of mHTT [4, 19, 20].

18.4.4 Targeting Mitochondrial Dysfunction, Oxidative Stress, and Neuroinflammation

mHTT induced impairment in mitochondrial electron transport chain results in the decreased level of ATP, excessive ROS production, and apoptosis. Therefore, the use of antioxidants drugs that decrease or stop excessive ROS production and enhance mitochondrial machinery may be instrumental as potential neuroprotective strategy to treat HD like symptoms. In this regard, till date, numerous antioxidants drugs have been tested in toxin and transgenic HD animal models. The use of these natural antioxidants met with excellent success in preclinical settings but they fail in clinical settings, possibly explanation may be wrong therapeutic dose, bioavailability or may be anything else we don't know. Creatine possesses mitochondrial protectant property and antioxidant properties. Preclinical studies using toxin and R6/2 mice have documented neuroprotective potential of creatine. In the translational study, administration of creatine to HD patients for 2 years proved to be effective only in preventing weight loss and improvement in the neurological scores in some patients and decreased serum levels of 8-hydroxy-2-deoxyguanosine (indicator of oxidative damage to DNA). These results suggest that creatine has some potential to counteract oxidative stress in HD subjects. Another mitochondrial protectant drug, Coenzyme Q10 plays important part in energy production in mitochondria. Coenzyme Q10 was successfully tested in R6/2 and in N171-82Q transgenic mice models of HD but human clinical trial with coenzyme Q10 revealed non-significant settlement for HD patients. There are numerous drugs with antioxidant potential which are successfully evaluated for their neuroprotective potential in animal models of HD like polyamines, curcumin, quercetin, green tea, EGCG, eicosapentaenoic acid (EPA), and many more. These drugs showed significant improvements in preventing motor deficits and behavioral abnormalities in different HD animal models. But, these drugs failed to prove themselves as good neuroprotective candidate in clinical settings. Future studies using these drugs need dose optimization, well-designed study protocol in multiple animal models of HD for proper evaluation of their neuroprotective potential, so that they can be taken to human clinical trials [1, 4, 8, 9].

18.4.5 Targeting Gene Transcription

Transcriptional dysregulation is now well-established mechanism underlying HD pathogenesis. Impairment in various transcriptional systems in HD is believed to involve downregulation of gene expression. This hypothesis has kicked off HD research to new level and is aimed at evaluating the potential of new drugs to restore gene transcription in transgenic HD models. In this regard as discussed earlier, HDAC inhibitors may restructure mRNA abnormalities by modifying chromatin structure. Therefore, the use of HDAC inhibitors has now become a center of attraction to target transcriptional dysregulation and increase gene transcription [21]. In this context, SAHA (HDAC inhibitor) was evaluated in mouse model of HD. This study yields positive results as SAHA successfully crosses blood–brain barrier and enhances histone acetylation, thereby reducing the motor deficits in R6/2 transgenic mice. In another study, pimelic diphenylamide (novel HDAC inhibitor) was found to prevent motor dysfunction and neurodegeneration with a low toxicity in vivo. In the same study, using microarray analysis pimelic diphenylamide treatment improved gene expression abnormalities in the HD mice. These instrumental findings have provided basis for the use of HDAC inhibitors in clinical trials. Sodium phenyl butyrate is another promising HDAC inhibitor and is regarded as safe and is well tolerated by HD patients. However, some serious side effects like inhibition of cell division and induction of apoptosis, were observed with these compounds. Preclinical studies with mithramycin and chromomycin (anthracycline antibiotics) have shown good DNA binding property, inhibition of apoptosis, and modulation of epigenetic histone modifications that affect gene transcription. It has been reported that administration of mithramycin to R6/2 transgenic HD mice increased their survival rate by 29.1%, highest in comparison to any drug reported till date [4, 22, 23].

Conclusions

At present, numerous therapeutic agents (memantine, tetrabenazine, minocycline, trehalose, C2–8, creatine, green tea, congo red, coenzyme Q10, ethyl-EPA, cysteamine, HDAC inhibitors, mithramycin, curcumin, quercetin) typically performing on the aforementioned pathogenic mechanism have revealed excellence on motor and/or cognitive dysfunction mostly in the 3-NP, QA, R6/2 and N171-82Q animal models of HD. So far, a number of compounds have been thoroughly evaluated in HD subjects at different stages of the disease. Only one is now available in several countries, i.e., tetrabenazine. There is no possible explanation for discrepancies existing between preclinical and clinical trials. These discrepancies emphasize the complexity in predicting the usefulness of new drugs in humans based on animal models of HD. Therefore, a cooperation has been made in the field that a particular drug or agent with impending therapeutic effects needs a systematic assessment in more than one animal model, possibly a short model (expressing the NH2-terminal portion of mutant huntingtin) and a full-length mouse model, either knock-in or YAC or BAC. Only afterwards, the selected compounds can be anticipated for use in clinical trials. Agreed with a fact that multiple pathogenic mechanisms are involved in HD pathogenesis, it is anticipated that a drug targeting multiple pathogenic mechanisms should have greater efficacy in targeting HD pathogenic mechanism.

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Amyotrophic Lateral Sclerosis: Current Therapeutic Perspectives

19

Vijay Kumar, Tara Kashav, and Md. Imtaiyaz Hassan

19.1 Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease which is characterized by a progressive loss of motor neurons in the brain and spinal cord. According to the National ALS Registry, almost 12,000 people in the USA diagnosed to ALS with a prevalence of 3.9 cases per 100,000 persons. This disease is more common among white males, non-Hispanics, and persons aged 60–65 years [1]. Ninety percent of ALS cases are sporadic form of ALS (sALS) occurring without known genetic cause and have no family history of the disease. The remaining 10% of cases are inherited and classified as familial ALS (fALS), which is associated with more than a dozen genes [2–5].

ALS is a complex disorder that could involve a single disease or represent several closely related disorders with different causes but similar clinical symptoms. The clinical manifestations include rapid muscle loss followed by muscle degeneration, paralysis, and respiratory problems [6–8]. Over 60% of patients die within 3 years of presentation, due to respiratory failure while about 10% survive for more than 10 years [9]. Although motor function declines, sensory, cognitive, and emotional capabilities are generally undamaged. Risk factors for ALS comprise gender, exposure to toxic chemicals, and trauma experienced during military service [10].

The etiology of ALS like other neurodegenerative diseases is multifactorial [2, 11, 12] and the pathogenesis is mediated by various cellular pathways including glutamate excitotoxicity, oxidative stress, neuroinflammation, mitochondrial dysfunction, apoptosis, and proteasomal dysfunction [13–16]. Targeting these different pathophysiological abnormalities remains a challenge in the ALS [17–21].

In this chapter, we summarize some of the neuroprotective agents targeting the proposed pathogenic mechanisms of ALS and discuss their neuroprotective efficacy in ALS mouse models (Table 19.1; Fig. 19.1). Furthermore, we discuss the potential for cell-based therapy to connect disease modeling and drug discovery (**see** Table 19.2). Finally, we provide a general overview of preclinical and clinical advances during recent years in the area of ALS therapy.

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_19

Table 19.1 Summary of pharmacological clinical trials in ALS

Target	Agent	Clinical trial	Survival ext	References
Anti-excitotoxic	Riluzole	Known treatment	10%	[192, 193]
	Ceftriaxone	Failed	8%	[34]
	Gabapentin	II	5%	
	Dextromethorphan	No clinical trial	10%	
	Talampanel	II	No effect	[38, 39]
	Memantine	II/III	5–7%	[44]
	Cobalamin	II/III	3%	
Anti-apoptotic	Minocycline	Failed	6–15%	[118]
	Pentoxifylline	Failed	10%	
	Rasagiline	Ongoing	14%	[55]
Anti-mitochondrial dysfunction	Creatine	Failed	14.6%	[111]
	Dexpramipexole	Failed	No effect	
	Melatonin	I/II	7.4%	[121]
	Olesoxime	III		[107]
Anti-oxidative	Vitamin E	Failed	No effect	
	N-Acetylcysteine	Failed	7%	
	Coenzyme Q	Failed	4%	[58]
	AEOL 10150	No clinical trial	26%	
	Edaravone	Ongoing	No effect	[56]
	Sodium Phenylbutyrate	Ongoing	21.9%	
Anti-inflammatory	Celecoxib	Failed	25%	[77, 78]
	Celastrol	No clinical trial	9–13%	[69]
	Glatiramer acetate	Failed	No effect	
	Thalidomide	Failed	12%	[73]
	Erythropoietin	Failed	10%	
	Pioglitazone	Failed	8–13%	

19.2 Pharmacological Approaches in ALS

Unfortunately, to date preclinical studies have not resulted in successful therapy against ALS. The main concern for developing new therapies is the lack of direct translation from preclinical findings to successful clinical results. Table 19.1 summarizes the most successful preclinical studies that recently performed on the animal model of ALS describing the survival extension and status of clinical trials. They are divided depending on the pathomechanisms targeted into the following sections: excitotoxicity, oxidative stress, neuroinflammation, mitochondrial dysfunction, protein misfolding, and apoptosis.

19.2.1 Anti-excitotoxic Drugs

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Accumulation of glutamate due to activation of glutamate receptors, absence of neurotransmitter clearance and increased sensitivity to glutamate, all leads to neuronal injury. Such neurotoxicity due to excitatory mediators is called excitotoxicity [22, 23]. This excitotoxicity induces cell death due to enormous calcium influx, reactive oxygen species, proteolysis, mitochondrial dysfunction, and energy imbalance [24–26]. Moreover, increase in the glutamate levels in ALS patients [27, 28] and the benefits of riluzole as an anti-excitotoxic drug [29] suggest the involvement of excitotoxicity in ALS. Few important anti-excitotoxic drugs are shown in Fig. 19.2.

Riluzole inhibits the release of glutamate, *N*-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, slowing down disease progression in SOD1G93A transgenic mice [30, 31] and increasing patient's survival by few months [32]. The efficacy of riluzole can be improved by

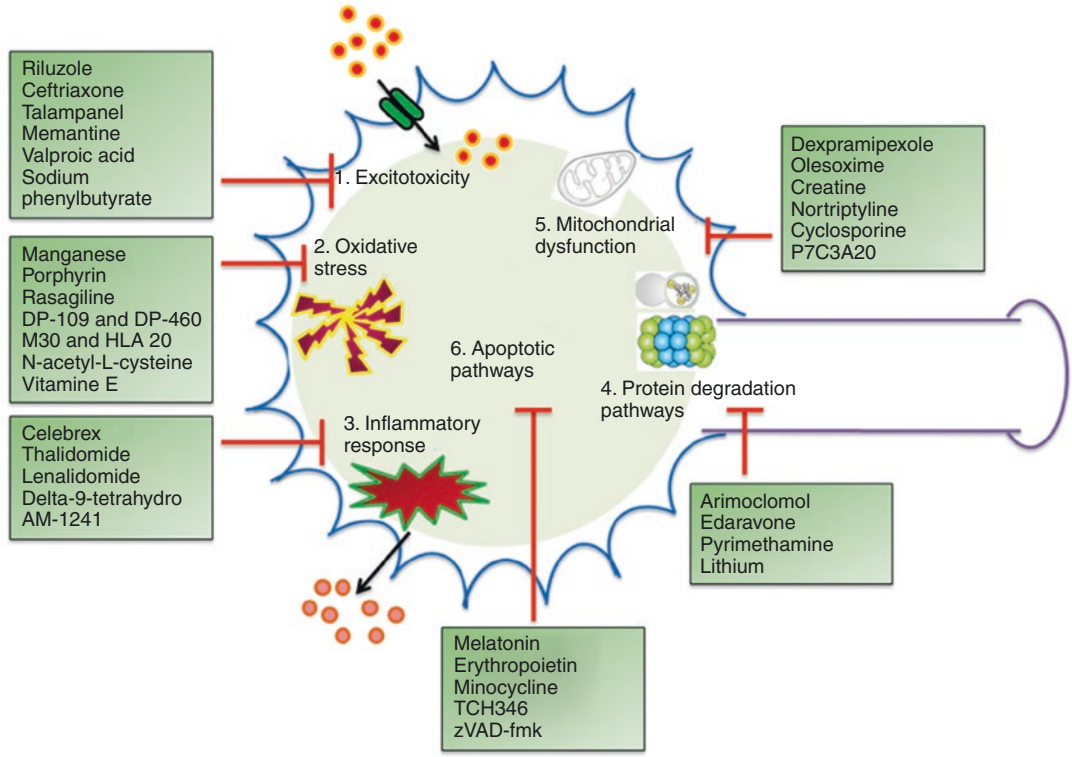

Fig. 19.1 Neuroprotective agents and their target in the pathogenic pathways in ALS. Adapted from Kumar et al. [12]

Table 19.2 Summary of stem cell-based clinical trials in ALS

Stem cell type	Number	Phase
Mesenchymal stem cell	NCT01494480	I/II
	NCT01609283	I
	NCT01777646	IIa
	16454-pre21-823	I
	NCT02017912	II
	NCT01759797	I
Bone marrow-derived stem cells	NCT01254539	I/II
	NCT01082653	I
	NCT00855400	I/II
	NCT01758510	I
	NCT01759784	I
	NCT01771640	I
Neural stem cell	NCT01348451	I
	NCT01730716	II
	NCT01640067	I
Hematopoietic stem cells	NCT01933321	II/III

combining with other drugs, suggesting the therapeutic advantages of combination therapy in ALS [33]. Besides riluzole, several anti-excitotoxic drugs like *gabapentin, topiramate, verapamil, lamotrigine,* or *dextromethorphan* have been tested but the overall results were discouraging [34].

Moreover, glutamate clearance from neuromuscular synapses is diminished in ALS patients because of the loss of glutamate transporter, GLT1 [35]. In the presence of β-lactam antibiotics, such as penicillin and cephalosporin, level of GLT1 gets upregulated. ***Ceftriaxone***, a third generation β-lactam antibiotics increases GLT1 promoter activity, thus reducing glutamate excitotoxicity [36]. However, ceftriaxone did not improve muscle strength and disability scores in a study of 108 ALS patients [37] and failed in phase III clinical trials despite being successful in phase I and II trials [38, 39].

Talampanel is also a non-competitive AMPA antagonist thus reducing calcium levels in SOD1 mice models and is effective during the early stage of the disease [40]. A phase II clinical trial in 60 ALS patients showed decrease in ALS Functional Rating Scale (ALSFRS), muscle strength, and hand movements [41].

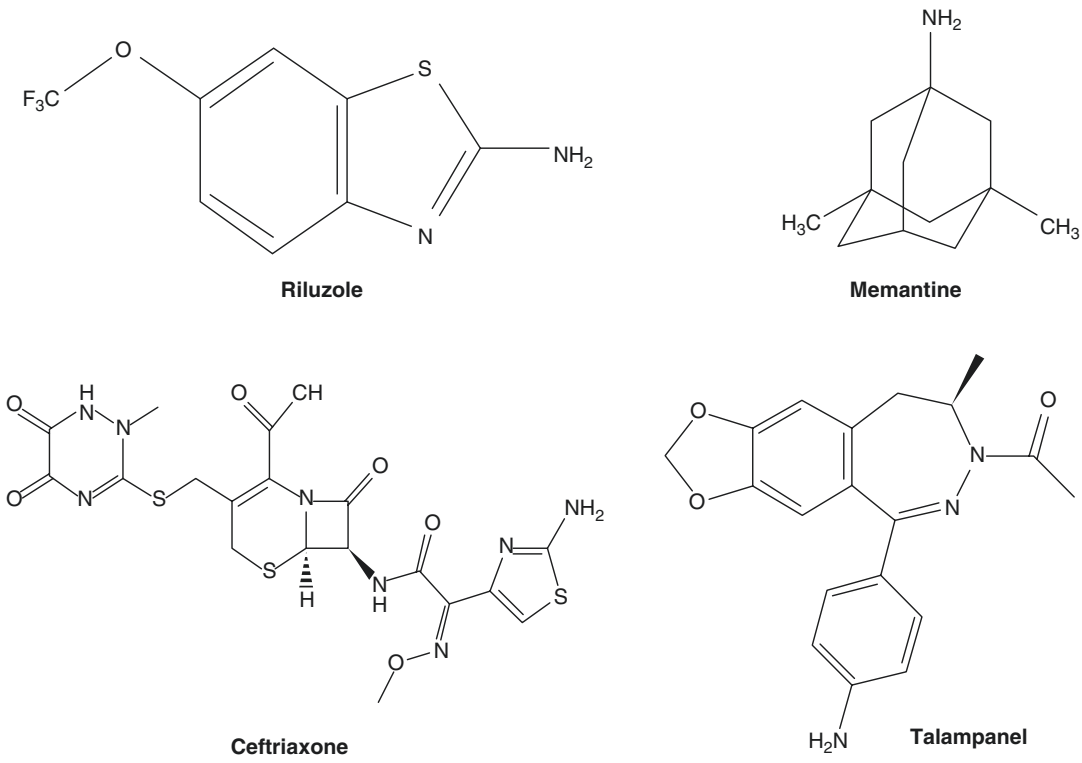

Fig. 19.2 Chemical structure of anti-excitotoxic drugs. Adapted from Kumar et al. [12]

Memantine is a FDA-approved drug which is targeting Parkinsonism and Alzheimer's disease [42–44]. It acts as a NMDA-receptor blocker, thus decreasing excitotoxicity [45] and prolonging survival to SOD1G93A mice [46].

19.2.2 Anti-oxidative Drugs

Oxidative injury has been studied in cellular and rodent models of ALS [47, 48], in spinal cord and motor cortex [49, 50], in postmortem tissue [51, 52], and in CSF [53, 54] of ALS patients. Several neuroprotective agents with antioxidant abilities have been studied in relation to ALS [55].

Rasagiline, a monoamine oxidase inhibitor, increases mitochondrial survival and is used to target Parkinson's disease [56]. Rasagiline, either alone or in combination with riluzole improves motor performance and survival in SOD1G93A mice [57]. Currently, it is undergoing phase II clinical trial to assess its safety and effectiveness in ALS patients [18]. Few important anti-oxidative drugs are shown in Fig. 19.3.

Edaravone, an antioxidant and a free-radical scavenger, delays disease progression, motor neuron degeneration, body weight loss, and reduces SOD1 aggregates in SOD1G93A mice [58].

MitoQ (mitochondrial coenzyme Q10) treatment increased survival time in SOD1G93A mice [59], but failed to translate successfully in human clinical trials [60].

Iron dysregulation also causes oxidative damage, and altered iron homeostasis has been found in ALS patients. The multifunctional iron-chelating drugs ***M30*** and ***HLA20*** delay disease onset and prolong the survival of SOD1G93A mice [61, 62].

Bromocriptine, a free-radical scavenger, delays declining of motor function and prolongs the survival of ALS-SOD1 mice [63].

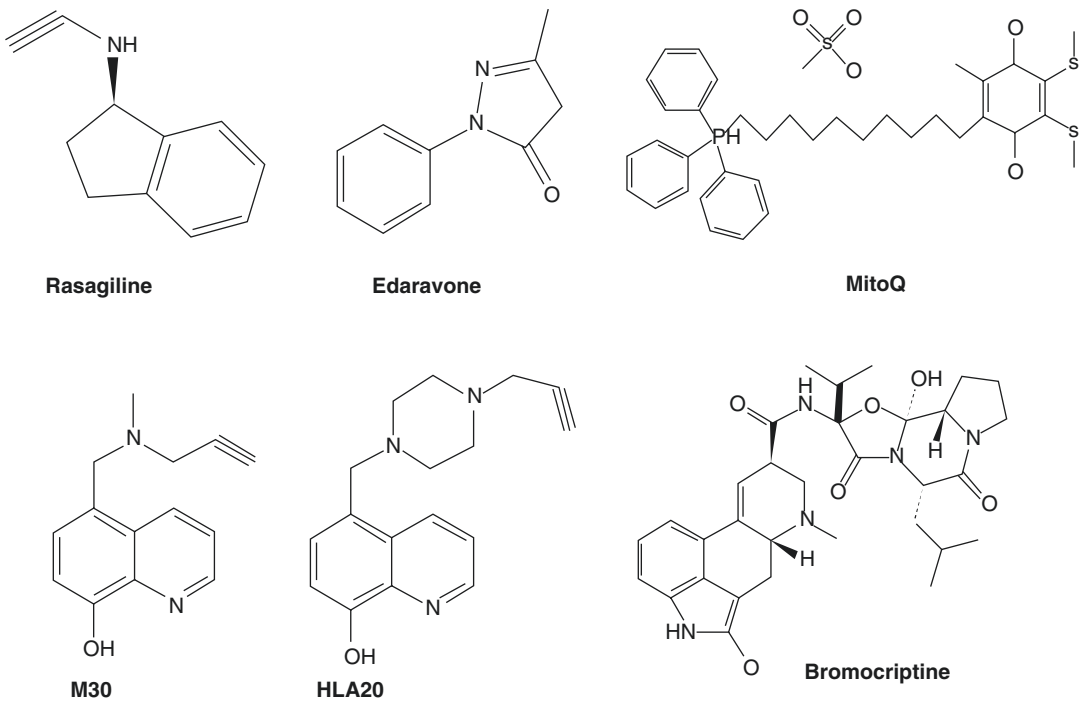

Fig. 19.3 Chemical structure of anti-oxidative drugs. Adapted from Kumar et al. [12]

19.2.3 Anti-neuroinflammatory Drugs

Neuroinflammation is a common pathological feature in ALS [64] representing an important possible therapeutic target [65]. Motor neurons damage in ALS leads to the activation of microglia, astrocytes, and the complement system [66, 67]. These activated astrocytes produce inflammatory mediators such as prostaglandin E2, leukotriene B4, nitric oxide and result in neuroinflammation in SOD1G93A mice [68] and in ALS patients [69]. Few important anti-neuroinflammatory drugs are shown in Fig. 19.4.

Celastrol is used as anti-inflammatory and anti-oxidative agents that also enhance the expression of heat shock protein (HSP) 70 [70]. It delays the disease onset, reduces neuronal cell loss, recovers motor function, and extends survival [71].

TNF-α activates microglia which then leads to neuronal apoptosis. High levels of TNF-α have been shown in the spinal cord of SOD1G93A mice and serum of ALS patients [72–74]. ***Thalidomide*** destabilizes the mRNA of TNF-α and other cytokines. Thalidomide and its analog, ***lenalidomide*** reduce the production of TNF-α, decrease weight loss and death of motor neurons, thus prolonging survival in SOD1G93A mice [75].

Cyclooxygenase-2 (COX-2) helps in the glutamate release from astrocytes via a calcium-dependent pathway [76–78], and thus represents an important drug target in ALS [78, 79]. ***Celecoxib*** is a COX-2 inhibitor and anti-inflammatory agent. It reduces weight loss and extends survival of ALS mice by inhibiting astrogliosis and microglial activation [79]. However, celecoxib didn't improve survival and failed in a clinical trial involving 300 ALS patients [80].

19.2.4 Protein Aggregates Clearing Drugs

Protein aggregates are characteristic feature of ALS [81–84]. Protein degradation pathways, the ubiquitin proteasome system (UPS) and autoph-

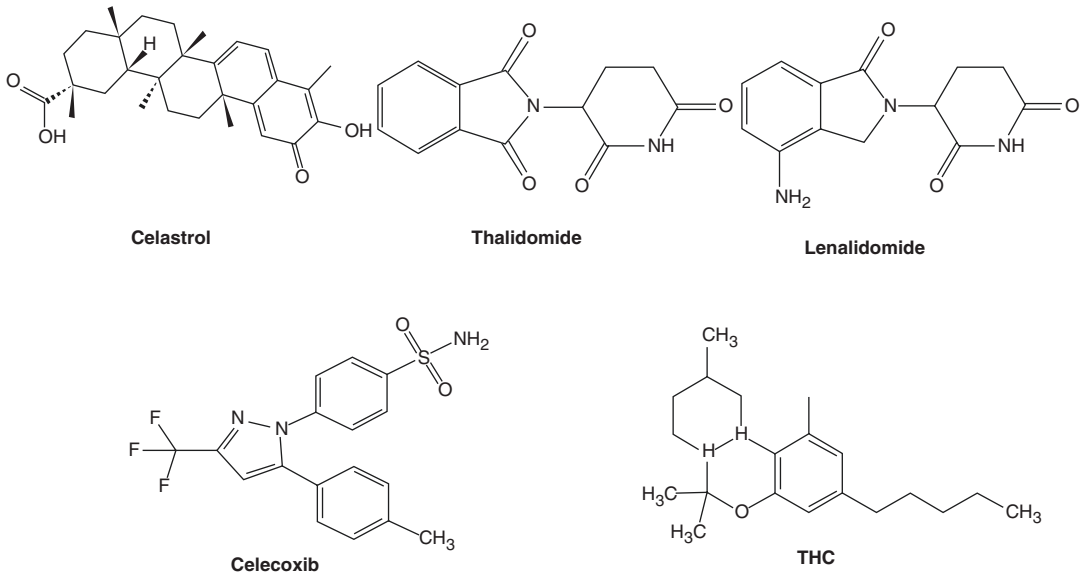

Fig. 19.4 Chemical structure of anti-neuroinflammatory drugs. Adapted from Kumar et al. [12]

agy are important in removing misfolded and aggregated proteins [85]. Many studies have demonstrated alteration in these pathways in ALS [84, 86].

Arimoclomol is an example of "smart drug" as it induces the expression of HSPs only under cellular stress conditions. It delays disease progression and increases survival in SOD1G93A mice [87, 88] and also reduced ubiquitin positive aggregates in the spinal cord of SOD1G93A mice [89]. Ongoing phase II/III clinical trials showed arimoclomol's good safety and efficacy [90, 91].

Pyrimethamine, an anti-malarial drug, has been shown to reduce mutant SOD1 levels in cultured cells, mice, and ALS patients [92].

Lithium exhibits neuroprotection in several studies. It decreases ubiquitin positive and SOD1 positive aggregates in motor neurons [93], inhibits motor neuron death due to excitotoxicity [94], and shows neuroprotection in cerebellar granule cells [95]. It also delays disease onset and duration and prolongs the survival in the SOD1G93A mice [93]. However, it failed to show neuroprotection in SOD1G93A mice also [96]. Combination therapy involving lithium and riluzole delayed disease progression in ALS patients [93], however failed in clinical trials [97].

19.2.5 Mitochondrial Defender

Several studies demonstrated the involvement of mitochondria in neurodegenerative diseases [98–100]. Mitochondrial dysfunction has been reported in the spinal cord of ALS patients [101], and in the skeletal muscle of ALS patients [102]. Moreover, calcium uptake and buffering capacity of mitochondria is also disrupted in the brain and spinal cord of SOD1G93A mice [103].

Pramipexole, a dopamine agonist and free-radical scavenger, improves the oxidative response by increasing ATP output, and reducing reactive oxygen species and apoptosis [104, 105]. ***Dexpramipexole***, the optical enantiomer of pramipexole, is more effective and displays neuroprotection both in vitro and in vivo [106], however recently failed in a phase III clinical trial.

Olesoxime, a cholesterol-like molecule, showed neuroprotection in animal and cellular models of ALS [107] by targeting mitochondrial membrane proteins and altering microtubule dynamics [108]. It also delays motor dysfunction and weight loss, and prolongs the survival of SOD1G93A mice [109].

Creatine reduces oxidative damage, motor neuronal loss, and mitochondrial dysfunction. It

improves motor performance and extends survival in SOD1G93A mice [110], but failed in human clinical trials [111–113].

19.2.6 Anti-apoptotic Drugs

A large number of studies have demonstrated the involvement of mitochondrial apoptosis in ALS [114, 115]. It has been shown that Bax/Bak pathways of mitochondrial apoptosis are removed in SOD1G93A mice, indicating the involvement of mitochondrial apoptosis in ALS [116]. Understanding and targeting mitochondrial apoptotic pathways will benefit ALS therapeutic studies.

Guanabenz, a FDA-approved drug for hypertension, exhibits neuroprotection in zebrafish and roundworm TDP-43 ALS model [117]. It reduces endoplasmic reticulum stress by activating the UPR pathway and reduces the amount of mutant SOD1 in SOD1G93A mice [118, 119].

Minocycline, an anti-bacterial, also works as an anti-apoptotic agent. It improves muscle strength, delays the onset of motor neuron loss, and extends survival in SOD1G93A mice [120] and SOD1G37R mice [121].

Melatonin is an anti-apoptotic agent, an antioxidant, and a free-radical scavenger [122]. It delays disease onset and prolongs survival in SOD1G93A mice. It also reduces superoxide-induced cell death and glutamate excitotoxicity in neuroblastoma-spinal cord cells [123].

Valproic acid promotes gene transcription [124] and inhibits neuronal cell death by reducing oxidative stress, excitotoxicity, and apoptosis [125, 126].

19.3 Non-pharmacological Approaches in ALS

Since the effect of current drugs is limited only to patient conditions, the idea of using cell-based therapy to replace the effected neurons came into picture. The development of cell-based therapy for ALS has shed light on the molecular pathways associated with the disease and has provided the much-needed alternative to animal models of ALS for high-throughput drug screenings. In this section, we provide an update on the current knowledge and human clinical trials using stem cell therapy, growth factors, and gene therapy for ALS.

19.3.1 Stem Cell Therapy

Stem cell-based therapies hold great promise for the treatment of ALS [127–129]. Because of their plasticity and ability to differentiate in response to extracellular signals, stem cells are exploited as therapeutic strategy in many neurological diseases [130]. Over the last years, several preclinical studies in ALS models have been performed to test the possibilities of different stem cells to reach the injury site, survive, and properly engraft [131–134]. The findings of the preclinical studies are promising and could lead to clinical trials in human patients (Table 19.2).

19.3.1.1 Mesenchymal Stem Cell

Mesenchymal stem cell (MSC) can be obtained from umbilical cord and adult bone marrow, which then naturally differentiated to osteoblasts, chondrocytes, and adipocytes. A large number of cell-based clinical trials for ALS are based on the use of MSCs. Clinical trials based on autologous MSC treatment have revealed the safety and achievability of intraspinal, intrathecal, and intracerebral MSC transplants [135, 136]. A phase 1 clinical study was conducted in Italy to evaluate the feasibility and toxicity of MSC transplantation in ALS patients [137].

19.3.1.2 Neural Stem Cell

Neural stem cell (NSC) can be obtained from the CNS of postmortem fetal samples and can be differentiated into astrocytes, as well as neurons and oligodendrocytes [138–140]. Phase I clinical trial for a NSC-based treatment of ALS was initiated in 2010 and completed in 2013. This clinical trial involved the transplantation of human spinal cord-derived NSCs into the spinal cord of 15 ALS patients [141–143].

19.3.1.3 Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) represent a novel source of autologous stem cells. They are isolated from fibroblasts and reprogrammed to an embryonic stem cell (ESC)-like pluripotent state. iPSCs were first generated from mouse fibroblasts in 2006 [144], followed by the generation of human iPSC in 2007 [145]. These cells are similar to ESC with respect to morphology, surface antigens, gene expression profiles, and differentiation ability, with the great advantage of bypassing rejection-related and ethical issues.

The major advantage of using iPSCs is that they facilitate the in vitro monitoring of disease initiation and progression as well as the screening and testing of drugs on the patient's own cells to understand the pathophysiology of neurodegenerative diseases [146, 147]. In 2008, human iPSCs from ALS patients were directed to differentiate into motor neurons [148]. Recently, fibroblasts from ALS patients have been reprogrammed to iPSCs and differentiated into motor neurons with reduced levels of vamp-associated protein B/C (VAPB) as observed in sporadic ALS patients [149].

We hope that stem cell therapy will enable high-throughput drug screening and testing in ALS, and stem cells in the long run express enough complexity (i.e., "organ on a chip") to dismiss the need for animal studies in general.

19.3.2 Gene Therapy

Gene therapy includes the delivery of genes encoding for neurotrophic factors, anti-apoptotic proteins or inhibiting the expression of harmful factors (e.g., utilizing viral vectors or small interference RNAs). The key advantage of gene therapy includes the administration of viral vectors to the CNS, overcoming the trouble of crossing the blood–brain barrier [150, 151].

19.3.2.1 RNA Interference Therapy

RNA Interference (RNAi) is a process by which short, non-coding micro RNA (miRNA) inhibits and regulates gene expression by binding to mRNA [152]. This endogenous gene-silencing mechanism is now being used in autosomal-dominant diseases to effective silencing of the dominant mutant allele, thus providing potential therapeutic applications.

The gene silencing can be achieved experimentally through the administration of small interfering RNA (siRNA) produced in vitro or through the transfection of short hairpin RNA (shRNA) or miRNA using viral vectors.

Gene therapy for ALS with siRNA has entered phase I clinical trials [153]. It has been shown that siRNA downregulates the human mutant G93A SOD1 gene in the spinal cord of ALS mice [154]. RNAi-mediated silencing of mutant SOD1 reduces cyclosporin A-induced death in neuroblastoma cultures [155]. RNAi through lentiviral vector also reduces SOD1 expression in brain and spinal cord [156]. These and other studies [157] demonstrated that RNAi can achieve allele-specific silencing and therapeutic benefits in SOD1G93A mice. Cationic nanoparticle-mediated targeted siRNA delivery has also demonstrated clinical importance [158].

19.3.2.2 Oligonucleotide Therapy

Antisense oligonucleotides have been exploited to reduce the amount of toxic proteins such as SOD1 and C9orf72, thus representing an alternative therapeutic strategy in ALS [159]. The decrease in accumulation of misfolded SOD1 using an antisense RNA has already been tested in SOD1 G93A rat. The antisense oligonucleotide, ISIS333611 reduced SOD1 mRNA and protein concentration in spinal cord and thus increased survival [160]. A phase I clinical trial with ISIS-SOD1RX in SOD1-related ALS patients is ongoing [161]. Moreover, ISIS Pharmaceuticals has developed "gapmers" against expanded C9orf72 RNAs and showed that it is well tolerated in mice [162], and RNA foci were reduced by nearly 50% [163–165].

19.3.3 Growth Factors

As the main hallmark of ALS pathophysiology is the loss of motor neurons [166], another possible

therapeutic approach has been the treatment with a variety of growth factors for example brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1).

GDNF maintains the survival and development of neurons. Increased levels of GDNF are found in the CSF of ALS patients [167]. It decreases motor neuron loss in mutant SOD1 mice, suggesting its possible role in ALS therapy [168]. It also significantly delayed the onset and progression of disease, and prolonged the life span of SOD1G93A mice [169].

BDNF is involved in growth and survival of neurons. BDNF production in the brain depends on neuronal activity and its levels are crucial for hippocampal neuronal functions [170]. Neeper et al. [171, 172] reported that mRNA level of BDNF increased in rat after general physical activity. It has been shown that exercise induces a 3–5-fold increase in mRNA expression of BDNF in mice and a 2–3-fold increase in human, thus improving the motor performance [173].

VEGF is an angiogenic growth factor and displays neuroprotective activity [174]. In SOD1G93A mice ALS model, it demonstrates regulatory effect on astrocytes and neuroprotection [175]. Moreover, it delays onset of paralysis, improves motor performance, and extends survival in SOD1G93A rat [176]. It also activates PI3-K/Akt pathway and reduces mutant SOD1-mediated motor neuron cell death in cell culture system [177].

IGF-1, a myotropic factor, demonstrates a positive impact on motor neuron survival and life span of SOD1 mice [178]. Various studies have documented that IGF-1 treatment delayed disease onset and extended the life span of ALS mice [179–181]. Even after the onset of symptoms, it prolonged the life of mice [182]. It has also been shown that brain damage was prevented by increased uptake of circulating IGF-I induced by exercise [183, 184]. IGF-1 injected through AAV vector in SOD1G93A mice resulted in improved muscle function, astrogliosis reduction, microglial activation, and prolonged life span [185, 186].

19.4 Precision Medicine

Precision medicine is a novel approach that works best along with recently developed biomedical technologies to optimize and individualize treatment to disease. Unlike personalized medicine, precision medicine emphasizes on specific characteristics including genetic, biomarker, and psychosocial of individual patient [187–189]. The application of precision medicine is perhaps best exemplified in cancer [190], while in neurological diseases, precision medicine still remains desirable [191–193].

Since the ALS Ice Bucket Challenge, there has been a sharp movement toward precision medicine programs in ALS. Advances in genomics, "OMICS" and iPSC technology have pushed the envelope to move precision medicine forward quickly. Precision medicine programs share common goals. They aim to identify new ALS genes and study their biology to identify new pathways involved in the disease process, which are potential therapeutic targets. iPSC lines isolated from people carrying the genetic mutations can be used in disease modeling and drug screening. Lastly, cell signatures of individual people can be established through genomic sequencing and "OMICS" analysis to understand the commonalities and differences between patients and also between patients and healthy people, which can be translated into potential therapeutic targets. Collectively, this information will ultimately help researchers understand the uniqueness of each person and to classify similar people living with ALS populations in more targeted ALS clinical trials to increase the chances of trial success.

19.5 Concluding Remarks

In 2016, ALS is still so rapidly progressing that it is physically and emotionally devastating for patients and families. There is an urgent need to identify and understand the molecular basis of ALS and find an effective cure. Numerous potential neuroprotective agents targeting pathophysiological processes have been studied, but there have been no successes.

Over the past decade, based on results using animal models of ALS, several new drugs were tested clinically, all of which failed to demonstrate efficacy. Ironically, some drugs, which effectively slowed disease progression in mice, accelerated the disease progression in humans. The constant failure of drug translation from animal models to humans as observed in ALS is very disappointing in terms of financial and human costs. These studies have cost nearly US$700 million over the past 10 years in the USA alone. The failure of clinical translation from animals to humans suggests that animal models are not an ideal system for studying ALS or for therapeutic interventions.

The rapid advancement in genetic discoveries points that ALS is much more a syndrome than a single disorder. Considering ALS as a single disorder is a major reason why previous therapeutic drug trials have failed. If we are to make therapeutic progress, the ALS research community needs to support the idea that one size does not fit all when we approach clinical trial designs in ALS. The therapeutic development of ALS now has strong academic, government, and industry involvement and hope seems almost around the corner.

Acknowledgements V.K. thanks the Department of Science of Technology, India for the award of DST-Fast track fellowship (SB/YS/LS-161/2014). M.I.H. thanks the Department of Science of Technology and Council of Scientific and Industrial Research (India) for financial support.

Conflict of interest: The authors have declared that there is no conflict of interest.

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20 Mechanistic Insights into Neurodegenerative Diseases: The Potential for the Development of Novel Therapeutics

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20.1 Introduction

Neurodegenerative diseases are conditions with complex etiologies resulting in progressive decline in functionality and, to date, have no cure. Significant progress has been made towards the mechanisms contributing to neurodegeneration in the past two decades, providing potential targets for the development of therapeutics. Not only are unique pathologies of specific neurodegenerative diseases becoming more clear, common features among different neurodegenerative diseases are also being elucidated. Perhaps the best illustration of this is our growing understanding of pathological steps that lead to amyotrophic lateral sclerosis (or motor neuron disease), little of which was understood until the identification of the first genetic link to the disease in 1990s. The goal of this review is to explore how novel mechanistic insights of neurodegenerative diseases may provide potential targets for the development of treatments. The primary focus will be on amyotrophic lateral sclerosis for the reasons stated above, but we will also address novel approaches for the treatment of Parkinson's disease, and various forms of dementia.

20.2 Amyotrophic Lateral Sclerosis

ALS is a progressive neurodegenerative disease that affects motor neurons in the brain and spinal cord. As with many other neurodegenerative diseases, the pathophysiology and etiology that leads to amyotrophic lateral sclerosis (ALS) remains elusive. ALS exemplifies the multifactorial nature of neurodegenerative diseases. Several genetic, molecular, and cellular factors have been implicated in the initiation and progression of the disease. That said ALS is also an example of how continued elucidation of factors that contribute to the neurodegenerative disease process can provide avenues for the development of treatments. As the disease progresses, patients lose the ability to initiate and control muscle movement. Individuals with ALS exhibit high mortality rates within 3–5 years of diagnosis as the loss in movement control progresses to paralysis. There is considerable complexity and heterogeneity in the onset, sites initially affected, and in progression of the disease that is likely a result of the numerous factors that contribute to etiology and pathophysiology of ALS.

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_20

One of the first insights into the pathology of ALS was the observation that altered superoxide dismutase 1 (SOD-1) activity is associated with familial ALS [1]. SOD-1 is a Cu/Zn-binding cytosolic enzyme that catalyzes the dismutation of the toxic superoxide anion to oxygen and peroxide, serving as cellular antioxidant. SOD-1 mutations are detected in approximately 20% of familial ALS and 3% of sporadic ALS cases [2]. Although numerous mutations of SOD-1 have been linked to ALS, its role in the pathophysiology is not clear. A common feature of the many SOD-1 mutations is the disruption of protein folding. This has led some to conclude that the misfolding and aggregation of SOD-1, but not loss of dismutase activity, contribute to its pathological role in ALS [3, 4]. Despite the lack of a defined role of SOD-1 in ALS, transgenic animal models expressing SOD-1 mutations have been a cornerstone of our growing understanding of other mechanisms that contribute to ALS [5, 6].

SOD-1 mutations remained the only known molecular link to ALS until causative mutations in TAR DNA-binding protein 43 (TDP-43) were found [7]. A pathological hallmark of ALS is the presence of ubiquitinated inclusions in surviving spinal motor neurons that results from proteasomal dysfunction [8, 9]. Some misfolded proteins are targets for ubiquitination, and the ubiquitinated forms of these proteins aggregate in various regions of the cell [10]. Both SOD-1 and TDP-43 mutations associated with ALS are known to be ubiquitinated and form aggregates in neurons, suggesting that as with other protein misfoldeding diseases (e.g., Alzheimer's disease) cellular aggregation of altered proteins may be the causal factor in ALS [3]. Consistent with this hypothesis, ubiquitinated TDP-43 is also observed in frontotemporal dementia [7], and more recently, TDP-43 has been shown to interact with the main characteristic pathologies of Alzheimer's disease, amyloid plaques and Tau tangles. The presence of TDP-43 in combination with the plaques and tangles was more likely to result in diagnosed Alzheimer's dementia than plaques and tangles alone [11]. Despite these similarities with other misfolding diseases, however, where SOD-1 is concerned protein misfolding and ubiquitinated cytoplasmic inclusions do not appear to be the primary casual factor in ALS. In three distinct transgenic SOD-1 animal models of ALS, enhancing the capacity of mitochondria to buffer calcium levels resulted in reduced aggregation of SOD-1 and suppression of motor neuron death; however, muscle denervation, motor axon degeneration, and disease progression and survival remained unaltered [12]. In addition, contribution of SOD-1 mutations to the ALS is about 10–20% of familial and 1–2% sporadic cases [13]. In contrast, TDP-43 proteinopathy is present in approximately 97% of all ALS cases [14–16].

TDP-43 is a widely expressed DNA/RNA-binding protein that has a nuclear localization signal and primarily localizes to the nucleus, but can also move between the nucleus and the cytoplasm [17, 18]. The identified biological roles of TDP-43 include: inhibition of retroviral replication, RNA splicing, and nucleocytoplasmic shuttling of messenger RNA [18–20]. TDP-43 localization to the cytoplasm is enhanced by mutations associated with ALS and appears to contribute to neurotoxicity [9, 21]. Expression of exogenous wild-type TDP-43 in rat cortical neurons results in higher levels of protein in the nucleus without producing neurotoxicity, whereas expression of mutant TDP-43 results in significantly higher accumulation in the cytoplasm with an associated increase in neurotoxicity [22]. Overexpression of wild-type TDP-43 in transgenic mice produces the same proteinopathy and disease observed with mutant forms of TDP-43, likely related to enhanced TDP-43 accumulation in the cytoplasm [23]. A feature of many neurodegenerative diseases is the prion-like spreading of underlying pathology into specific regions of the central nervous system as the disease progresses [24–27]. In ALS, the spread of TDP-43 proteinopathy has been used to stage the course of the disease into four distinct steps based on the brain regions affected [28]. The brain regions affected correlate with the neurological deficits that manifest in ALS suggesting a link between TDP-43 proteinopathy and disease progression. Elevated levels of cytoplasmic TDP-43, as a result of overexpression or mutant forms of

the protein, clearly contribute to pathogeneses of ALS, but TDP-43 is also essential for viability and motor neuron function. Complete knockout of TDP-43 in transgenic animals is lethal, and selective knockout in motor neurons, muscle, or glia alone precipitates ALS promoting some researches to refer to TDP-43 as the "Goldilocks" protein [29–32]. The role of TDP-43 in ALS appears to result from either gain or loss of function suggesting that potential therapeutics for ALS are ones that can prevent TDP-43 aggregation (Fig. 20.1) [15]. What are the factors that contribute to aggregation? In addition to mutations of TDP-43, aggregation can result from cellular stress and altered protein degradation, perhaps serving as a link between genetic and environmental factors [33, 34].

A protein that regulates TDP-43 levels is human up-frameshift protein 1 (hUPF1), an RNA helicase and regulator of nonsense-mediated mRNA decay (NMD) (Fig. 20.1) [32]. NMD is a surveillance mechanism that serves to mitigate errors in translation by recognizing anomalous mRNA transcripts and is thought to have evolved to eliminate abnormal transcripts due to routine errors in gene expression. Messenger RNAs that prematurely terminate translation because of a frameshift or nonsense mutation are selectively degraded by NMD [35]. In mammalian cells, NMD works on newly synthesized mRNA and is dependent on pre-mRNA splicing. NMD has been shown to modulate the severity of a number of diseases pointing to a possible mechanism for the development of therapeutics [36]. TDP-43 has been shown to autoregulate its synthesis by triggering nonsense-mediated RNA degradation that results from direct binding of TDP-43 to the 3′ untranslated region of its own mRNA and enhancing splicing of an intron region [37]. Barmada et al. tested the hypothesis that nonsense-mediated RNA degradation of TDP-43 was mediated by NMD [38]. In rat cortical

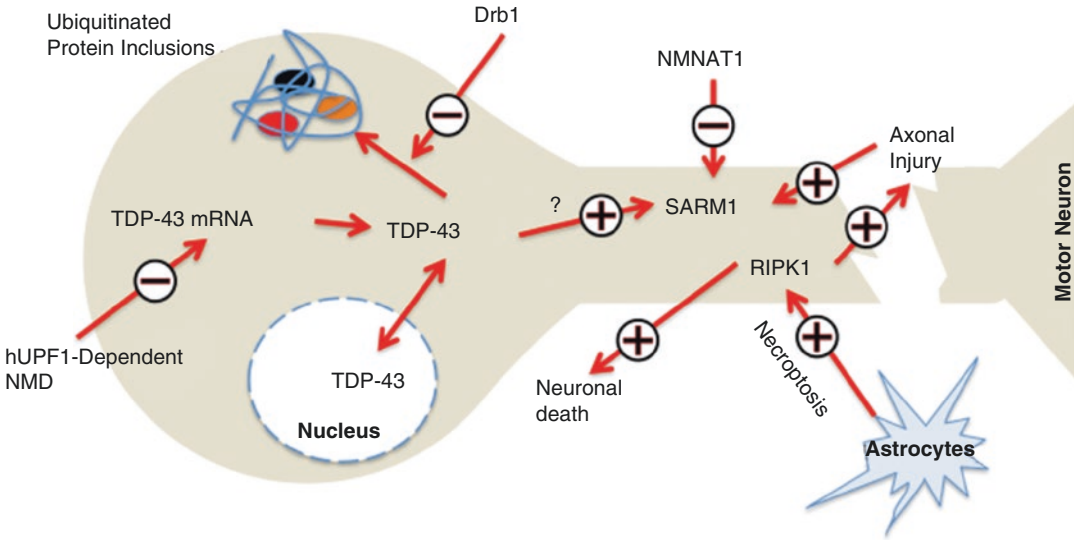


Fig. 20.1 Factors that contribute to axonal damage and death of motor neurons in ALS. One of the major factors linked to ALS is TDP-43. TDP-43 is primarily a nuclear protein involved in RNA processing. In ALS TDP-43 cytosol accumulation, ubiquitination, and incorporation into protein inclusions is thought to result in motor neuron loss. Mechanisms that decrease cytosolic TDP-43 such as hUPF1-dependent nonsense-mediated decay (NMD) and Drb1 produced products (see text) that sequester and prevent its aggregation provide potential therapeutic approaches. In *C. elegans* model of ALS, TDP-43-induced neuronal damage and paralysis is blocked by an ortholog of human SARM1. SARM1 plays a critical role in axonal degeneration (Wallerian degeneration) that follows axonal injury. SARM1 is directly inhibited by nicotinamide nucleotide adenylyl transferase 1(NMNAT1). It remains to be seen if TDP-43-induced neuronal damage is tied to SARM1. Another protein that can promote axonal damage and astrocyte-induced necroptosis is RIPK1, providing a link between inflammation and neuronal damage

neurons expressing wild-type or mutant forms of TDP-43, they were able to demonstrate that co-expression of hUPF1 reduced neuronal death by 40–50% through a mechanism that incorporates NMD. Similar results were observed with primary neurons expressing fused in sarcoma (FUS) protein, mutants of which are associated with familial, but not sporadic ALS [39]. FUS and TDP-43 are associated with multiple steps of RNA processing, especially in processing of long pre-mRNAs, but have largely non-overlapping RNA targets [40]. Although the results with hUPF1 are limited to a cellular model of ALS, they provide insight into a novel mechanism that can be exploited to modulate TDP-43 levels by enhancing hUPF1 activity or by targeting another component of NMD.

An alternative mechanism to preventing TDP-43 aggregation involves the protein Dbr1 (Fig. 20.1). Dbr1 is an intron lariat debranching enzyme, essential for normal processing of mRNA [41]. Reducing Dbr1 activity can block the toxic effects of TDP-43 in human neuronal cell line or in primary rat cortical neuron models of ALS [42]. An increase in the cellular pools of lariat RNA is thought to sequester TDP-43, thereby preventing its aggregation. Inhibitors of Dbr1 could be useful in the treatment of TDP-43-mediated ALS and the related neurodegenerative disease frontotemporal dementia. To that end, Montemayor et al. have solved the crystal structure of Dbr1, which should greatly aid in the development of selective small molecules with the capacity to inhibit the enzyme [43]. The feasibility of this approach is yet to be determined, however, because deletion of the DBR1 gene results in growth and morphological defects in yeast [43].

Whether protein aggregation is the pathological process that contributes to ALS is debated [44–46]. A long held view on the pathogenesis of Alzheimer's disease was that amyloid plaque formation was the casual factor in the disease; however, there is evidence to suggest that amyloid plaque formation may serve a protective function [46]. Similar process may occur in ALS. A study by Yonashiro et al. has uncovered a mechanism by which transcripts that stall ribosomal activity result in the tagging of the nascent polypeptide chain for aggregation, and a protein (Listerin/Ltn1) implicated in ALS-like symptoms is integral to this process [47]. What isn't known is whether the aggregates produced by ribosome-associated quality control are the same aggregates as those that result from mutants of TDP-43, FUS, or SOD-1.

Observations that link immune function to development of the disease provide another avenue for the development of potential therapeutics for ALS. Although ALS lacks the hallmark signs of autoimmune disease, the infiltration of circulating lymphocytes, factors that stimulate inflammation are present in central nervous system of people with ALS [48]. The role of immune response in ALS, however, appears to be mixed: protective or potentially destructive [49–51]. Recent studies in a *C. elegans* model of ALS, in which the animals were induced to undergo motor degeneration by the expression of TDP-43 or FUS, degeneration of motor neurons was induced by an innate immune response mediated by TIR-1 [52]. TIR-1 plays an integral role in response to microbial infection as part of the innate immune system in *C. elegans* [43]. Deleting the tir-1 gene in *C. elegans* results in significantly reduced neurodegeneration and paralysis induced by expression of TDP-43 or FUS, which demonstrates the link between immune function and ALS disease progression [53]. The human ortholog of the tir-1 gene, SARM1 (sterile alpha and TIR motif containing 1), plays a role in maintaining the integrity of neurons [54, 55]. Mitochondrial dysfunction, a feature of multiple neurodegenerative diseases, in sensory neurons causes neuron death through a SARM1-dependent mechanism [56]. SARM1 has also been implicated in Wallerian degeneration, a localized form of programmed axon destruction that occurs in response to axon trauma or disease (Fig. 20.1) [57]. Axon degeneration is an early pathological event in many neurological disorders, including ALS [58]. In response to injury, SARM1 promotes axonal degeneration by depleting cellular levels of NAD^+ (nicotinamide adenine dinucleotide) limiting energy generation in axons [59]. In SARM1

knockout mice mechanical severed axons remained intact 24 h post injury, whereas axonal degeneration was evident in mice carrying functional SARM1. Yang et al. have provided insight into the mechanisms by which axonal damage results in energy deficit near the sight of injury [60]. They show that SARM1 must activate the MAPK kinase pathway to initiate the process, and that SARM1 activation of the MAPK kinase pathway can be blocked by cytosolic version of nicotinamide mononucleotide adenylyl transferase 1 (NMNAT1) (Fig. 20.1). NMNAT1 is one of three homologous enzymes involved in the synthesis of NAD+ that is known to have neuroprotective effects [61–63]. SARM1 and NMNATs appear to have apposing effects in neurodegenerative process [64]. NMNAT2 deletion causes neurodegeneration that is reversed by eliminating the expression of SARM1 [64, 65]. Surprisingly, NMNAT1 counters SARM1 effects on NAD^+ depletion not by increasing NAD^+ synthesis but by blocking injury-induced SARM1-dependent NAD^+ depletion through a mechanism that is still to be determined [66]. Inhibitors of SARM1 and activiators of NMNATs make for intriguing therapeutic approaches for ALS and other axonal degeneration diseases. SARM1 contains a SAM (sterile α motif) or TIR (Toll-interleukin-1 receptor) domains that must interact for SARM1-induced neurodegeneration [54]. In addition, unique motifs in the SARM1 domain have been identified that are essential for its prodegeneration effects pointing to possible mechanisms that could be targeted by small molecules [67].

If SARM1 contributes to axonal degeneration, what contributes to motor neuron death? Are there other inflammatory processes that contribute to ALS? The answer to these questions comes from studies that measured the effect of astrocytes derived from patients with either familial or sporadic cases of ALS on co-cultured motor neurons [68]. When cells were co-cultured, the derived astrocytes produced toxic effects on the motor neurons in culture. This effect is specific to astrocytes isolated from ALS patients and occurs through necroptosis, a form of programmed necrosis [69, 70]. Necroptosis can be reduced by inhibition of RIPK1 (receptor-interacting kinase 1) (Fig. 20.1) [71]. RIPK1 is a critical regulator of cell death and inflammation [72]. Recent work by Ito et al. has linked RIPK1 to ALS; in transgenic mice with an optineurin (OPTN, mutations of which have been linked to familial and sporadic forms of ALS) knockout, the loss of OPTN resulted in demyelination and axonal degeneration through processes that required RIPK1 [73, 74]. Selective inhibitors of RIPK1 have already been identified and characterized, and have shown the ability to block necroptosis, providing the necessary tools to further explore the potential of RIPK1 as a therapeutic target [75, 76].

20.3 Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by a wide range of motor symptoms and pathological features, some or all of which may be present in any individual patient [77]. While the ultimate cause of PD is unknown, both genetic and environmental factors appear to be involved, and PD etiology suggests multiple potential mechanisms involving dopaminergic neurons and disruptions in neurotransmitter metabolism [78]. Currently, there is no cure for PD, and symptoms are managed primarily through routes that attenuate the effects of disruptions in dopamine metabolism [79]. As more underlying mechanisms are elucidated, additional targets for potential pharmacotherapy are coming into focus. One such target is protein deglycase (DJ-1, alternatively called Parkinson disease protein 7 or PARK7) [80].

Mutations of the PARK7/DJ-1 gene are responsible for one form of recessive early-onset Parkinson's disease, making PARK7 an attractive target for pharmacotherapy [81]. There is growing evidence that the protein product of this gene, PARK7, along with homologs, functions as glyoxal- and methylglyoxalases, thus attenuating the maturation of early glycation products into advanced glycation end products (AGEs) [82, 83]. Over time, AGEs promote the cross-linking of several classes of biomolecules, including proteins, lipids, and nucleic acids. These cross-linked biomolecules result in vascular and tissue damage and contribute to the

underlying pathology of not only Parkinson's disease, but other disorders including other neurodegenerative disorders, diabetes, atherosclerosis, cardiovascular disease, and chronic renal failure [84–86].

One significant protein that exhibits extensive cross-linking by AGEs is α-synuclein [87], which is the primary protein component of Lewy bodies [88]. While found in other tissues, most notably at the neuromuscular junctions of cardiac and skeletal muscles, the primary location of expression is in the presynaptic neuron [89]. The native function of the α-synuclein appears to be the regulation of neurotransmitter transport, acting at the early stages of vesicular trafficking and possibly dopamine re-uptake [90, 91]. There is a high degree of homology among the synuclein protein family and they appear to have at least some functional redundancy [90, 92]. The aggregation of α-synuclein into a Lewy body structure is kinetically mediated by the equilibrium which exists between the membrane-bound, helix-rich monomer and a pathologic α-sheet-containing conformer which can aggregate into insoluble fibrils, making up the fibrous protein component of the Lewy body [93–95]. Even prior to Lewy body formation, the synuclein aggregates exhibit neurotoxicity [95–97]. AGE-cross-linking presumably increases toxicity by stabilizing the aggregation of the ß-sheet conformer, driving the equilibrium towards oligomer formation [98, 99].

Targeting PARK7 would presumably be a viable route towards clearing certain early glycation products, thus attenuating the formation of AGE-cross-linked synuclein aggregates. Methylglyoxal forms as a spontaneous by-product of triose degradation during glycolysis and covalently links to various amino acid side chains, including cysteine, arginine, and lysine [100]. The covalent modification of proteins by methylglyoxal can be reversed at all three of these amino acids by the actions of PARK7 and its prokaryotic homolog, YajL, releasing the methylglyoxal as lactate [82, 101, 102].

PARK7 deficiency has been shown to result in increased oxidative stress through the loss of ROS quenching capacity [103, 104]. This effect on neuron oxidative state may occur through the regulation of amino acid uptake specifically that of glutamate/glutamine and serine [105, 106]. Serine can potentially act as a precursor to glycine and cysteine synthesis. Glycine and cysteine, along with glutamate, which can be taken up directly or synthesized from glutamine, are in turn the precursors to glutathione (GSH). Decreased uptake and synthesis capacity of these amino acids could result in a GSH deficiency [107]. Indeed, model systems of PARK7 deficiency have been shown to exhibit decreased biosynthesis of serine and GSH as well as a downregulation in the expression of key enzymes involved in cysteine, glutamate, and GSH metabolism [106, 108, 109].

PARK7 may exert influence through its actions as a sensor for oxidative stress. Under oxidative conditions, alterations to PARK7 cysteine residues lead to nuclear and mitochondrial translocation, where it may act as a transcription factor [110, 111]. In addition to the aforementioned effect on glutathione pathways, PARK7 deficiency results in decreased expression of the lipid raft proteins flotillin-1 and caveolin-1, which have been shown to be involved in functioning of dopamine transporter (DAT) and excitatory amino acid carrier (EAAT) [105, 112–114]. The transcription activity of PARK7 also influences dopamine synthesis directly, as tyrosine hydroxylase is also upregulated [110].

The involvement of PARK7 by these mechanisms in the pathogenesis of PD is supported by the observation that amphetamine use increases the risk of developing PD [115, 116]. Amphetamines exert their effect through disruption of dopamine transporter and endocytosis of cell membrane EAATs, which would disrupt glutamate metabolism in much the same way as PARK7 dysfunction [117, 118].

PARK7 may also influence protein function directly. Oxidation-triggered translocation to the mitochondria results in PARK7 interactions with Complex I and Complex II activity, which is diminished with PARK7 deficiency [111, 119]. PARK7 potentially acts as a protein chaperone for cysteine-rich proteins by forming mixed disulfides with the thiol proteome [102]. The prokaryote PARK7 homolog YajL has been shown to

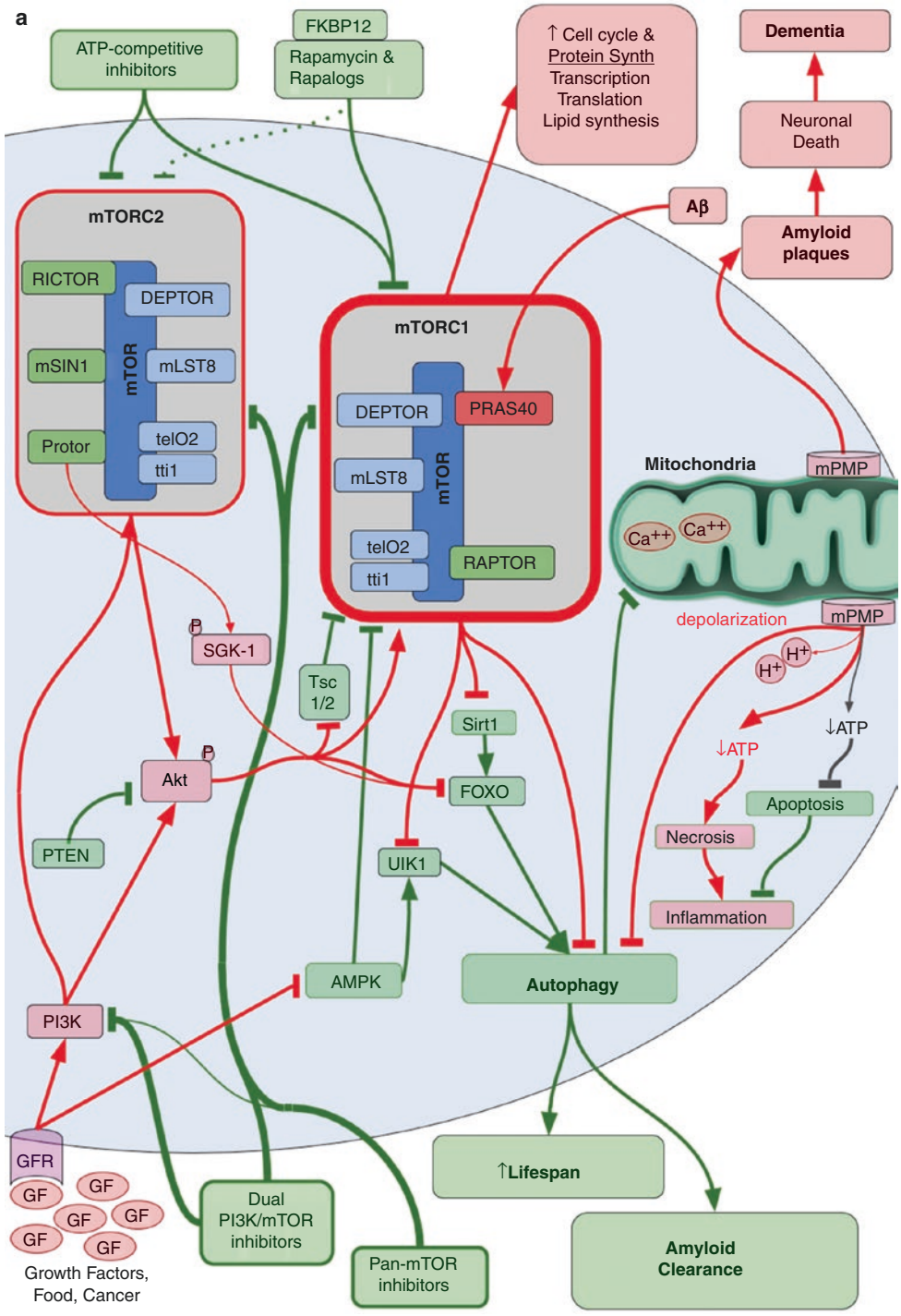
chaperone and other thiol containing biomolecules as well, and this activity may allow PARK7 to participate in the reduction of oxidized thiols and may be a mechanistic route towards the observed attenuation of α-synuclein aggregation [102, 120, 121].

Mechanisms of other neurodegenerative disorders may also be influenced by PARK7. In addition to its role as a general thiol chaperone, PARK7 has also been identified as a copper chaperone with peroxidase activity involved in the transfer of copper ion to activate SOD-1 [122, 123]. The interaction between PARK7 and SOD-1 may be an important key to ALS treatment, as cell culture studies and animal models have shown PARK7 overexpression attenuates oxidative damage and increases cell viability in SOD-1 mutant neurons and mice [124].

20.4 Dementia and mTOR

A more recent therapeutic target for multiple dementias has been the inhibition of the serine/threonine protein kinase mechanistic target of rapamycin (mTOR). Both dementias and increased mTOR activity seem to create cells with an inability for cellular housekeeping of macromolecules, leading to protein aggregates in the case of dementias. Research into the mTOR pathway was launched in 1975 when rapamycin, an inhibitor to mTOR found in soil on Easter Island, was shown to act as an antifungal antibiotic [125]. Since that time, it has been elucidated that mTOR acts as a central detector coordinating cell action based on nutrient and stress sensing (Fig. 20.2). Rapamycin, hailed as a fountain of youth compound, has been found to increase lifespan in organisms ranging from yeast, to nematodes, to fruit flies, and in mice [125–129]. Lifespan increased by as much as 100% in mice, with an average increased lifespan of ~20% [130]. Caloric restriction, which is also known to decrease mTOR activity, likewise has been shown to increase lifespans across many genera, and recently a 30% calorically restricted diet has been shown to increase lifespan in rhesus monkeys by 15% [131, 132]. Resveratrol, a compound found in red wine among other foods, has been extensively investigated as a compound that promotes longevity and has been found to inhibit the mTOR pathway. While most of the work with resveratrol has investigated its ability to decrease DNA methylation thereby preventing genes such as tumor suppressors from being shut off, it has recently been shown the resveratrol directly binds to mTOR at the same site as ATP, which presents an elegant mechanism to mimic caloric restriction at a molecular level [133–138]. There have been 14 clinical trials of resveratrol for various dementias with eight in phase 1, three in phase 2, and three in phase 3 (clinicaltrials.gov as of February 21st, 2017). In general, resveratrol has proven safe but not very effective. Two of the phase 3 trails were targeted at AD, with one being withdrawn (clinicaltrails.gov NCT00743743) and the other completing but not yet reporting results (clinicaltrails.gov NCT00678431). There are promising phase 2 trials targeting AD. A 2015 study showed positive resveratrol dose-dependent effects even though only 1% of resveratrol passed the blood brain barrier to reach the nervous system (NCT01504854) [139]. More recently, a phase 4 study completed in December 2016 is comparing dietary interventions of resveratrol supplementation, omega-3 supplementation, and caloric restriction. In a second phase, the addition of physical/cognitive training in conjunction with the supplements is being assessed. Outcomes will measure any change in the Alzheimer Disease Assessment scale, functional/structural brain changes, and plasma biomarkers, but results have not been released yet (clinicaltrails.gov NCT01219244). The last phase 3 trial of resveratrol is targeting HD and is still in the recruiting phase (clinicaltrials.gov NCT02336633).

mTOR is actually a component of two protein complexes termed mechanistic target of rapamycin (mTORC1 and mTORC2), both of which contain DEPTOR, mLST8, telO2, and tti1 (DEP domain containing mTOR-interacting protein, mammalian lethal with sec-13 protein 8 [also known as Gβ*L*], telomere maintenance 2, and telO2-Interacting Protein 1, respectively). The mTORCs differ in that mTORC1 alone contains mSIN1, RICTOR, and Protor (mammalian


a
ATP-competitive inhibitors
FKBP12
Rapamycin & Rapalogs
↑ Cell cycle & Protein Synth Transcription Translation Lipid synthesis
Dementia
Neuronal Death
Amyloid plaques
Aβ
mTORC2
RICTOR
DEPTOR
mTOR
mSIN1
mLST8
Protor
telO2
tti1
mTORC1
DEPTOR
PRAS40
mTOR
mLST8
telO2
tti1
RAPTOR
Mitochondria
mPMP
Ca++
Ca++
depolarization
mPMP
H+
H+
↓ATP
↓ATP
Apoptosis
Necrosis
Inflammation
P
SGK-1
Tsc 1/2
Sirt1
FOXO
P
Akt
PTEN
ULK1
AMPK
Autophagy
PI3K
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Dual PI3K/mTOR inhibitors
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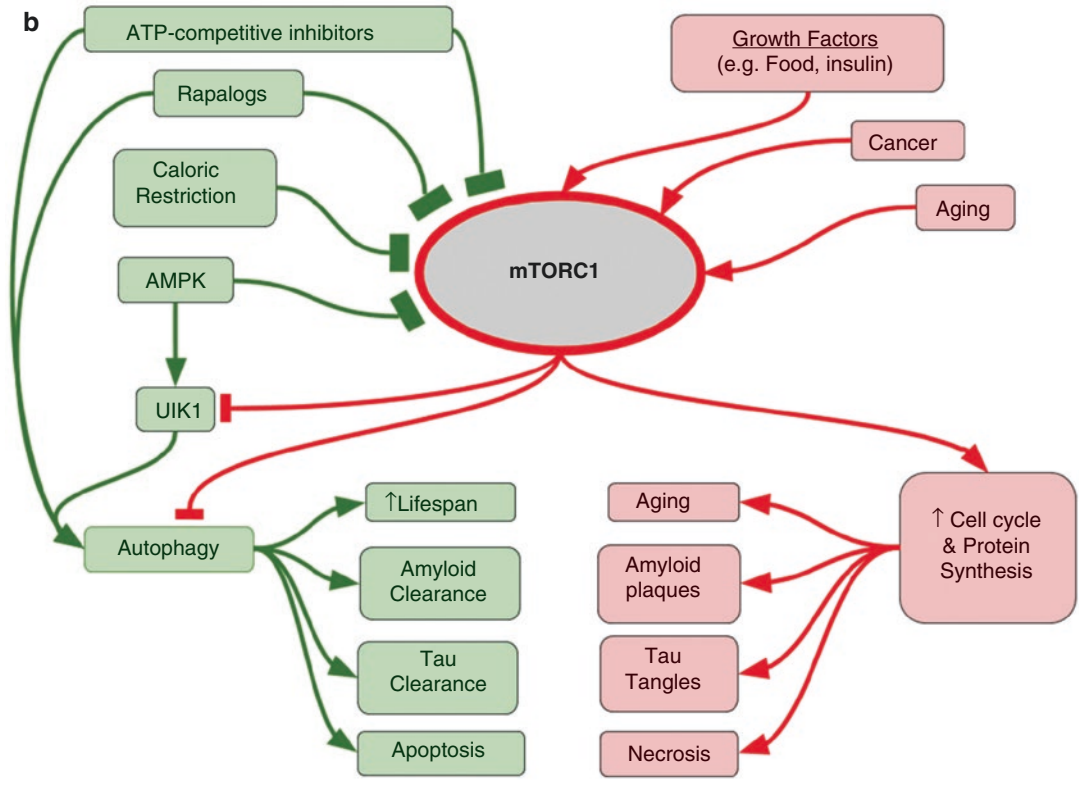

Fig. 20.2 (continued)

Fig. 20.2 mTOR signaling and inhibitors: (**a**) Growth factors, food, and cancer all cause activation of PI3K and inactivation of AMPK which cause an increase in mTOR activity in both complexes mTORC1 and mTORC2 and decrease the level of cellular autophagy. Restoring autophagy either through mTOR inhibitors (rapalogs, ATP-competitive inhibitors, pan-mTOR inhibitors, or dual PI3K/mTOR inhibitors) or reduced caloric intake (growth signals) all restore autophagy which can clear Aß and Tau, thereby reducing amyloid plaques and tangles, respectively. (**b**) A simplified mTORC pathway showing the positive effects rapamycin and its analogs have by increasing autophagy compared to the detrimental outcomes of increased mTORC signaling. Beneficial and deleterious interactions or macromolecules are shown in green and red, respectively. Proteins found in both mTOR1 and mTOR2 are colored blue. Abbreviations: *AMPK* AMP-activated protein kinase, *DEPTOR* DEP domain containing mTOR-interacting protein, *FKBP12* FK506/rapamycin binding protein, *FOXO* Forkhead box protein, *mLST8* mammalian lethal with sec-13 protein 8 (also known as GβL), *mPMP* mitochondrial permeability transition pore, *mSin1* Mammalian Stress-activated map kinase-Interacting protein 1, *mTOR* Mammalian Target Of Rapamycin, *mTORC* Mammalian Target Of Rapamycin Complex, *PTEN* Phosphatase and tensin homolog, *PRAS40* proline-rich Akt substrate 40 kDa, *protor1/2* protein observed with rictor 1 and 2, *RAPTOR* Regulatory-Associated Protein of mammalian Target Of Rapamycin, *RICTOR* Rapamycin-Insensitive Companion of mTOR, *Sirt1* Sirtuin-1, *telO2* telomere maintenance 2, *tti1* telO2-Interacting Protein 1

stress-activated map kinase-interacting protein 1, and rapamycin-insensitive companion of mTOR, and protein observed with rictor 1 and 2, respectively), while mTORC2 alone contains PRAS40 and RAPTOR (proline-rich Akt substrate 40 kDa, and regulatory-associated protein of mammalian target of rapamycin, respectively) (Fig. 20.2a). Much more is known about mTORC1 signaling compared to mTORC2; however, mTORC2 can activate mTORC1 through phosphorylation and activation of AKT. It has been reported that both amyloid-beta (Aß) increases mTOR activity and that mTOR increases Aß levels [140, 141]. Interestingly, Aß has been shown to induce mTOR hyperactivity through PRAS40 that is unique to mTORC1 [142].

Growth factors activate mTORCs by binding growth factor receptors, which activate PI3K. While rapamycin inhibition of mTORC1 complex has proven beneficial for many disease states, long-term rapamycin use also inhibits mTORC2 which some have suggested could cause unforeseen deleterious side effects [143]. However, pan-mTOR inhibitors (which block both mTORC1 and mTORC2) have been shown to be beneficial [143]. There are now compounds which preferentially target mTORC1 (rapalogs), both mTORC1 and 2 over PI3K (pan-mTOR inhibitors), or target all three components equally (dual PI3K/mTOR inhibitors) (Fig. 20.2a).

A cellular theme that has been repeatedly supported is that, while mTOR and aging in general inhibit autophagy (cleaning up of macromolecules in a cell), maintaining a high level of autophagy via caloric restriction, sirtuin, or rapalogs promotes longevity [144]. Neurodegenerative diseases seem particularly susceptible to low autophagy activity, increasing the speed with which amyloid aggregates form. Calorically restricted cells increase autophagy via two well-established pathways. Cells in a low energy state have decreased PI3K activity, thereby lowering Akt activity, which in turn lowers mTORC1 (Akt inhibits Tsc1/2 which inhibits mTORC1). Normally mTORC1 inhibits sirtuin (Sirt1) which in turn activates FOXO, increasing, cellular autophagy. Calorically restricted cells also have increased AMPK activity, which activates Ulk1 also leading to increased autophagy. The increased cellular autophagy has multiple beneficial effects. Autophagy offers a molecular mechanism to alleviate the burden posed by the general phenomena of amyloid proteins in the various dementias. A low level of autophagy also leads to necrosis over apoptosis, which increases inflammation via the immune system. Intracellular stress is known to signal through Bcl-2 and cause increased calcium levels opening the mitochondrial permeability transition pore (mPTP) which can lead to caspase-dependent intrinsic apoptosis [145, 146]. The mPTP can exist in three states: closed, transiently open in low conductance, and permanently open in high conductance [147–149]. When excessively open, the mPTP causes an almost complete loss of ATP production, due to depolarization of the mitochondria, and leads to caspase-independent necrosis due to lack of energy to follow the controlled apoptotic path (Fig. 20.2a) [150].

While mitochondrial dysfunction has been implicated in various dementia including Parkinson disease, amyotrophic lateral sclerosis, Huntington disease, and Alzheimer disease, methods to target it have trailed [151–154]. Upregulation of the mTOR pathway is now known to increase oxidative stress. Oxidative stress has been targeted with antioxidant therapy and it has been found conjugating a cation compound to the antioxidant can greatly increase localization to the mitochondria due to its negative potential of 165 mV across the inner membrane [155]. Uptake of antioxidants has been shown to increase up to 80-fold while potency has increased up to 800-fold [156]. Most of the early targeting of the mTOR pathway was for cancer patients with many cancers showing increased mTOR pathway signaling. Not only is rapamycin FDA approved (Sirolimus) but follow on rapamycin analogs (rapalogs) are also FDA approved. For example, everolimus (Afinitor), temsirolimus (Torisel), and ridaforolimus are used for various cancers, with sirolimus and everolimus also particularly being used as immunosuppressants for use after organ transplants [130, 157]. There are 417 clinical trials listed as targeting mTOR, and of those 23 are targeting neurodegenerative diseases (clinicaltrials.gov as of February 21st, 2017). For example, tamoxifen has been shown to increase autophagy [158] like the mTOR inhibitors mentioned earlier, which is

why there is a phase 1–2 clinical trial using tamoxifen for ALS (clinicaltrials.gov NCT02336633). The increased autophagy should decrease TDP-43 accumulation seen the in multiple neurogenerative diseases, but results have not yet been published for this trial. Interestingly, the combination of an anticancer therapeutic (CSC-3436) and tamoxifen was shown to synergistically kill cancer cells but switch cells from an autophagic to apoptotic state [159], suggesting combination therapies involving mTOR will likely take more time to elucidate. The fact that inhibition of mTOR seems to reduce dementia and extend lifespan raises the hope of not only longer life but also an increase in years of quality.

Conclusions

While the aforementioned disorders, on the surface, appear to be distinct and unrelated, many of the underlying mechanisms can offer insights into other diseases of neurodegeneration. The mitigation of oxidative stress has long been a focus in preventing the pathogenesis of many classes of neurodegenerative dysfunctions. In the past, with limited knowledge of mechanistic pathways leading to increased oxidative conditions, elevation of antioxidants, both exogenous and endogenous, has been the point of concentration. As seen above, a greater understanding of the pathways that either exacerbate or attenuate oxidative stress is revealing a number of regulation points to target for future therapy. A generation ago, the analogy of antioxidants being akin to a bullet-proof vest to intercept free-radicals was often evoked. Using this same analogy, we can equate the increasing understanding of signaling mechanisms and regulating pathways of oxidative stress as identifying the shooter, thus rather than trying to deflect damaging oxidative agents, by targeting these pathways, we can instead decrease the generation of these agents. As a greater understanding of the regulating pathways of neurodegeneration comes to light, the ability to develop pharmacotherapy to manipulate these pathways and improve treatment options will follow.

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21 Neural Stem Cell-Based Therapeutic Approaches for Brain Repair

Cláudia Saraiva, Tiago Santos, and Liliana Bernardino

21.1 Introduction

The central nervous system depends on a small number of neural progenitors/neural stem cells (NSCs) to generate multiple cell types. During development, these progenitors proliferate, self-renew and differentiate into neurons and glia, relying on spatial and temporal cues [1]. The onset of neurogenesis is accompanied by a phenomenon called radial glia differentiation, where most neurons, if not all, are derived from these glial-like cells located at the ventricular zone [2, 3]. In mammals, radial glial cells disappear soon after birth. The role of NSCs is occupied by a closely related glial fibrillary acidic protein (GFAP)-expressing astrocyte-like cell [4, 5]. These astrocyte-like adult NSCs are principally found in the subventricular zone (SVZ) lining the lateral ventricle wall and the subgranular zone (SGZ) in the hippocampal dentate gyrus (Fig. 21.1a); outside these two regions, neurogenesis is limited, with the SVZ being the largest germinal center [6]. NSCs are known to be responsive to several brain injury scenarios, where they markedly increase both in terms of proliferation and migration [7]. The potential of having a virtually unlimited source of new cells that, in response to injury, proliferate, migrate towards the injured site and differentiate into new neurons raises many expectations for the treatment of brain injury and degeneration. Nevertheless, no therapies that fully restore loss of brain function are yet available. On the other hand, micro- and nanoparticles are also promising platforms to support the integration, differentiation and activity of NSCs since they are very versatile allowing protection, stability and spatio-temporal control release of factors to target cells [8, 9]. Recent advances in this field that are relevant for the treatment of neurodegenerative diseases will be discussed. We will give an overview of the cellular and molecular mechanisms of neurogenesis in neurodegenerative disorders like Parkinson's disease (PD) and Alzheimer's disease (AD) as well as some promising stem cell-based therapies.

21.2 Neural Stem Cell Niches and Adult Neurogenesis

21.2.1 The Subventricular Zone

The SVZ stem cell niche (referring to both ventricular and subventricular regions) is the largest neural stem cell pool of the adult mam-

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_21

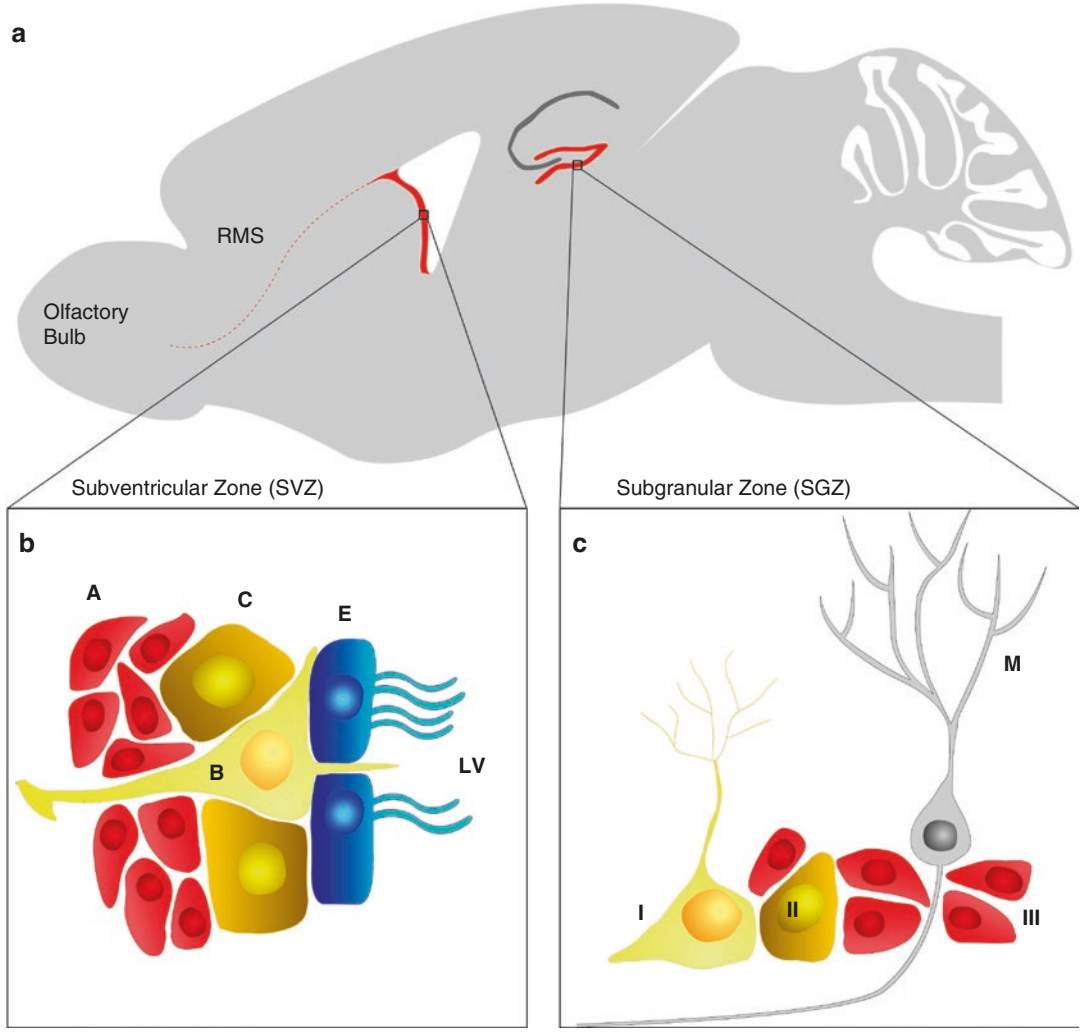

Fig. 21.1 Localization and composition of neural stem cell (NSC) niches in the adult rodent brain. (**a**) Sagittal rodent brain slice displaying the localization of the two main neurogenic niches: the subventricular (SVZ) and subgranular (SGZ) zones. (**b**) At the SVZ, type B NSCs cells contact with the lateral ventricles and give rise to C progenitor cells that differentiate into neuroblasts (type A cells). Physiologically, neuroblasts migrate through the rostral migratory stream (RMS) towards the olfactory bulb where then differentiate into mature neurons; E, ependymal cells. (**c**) Similarly, type I (or B) stem cells at the SGZ give rise to type II (or D) progenitor cells that in turn generate neuroblasts (type III or G cells) which ultimately differentiate into mature granule neurons (M)

malian brain. In this region, there are three types of cells: B, C, and A (Fig. 21.1b). Type B cells have been hypothesized to be quiescent astroglial stem cells, expressing phenotypic markers of immaturity such as nestin and sex determining region Y-box 2 (Sox2) and the glial cell marker GFAP. These cells generate non-radial actively proliferating type C progenitor cells. These intermediate progenitors then originate doublecortin (DCX)-positive neuroblasts, or type A cells, that migrate towards the olfactory bulb (OB) in rodents [10]. Type B stem cells are responsive to external cues present in the SVZ niche (Fig. 21.2): the cerebrospinal fluid (CSF) from the ventricular lumen (through a small apical process that culminates in a single cilium); other cells in the SVZ (type C and A cells, microglia, astrocytes); and the vasculature at dedicated sites that lack glial end feet and pericyte coverage (via a long basal process) (reviewed in [11]). The migration towards the OB occurs in chains of condensed neuroblasts through the

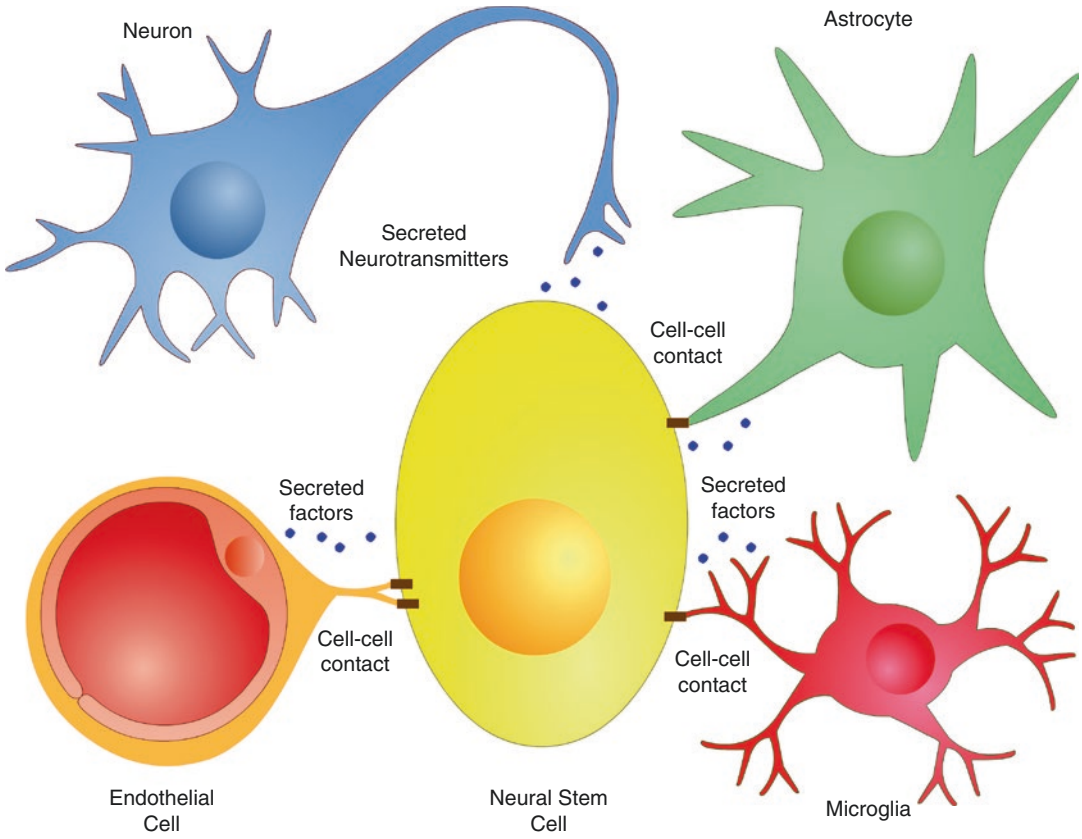

Fig. 21.2 Factors modulating neural stem cells. Microglia, astrocytes, and basal lamina extensions (fractones) from endothelial cells can establish contact with neural stem cells and trigger signaling pathways involved in survival, proliferation, and/or differentiation. Signaling can also be induced by factors secreted by these cells plus neuron-derived neurotransmitters

rostral migratory stream (RMS) [12, 13]. At the rodent OB, neuroblasts integrate the cortical layers and differentiate, mainly as new GABAergic granule-interneurons and GABAergic or dopaminergic periglomerular-interneurons [14]. These newborn neurons contribute to olfactory discrimination and memory [15].

Regarding the human brain, there is no consensus for the existence of a RMS. A ventral extension of the lateral ventricle presenting DCX- and GFAP-positive cells, that resemble the RMS, was described during the second trimester of gestation. However, no neuroblast chain-like structures were detected in the OB, indicating that migration might occur to other destination [16]. Accordingly, new neurons generated from the SVZ were found to migrate to the prefrontal cortex in children up to 18 months old [17]. After birth, it has been described that SVZ proliferation and migration tends to decrease, with no detectable RMS during adulthood [17, 18], or a shift of the putative human RMS to a more caudal positioning. This unpredictable organization is likely due to the overdeveloped human frontal lobe [19, 20]. Nevertheless, the migration and incorporation of new neurons into the adjacent striatum was detected in the human adult brain [21].

21.2.2 The Subgranular Zone

Neurogenesis also persists in the hippocampal dentate gyrus, however with a smaller pool of NSCs [22, 23]. Type I (or B) and type II (or D) cells correspond to the type B and C cells present in the SVZ, respectively (Fig. 21.1c). Similarly to

SVZ type B cells, SGZ type I cells are quiescent astroglial stem cells [24]. These cells usually have a radial process that projects throughout the niche bridging the entire granule cell layer and ramifying into the inner molecular layer. Type I cells also express markers of immaturity such as Sox2 and nestin and the glial marker GFAP and also establish physical and chemical contact with blood vessels. Type II cells, or intermediate progenitors, no longer have the radial morphology nor express GFAP. They give rise to type III (or G) neuroblasts that, unlike SVZ, do not migrate a long distance. Instead, SGZ neuroblasts migrate locally and differentiate into glutamatergic dentate granule cells with axonal projections towards CA3 [25–27]. These new neurons are involved in the process of learning and memory [28, 29].

In the human brain, Spalding and colleagues have confirmed that substantial neurogenesis occurs throughout life [30]. In this integrated study, they report that the size of the cycling neuronal population constitutes 35% of hippocampal neurons corresponding to a vast number of the dentate gyrus neurons. Additionally, 0.004% of the dentate gyrus neurons are exchanged daily in adult humans, corresponding to approximately 700 new neurons *per* day in each hippocampus. More importantly, there is only an approximately fourfold decline in hippocampal neurogenesis during the entire adult lifespan in humans. Altogether, these data undeniably confirm that adult hippocampal neurogenesis occurs in the human brain and that it may contribute to relevant brain functions.

21.3 Neurogenesis in Neurodegenerative Diseases: Cellular and Molecular Mechanisms and Potential Regenerative Therapies Using Nanomaterials

21.3.1 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder mainly characterized by the progressive degeneration of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) leading to striatal dopamine (DA) depletion and the incidence of alpha (α)-synuclein aggregates known as Lewy bodies [31, 32]. Common motor symptoms that clinically define PD include rigidity, tremor, bradykinesia, among others. Currently the standard treatment for PD is based in DA replacement (using a precursor of DA, levodopa) with high efficacy in the early stage of the disorder. Nevertheless, over time these drugs lose effectiveness and cause dyskinesias and severe psychiatric complications. Deep brain stimulation is also used in some patients, in more advanced stages of the disorder, with successful suppression of motor symptoms; however, it does not stop the disease progression.

Several regenerative medicine approaches are under intense examination to address the impact of stem cells in PD. However, the low amount of data in post-mortem brain tissue from PD patients, together with contradictory experimental findings, make the role of adult neurogenesis in PD a high controversial subject among the scientific community. It was firstly shown that PD patients display impaired neurogenesis since they presented lower levels of cells expressing the marker for proliferation PCNA (proliferating cell nuclear antigen) in the SVZ, a decrease of nestin-positive cells (immature neural precursor cells) both in the OB and in the dentate gyrus of the hippocampus, as well as a reduction in β-III-tubulin-positive cells (neuronal marker) in the SGZ [33]. DA seems to play a critical role in neurogenesis impairment. Indeed, PD progression negatively correlates to NSCs numbers while cumulative use of L-Dopa in PD patients seems to result in increased numbers of proliferating NSCs in the SVZ [34]. The SNpc and the ventral tegmental area (VTA) project dopaminergic fibers that innervate the neurogenic niches in a specific pattern [35], and they are in close proximity to epidermal growth factor receptor (EGFR)-positive cells that include all C cells and a subset of B cells [33, 36]. Advanced PD patients present not only significantly less amount of EGFR-positive cells in the SVZ but also weaker expression of EGFR [37]. In addition, EGF and EGFR levels were also found to be decreased in the striatum of

PD patients [38]. SVZ type C and A cells express both, D1-like and D2-like, DA receptors [33, 39]. DA reduction in animal models leads to impairments in NSCs proliferation and EGFR expression that are D2-like receptor-mediated [33, 37, 40, 41]. Alpha-synuclein also seems to be involved in neurogenesis impairment. Interaction between accumulated α-synuclein and p53 culminated in Notch1 signaling dysregulation in the SGZ of rats that potentially trigger some of the non-motor symptoms associated with the PD pathology [42, 43]. Neural-committed induced pluripotent stem cells (iPSCs) obtained from fibroblasts of patients with triplication of the α-synuclein gene (SNCA; associated with early onset of PD) were unable to develop neuronal complex networks when compared with control neural committed iPSC, also showing a correlation between α-synuclein expression and neurogenesis impairment [44]. Hypermethylation of thousands of genes has been found in brain tissue of PD patients, specifically neurogenic-related genes such as Wnt, suggesting a critical role for Wnt-associated neurogenesis in PD [45]. Inflammation is also a major player in neurodegenerative disorders and higher expression of inflammatory molecules in PD patients, such as tumor necrosis factor (TNF-α) or cytokines (e.g., interleukin (IL)-6), correlates with non-motor symptoms, namely anxiety and depression, that precede the motor symptoms of the pathology [46, 47]. Similar symptomatology is found in animal models of impaired neurogenesis [48] leading some groups to defend a robust developmental component in PD onset and progression.

On the other hand, neurogenesis impairment hypothesis in PD was challenged by Hol's group that analyzed brain tissue from healthy controls, PD patients and incidental PD (did not receive L-Dopa treatment) in terms of SVZ proliferation using two markers for proliferation: PCNA and phosphohistone H3 (pHH3). No significant differences neither in terms of proliferation in the SVZ between groups or in glial fibrillary acidic protein delta (GFAPδ)-positive cells (radial glia marker) in the OB were found in the study. Cultivation of neurospheres, obtained from the post-mortem tissue of the three analyzed groups, was achieved with similar efficiency and differentiation potential into neurons and glial cells. Moreover, treatment of human NSC lines with DA and DA agonist did not result in stimulation of NSCs proliferation [49], indicating that DA depletion may not affect the neurogenic capacity of the PD brain. Isolation and culture of human-derived NSC lines from the SVZ, cortex or SNpc of post-mortem PD patients was confirmed by Wang et al. [50], although there are high variability in the amount of SVZ proliferating cells isolated from post-mortem tissue of different donors [51]. The contradictory results obtained from different groups both in PD patients (mentioned above) and animal models of PD (reviewed at van den Berge et al. [52]) are being under debate in order to achieve the best analytic methodology that shine light into neurogenesis in PD.

Major advances have been made in the characterization and molecular understanding of PD although it did not result in the development of any effective treatment. Cell replacement strategies are a promising approach since they are based on the introduction of new cells that can replace the lost dopaminergic neurons in PD patients. Transplantation studies in rodents using SVZ tissue explants, neurospheres or dissociated cells are being made since the early 1990s (reviewed at Cave et al. [53]). Although they achieve some recovery in PD animal models after transplantation, the low survival of grafted cells together with a high differentiation into glial cells are still challenges that need to be surpassed. The development of new biocompatible biomaterials that provide adequate physical and chemical support to grafted cells might allow the use of this type of therapeutic strategies. For example, Wang and colleagues developed scaffolds bio-functionalized with glial cell-derived neurotrophic factor (GDNF) that were able to promote NSC proliferation or support ventral midbrain DA progenitors as well as to improve their survival and axon growth in vitro. It also improved survival, proliferation, migration and neurite growth of grafted cells increasing striatum reinnervation without affecting the host immune system [54–56]. A hyaluronic acid-laminin based hydrogel that promotes the expression of chemokine stro-

mal cell-derived factor-1α (SDF-1α) receptor in NSCs, promoted the retention and migration of transplanted stem cells in response to SDF-1α [57], demonstrating the potential of biomaterials to support and/or improve cell engraftment.

Another promising therapeutic approach involves the modulation of endogenous NSCs from the SVZ niche in order to obtain new dopaminergic neurons able to repopulate the lesioned striatum of PD patients. This strategy lacks ethical issues, bypasses teratoma formation risk and represents a less invasive approach. This type of modulation may be mediated by growth or transcription factors and some biomaterials that provide support to the cells. Transforming growth factor alpha (TGF-α) infusion in a 6-hydroxydopamine (6-OHDA) rat model of PD resulted in dopaminergic differentiation in the striatum [58], while the brain-derived neurotrophic factor (BDNF) showed to be able to recruit NSCs from SVZ into the striatum and SNpc [59]. An *in vitro* study with polymeric films made of poly(lactic acid) PLA was able to mimic some physical and biochemical characteristics of the NSC niche, maintain NSC progenitors and restrict glial progenitor phenotypes [60]. Recently, the administration of microRNA-124 (miR-124) loaded poly(lactic-*co*-glycolic acid) PLGA nanoparticles (NPs) into the lateral ventricles of 6-OHDA challenged mice proved to be efficient in promoting migration of new neurons into the lesioned striatum leading to behavior improvements in the 6-OHDA mice treated with miR-124 NPs when compared with saline ones [61]. Multifunctional biomaterial comprising injectable multifunctional gelatin-hydroxyphenylpropionic acid hydrogels and dextran sulfate/chitosan polyelectrolyte complex nanoparticles able to deliver SDF-1α provided cues and structural support to NSCs, being a promising biomaterial for injection into cavity brain lesions to recruit endogenous NSCs and enhance neural tissue regeneration [62]. Altogether, these studies validate the importance of biomaterials to enhance the therapeutic potential of NSC-based therapies.

Several trials based on the transplantations of stem cells obtained from different sources have been made over the years in PD patients and, besides the drawbacks in these studies, they demonstrated an overall beneficial effect in the quality of life of PD patients (reviewed at Barker et al. [63]), resulting in a new European clinical trial based on human fetal ventral mesencephalic (hFVM) tissue—TRANSEURO. A clinical trial based on neural stem cells (ISC-hpNSC®) from human parthenogenetic stem cells (hpSC), from International Stem Cell Corporation (ISCO) was also recently approved by the Australian authorities to treat PD moderate to severe patients.

21.3.2 Alzheimer's Disease

Alzheimer's disease (AD), the most prevalent type of dementia, is characterized by synaptic and neuronal loss mainly in the entorhinal cortex, hippocampus and neocortex. The hallmarks of AD are neurofibrillary tangles, intraneuronal lesions composed of aggregated hyperphosphorylated tau, and amyloid deposits composed of aggregated amyloid beta (Aβ), that give rise to behavioral and physical impairments, namely olfactory deficits, memory impairments, cognitive and functional decline. The treatments available for AD patients are merely symptomatic and belong to two different categories: cholinesterase inhibitors and *N*-methyl-D-aspartate (NMDA) receptor antagonists. These drugs act by reducing the rate at which acetylcholine (ACh) is broken down, and by inhibiting the glutamate overstimulation in the glutamatergic system, respectively [64].

Molecular players in AD, such as acetylcholine, presenilin-1 (PS1), and soluble amyloid precursor protein α (sAPPα), are also modulators of neurogenesis. Indeed, proteases involved in PS1 and sAPPα pathways are able to cleave Notch1 and components of EGF signaling, essential factors for neurogenesis [65–67]. Despite the enormous amount of data in animal models of AD (reviewed at Chuang [69]; Mu and Gage [68]), how neurogenesis is altered in AD patients is still unclear with more studies in post-mortem human tissue needed. Conflicting results have been released over the years with some authors defending that there are AD-related neurogen-

esis impairments that contribute to cognitive deficits while others argue that there are either increased or no alterations in neurogenesis in AD pathology.

An increase in the levels of DCX, polysialylated neuronal cell adhesion molecule (PSA-NCAM; migrating neuronal and synaptogenic marker), TUC-4 (Turned On After Division/Ulip/CRMP-4; early neuronal marker) and NeuroD (basic helix-loop-helix transcription factor; neuronal marker) was found in the dentate gyrus of AD patients when compared with healthy controls [70]. An increase in Ki67-positive cells (marker of proliferation) was also found in the hippocampus of pre-senile AD patients, nevertheless it was a result of increased proliferation of glia and vascular cells rather than a result of enhanced neurogenesis (no alteration in terms of DCX expression) [71]. A more recent study showed that in AD patient neurogenesis varies between neurogenic niches, type of cells and AD phase. The authors showed a reduction in Musashi-1 (a progenitor/stem cell marker) and an increase in nestin and PSA-NCAM that correlated with AD progression (namely the severity of taupathology) in SGZ and increased levels of nestin in the SVZ. DCX was also increased in the SGZ of AD patients although no correlation with the pathology progression was observed. However, no alterations were found in the neuronal marker β-III-tubulin [72]. This study can in part explain the contradictory results seen by others while clearly indicating that the cholinergic pathology can have a detrimental influence on neurogenesis, but also that a compensatory increase in proliferation that is not followed by an increase on the migratory neuroblasts and differentiated neurons may exist. Recently, a study compared the expression of Sox2 (neural stem cell marker) and NeuN (mature neuronal marker) in the post-mortem tissue of non-demented with Alzheimer's disease neuropathology (NDAN), mild cognitively impaired (MCI), AD and healthy individuals. Contrary to what happens in mice, there are a subset of cells that expressed at same time Sox2 and NeuN. They found that Sox2-positive and Sox2/NeuN-double positive cells are increased in NDAN when compared with AD and MCI and are even expressed in higher levels than in controls, while Sox2 expression is reduced in AD comparing with healthy controls. Moreover, Sox2 expression correlated positively with preserved cognitive function. This study seems to indicate that neurogenesis is affected even in early stages of the pathology and that enhancement of neurogenesis driven by a differentiated microRNA expression is a viable approach, since it could minimize the cognitive impairments associated with AD pathology [73].

Many advances have been made in the understanding of AD pathology; nevertheless, pharmaceutical companies are repeatedly failing at developing new effective treatments for AD. Stem cell-based therapies are a promising approach that could compensate for the massive and progressive neuronal and synaptic loss. Transplantation of human NSCs overexpressing choline acetyltransferase showed improvements in learning and memory deficits in rat and mouse models of AD [74, 75]. Bilateral transplantation of NSCs into the hippocampus of APP/PS-1 mouse model of AD demonstrated to be able to integrate into the circuits, replacing some of the damaged/dead neurons; to promote brain plasticity (increasing synaptogenesis and long-term potentiation); as well as to reduce inflammation, by regulating microglia and astrocyte activation, culminating in improvements in spatial learning and memory function, despite no alteration in terms of Aβ pathology were observed [76, 77]. These studies validate the potential of cell transplantation as a possible therapeutic approach, although advances in terms of transplant survival and engraftment are needed. Cell replacement in AD might also be achieved through the induction of endogenous NSCs. However, in both strategies several challenges need to be overcome: functional integration, recruitment and modulation of NSCs, micro-environmental control, to name a few. The use of biomaterials can be a useful tool to overcome some of these challenges. One example is the transplantation of NSCs alone or in combination with nerve growth factor (NGF)-poly(ethyleneglycol)-poly(lactic-*co*-glycolic acid)-nanoparticles (NGF-PE-PLGA-NPs) in the intra-hippocampal and basal forebrain of 192IgG-

saporin-induced AD rats. The authors showed that both NSC transplants and combination treatment led to learning and memory improvements. Nevertheless, the combined treatment was more efficient in promoting synaptogenesis, in differentiating cells into cholinergic neurons, and in forming acetylcholine-esterase fibers. This can be explained by the presence of NGF, a supporter of cholinergic neuronal differentiation, survival, and growth that was released in a controlled way for a long period of time by the NP formulation [78]. NSCs obtained from postnatal mice were grown *in vitro* and transfected with: poly(2-hydroxyethyl methacrylate)-retinoic acid-poly(carboxybetaine)-cell penetrating peptide (PHEMA-RA-PCB-CPP) polymers followed by the conjugation with superparamagnetic iron oxide nanoparticles (SPIONs) and complexation with small interfering (si)RNA against SOX9 (siSOX9), from now on designated as ABC/SPIONs/siSOX9. This formulation has theranostic properties that allow not only the tracking of NPs *in vivo* due to the SPIONs but also the enhancement of neuronal differentiation due to the dual release of siSOX9 in an initial phase (inhibiting translation of Sox9, a critical protein in NSC maintenance and glial fate determination), and retinoic acid in a later phase (a well-described promotor of neurogenesis). Transplantation of these NSCs transfected with ABC/SPIONs/siSOX9 in a double transgenic mouse model (2x-tg) of AD resulted in greater levels of neurons that culminated in a decrease in memory and cognitive deficits when compared with non-treated 2x-tg AD mice. Moreover, the migration pattern of transplanted NSCs could be traced non-invasively by magnetic resonance imaging (MRI) [79]. On the other hand, the intraperitoneal administration of curcumin-loaded PLGA NPs resulted in higher levels of both proliferating cells (BrdU-positive cells) and new neurons (BrdU/NeuN-double positive cells) in the SGZ of a rat model of AD, induced by the injection of $A\beta_{1-42}$ in the hippocampus. Most importantly, the curcumin-loaded NPs treatment culminated in the amelioration of learning and memory deficits of AD model rats by enhancing endogenous neurogenesis through activation of the Wnt/β-catenin pathway. Similar effects were observed with bulk curcumin, nevertheless the use of NPs reduced in 40-times the amount of compound need to obtain a similar physiological response [80]. These results endorse the potential of NPs in the transport and controlled release of drugs and its benefits to the field of neurodegenerative disorders.

Although all the progress and knowledge that we have acquired throughout all these years in stem cell-based therapies, there is still no clinical trial in progression using NSCs or nanoparticles to treat AD. A pilot study was done using human granulocyte colony-stimulating-factor (G-CSF) to mobilize endogenous NSCs in AD patients and placebos. This compound proved to be safe and showed promising results in cognitive performance of AD patients [81].

Conclusions

The discovery of NSCs in the adult mammalian brain opened new avenues in understanding brain plasticity over the past decades. Adult neurogenesis seems to be mainly restricted to specific areas of the brain (SVZ and SGZ neurogenic niches), that display a specific microenvironment. Scrutinization of the composition and function of these neurogenic regions gave us the understanding that adult neurogenesis is essential for proper brain function. Remarkably, neurogenesis is enhanced in cases of brain lesions as stroke in an attempt of the brain to repair itself. In neurodegenerative disorders the role of neurogenesis is still under debate, although a clear relationship between aging and a decline in neurogenesis has been shown. Nevertheless, the multipotent and self-renewing abilities of NSCs together with their ability to integrate pre-established circuits make them good candidates for possible interventions in neurodegenerative disorders. This can be achieved by using strategies such as transplantation or enhancement of endogenous neurogenesis. In both cases, several challenges have to be overcome, including the delivery of factors and/or cells into the

brain, the differentiation and integration of the cells into the pre-established circuits, and their survival, to name a few. Recent developments in the field of nanomedicine made nanoparticles as one of the best options, not only to enhance the delivery of drugs into the brain but also to act as scaffolds to improve survival and adaptation of new cells into the damage areas. Despite the advances in the field, there is still a big gap between pre-clinical studies and their translation into the clinic. So, a better understanding regarding the mechanism behind cell replacement, the development of animal models that better resemble the pathologies, as well as the standardization of protocol/methodologies (namely in the case of transplantation) together with the evolution of smarter nanomaterials are essential to successfully implement novel clinical therapies for neurodegenerative pathologies such as Parkinson's and Alzheimer's disease.

Acknowledgments This work was supported by Fundação para a Ciência e a Tecnologia (FCT) (SFRH/BD/90365/2012), by FEDER funds through the POCI—COMPETE 2020—Operational Programme Competitiveness and Internationalisation in Axis I—Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491), the National Funds by FCT—Foundation for Science and Technology (Project UID/Multi /00709/2013) and "Programa Operacional do Centro, Centro 2020" through the funding of the ICON project (Interdisciplinary Challenges On Neurodegeneration; CENTRO-01-0145-FEDER-000013)". The authors declare no conflicts of interest to disclose.

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22 Recent Advances in the Antioxidant Therapies for Alzheimer's Disease: Emphasis on Natural Antioxidants

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22.1 Alzheimer's Disease and Antioxidants

Alzheimer's disease (AD) is the foremost widespread neurological disorder and most common contributor of dementia and reduced cortical functions (e.g., judgment, orientation, behavior). The increment in AD cases, the lack of a treatment for the disease, and the current intricacy in diagnosing its preclinical phase comprise three major challenges that mark AD, a social threat. Distinct markers of AD pathology include marked loss of cholinergic neurons, hyper-phosphorylated tau protein, intracellular neurofibrillary tangles, β-amyloid (Aβ) deposits, and extracellular senile plaques [1, 2]. Oxidative stress is a prime trait of the AD and has been measured as remedial target for its treatment. Various factors could contribute to oxidative stress in brains of AD patients. Clinical characterization of AD relates to cognitive impairment and pathological characterization is marked by aggregation of β-amyloid plaques and neurofibrillary tangles, and the deficit of the cholinergic basal forebrain neurons [3–5]. β-amyloid toxicity may be responsible for neurodegenerative process in AD. Several studies have been performed to explore the therapeutic properties of natural antioxidants in last decade and recently [6, 7]. Byproducts of cellular physiology especially hydrogen peroxide, hydroxyl radicals, superoxides, etc. collectively form reactive oxygen species (ROS) which at low concentrations play significant role in cellular signaling (apoptosis, gene expression, etc.). ROS can act as intra as well as intercellular messengers. Production and elimination ratio of ROS is balanced through various defense mechanisms; however, few factors may be responsible for ROS overproduction and this imbalance may lead to oxidative stress [8]. Aggregation process of Aβ requires oxygen and generates hydrogen peroxide and this mechanism is assisted by Fe^{2+} and Cu^{+} ions. The deterioration of synapses in AD may involve Aβ-induced oxidative stress as previously reported that exposure of synapses to Aβ spoils the function of membrane ion and glutamate transporters and impairs mitochondrial function by an oxidative-stress-mediated mechanism [9, 10]. There can be various sources of ROS; hence, cells need to develop an effective antioxidation system to resist ROS damage. Since AD is a complex multifactorial disease, "one drug strategy" doesn't seem to work well in AD. Variety of compounds (acetylcholinesterase inhibitors, cognitive enhancers, natural products, etc.) has been well investigated for effective AD treatment but exact therapeutic solu-

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_22

tion is still lacking. The treatment options currently available for AD treatment such as group of acetylcholinesterase inhibitors (e.g., donepezil, rivastigmine, memantine, and galantamine) remain severely narrow and provide only minimal symptomatic relief rather than altering the disease progression. Clinically memantine is given to AD patients dealing with moderate to severe forms of disease.

Antioxidants when available at low concentrations in comparison to utilizable ROS levels, appreciably hinder the oxidation of that substrate [11]. Recent findings suggest that dietary antioxidants have therapeutic potential towards AD and its related symptoms. Antioxidants can be considered for prevention of degenerative disease and economical strategy to reduce the harmful consequences of augmented free radicals. It is vital to regulate the generation of free radicals in a given condition and this can be achieved by supplying antioxidants since they are capable of minimizing cellular damage. Amyloid plaques are generated due to imbalanced production and reduction of Aβ. Mitochondrially targeted antioxidant treatments for AD have been explicitly reviewed [12]. The author has discussed how the mitochondrially targeted antioxidants enter mitochondria. Development of MitoQ, MitoVitE, MitoPBN, MitoPeroxidase, and amino acid and peptide-based SS tetrapeptides as mitochondrially targeted antioxidants have been formulated and their incorporation into mitochondria has been reported as numerous 100-fold more than the natural antioxidants. These targets are known to quickly counteract the free radicals in mitochondria and diminish the toxicity levels. In this chapter, we shall deal with the magnitude of natural antioxidant treatments in neurological diseases and in particular for Alzheimer's disease. Neuroprotective effects of natural antioxidants and their therapeutic potential for AD have been explicitly explained. We have shed lights upon the significance and impact of most common dietary antioxidants in therapeutics of AD.

Based on the chemical structures and ability to directly interact with ROS, these compounds may be classified as direct or indirect antioxidants. To exert their primary action, direct antioxidants are independent of cellular macromolecules like enzymes but they react with free radicals at molecular level. Direct antioxidants can be distinguished as (1) monophenolic compounds (vitamin E and 17β-estradiol) and (2) polyphenolic compounds (wine-related polyphenols, stilbenes, and hydroquinones). Direct antioxidants (vitamin C and E) are inferior because they deactivate after they quench one free radical whereas indirect antioxidants can actually push the body to produce its own store of antioxidants. Certain flavonoids confer direct antioxidant protection to cells, others induce enzymes that protect cells against oxidative and other insults ("indirect antioxidants"), and others appear to be protective by both mechanisms. It has been reported that direct antioxidants are short lived and utilized in the process of antioxidative activity, thus need to replenish from their precursors while indirect antioxidants could or could not contain redox activity [13].

22.2 Natural Antioxidants

Enzymatic and nonenzymatic antioxidants (vitamin E and C) can control the ROS overproduction. Among all available antioxidants employed in past research, the extensive attention has been given to vitamins containing antioxidant activity, particularly vitamin E and also towards flavonoids, specifically obtained from the Ginkgo biloba leaves. In AD patients, the decreased levels of plasma antioxidants and total plasma antioxidant activity were observed [14, 15], suggesting that natural antioxidants might provide beneficial effects. Phenolic acids and flavonoids also have free radical scavenging properties and have shown health benefits in chronic and degenerative diseases [16].

Polyphenols are categorized into several categories like vitamins (β-carotene, α-tocopherol), phenolic acids (phenylacetic acid and benzoic acid), flavonoids (isoflavone and flavanone), and other miscellaneous polyphenols (thymol, sesamol, eugenol, ellagic acid, etc.).

22.2.1 Vitamins

Intake of vitamins can potentially modify the deposition or toxicity of β-amyloid. Vitamins that are widely available and can significantly affect AD include vitamin E (α-tocopherol), vitamin C, etc. Vitamin C blocks the production of nitrosamines by reducing nitrites [17]. Demographic studies show that persons with high intakes of vitamin C have lesser risk of a number of chronic diseases, including cancer, heart disease, eye diseases, and neurodegenerative conditions [17]. Vitamin D deficiency upto a great extent has been involved in pathogenesis, progression, and clinical manifestations in neurodegenerative disorders [18].

Group of patients with AD and PD have been investigated for vitamin D deficiency and results were compared with healthy controls. It was observed that vitamin D was deficient in more than half of PD patients as compared to controls (one-third). PD patients were reported with higher vitamin D insufficiency than AD patients. Vitamin E is lipophilic in nature and utilized in antioxidant activity, thus acts as direct antioxidant. Being monophenolic in nature, hydroxyl group of vitamin E can donate proton to impregnate and detoxify the unpaired electron. It has been reported [19] that vitamin E has the potency to reduce Aβ neurotoxicity and ROS production and hence can contribute to AD prevention and control. Vitamin E has also been observed to minimize cognitive functions in AD patients due to its antioxidation properties. It has been observed that vitamin E and C supplements when used in combination have the ability to slow down AD advancement [20] (Fig. 22.1).

It has been also reported that AD can be associated with vitamin B_{12} and folate [21]. Incident AD cases were studied for 3 years by selecting 370 non-demented aged persons who were not treated with B_{12} and folate. People with low levels of B_{12} or folate had twice the risk of developing AD as judge against those with normal levels. Aisen and research group [22] reported a clinical trial study for high-dose folate, vitamin B_6, and vitamin B_{12} supplements in individuals with mild to moderate AD. The authors inferred that the high-dose vitamin supplement successfully reduced the homocysteine levels in the patients but did not help the cognitive decline among the patients. The association of mild cognitive impairment, AD, and vascular dementia with blood homocysteine, folate, and vitamin B_{12} has been probed by Quadri et al. [23]. Authors have concluded that folate deficiency may be responsible for AD development and commencement of dementia. When a clinical trial on 197 participants was followed for 6 years with use of antioxidants supplement, it was observed that high risks of vitamin C and E were linked with minor risks of AD [24]. Morris et al. [25] have studied

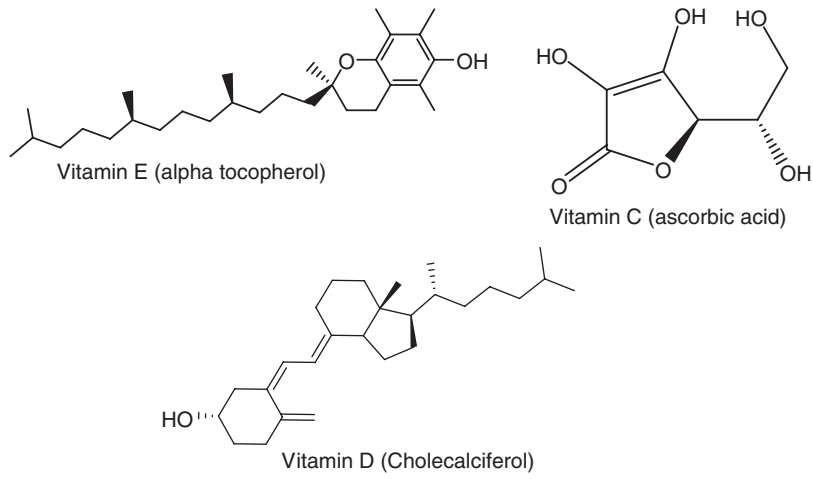

Fig. 22.1 Chemical structure of vitamins

the effect of vitamin E and vitamin C on incident Alzheimer disease and accounted that low dose of multivitamins cannot be related to AD however; high dose of vitamins C and E can reduce the AD risk. In a similar attempt, trials were carried out [26] to study the role of vitamin E towards the deterrence of AD and mild cognitive impairment (MCI). Authors found no evidence involving the role of vitamin E on AD prevention and control. It has been documented [27] that few specific AD associated areas of brain have reduced degeneration due to the effect of high dose of B-vitamin (folic acid, vitamin B6, vitamin B12: 0.8, 20, 0.5 mg, respectively). Overall contraction of brain volume was also controlled. Another study reported the effect of monoamine oxidase inhibitor selegiline and alpha-tocopherol (vitamin E) and also their combination on the AD development and progress [28]. It was observed that selegiline or alpha-tocopherol slowed down the advancement of disease.

22.2.2 Ginkgo Biloba

The extract of *Ginkgo biloba contains antioxidant capacity and could* improve cognitive function in patients of AD [29]. This plant is harvested in the remote mountainous valleys of Eastern China, and also named as "living fossil." An article published by Dr. Edward R. Rosick commented on multiple effects of Ginko Biloba on AD [30]. Author has claimed that Ginko improves the cholinergic function, protects brain neurons, and improves antioxidant activity (Fig. 22.2). Randomized placebo-controlled clinical trials to study the efficiency of formulated Ginko biloba extract (GbE) on cognitive symptoms of dementia with the management duration of approximately 6 months were conducted by Wang et al. [31]. Data interpreted by meta-analysis method revealed that GbE was found to be efficient for cognition in dementia with the treatment time of approximately 6 months. Similar investigations were done to assess the effect of Ginkgo biloba extract on AD using meta-analysis by Yang et al. [32]. Shi et al. [33] have reviewed the possible mechanisms underlying neuroprotective actions of GbE collectively with a concise dialogue of the problem of analyzing this herb clinically to validate its efficiency in the treatment and prevention of AD pathology. Discussion regarding its dosage and its permeability through blood brain barrier

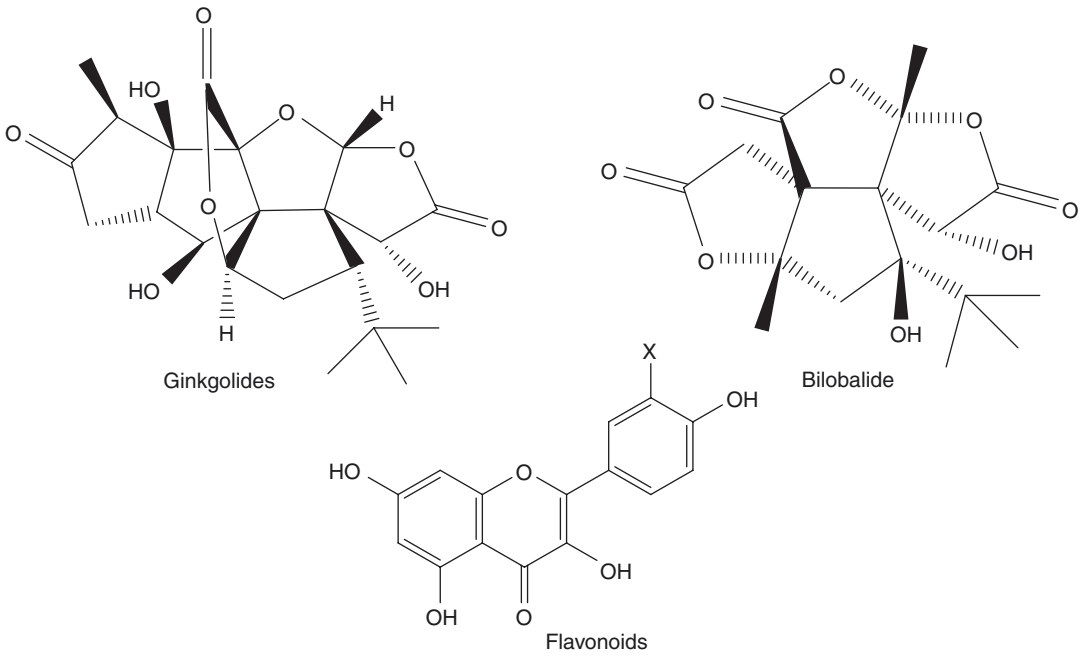

Fig. 22.2 Active components of Ginkgo biloba

(BBB) impacting its outcome in the clinical effectiveness were suggested by authors and suggested that the different factors that could interfere with the effect of GbE should be considered. In a clinical trial performed by Vellas et al. [34], it was found that extended use of formulated GbE in this trial did not reduce the risk of AD progression in comparison to placebo.

22.2.3 EGCG (Epigallocatechin-3-Gallate)

Natural polyphenolic compounds have antioxidant properties and can eliminate free radical species. Fenton reaction accounts for the production of hydroxyl radical but green tea extracts (catechins and polyphenols) can chelate with metals like iron (Fe^{2+}) and copper (Cu^{2+}) and prevent free radical formation [35] (Fig. 22.3). EGCG (a major green tea constituent) has been reported to show shielding effect against amyloid-beta-induced apoptosis in hippocampal neuronal cells [36]. In an investigation, EGCG has been found to treat memory dysfunctions in mice caused by amyloid-beta peptide [37]. Moreover, inhibition of amyloid fibrils was also observed under the effect of EGCG. According to an in vivo study [38], the oxidation of proteins and lipids in rats can be controlled upon green tea administration in rats. Superoxide dismutase and catalase are the protective enzymes that are activated by polyphenols in green tea to impart them neuroprotection properties [39]. It has been documented that EGCG promotes the antioxidant enzymes activity in mice striatum [40]. Although recently catechins have emerged as an effective neuroprotective agent [41], EGCG has numerous advantages over it. Free radical scavenging, antioxidant actions, and metal-chelating properties of EGCG define their biological and pharmaceutical strength [42]. It has been observed that PC12 cells can be protected against amyloid β-induced neurotoxicity by EGCG [43, 44]. Kuroyama and research group [45] have concluded in their study that cognitive impairment may be controlled by high green tea consumption. Also Parkinson's disease risk can be checked upon regular green tea consumption (two cups/day or more) [46].

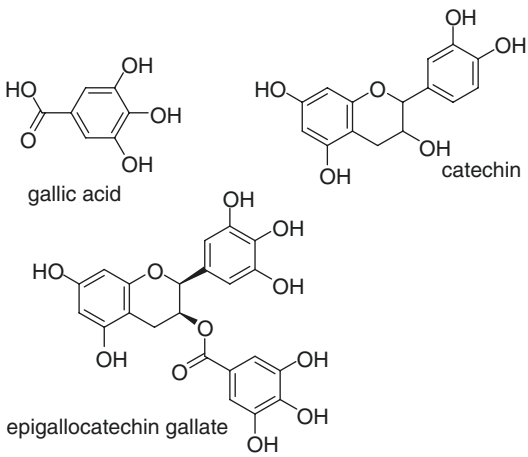

Fig. 22.3 Active components of green tea

22.2.4 Blue Berries Extract

Blueberries are rich in flavonoids, tannins, and phenolic acids, and have several beneficial health properties associated with the presence of such bioactive compounds, especially anthocyanins [47] (Fig. 22.4). In several in vitro studies, blueberry extract has revealed neuroprotection via antioxidant and anti-inflammatory activities [48]. Blueberries are rich in polyphenolic compounds involving flavonoids and catechins are the major components [49–51]. Authors [52] have claimed that it is possible to overcome genetic predisposi-

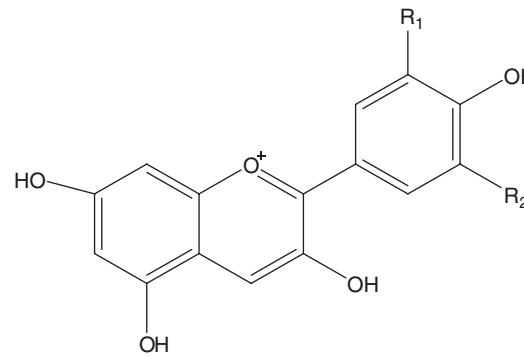

Fig. 22.4 Structure of major anthocyanin

tions to Alzheimer disease through blue berries extracts.

22.2.5 Tannic Acid

Tannins/tannic acid (TA) is water-soluble polyphenols which is different from other natural phenolic compounds in their capability to precipitate proteins such as gelatin from the solution [53] (Fig. 22.5). Similar to polyphenols, TA also has been shown to have antioxidant property [54]. Small molecule polyphenols like catechin, quercetin, and kaempferol are less capable of scavenging ROS as compared to tannins. Tannic acid dose dependently inhibits β-amyloid fibrils (fAβ) formation from fresh Aβ and also destabilizes the preformed fAβ in vitro [55]. Hence, TA has the therapeutic potential towards AD by scavenging ROS and inhibiting accumulation of fAβ in the brain [53, 56].

22.2.6 Curcumin

A polyphenol extracted from the rhizome of Curcuma longa Linn (family Zingiberaceae) is named as Curcumin, chemical name: 1,7-bis[4-hydroxy 3-methoxy phenyl]-1,6-heptadiene-3,5-dione (Fig. 22.6). It is recognized for various medicinal properties including anti-inflammatory and antioxidant property [57]. It is a key ingredient of Indian spices. Curcumin has been studied in vitro for their ability to protect against β-amyloid toxicity in PC-12 cells and human umbilical vein endothelial cells [58]. Authors claimed that curcumin can manifest better antioxidant properties than α-tocopherol. Synaptic plasticity due to amyloidβ in rat has also been recovered with curcumin [59]. Lim and co-workers have documented that inflammation and oxidative loss in mice brain can be recovered by dietary curcumin [60]. Alloza et al. have claimed that curcumin can cross blood brain barrier and

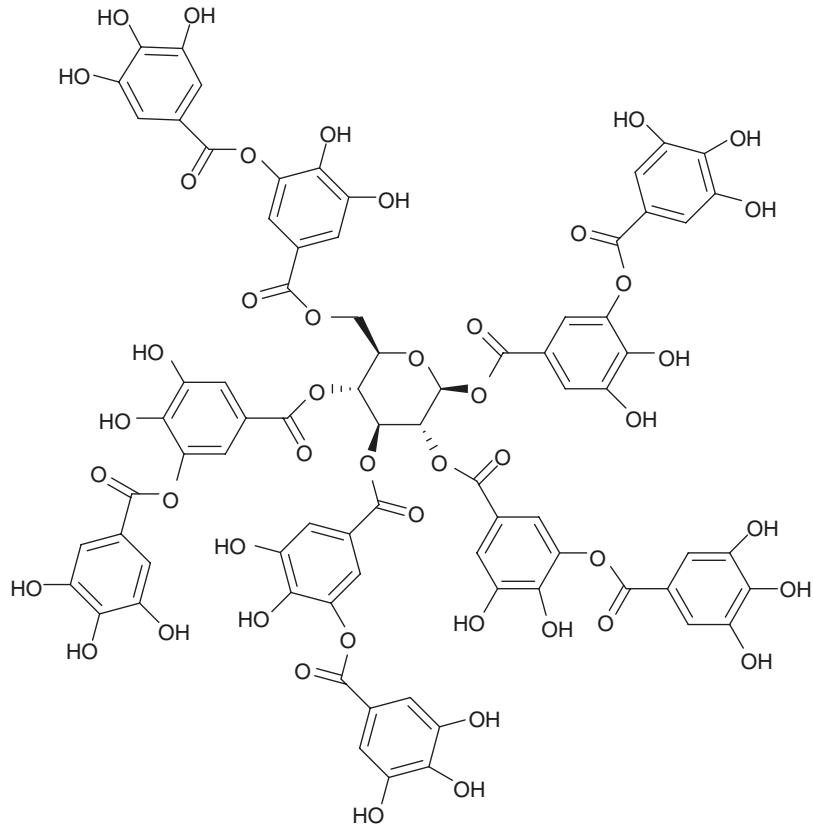

Fig. 22.5 Tannic acid

bind to β-amyloid aggregates which can further aid in their elimination. In spite of these observations, the protective role of curcuma in AD has not been validated so far.

22.2.7 L-Carnitine and Derivatives

Acetyl-L-carnitine (ALC) can benefit the functional loss in brain activity typically observed in neurodegenerative disorders (Fig. 22.7).

Dietary LC synthesized from the intake of red meats, but the cellular synthesis of LC from the lysine and methionine amino acids has also been reported [61]. ALC must be measured as neuroprotective agent, due to following properties: (a) antioxidizing property, (b) mitochondria1 energy supply, (c) membrane stability and its function, (d) enhancement of cholinergic transmission [62]. Treatment of rat pheochromocytoma (PC12) cells with ALC stimulates the synthesis of nerve growth factor receptors [63]. ALC and R-α-lipoic acid have been explored to confirm their role towards improvement in cognitive functions (special and temporal memory) in mice [64].

22.2.8 Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a stilbenoid, a class of polyphenolic compounds found in grapes and red wine. Studies have accounted its antioxidant and anti-inflammatory activities [65]. Resveratrol may undergo antioxidant mechanisms and cause vasodilation (increased blood flow); however, any contribu-

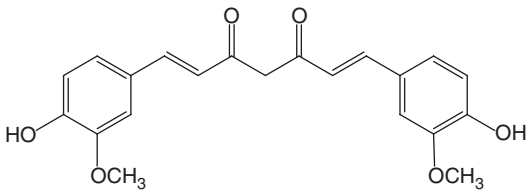

Fig. 22.6 Curcumin

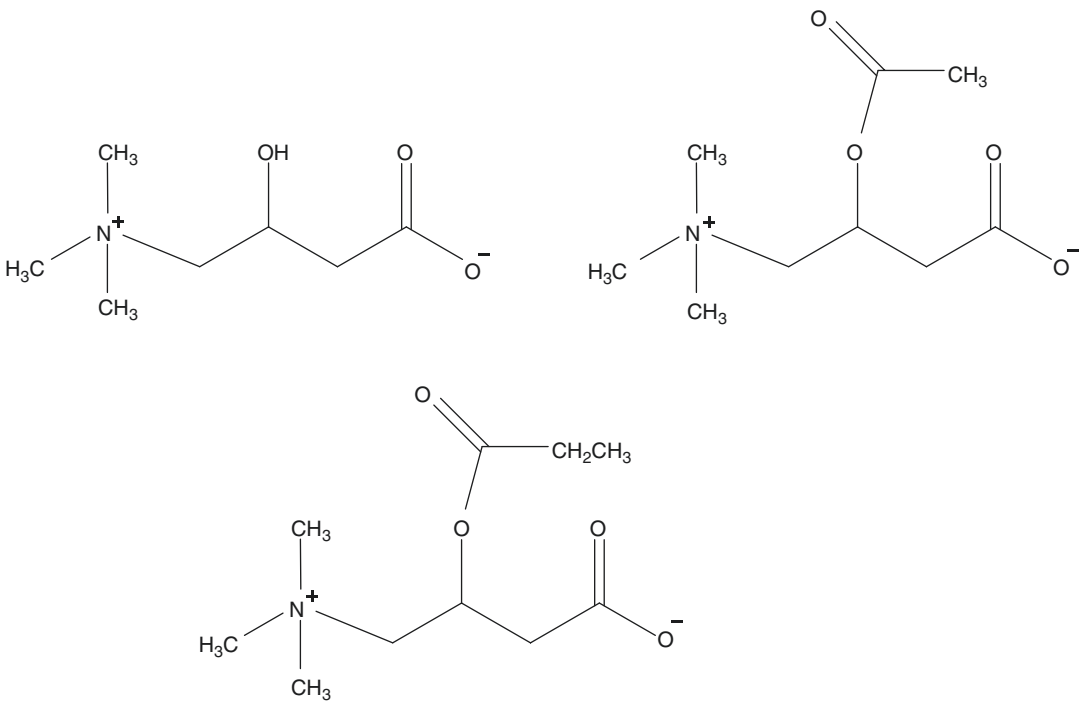

Fig. 22.7 Chemical structure of L-Carnitine and derivatives

tion towards cognitive recovery has not been yet concluded [66].

In the context of the present review, direct antioxidants with phenolic group like 2,4,6-trimethylphenol (TMP), cannabidiol, and (−)Δ^9-tetra-hydrocannabinol (THC) are worth mentioning. These have been shown to exert antioxidative neuroprotective activities along with their regular physiological task. Also aromatic amines are evolving as a new group of direct antioxidants that can effectively provide neuroprotection. These can act effectively in opposition to oxidative glutamate toxicity, glutathione deficit, and hydrogen peroxide toxicity [67]. Studies have shown that polyphenols present in skin and seeds of grapes have neuroprotective effects as they are able to scavenge ROS [68]. Reigi et al. have extensively reappraised the neuroprotective role of resveratrol in AD pathology [69].

22.2.9 LA (α-Lipoic Acid)

The innate antioxidant, thioctic acid is normally known as α-lipoic acid (LA) play various pharmacological and antioxidant properties [70] (Fig. 22.8). LA has been shown to contain diverse properties which may cause hinder in pathogenic principles of AD [71]. Authors have well documented that LA can chelate redox-active transition metals, inhibit the generation of hydrogen peroxide and hydroxyl radicals; salvage ROS, augment the level of reduced glutathione; scavenge ROS, downregulate inflammatory processes; inhibit formation of lipid peroxidation mediated products; and induce the levels of enzymes of glutathione synthesis pathway and other antioxidant protective enzymes. LA has shown to reduce markers of oxidative stress and to improve cognitive function in aged animals [72].

22.3 Therapeutic Approach

Numerous therapeutic strategies have been recommended so far for the treatment of amyloidogenic diseases. Several antioxidants have

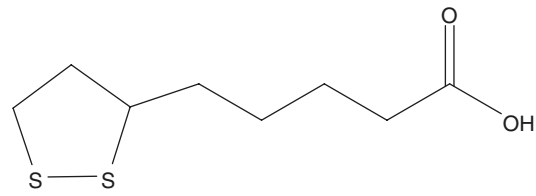

Fig. 22.8 Lipoic acid

manifested advantageous roles in diverse biological systems, in which they were capable to inhibit the age-associated damage. Number of these agents has shown to protect cells from Aβ-induced neurotoxicity [7]. Free radicals implicated their pathophysiological role in many diseases. Studies have shown that ROS elimination can be performed by antioxidants like lipoic acid, vitamin E and C, and β-carotene [73]. Pathogenesis of many neurodegenerative diseases can be explained on the basis of mitochondrial imperfections specially AD and PD. Hence, effective measures to combat mitochondrial dysfunctions may open the doors for AD treatment. Antioxidants that may treat imperfections in mitochondria especially ROS production need to be investigated thoroughly. Several groups have demonstrated different types of antioxidants which inhibited the formation of fAβ from Aβ and/or destabilized β-amyloid fibrils (fAβ) involving both in vitro and in vivo test systems. Few recent developments in antioxidants systems cannot be ignored specially microparticles with natural antioxidants for controlled release studies [4]. Deferiprone-resveratrol hybrids as antioxidants have been recently studied as $A\beta_{1-42}$ aggregation inhibitors and metal-chelating agents for AD [7]. Pyridoxine-resveratrol hybrids mannich base derivatives with antioxidant and metal-chelating property for AD treatment can be counted as another milestone in recent advances [74]. AChE inhibitor, Tetrahydropyranodiquinolin-8-amines with antioxidant property against AD therapy has been recently designed and explored for SAR activities [75]. Thus, it is clear that antioxidant-based drugs have evolved as effective agents for treatment of AD and are currently a topic of extreme interest. The key role of antioxidants can be summed up as (1) the attenuation of

free radical generation, (2) the nonenzymatic scavenging of endogenously or exogenously generated free radicals, and (3) the enzymatic neutralization of accumulating ROS.

22.4 Conclusion and Future Perspectives

Various natural antioxidants and their potential to inhibit ROS formation has been reviewed in this chapter and relevant clinical information have been provided. Due to neuroprotective tendency of natural antioxidants, these may be considered as significant tools to fight neurodegeneration. However, in-depth investigation of their structure and mechanism needs to be done to gain better insights. In spite of plethora of research done in this context, the conclusion for potential of natural antioxidants against amyloid beta toxicity and free radical production is still not satisfactory. Clinical trials especially with dietary antioxidants have confusing inferences indicating a much needed effort in this direction. Experimental hindrances like low solubility and poor blood brain barrier penetration of antioxidants can be the possible problem of the investigation. Hence, efforts need to be directed for development of a much potent and versatile antioxidant that may be effective against most of the neurodegenerative disorders.

Acknowledgements The authors acknowledge Department of chemistry, Pt. Ravishankar Shukla University, Raipur and Department of Chemistry, Indian Institute of Technology, Bombay.

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Dietary Directions Against Dementia Disorders

23

Helmut M. Hügel, Anthony R. Lingham, Neale Jackson, and Trevor Rook

23.1 Introduction: Neurodegeneration and Polyphenol Microbial Metabolites

There are 86 billion neurons/reasons why it is important to study the science of neurodegeneration. With the global aging population increasing, the suffering from dementia is anticipated to rise to over 115 million by 2050. Alzheimer's disease (AD) that is the foremost widespread dementia is a progressive age-risk-related disorder of the brain cortex and hippocampus regions, and involves the cognitive impairment along with reduced capacity/function. It is the generation of Aβ-oligomers-fibrils, hyper-phosphorylated tau-proteins, and oxidative stress that contribute and cause Alzheimer's disease. Studies have suggested that a restricted diet can contribute extra years of healthy living. However, many dietary foods, phytochemicals, herb plant secondary metabolites, and polyphenols [1–3]have been shown to possess pharmacological benefits against human diseases and are outlined in Scheme 23.1. How complex foods/beverages interact with gut microbes generating diverse health benefits and longevity by the many microbial metabolic products that are available to be recycled and effectively used awaits further research. The in vivo fitness of the human body is also a consequence of the degree of bioavailability of dietary foods, the extent of microbiota metabolic responsiveness to the nature and diversity of foods consumed, lifestyle and personal choices. The dream delivery and destination of phytochemicals against neurodegenerative diseases like AD, Parkinson disease [PD], Huntington disease [HD], and Amyotrophic Lateral Sclerosis [ALS] is via their swift uptake, rapid absorption, unimpeded brain entry, and neuro-activity.

23.1.1 Gut Microbiota: A Health Barometer

Variation in the composition of gut microflora is now apparently linked to health and disease by multiple interactive factors [4] such as diet, food supplements, drug therapy [5], use of medication, red blood cell counts, fecal chromogranin A, and stool characteristics that summarily may represent/reflect the potential biomarkers of healthy/normal gut communities [6]. The manipulation [7] of the microflora balance in the gut serving as

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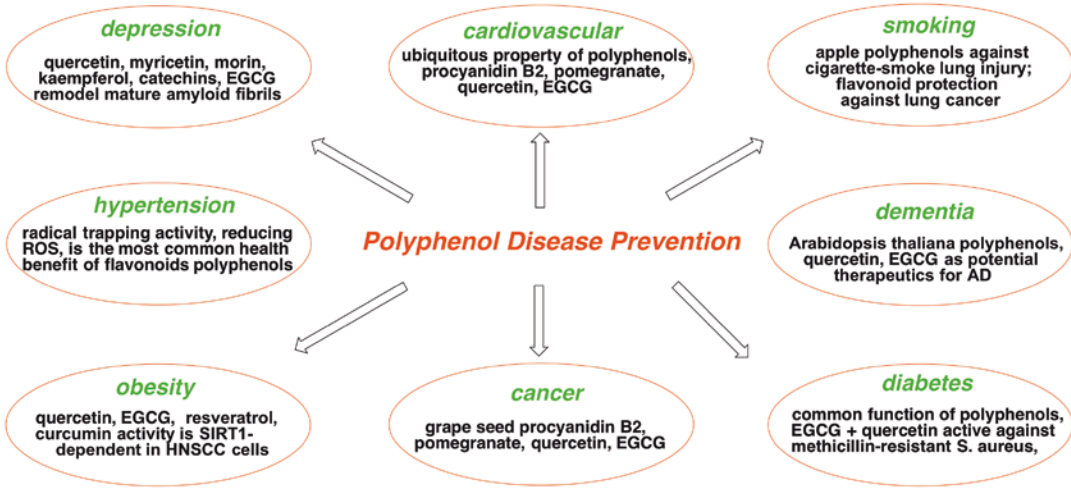
S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_23



Scheme 23.1 Disease-health profile of polyphenols

a way to alter microbiota fitness levels whereby its profile may have substantial reversible influence on the CNS and brain function to improve health [4, 8]. This has been revealed by the advances made in the technologies of high throughput gene sequencing to define/measure the diversity of the microbiota that has illuminated this field. Aging is associated with physiological and mental decline whereby lifestyle plus changes in diet-related microbiota [8–11] manipulations may transform aging-dementia diseases into sustainably modifiable conditions that focus greater bioactivity into delaying the declines in healthy human homeostasis.

23.1.2 The Microbiome in Cognitive Decline

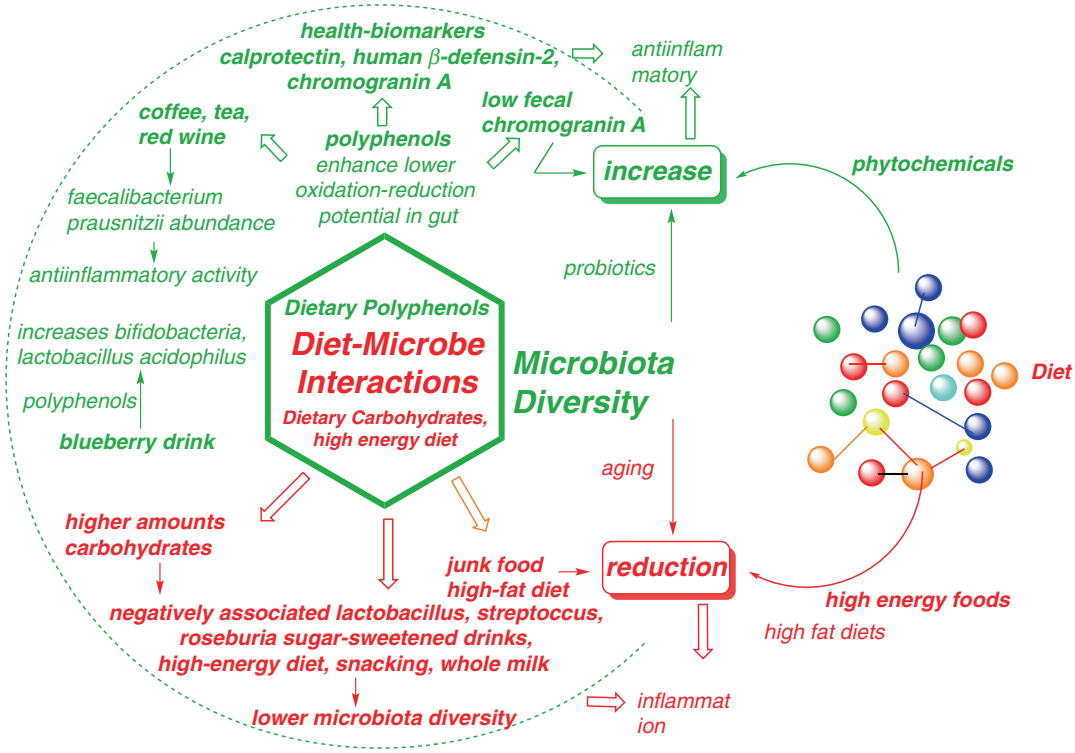
As outlined in Scheme 23.2 dietary choices direct and tune the degree of microbiota diversity. Age-related gut microbiota changes trend towards a diminishing microbiota diversity reflected by increased concentrations of proteobacteria that may be coupled to chronic inflammation [12] and declines in bifidobacteria which decrease the synthesis of pro-inflammatory cytokines [13] and lower short chain fatty acid production conditions [14] that all contribute towards compound aging, obesity, diabetes, AD, and dementia diseases [15–20].

Foods that contain biologically active compounds and polymeric/polyphenols, complex carbohydrates, unsaturated fats/fatty acids, and antioxidants are now known to also have health benefits for the host through catabolism and by anaerobic fermentation by gut bacteria that produce small molecules that can be further used by entering the circulation system of the host and may be neuroprotective [15].

The health of elderly people on diets of low meat is positively linked with increase in brain volume and cognitive function [21]. This is in contrast to the deficit of microbiome purpose including genes which instruct short chain fatty acids and augmented levels of circulating pro-inflammatory cytokines [22] that has been found in healthy elder humans. Mature mice fed a high fat/energy diet promoted physiological and anxiety syndromes, whereas aged mice displayed decrease in spatial cognition [23] indicating the involvement of multiple targets in aging.

23.1.3 Polyphenol Derived Brain Permeable Metabolites

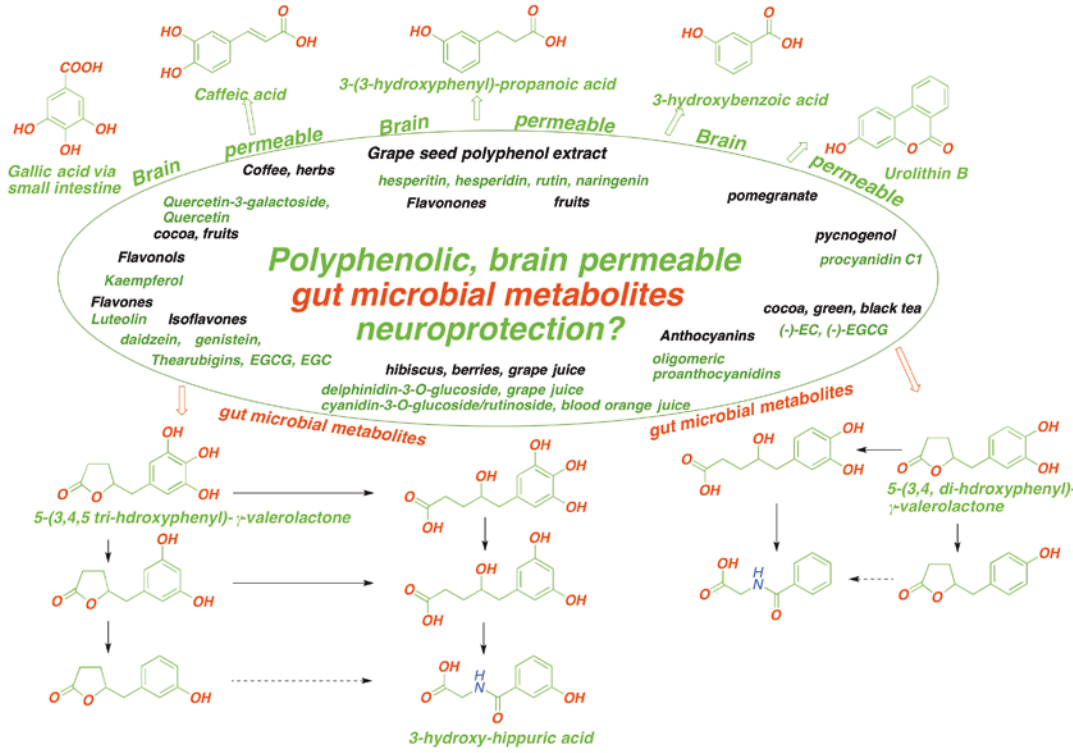
When rats were orally administered grape seed polyphenol extract, the bioavailability of 12 phenolic acids acknowledge to be derived via the gut microbiota metabolism/fermentation of



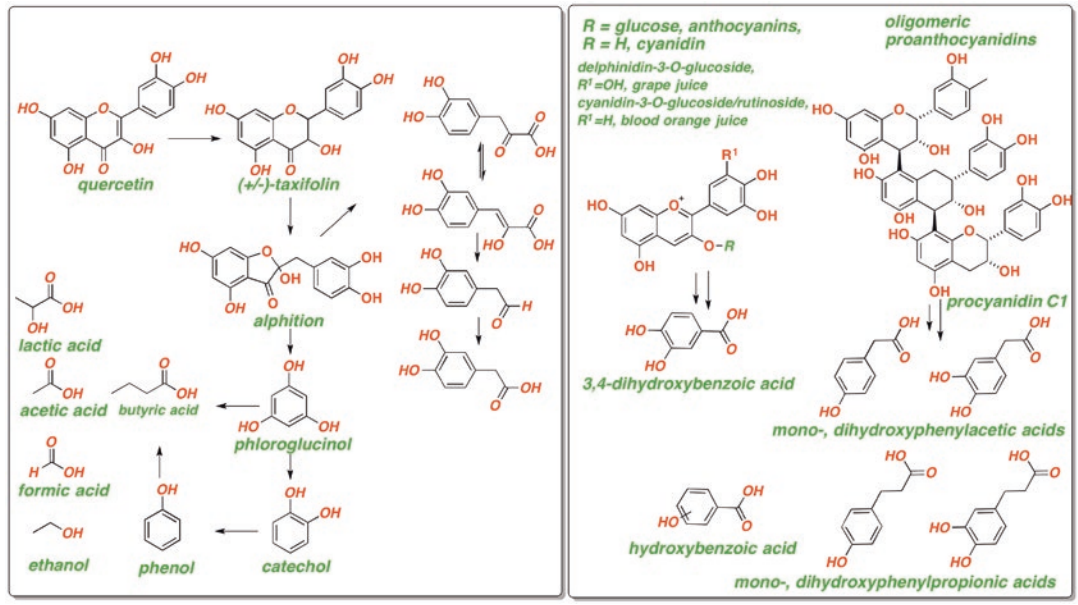
Scheme 23.2 Dietary directions and microbiota diversity responsiveness-health axis

anthocyanidins, and the phenolic acid metabolites 3-hydroxybenzoic acid (3-HBA) and 3-(3′-hydroxyphenyl) propionic acid (3-HPP) were significantly observed to build up in the brain in a dose dependent manner [24]. The in vitro thioflavin-T (ThT) assay, circular dichroism, and other studies reveal that both above said brain-accumulating phenolic acids potently interact with the aggregation of β-amyloid (Aβ) peptides in reducing Aβ sheet formation by generating Aβ random coils. The outcomes from such studies need further research, clinical evaluation and substantiation to confirm that intestinal microbiota can provide protection against the onset/progression of AD and other neurodegenerative diseases involving aberrant, pathological protein aggregations. The structures of representative metabolites generated from microbial metabolism of black [25] and green [26] tea include catechin, procyanidins, thearubigins, and theaflavins, which are shown in Scheme 23.3. Since the bioavailability of simple and complex polyphenols found in fruits, vegetables, and herbs is generally low, their small intestine absorption into the blood stream is also low with the majority polyphenols being extensively metabolized by microbial bacteria in the colon. Short chain and predominantly phenolic acids are abundantly generated from flavonoid polyphenols on gut microbial metabolism [27] illustrated in Scheme 23.4 that are highly bioavailable, are circulated to target organs and considered to actually provide the many hugely healthy and protective functions of ingested plant polyphenols. The extent of their neuroprotective efficacy against neurodegeneration is not known.

The intravenous injection of a single bolus comprising 23 known dietary polyphenol metabolites [28] into rats was performed to monitor and probe their organ destination. As was expected most of the injected metabolites were excreted into the urine. Surprisingly, 10 (shown in Fig. 23.1) of the 23 phenolic components injected reappeared in the brain. The kinetic absorption



Scheme 23.3 Some natural product containing polyphenol—gut microbial metabolites



Scheme 23.4 The phenolic microbial metabolites from flavonoid polyphenols

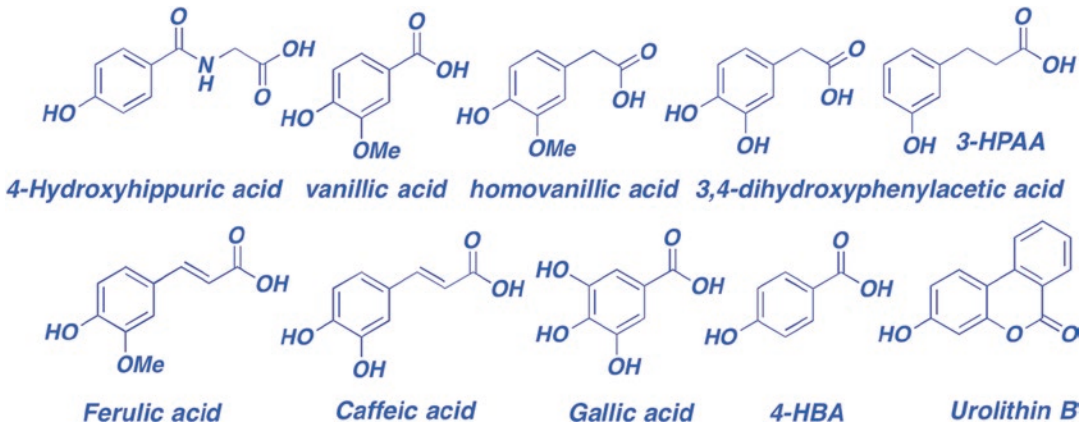


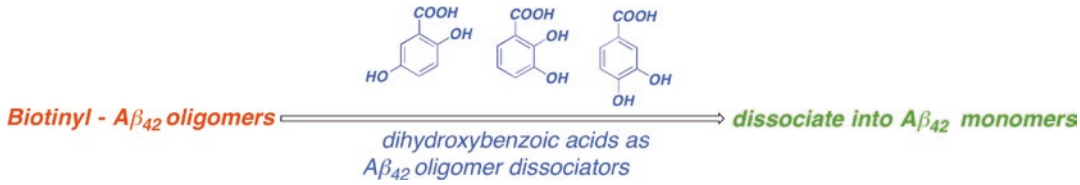
Fig. 23.1 The ten phenolic acid microbial metabolites found in the brain of rats

distribution found that four compounds were detected after 2 min, and others were identified at 5 min with nine compounds persisting after 15 min. This precise investigation illustrated the facile brain bioavailability of small phenolic metabolites and supports the direct role of dietary polyphenols on brain biochemistry and the potential to modulate brain function.

23.1.4 Anti-amyloid Activity of Polyphenols

The three dihydroxy benzoic acid (DHBA) positional isomers [29] shown in Scheme 23.5 were each found to actively convert biotinyl-Aβ (42) oligomers progressively into Aβ monomers.

We have investigated a class of 21 polyphenolic and related compounds which are found naturally in the Chinese traditional medicine *Salvia miltiorrhiza and* also known as danshen. These were isolated and screened for in vitro anti-amyloidogenic activity [30] in test system exposed to synthetic amyloid beta peptide ($A\beta_{42}$) of AD. Incubation of these compounds with $A\beta_{42}$ for 24 h resulted in reduced ThT fluorescence of 8 of these phenolic acids, the structures of which are illustrated in Fig. 23.2. This reflects the anti-amyloid-fibrillation propensity ($p<0.001$) of these 8 phenolic acids. Transmission electron microscopy and western blotting analysis also revealed that specified compounds have ability of hindering the fibril formation even after prolonged incubations. Furthermore, we observed that these isolated compounds also have capability to rescue yeast cells against chemically synthesized $A\beta_{42}$ induced toxicity. The assays were performed by utilizing a yeast *Saccharomyces cerevisiae (AHP1* deletant strain transformed with GFP) fused to $A\beta_{42}$ was treated with these compounds and analyzed for estimation of fluorescence levels. Flow cytometry data showed the significant reduction in the green fluorescence intensity for 14 out of 21 phenolic compounds which suggested that these 14 compounds contain an anti-amyloid-fibrillation tendency in the yeast and such GFP-$A\beta_{42}$ was deleted by proteolysis. Since the relative position (not the number) of the hydroxyl groups on the aromatic ring was found to be the major determinant for their interaction with amyloid protein, we propose that the observed differential interaction of the hydroxyl-polyphenol positional isomers with the amyloid protein may be accounted by the nature of the increased chemical reactivity attributed to the positional influence of the hydroxyl groups and the resultant electrophilic or nucleophilic chemical interactions or by their antioxidant/redox reaction mechanisms.



Scheme 23.5 The DHBA mediated dissociation of soluble biotinyl-Aβ(42) oligomers

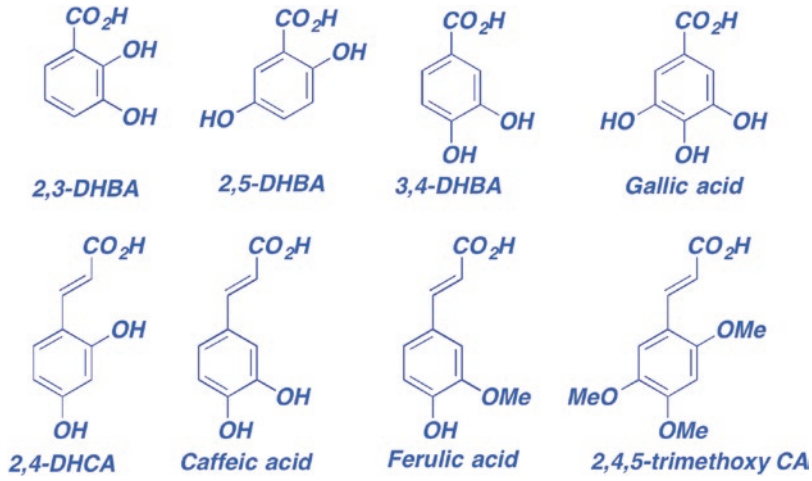


Fig. 23.2 Danshen-related polyphenolic compounds that inhibited $A\beta_{42}$ aggregation

23.1.5 Dietary Products Including Herbs that Provide Neuroprotection Against AD

Some of the most commonly consumed foods/beverages with their brain-bioactive ingredients that may provide neuroprotection against AD are presented in Table 23.1.

Ginseng belongs to the Araliaceae family, is one of the most widely consumed food and medicine and is regarded as a panacea, a "cure all" to enhance the quality of life [51] from children to the elderly. Asian or Korean ginseng (*Panax* ginseng), American ginseng (*P. quinquefolius*), *P. notoginseng*, and Siberian ginseng (*Eleutherococcus senticosus*) are all utilized. The main active principles of ginseng extracts are ginsenosides [52] and glycosylated derivatives of the triterpene dammaranes. *P.* ginseng are used as an invigorant to combat memory lapses/loss by improving blood and oxygen flow to the brain and is considered to stimulate mental activity. The influence of feeding single doses of the ginsenosides Re, Rg_1, and Rg_3 at 50 μM in conditioned medium of CHO 2*B7* cells resulted in a reduction of 32.2%, 19.4%, and 69.3%, respectively, of $A\beta_{42}$ after 3 h of treatment [53] and for Rg_3 the apparent IC_{50} was found to be <25 μM. This was supported by further evidence from the administration of 25 mg/kg of the ginsenosides which resulted in 20–30% reduction in $A\beta_{42}$ in vivo studies in a Tg2576 mouse model after 18 h.

Computational modeling of 12 ginsenosides to screen their ginsenoside-BACE1 receptor inhibition [54, 55], BBB permeability, and ADMET analysis specifically predicted that the monoglucosylated ginsenosides Rh_1, Rh_2, F_1, and CK shown in Scheme 23.6 were the best candidates. This suggests that microbial metabolism may be essential for the neuroprotective and pharmacological effectiveness of many highly glycosylated ginsenosides and this is further exemplified whereby compound K promoted the

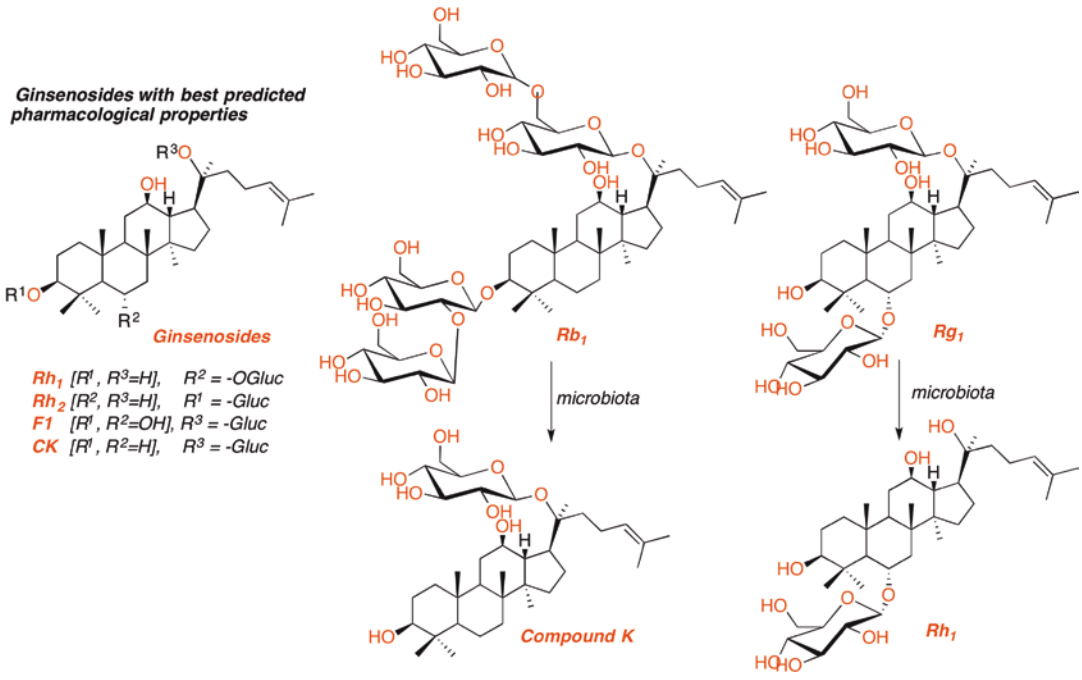
Table 23.1 Dietary products that alleviate AD brain-related disorders

Food	Bio-actives	AD Neuroprotection
Pomegranate fruit extract PE [31, 32]	Methyl-urolithin B from PE-ellagic acid-gut microflora biotransformation, and found in brain	The brain permeable methyl-urolithin B showed protective effect in *C. elgans* post induction of β-amyloid induced neurotoxicity.
Buckwheat [33]	Flavonoids rutin, quercetin, also many phenolic acids	Anti-amyloidogenic, antioxidant
Caffeoylquinic acid-rich purple sweet potato extract [34]	Caffeoyquinic acid, anthocyanidine	Increased cell viability relative to $A\beta_{42}$ treated cells
Apple [35] and berries,	Some bioavailable flavonoids, gut metab. phenolic acids	Antioxidants, protection against Aβ toxicity
Curry [36] is only slightly bioavailable	Curcumin [C] see also below	Modulates Aβ to non oligomer aggregates
Grape seed polyphenol extract [24]	Flavanols, flavonoids, gut metabolites: 3-hydroxybenzoic acid [3-HBA], 3-(3′-hydroxyphenyl) propionic acid [3-HPP]	GSPE gut microbial metabolites brain active 3-HBA, 3-HPP have anti-amyloidogenic activities and may confer neuroprotective activity against AD
8 week, CRT consumption [37] of 100% orange juice, by 37 healthy adults [mean age 67 years] with MCI, neurodegenerative disease	305 mg flavanones, hesperitin, naringenin	Global cognitive function was significantly better after 8 week daily orange juice consumption
3 months CRT study healthy 50–69 years consumed high or low cocoa flavanol-diet [38]. Gyrus function [DG] dysfunction is a driver of age-related cognitive decline	Dietary cocoa contains flavanols, catechin, epicatechin, proanthocyanidins are reported to improve vascular function [39]	The high flavanol drink intervention enhanced DG and significantly improved age related cognitive decline, provided a non-pharmacological agent against dementia
Black, green tea [40–42] coffee [43]	Flavanols, flavonoids, EGCG, C, EC, gut metabolites: polyphenolic acids, 5-(3,4,5-trihydroxyphenyl)-γ-valerolactone, 5-(3,5-dihydroxyphenyl)-γ-valerolactone, caffeine chlorogenic acids	Many dietary polyphenolic compounds have anti-amyloid, antioxidant, anti-inflammatory activity; galloylated catechins exerted greater hypotensive effects in vivo than catechins
Extra virgin olive oil [44–47]	Phenolic component oleocanthal, reduced AD risk	Upregulation of major Aβ transport/clearance proteins: P-gp, LRP1 in cultured mice brain cells increased 15.9%
S. miltiorrhiza Danshen [48]	Salvianolic acid B plus borneol enhanced oral bioavailability, tanshinones	Tanshinones inhibit Aβ aggregation [49] disaggregate Aβ fibers and reduce Aβ-induced cell toxicity in vitro by 57.5–71.3%
Curcumin [C] degradation in a human faecal fermentation model [50]	Cur, DMC, bis- DMC, THC, TH-DMC, TH-bis-DMC, DFA metabolites were found	C has anti-amyloidogenic, antioxidant, anti-inflammatory properties.

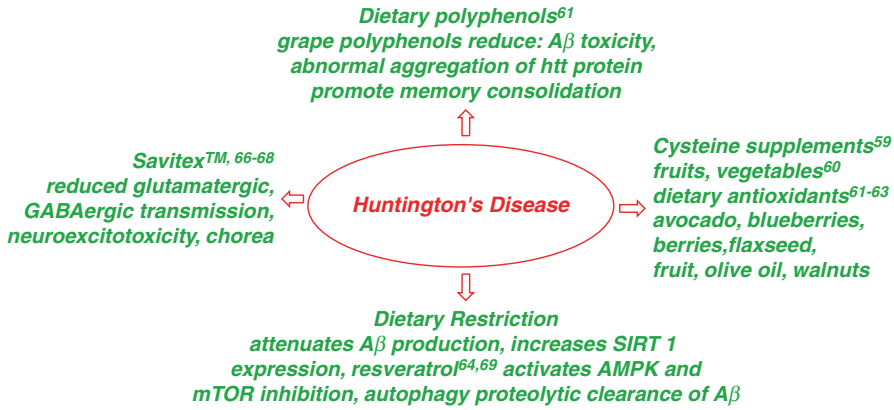
clearance of Aβ by enhanced autophagy via inhibition of mTOR phosphorylation in primary astrocytes. This implies that some ginsenosides serve as prodrugs and are metabolized in the gut to brain permeable compound K, Rg_3, or related compounds [56, 57].

23.2 Natural Products Against Huntington's Disease

Huntington's disease (HD) is fatal genetic neurodegenerative disorder affecting the populations having the mutant huntingtin (mHtt) gene



Scheme 23.6 AD protective *Panax* ginsenoside from microbial metabolism



Scheme 23.7 A range of natural product interventions against Huntington's disease

[58]. For HD, like other neurodegenerative diseases, no curative treatments are known or are available. This disease is characterized by a polyQ, [CAG] and more than 30 CAG repetitions render the Htt gene mutated, resulting in corticostriatal neurodegeneration, aberrations in glutamatergic and GABAergic transmission, excitotoxicity, involuntary movements [chorea], and dementia. PolyQ proteins are susceptible to misfolding, therefore HTT misfolding may contribute to HD neuropathology. Scheme 23.7 outlines some of the dietary approaches and phytochemical products that have considerably alleviated HD. Furthermore, it has been shown that significant reduction/depletion of cystathionine-γ-lyase enzyme that converts cystathionine into cysteine en route to glutathionine and/or H_2S in HD tissues may be pathogenic [59]. Cysteine

and *N*-acetylcysteine supplementation in the consuming water coupled with a high cysteine containing diet delayed the commencement of motor abnormalities and reduction in brain weight were also partially upturned by treatment with cysteine-enriched diet in mice with HD and thus their survival was extended. This suggests that cysteine supplementation and certain dietary fruits/vegetable intake to augment endogenous cysteine/H_2S can be of therapeutic benefit against HD.

Table 23.2 lists the fruits and vegetables with the highest concentrations of *N*-acetylcysteine and *L*-cysteine. Red pepper has shown to consist of appreciable amounts of *L*-Cysteine (349 nM/g wet weight). Other cysteine rich vegetables include asparagus, spinach, green beans, and tomato. The commonly consumed fruits/juices like oranges, strawberry, and papaya also contain significantly high *L*-cysteine levels [60].

Since the endocannabinoid system [ECS] in the CNS participates in many neuromodulatory functions [65] including the glutamatergic system, the pharmacological manipulation of which offers a mechanism to control excitotoxic glutamate episodes related to HD. The activation of specific receptors within ECS is a promising therapeutic agent in HD. Recently, the therapeutic benefits [66–68] of Sativex® that is composed of equimolar combinations of the phyto-cannabinoids δ-9-tetrahydrocannabinol and cannabidiol as a cannabinoid medicine treatment for HD have undergone a clinical pilot trial as it exhibits anti-hyperkinetic, anti-inflammatory, neuroprotective, and neuroregenerative activities at the pre-clinical level.

Table 23.2 *N*-Acetylcysteine and/or *L*-Cysteine rich fruits and vegetables

Fruits/vegetables	*N*-acetylcysteine (nM/g) [wet/weight]	*L*-Cysteine (nM/g)
Orange	ND [not detected]	41 ± 2
Strawberry	5 ± 1	59 ± 5
Papaya	ND	58 ± 5
Grapefruit	4 ± 0	15 ± 2
Mango	ND	10 ± 0
Red pepper	**25 ± 4**	**349 ± 18**
Asparagus	46 ± 1	122 ± 1
Spinach	ND	84 ± 2
Green beans	ND	67 ± 11
Tomato	3 ± 1	55 ± 2

23.3 Natural Products Against Parkinson's Disease

Parkinson's disease [70, 71] (PD) is an age-related neurodegenerative disease, initially affecting the pigmented neurons of the substantia nigra region of the brain. Typical motor impairment is related to the neurodegeneration/loss of over 60% of dopaminergic nigral cells that is commonly diagnosed at an age of 50–60 years. Clinical diagnostic criteria include bradykinesia [poor voluntary movements, velocity, and sustainability] rest tremor, muscular rigidity, and postural instability. While sporadic PD is the most frequent occurrence of the disease, genetic factors of PD include inherited mutations in the α-synuclein gene leading to formation of Lewy body accumulation as the major component of α-synuclein protein fibrils/aggregates, mitochondrial disorders, oxidative stress, environmental, and neurodegeneration/cognitive impairment are also contributing risk factors. Although not a cure, levodopa therapy, nicotine and derivatives, and coffee/caffeine alleviate PD. Some dietary phytomedicines that contribute to therapeutic treatment of PD are listed in Table 23.3. In vivo studies of a surfactant formulated mixture of curcumin-piperine-glyceryl monooleate nanoparticles [71] that improved BBB permeation/bioavailability of curcumin when administered to a PD mice model, demonstrated an inhibition of α-synuclein protein into oligomers and fibrils, reduced the rotenone mediated impairment in motor responses, oxidative stress and also restrained dopaminergic neuronal degeneration. Saponins are active ingredients in many herbs, and some have neuroprotective effects. It is found that ginsenoside Rg1 can reverse the changes of neurotransmitters occurred due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [72] (MPTP) administration, and ginsenoside Re also has significant inhibition [73] on apoptosis of nigra neurons in mouse caused by

Table 23.3 Herbal products with neuro-activities against PD

Natural product	Bio-actives	PD neuroprotection
Curcumin-piperine formulated lipid/ surfactant nanoparticles [72]	Curcumin-piperine	The brain permeable formulation showed multiple protective effects against dopaminergic neuronal degeneration
Ginseng triterpenoids [73, 74]	Ginsenoside Rb1 Ginsenosides Rb1, Rg1 effectively inhibited increase of neuron Ca^{2+} levels	Reversed/ disaggregated α-synuclein protein Increased the survival of dopaminergic cells exposed to glutamate excitotoxicity
Sasanqua saponins [75] from the seeds of *C. oleifera*	Sapogenin and amino derivative	Have anti-inflammatory, analgesic, neuroprotection of dopaminergic neurons increased levels of DA in striatum
Licorice [76–78]	Brain permeable glycyrrhizic acid and 18β-glycyrrhetinic acid	Counteract brain damage induced by ischemia and 6-hydroxydopamine, and PD

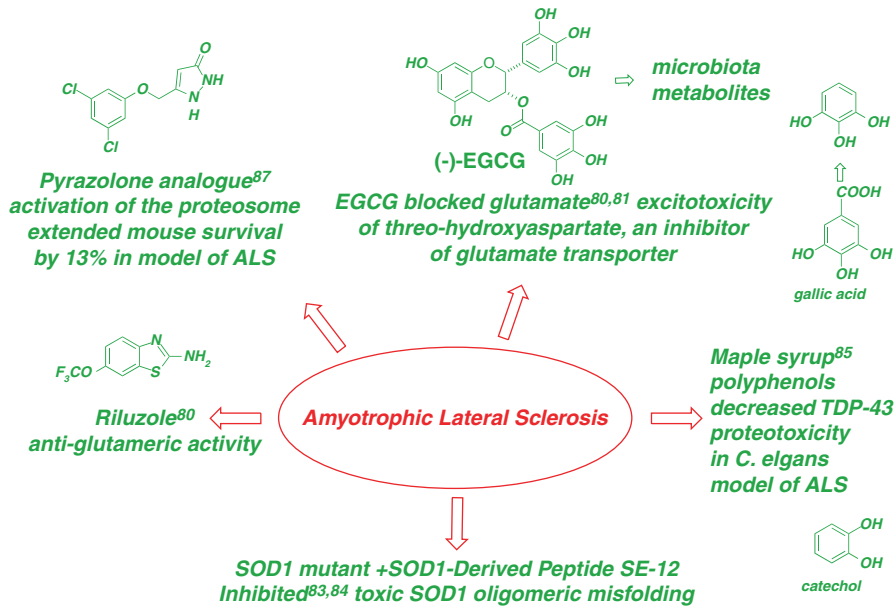
MPTP. Unlike the triterpenoid ginsenoside, the sasanqua saponin [74] from seeds of *C. oleifera* is a pentacyclic triterpenoid whose main bioactive compound is sapogenin. Previous research provided evidence of its anti-inflammatory and analgesic activities. The protective role of this sapogenin and derivatives on dopaminergic neurons awaits further investigation.

23.4 Natural and Designed Synthetic Products Against Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis [78](ALS) is a neurological disorder resulting in motor neuron loss (MND) and muscle wasting/paralysis, symptomatic of weak respiratory muscle performance, and respiratory failure. The survival time of ALS patients is 2–5 years and the only known effective treatment/medication is riluzole that blocks glutamate release from neurons.

Cochrane review [79] data results indicated that riluzole 100 mg possibly extends median survival in ALS patient by 2–3 months and thus the safety of the drug is not a main concern. Epigallocatechin-3-gallate (EGCG) is a main constituent of green tea polyphenols that has been found to inhibit glutamate excitotoxicity augmented by *threo*-hydroxyaspartate, an inhibitor of glutamate transporter studied in organotypic culture of the rat spinal cord [80, 81]. This suggests that the neuroprotective effects of EGCG may be used against ALS. Superoxide dismutase 1 (SOD1) is an ALS pathogenic protein, whose misfolding results in the formation of amyloid aggregates, although the cause of this abnormal protein–protein interaction is not understood [82, 83]. However, mutant SOD1 together with SE-12 a synthetic SOD1-derived peptide was found to bind SOD1 proteins and redirect the toxic amyloid aggregation towards a more benign, less toxic amorphous aggregation pathway.

The natural polyphenols in maple syrup [84] have been found to exhibit significant protection against the TDP-43 pathogenic protein in a *C. elegans* model of ALS. In particular, gallic acid (GA) and catechol illustrated in Scheme 23.8 exerted the same affect on the toxic protein as maple syrup. GA is known to have neuroprotective effects against neural damage, inhibition of β-amyloid oligomerization, and α-synuclein proteotixicity [85].Since GA is a constituent of many fruits, nuts, and herbs and herbal beverages, the chemoprotective benefits could be derived from the intake of these dietary foods. The generic activation of the proteasome is an attractive and valid therapeutic target to facilitate the disposal and degradation of multiple misfolded and aggregated proteins that are associated with ALS pathology. This strategy has generated evidence that pyrazolone-containing compounds [86]



Scheme 23.8 Some molecular interventions that ameliorate ALS pathology

exhibit therapeutic activity in ALS cellular and animal models via activation of the proteasome by direct binding to constituent proteins of the 26S proteasome. The bioactivity of the pyrazolone derivative shown in Scheme 23.8 in a SOD1G93A mouse model of ALS extended the average endurance by 13%.

Conclusion

It has been established that dietary foods, fruits, vegetables, herbs, all contain phytochemicals in variable concentrations of monomer and polymeric-phenolic natural products that can be ideally directly absorbed or are transformed and metabolized by gut microbes into small compounds, many of which are neuro-available. However, their therapeutic efficacy, potency of their multi-purpose body-brain pro-health-activities including preventing peptide/protein misfolding diseases, antioxidant, anti-inflammatory functions to actively overcome neuropathologies, maintain and sustain brain cognition required for human health and against dementia disorders is unknown. Future neuro-nutritional studies will discover/tune the molecular mechanisms and metabolic processes of food consumption on body-brain and will determine and provide new strategies of how to optimize and select dietary constituents that may sustainably provide generic benefits for holistic health including neurochemical mechanisms, enabling neurons to better defend against insults and damage, and sustain mental fitness against AD, HD, PD, and ALS neurodegenerative diseases.

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24 Use of Herbal Products/Alternative Medicines in Neurodegenerative Diseases (Alzheimer's Disease and Parkinson's Disease)

Omar M. E. Abdel-Salam

24.1 Introduction

The most common neurodegenerative diseases are Alzheimer's disease (AD) characterized by a devastating memory loss and cognitive dysfunction and Parkinson's disease (PD), characterized by slowing and difficulty in initiating movements. The list also includes diseases such as Huntington's disease, fronto-temporal-dementia, amyotrophic lateral sclerosis, motor neuron disease, and other rare genetic forms of neurodegeneration. Alzheimer's disease and Parkinson's disease are age-related progressive disorders. Alzheimer's disease, the most common cause of dementia in the elderly [1] affects subjects over the age of 65 years with a prevalence of 11% and which increases to ~30% in those aged 85 and older in the United States [2]. Similarly, the prevalence of PD worldwide rises as age advances with 0.4% prevalence in subjects aged 65–74 years and 1.1% in those aged 70–79 years [3]. In these diseases, the process of neurodegeneration usually involves selective areas in the brain, e.g., the hippocampal region and cortical parenchyma, in AD [4], and the midbrain dopaminergic neurons in PD [5]. These disorders also share common pathogenetic mechanisms such as oxidative damage [6–8], neuroinflammation [9–11], and the presence of abnormal proteins; intracellular accumulation of α-synuclein in PD [12], and extracellular deposits of amyloid-β-peptide (Aβ) in the parenchyma (senile plaques) and neurofibrillary tangles made of hyperphosphorylated form of the microtubule associated protein (tau) in the neuronal cell body in AD [4]. Until now there is no treatment to stop either disease and although dopaminergic replacement therapy with L-dopa, the precursor of dopamine and dopamine receptor agonists have eased the life of many patients with PD, the natural history of the disease is not altered [13]. Similarly, in AD, the use of cholinesterase inhibitors to boost cholinergic neurotransmission results in modest improvements in memory [14].

In the search for novel therapies for these neurodegenerative disorders, research in the field of botanicals and phytochemicals suggested promising molecules. The most common of these herbal remedies are *Ginkgo biloba*, *Panax ginseng*, and curcumin. Other dietary components, e.g., polyphenols, black or green tea and their catechins, and coffee, have also been shown to exert beneficial effects. The aim of this chapter is to review the evidence pertaining to the action of these herbal preparations and some dietary components and their biologically active constituents.

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S. Singh, N. Joshi (eds.), *Pathology, Prevention and Therapeutics of Neurodegenerative Disease*,
https://doi.org/10.1007/978-981-13-0944-1_24

24.2 Dietary Polyphenols

There is evidence that some dietary patterns or specific components in diet are able to modulate the risk of PD. In a study on 805 subjects who developed PD over a period of 20–22 years, those with highest intake of total flavonoids showed 40% lower risk for PD compared with subjects with the lowest intake. The protective effect for flavonoids was significant in men but not in women. In particular, significant associations were found between increased intakes of berries (rich in anthocyanins) and apples (though not tea) and a lower PD risk [15]. In their study on 257 subjects with PD, Alcalay et al. [16] found that higher adherence to Mediterranean-type diet was associated with reduced risk for PD. In contrast, lower intake of such diet was associated with earlier PD age-at-onset. The study showed that PD subjects were less likely to adhere to Mediterranean-type diet compared with controls which might be associated with earlier age-at-onset. Such diet is rich in polyphenols and the antioxidants ascorbic acid and α-tocopherols derived from vegetables, fruits, cereals, and olive oil (contains unsaturated fatty acids) [17, 18]. Studies also indicated that consumption of specific nutrients or some dietary patterns might have a beneficial impact on the cognitive status of the individual. In this context, increased consumption of fish, mono- and polyunsaturated fatty acids was found to be associated with decreased risk for cognitive impairment and dementia [19]. Several studies also found that adherence to a Mediterranean-type diet delayed the development of cognitive dysfunction in the elderly [20–22]. Feart et al. [20] found that higher adherence to Mediterranean diet in older persons was associated with fewer errors on Mini-Mental State Examination (though not in other cognitive tests). The study included 1410 individuals, with average age of 75.9 years and a median follow-up of 4.1 years. In a follow-up study of 1393 cognitively normal subjects for 4.5 years, moderate and high intake of Mediterranean diet was associated with 17% and 28% less risk of developing mild cognitive impairment as compared to subjects with low intake. The risk for progression of mild cognitive impairment to AD was also decreased by 45% and 48% in subjects with moderate and high intake of Mediterranean diet compared with those with low intake [21]. In a randomized clinical trial of 334 cognitively healthy volunteers with a mean age of 66.9 years, participants allocated to a Mediterranean diet plus olive oil or nuts for 4.1 years showed better cognitive function compared with controls [23]. In the elderly, adherence to Mediterranean diet was also found to reduce the likelihood of developing depressive symptoms over an average follow-up of 7.2 years [24].

24.3 Tea Catechins

Tea is a water infusion from the dried leaves of *Camellia sinensis* (L.) and one of the most popular beverages worldwide. Green tea is made by steaming the leaves, preventing oxidation of the polyphenols. Polyphenols account for up to 40% of the dry weight of green tea. These are mostly flavanols, known as catechins of which (−)-epicatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin, and (−)-epigallocatechin-3-gallate are the most important. Epigallocatechin-3-gallate is the most abundant and most bioactive catechin of green tea. Other constituents of tea are caffeine, theophylline, theobromine, amino acids and phenolic acids, such as gallic acid. Black tea is a fermented tea where flavanols are oxidized to theaflavins and thearubigins [25, 26]. Studies in humans, however, showed limited bioavailability of black tea catechins with ~ 1.68% of the ingested catechins: (−)-epigallocatechin, (−)-epicatechin, (−)-epigallocatechin gallate, and (−)-epicatechin gallate being present in plasma. Moreover, bioavailability of the gallated catechins was lower than that of the non-gallated catechins [27]. In subjects given a single oral dose of a "decaffeinated green tea catechin mixture", epigallocatechin, epicatechin, were found in plasma mainly in their glucuronide/sulfate conjugates [28]. In subjects given single oral dose of green tea (20 mg tea solids/kg), the maximal plasma concentrations of (−)-epigallocatechin-3-gallate, (−)-epigallocatechin, and (−)-epicatechin were 77.9, 223.4, and

124.0 ng/ml, respectively. These concentrations were achieved 1.3–1.6 h following tea ingestion. In the plasma, (−)-epigallocatechin-3-gallate was present mainly in the free form, while (−)-epigallocatechin and (−)-epicatechin existed mostly in the conjugated form [29]. The effect of epigallocatechin gallate on brain activity was examined in a double-blind, placebo-controlled study. When given at 300 mg, the flavonoid increased overall electroencephalogram activity and calmness while reducing stress [30].

Consumption of tea has been associated with decreased risk for developing PD. Reduced risks were observed after consumption of 2 cups/day or more of tea [31]. A study among Chinese subjects suggested that intake of 3 cups of tea/day for 10 years would result in 28% decrease in the risk of developing PD [32]. Another study from Japan indicated an inverse relationship between intake of black tea, Japanese tea, or Chinese tea, and the risk for developing PD [33]. Similarly, Kandinov et al. [34] found in subjects with PD that consumption of more than 3 cups of tea/day delayed the age by which motor symptoms appear by 7.7 years. Studies also examined the effect of tea catechins on the response to antiparkinsonian drug therapy. (−)-epigallocatechin-3-gallate, (+)-catechin, and (−)-epicatechin were found to inhibit catechol-O-methyltransferase (COMT)-mediated O-methylation of L-DOPA in vitro [35]. In rats, only (+)-catechin significantly inhibited L-DOPA methylation in periphery and striatum, with this effect being attributed to better bioavailability [35].

Tea polyphenols are potent scavengers of reactive oxygen and nitrogen species and also inhibit redox-sensitive transcription factors such as nuclear factor-kappaB and activator protein-1 [36]. Tea polyphenols are also efficient chelators of Fe^{++}. In this latter study, the authors investigated the metal chelating and antioxidant properties of a number of dietary constituents thought to be of value to brain function. Phenolic compounds containing the pyrogalol moiety gallic acid, propylgallate, gallamide and epigallocatechin gallate were all strong chelators of Fe^{++}. Epigallocatechin gallate was also potent chelator of Cu^{++} and Zn^{++} [37]. The experimental data on the neuroprotective effect of tea or catechins are shown in Table 24.1.

Table 24.1 Neuroprotective effect of tea or tea constituents in models of Parkinson's disease and Alzheimer's disease

Model	Tea or individual constituents	Neuroprotection	Mechanism (s)	Study
6-hydroxydopamine (6-OHDA) toxicity in PC12 cells	(−)-epigallocatechins gallate (200 μM) (−)-epicatechin gallate (200 μM)	↓ Cell death	↓ Apoptosis	[38]
1-methyl-4-phenylpyridinium (MPP+) toxicity in embryonic rat mesencephalic dopaminergic neurons	Green tea polyphenols (10–30 μg/ml)	↓ Loss of tyrosine hydroxylase (TH)-positive cells Block MPP(+) uptake into dopaminergic neurons	Inhibitory effect on dopamine transporter	[39]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- nonhuman primates model of Parkinson's disease	Tea polyphenols given orally	↓ Motor impairment ↓Dopaminergic neuronal injury in the substantia nigra	↓ α-synuclein oligomers	[40]
PC12 cells treated with Aβ (25–35) (10–50 μM)	Green tea extract (10–50 μg/ml)	↓Aβ (25–35) (50 μM)-induced cell death ↓ Intracellular reactive oxygen species ↓ 8-oxodG formation ↓ p53, Bax, and caspase-3 expression	↓Activation of NF-κB and ERK and p38 mitogen-activated protein kinase pathways	[41]

(continued)

Table 24.1 (continued)

Model	Tea or individual constituents	Neuroprotection	Mechanism (s)	Study
Aβ (25–35) toxicity in primary cultures of rat hippocampal cells	Green and black tea extracts (5–25 μg/ml) Gallic acid (1–20 μM) Epicatechin gallate (1–20 μM) Epigallocatechin gallate (1–10 μM)	↓Apoptosis (Epicatechin gallate and Epigallocatechin gallate)	↓Aβ aggregation (epigallocatechin gallate and gallic acid)	[42]
Aβ (1–42) (2 μg/mouse, i.c.v.) Alzheimer's disease mouse model	l-theanine (2 and 4 mg/kg) for 5 weeks in the drinking water	↓ Neuronal cell death in cortex and hippocampus ↓ Memory impairment	↓ ERK ↓ p38 mitogen-activated protein kinase ↓ NF-κB ↓ Oxidative damage	[43]
Neuroinflammation (LPS 1 μg/mouse, i.c.v.)	(−)-epigallocatechin-3-gallate 1.5 or 3 mg/kg in drinking water for 3 weeks	↓ Aβ levels ↓ Apoptotic neuronal cell death	↓ Brain β- and γ-secretase activities ↓ Inducible iNOS expression ↓ COX-2 expression	[44]
Aβ (1–42) (0.5 μg/mouse, i.c.v.) Alzheimer's disease mouse model	(−)-epigallocatechin-3-gallate 1.5 or 3 mg/kg in drinking water for 3 weeks	↓Apoptotic neuronal cell death ↓ Memory impairment	↑ Brain α-secretase activity ↓ Brain β- and γ-secretase activities ↓ ERK ↓ p38 mitogen-activated protein kinase ↓ NF-κB	[45]
Preseniline 2 (PS2) mutant Alzheimer's disease mouse model	(−)-epigallocatechin-3-gallate 3 mg/kg in drinking water for 3 weeks	↓ Aβ brain levels ↓ Memory impairment	↑ Brain α-secretase activity ↓ Brain β- and γ-secretase activities	[45]
Swedish double mutation in the APP gene (APPsw) transgenic Alzheimer's disease mouse model	(−)-epigallocatechin-3-gallate (50 mg/kg) in drinking water for 6 months	↓ Aβ levels in brain ↓ Memory impairment	↓ Phosphorylated tau isoforms	[46]
Primary neurons from APPsw transgenic mice	(−)-epigallocatechin-3-gallate	↓ Aβ brain levels	Enhanced non-amyloidogenic α-secretase proteolytic pathway	[47]
PSAPP transgenic Alzheimer's disease mouse model	Tannic acid (tea) orally for 6 months	↓ Cerebral vascular β-amyloid deposits ↓ Behavioral impairment ↓ Memory impairment	↓ Neuroinflammation	[48]
Transgenic Alzheimer's disease mouse model	Catechin (green tea) 1 mg or 10 mg for 6 months	↓ Aβ-42 production ↓ Behavioral impairment	↓ γ-secretase activity ↑ α-secretase activity	[49]
Neuroinflammation (lipopolysaccharide 250 μg/kg, i.p.) for 7 days	(−)-epigallocatechin-3-gallate 1.5 or 3 mg/kg in drinking water for 3 weeks	↓ Aβ levels ↓ Amyloid precursor protein (APP) expression ↓ Apoptotic neuronal cell death ↓ Memory impairment	↓ Astrocyte activation ↓TNF-α ↓ IL-1β ↓ Macrophage colony-stimulating factor ↓ Soluble intercellular adhesion molecule-1 ↓ IL-6 ↓ Inducible iNOS expression ↓ COX-2 expression	[50]

Abbreviations: *i.c.v.* intracerebroventricular, *NF-κB* nuclear factor kappaB, *ERK* extracellular signal-regulated kinase, *IL-1β* L interleukin-1beta, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-alpha, *COX-2* cyclooxygenase-2, *iNOS* inducible nitric oxide synthase, *i.p.* intraperitoneal

24.4 Coffee

Coffee is a popular beverage produced from the ground roasted beans. Coffee contains the alkaloids caffeine and trigonelline, chlorogenic acid, and the diterpenes cafestol and kahweol [51]. Coffee is the main source of caffeine (1,3,7-trimethylxanthine) intake in many parts of the world. In North America, coffee followed by tea provides most of caffeine in the adult diet. Brewed coffee contains 56–100 mg caffeine/100 ml while instant coffee and tea provide 20–73 mg caffeine/100 ml. Other sources of caffeine are cola, cocoa, and chocolate [52]. It is estimated that for adults consuming 3–4 cups of coffee/day, this will provide 300–400 mg of caffeine [53]. In the United States, studies suggested an average caffeine intake of 193 mg/day in caffeine consumers, with the highest intake being in men and women aged 35–64 years. In this group, coffee represented the major source of caffeine in the diet. Coffee also accounted for most of caffeine in diet (71%) to be followed by soft drinks (16%), and tea (12%) [54]. Data from a recent study indicated that 98% of the adult US population consumed caffeine. The prevalence was equal in men and women. The average caffeine intake was 211 and 183 mg/day for men and women consumers, respectively, with consumption being highest in men aged 31–50 years [55].

24.4.1 Coffee and the Risk of Parkinson's Disease

Studies indicated an inverse association between consumption of coffee and the risk of PD, with the effect of coffee being a dose-dependent one [56–66]. Sääksjärvi et al. [62] reported decreased risk for PD in subjects consuming 10 or more cups of coffee/day compared with non-drinkers. In a study on 304,980 participants, higher coffee intake in 1995–1996 was associated with lower PD risk in both men and women over about 10 years of follow-up [65]. In a study in 1808 patients with idiopathic PD, moderate intake of caffeine (3.1–5 cups/day) was associated with a lower risk for PD [66]. The effect of coffee in reducing the risk for PD appears to be limited to those who drink caffeinated coffee. Other sources of caffeine, e.g., soft drinks, hot tea, and iced tea, were not associated with the risk of PD [65]. The effect of coffee on PD could be observed for both men and women [58, 59, 62, 64, 65], but is likely to be attenuated in women by hormonal replacement therapy post-menopause. In their study on post-menopausal women, Ascherio et al. [58] found that caffeine reduces the risk for PD among those who do not use estrogen replacement therapy. In contrast, coffee increases risk among hormone users where the risk of PD increases by fourfold in women who consumed 6 or more cups of coffee/day compared with non-drinkers. In a prospective study on the relation between coffee consumption and Parkinson's disease mortality, coffee consumption was inversely associated with Parkinson's disease mortality in men but not in women. The failure of coffee to reduce mortality from PD in women was attributed to the use of estrogen replacement therapy after menopause [59]. In another prospective study of caffeine intake and risk of PD, high caffeine consumption was associated with a reduced risk of PD. Women who never used estrogen replacement therapy showed stronger association between coffee and decreased risk of PD compared with ever users [64].

In PD, dyskinesia refers to involuntary movements, most commonly chorea, that develops several years after treatment with L-dopa. It occurs at the time of peak L-dopa effect and the risk of developing dyskinesia is L-dopa dose-dependent [67]. In their study, Wills et al. [68] found that subjects who consumed >12 ounces of coffee/day were less likely to develop dyskinesia compared with those who consumed <4 ounces/day. Similarly, Nicoletti and Zappia [69] reported a negative association between coffee drinking and the presence of dyskinesia in subjects with PD on dopamine replacement therapy. The study also showed a dose-dependent effect for coffee in decreasing PD risk. It has also been shown in patients with idiopathic PD that caffeine improves L-dopa pharmacokinetics [70]. This is likely to reduce the development of dyskinesia due to L-dopa by decreasing the effective dose required.

The effect of coffee in PD could also be attributed to adenosine A2A receptor antagonism by the caffeine content. Istradefylline is a nonselective adenosine A2A receptor antagonist which can be used as adjunct to L-dopa [71].

24.4.2 Coffee and Cognition

Caffeine intake results in a decrease in mental fatigue and increased alertness while improving memory processes. These effects are observed in both habitual caffeine consumers and habitual non-consumers [72]. Coffee/caffeine intake has also been found to be associated with better cognitive performance in elderly. It was noted that in women with a mean age of 72.6 years, higher lifetime and current consumption of coffee resulted in better scores in many tests for cognitive functions. This effect of coffee was not observed in men with a mean age of 73.2 years or women aged 80 years or more. The study also found no effect for decaffeinated coffee on cognitive function in older men or women [73], thereby suggesting that caffeine content mediated the improvement in cognitive function. In their study, Eskelinen et al. [74] found that subjects who drink 3–5 cups of coffee/day at mid-life had 65% lower risk of dementia and AD in late-life compared with individuals who drink no or little coffee. This study included 1409 individuals aged 65–79 years. In a community-based sample of 4197 women and 2820 men aged 65 years and over, coffee consumption was associated with less degree of cognitive decline in women without dementia who consumed >3 cups of coffee/day. Verbal retrieval and visuospatial memory showed fewer declines over 4 years of follow-up compared to women consuming one cup or less of coffee. The cognitive protective effect of coffee was more evident as the age increases. The study found no association between coffee intake and cognitive decline in men [75]. In a cohort of 648 subjects aged 65 years or more, caffeine intake of >62 mg/day was associated with a lower risk for cognitive decline as compared with an intake of <22 mg/day. The effect of coffee was significant only in women but not in men [76]. Similarly, Vercambre et al. [77] observed slower rates of cognitive decline with increasing caffeine intake over 5 years in women. The study included 2475 women aged >65 years with vascular disorders.

The effects of caffeine on cognitive performance and mood would be also important for subjects with PD for they also suffer from fatigue [78], apathy [79], cognitive dysfunction [80], and depression which affect approximately 20–40% of patients [81, 82]. The effect of caffeine on day somnolence, motor activity, and other non-motor manifestations of PD were evaluated in a 6-week randomized placebo-controlled trial of 61 patients with PD. Caffeine improved somnolence and objective motor measures. There was no effect, however, on the quality of life, sleep, or depression [83].

Studies in a transgenic mouse model of AD showed that caffeine given from young adulthood till aging protected against memory impairment and reduced Aβ-peptide levels. Moreover, old transgenic mice showed improved memory and decreased Aβ-peptide burden upon giving caffeine [84, 85]. Table 24.2 summarizes the results on the protective effect of coffee or caffeine in models of Parkinson's disease or Alzheimer's disease.

24.5 Ginseng

Panax ginseng (*P. ginseng,* Fam. *Araliaceae*) is a perennial herbaceous plant widely cultivated in China, Korea, and Japan. The root of the plant is valued for its medicinal properties and has been used in traditional Chinese medicine since antiquity. Ginseng is usually described as being an adaptogen or restorative tonic [89]. There are several different *Panax* species, including *Panax ginseng* Meyer (Chinese or Korean ginseng), *Panax pseudo-ginseng* (Japanese ginseng), *Panax notoginseng* (China), *Panax vietnamensis* (Vietnamese ginseng), and *Panax quinquefolium* (American ginseng) [90]. The latter is native to eastern North America. It is currently grown in Eastern USA and Canada [91]. Siberian or Russian ginseng (*Eleutherococcus senticosus*) is

Table 24.2 Neuroprotective effect of coffee or caffeine in models of Parkinson's disease and Alzheimer's disease

Model	Coffee, tea, or individual constituents	Neuroprotection	Mechanism (s)	Study
APPsw transgenic mouse model of Alzheimer's disease	Daily caffeine (1.5 mg/mouse) in drinking water, starting from young adulthood to old age (equivalent to 500 mg of caffeine in humans or 5 cups of coffee/day)	↑ Memory performance ↑ Brain adenosine levels ↓ Aβ-peptide production ↓ Aβ-peptides in hippocampus	↓ Expression of Presenilin 1 (PS1) and β-secretase	[84]
Aged APPsw mice with cognitive impairment	Daily caffeine (1.5 mg/mouse) for 4–5 weeks	↓ Aβ-peptides in hippocampus and entorhinal cortex (40% and 46%). ↓ Soluble Aβ-peptides in brain	cRaf-1/ NF-κB mediated	[85]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-toxicity in mice striatum	Caffeine (20 mg/kg, i.p.) daily for 9 days <MPTP	↓Loss of striatal dopamine ↓ Loss of dopamine transporter binding sites	–	[86]
SH-SY5Y cells exposed to lipopolysaccharide + interferon-γ or interferon-γ released from activated microglia and astrocytes	Quercetin, flavones, chlorogenic acid, and caffeine	↑ Cell viability (MTT assay)	↓ TNF-α, and ↓ IL-6 from the activated microglia and astrocytes. ↓ Activation of proteins from P38 mitogen-activated protein kinase ↓ NF-κB ↓ Oxidative/ nitrative damage (quercetin).	[87]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-toxicity in mice striatum	Caffeine (20 mg/kg, i.p.) daily for 8 weeks <MPTP co-treatment once in a day for 2–4 weeks	↓ Loss of striatal dopamine ↑ Dopamine transporter mRNA expression	↓ Adenosine A2A receptor mRNA expression	[88]

Abbreviations: *NF-κB* nuclear factor kappaB, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-alpha

not a true ginseng but belongs to a different genus in the family *Araliaceae* and does not contain ginsenosides [92]. White ginseng refers to the air-dried root after being harvested while red ginseng is produced by steaming the fresh, unpeeled root at 98–100°C for 2–3 h before drying [93]. The chemical constituents of *P. ginseng* are polysaccharides, phenolics and flavonoids, mostly quercetin and kaempferol, and triterpene saponins known as ginsenosides which account for most of the biological activity of ginseng. The root contains 3–6% by weight of ginsenosides [94]. The ginsenoside content of American and Asian ginseng differs, e.g., Rf is absent in American ginseng while present in Asian ginseng. Siberian or Russian ginseng is devoid of ginsenosides [91]. *Panax notoginseng* is another species of the genus *Panax*. The root of *P. notoginseng* is a widely used traditional Chinese medicine. The major constituents are ginsenosides, notoginsenosides, gypenosides, flavonoids, cyclopeptides, and sterols [95]. Commercially available standardized extracts of ginseng are G115 from *P. ginseng* (Pharmaton

SA, Switzerland) and NAGE from *P. quinquefolius* (Canadian Phytopharmaceutical Corporation, Canada).

Whether ginseng would improve cognition in healthy subjects has been examined by several authors. One study that involved 3500 healthy volunteers found that neither ginseng nor *Ginkgo biloba* was able to enhance memory performance. The participants reported up to 2 years of regular use of either herb. The kind of herbal preparations used is, however, not specified in the study [96]. In this context, it should be noted that herbal preparation, especially those of ginseng vary widely in their content of ginsenosides [97]. Wesnes et al. [98], however, found the combination of ginseng and *Ginkgo biloba* to be superior to placebo in improving working and long-term memory. In this study, 256 healthy middle-aged volunteers received standardized extracts of *Ginkgo biloba* (GK501) and of *Panax ginseng* (G115) at doses of 60 mg and 100 mg, respectively, for 14 weeks. Similarly, in healthy, young adult volunteers, Kennedy et al. [99] reported improvements in memory function following 360 mg of *Ginkgo biloba*, 400 mg of *Panax ginseng*, or 960 mg of the two extracts as compared to placebo. In thirty healthy young adult volunteers, acute administration of G115® (400 mg) improved speed of attention tested 90 min after drug ingestion [100]. Sutherland et al. [101] used HT1001, a standardized North American ginseng (*Panax quinquefolius*) extract in healthy young adults and middle-aged volunteers. The extract is standardized to contain 13–20% of active ginsenosides. HT1001 given at 100 mg (equivalent to 500 mg of North American ginseng dried root) twice daily resulted in significant improvement of several aspects of memory. Scholey et al. [102] studied the effect of highly standardized extract of *P. quinquefolieus* (Cereboost™) on cognitive function in 32 healthy young subjects. Cereboost™ which is standardized to contain 10.65% ginsenosides was given at doses of 100, 200, and 400 mg. The authors reported significant improvement of working memory performance as well as an increase in calmness by ginseng. There was no change in blood levels of glucose after the intake of ginseng. In 52 healthy volunteers with a mean age of 51 years, Cereboost™ 200 mg improved working memory 3 h after dosing as compared to placebo. Cereboost™ at this dose showed no significant effects on mood or blood glucose levels [103]. Reay et al. [104], however, suggested a glucoregulatory mechanism to account for the effect of ginseng on cognitive performance. In their study, 27 healthy young adults received either 200 mg G115, 25 g glucose, or their combination. Interestingly, either ginseng or glucose increased the performance of a mental arithmetic task and alleviated the subjective mental fatigue in late stages of a sustained mental exercise.

In mice, memory impairment induced by the use of the cholinergic agent scopolamine could be prevented by pretreatment with a ginsenoside Rg3-enriched ginseng extract. Ginseng inhibited acetylcholinesterase activity and suppressed NF-κB signaling in the hippocampus [105]. Other mechanisms by which ginseng or individual ginsenosides enhance memory involve increased expression of choline acetyltransferase and trkA mRNAs in the basal forebrain and nerve growth factor mRNA in the hippocampus by ginsenoside Rb1 [106], and increased proliferation of hippocampal progenitor cells by Rg1 [107].

Ginseng exerts a number of important pharmacological effects which are likely to contribute to the observed neuroprotective effects of ginseng or ginsenosides and these include:

1. Antioxidant properties: inhibition of metal-induced lipid peroxidation (chelation of transitional metal ions Cu^{++} and Fe^{++}) by *P. quinquefolius* extract CNT2000 (standardized to 8% ginsenosides) [108]. Decreased intracellular reactive oxygen species and malondialdehyde, and increased glutathione and antioxidant enzyme activities of catalase, superoxide dismutase and glutathione peroxidase by ginsenoside Rd. [109].
2. Inhibition of caspase-3 mediated apoptosis by G115 [110], Rg1 [111], and ginsenoside Rd. [109].

3. Inhibition of cyclooxygenase-2 expression by panaxatriol saponins (P. notoginseng) [112] and Rg3 [113].
4. Inhibition of glia activation by G115 [114], ginsenoside Re [115], and ginsenoside Rg3 [113].
5. Increased BcL-2 expression and decreased Bax and HSP70 expression by ginsenoside Rg2 [116].
6. Increased expression and secretion of the neurotrophic factors nerve growth factor and brain-derived neurotrophic factor by ginsenosides Rb1 and Rg1 [107, 117].
7. Inhibition of glutamate-induced intracellular Ca^{++} influx by ginsenoside Rd. [118].
8. Decreased production of interleukin-6 by *P. notoginseng* (NotoG™) [119] and decreasd interleukin (IL)-1β and IL-6 mRNA by ginsenoside Rb1 [120].
9. Inhibition of tumor necrosis factor-alpha (TNF-α) release by ginsenoside Rg3 [113], ginsenoside Rb1 [120], and by *P. notoginseng* (NotoG™) [119].
10. Inhibition of nitric oxide release by ginsenoside Rd. [121] and *P. notoginseng* (NotoG™) [119].
11. Activation of phosphatidylinositol 3-kinase and Nrf2 signaling pathway by panaxatriol saponins from *P. notoginseng* [122].
12. Modulation of cerebral monoamine transmitters by ginsenoside Rb1 [123] and increased choline uptake by ginsenoside Rb1 by central cholinergic nerve endings [124].

Ginseng or individual ginsenosides were shown to exert protective effects in different experimental models of PD or AD (Table 24.3).

Table 24.3 Neuroprotective effect of ginseng or ginsenosides in models of Parkinson's disease and Alzheimer's disease

Model	Ginseng or ginsenosides	Neuroprotection	Mechanism (s)	Study
Scopolamine-induced memory impairment in mice	Ginsenoside Rg3-enriched ginseng extract (50 and 100 mg/kg) orally for 14 days	Alleviation of memory impairment	↓ Acetylcholinesterase activity ↓ NF-κB signaling in hippocampus	[105]
Parkinson's disease caused by feeding rats with dietary phytosterol glucoside β-sitosterol β-D-glucoside	G115 orally 100 mg/kg/day	↓ Locomotor deficits ↓ Tyrosine hydroxylase-immunoreactive cells loss in substantia nigra ↓ Microgliosis ↓ α-synuclein aggregates	↓ Caspase-3 activation ↓ Glia activation	[110]
1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-treated mice.	Ginsenoside Rg1 (5.0, and 10.0 mg/kg) 3 days prior to MPTP	↓ Apoptosis	↑ Bcl-2 expression ↓ Bax expression ↓ iNOS expression ↓ Caspase-3 activation	[111]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in mice	Panaxatriol saponins from *Panax notoginseng*	↓ Behavioral impairment ↓ Neuronal death in substantia nigra	↑ Thioredoxin-1 (Trx-1) expression ↓ COX-2 over-expression ↓Mitochondria-mediated apoptosis.	[112]
Systemic lipopolysaccharide injection in mice (3 mg/kg, i.p.)	Ginsenoside Rg3 20 and 30 mg/kg orally 1 h prior to the lipopolysaccharide	↓ Neuroinflammation	↓TNF-α, IL-1β, IL-6 mRNA ↓ COX-2 expression ↓ iNOS expression ↓ Microglia activation	[113]

(continued)

Table 24.3 (continued)

Model	Ginseng or ginsenosides	Neuroprotection	Mechanism (s)	Study
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice 1-methyl-4-phenylpyridinium (MPP+) in rats	G115 orally prior to and/or following exposure to MPP+	↓ Locomotor changes Prevented tyrosine hydroxylase-positive cell loss in the substantia nigra	–	[114]
Methamphetamine-induced dopaminergic toxicity in mice	Ginsenoside Re (10 and 20 mg/kg, p.o.)	↓ Behavioral changes ↓ Dopaminergic degeneration	↓ Oxidative stress ↓ Microglia activation (effects mediated by inhibition of protein kinase C (PKC) δ)	[115]
Mesencephalic primary cultures treated with lipopolysaccharide (100 microg/ml)	Ginsenoside Rd	↓ Cell death	↓ Neuroinflammation (↓ Nitric oxide and ↓ PGE2 synthesis)	[121]
Human SHSY5Y cells treated with MPP+ (1-methyl-4-phenyl-pyridinium)	Rg1(10 and 20 μM)	↓ Apoptosis	↓ Reactive oxygen metabolites ↓ c-Jun N-terminal kinase (JNK) activation ↓ Cleaved caspase-3 expression (anti-apoptotic effect)	[125]
Glutamate toxicity in embryonic mouse mesencephalic cells	Ginsenosides Rb1 and Rg1	↑ Number and length of neurites of surviving dopaminergic cells	Neurotrophic effect	[126]
Embryonic mouse mesencephalic cells treated with 1-methyl-4-phenylpyridinium-iodide (MPP).	Ginsenoside Rb1 (10 μM)	↑ Survival of dopaminergic neurons by 19%	Neurotrophic effect	[126]
6-hydroxydopamine (6-OHDA) toxicity in human neuroblastoma SK-N-SH cells	Ginsenoside Rg1	↑ Cell survival	↓ Bax ↑ Bcl-2 mRNA and protein expression ↑ Mitochondrial membrane potential (mediated by activation of insulin-like growth factor-I receptor-dependent pathway and estrogen receptor-dependent pathway).	[127]
6-hydroxydopamine (6-OHDA)-induced toxicity in MES23.5 cells	Ginsenoside Rg1	↑Cell viability	↑ Gene and protein expressions of Bcl-2 ↑Akt phosphorylation ↓ ERK1/2 phosphorylation induced by 6-OHDA	[128]
6-hydroxydopamine (6-OHDA)-treated MES23.5 cells	Ginsenoside-Rg1	↓ Cellular iron accumulation	↓ 6-OHDA-induced upregulation of iron importer protein divalent metal transporter 1 with iron responsive element.	[129]

Abbreviations: *Akt* Protein kinase B, *PGE2* prostaglandin E2, *IL-1β* L Interleukin-1beta, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-alpha, *COX-2* cyclooxygenase-2, *iNOS* inducible nitric oxide synthase

24.6 Ginko biloba

Extracts from the dried green leaves of *Ginkgo biloba* L. (Fam. *Ginkgoaceae*) have been used in traditional Chinese medicine over thousands of years. Ginkgo is one of the best-selling herbs in the United Sates, being used as a complementary therapy for cognitive impairment such as that associated old age or AD and vascular dementia [130]. Other uses of the extract are in the treatment of intermittent claudication, schizophrenia, and vertigo [131, 132]. EGb 761® is a water–acetone extract of the dried green leaves of *Ginkgo biloba*, standardized to contain 24% flavonoid glycosides (including quercetin, kaempferol, isorhamnetin), 6% terpene lactones (containing 3.1% ginkgolides A, B, C, and J and 2.9% bilobalide), and less than 9% proanthocyanidins and organic acids (<5 ppm ginkgolic acid) [131, 133]. When used to treat dementia syndromes, the dosage is 240 mg/day [134].

Studies in young healthy volunteers suggested that the administration of *Ginkgo biloba* extracts results in better performance in cognitive demanding tasks. When given to healthy young volunteers (mean age 19.9 years), GK501 (320 mg) increased cognitive function. GK501 (Pharmaton SA) is standardized to 24% ginkgoflavone glycosides and 6% terpene lactones [135]. In 78 healthy young volunteers aged ~20 years, compared with placebo, EGb at a low dose of 120 mg/day improved the quality of memory at 1 and 4 h post-dosing. This dose, however, was observed to impair performance on the "speed of attention" task performance [136]. Students (18–26 years) who received a single dose of standardized *Ginkgo biloba* extract (120 mg) and tested 4 h later demonstrated increased performance on the sustained attention and pattern recognition memory tasks. However, after 6 weeks of treatment, there was no effect for *Ginkgo biloba* for memory compared with controls who received placebo, suggesting that tolerance has developed. GK501 (Pharmaton SA) is standardized to 25% total ginkgoflavone glycosides and 6% terpene lactones [137].

The effect of *Ginkgo biloba* on cognitive decline in the elderly is somewhat less clear. Improvement in cognitive function has been reported after 24 weeks of treatment with EGb 761 (240 mg/day) in patients with dementia [138]. DeKosky et al. [139] found *Ginkgo biloba* (EGb761120-mg twice a day) to be no better than placebo in reducing the rate of progression to dementia or AD in elderly individuals with normal cognition or those with mild cognitive impairment. Moreover, an increasing rate of AD was noted in individuals with cerebrovascular disease given *Ginkgo biloba.* Similar observations were reported by Snitz et al. [140] who found that EGb761 (120-mg extract twice a day) did not lessen cognitive decline in the elderly with normal cognition or with mild cognitive impairment. In contrast, dementia patients with neuropsychiatric symptoms who received EGb761 for 22 weeks at the dose of 240 mg/day showed improvement in cognition as compared to placebo. The patients aged 50 years or above included those with AD and vascular dementia [141]. Patients with dementia and neuropsychiatric manifestations treated with EGb761 (240 mg/day) for 22 weeks exhibited improvements in apathy, indifference, anxiety, irritability, depression, dysphoria, and sleep [142]. In subjects with cognitive complaints and low functioning, EGb761 (240 mg/day) given for 12 weeks improved cognitive function and the quality of life compared with placebo. The subjects aged 45–65 years showed improvements in concentration and working memory as well as in memory tasks related to everyday life [143]. In a meta-analysis of nine trials on the use of ginkgo in patients with cognitive impairment and dementia, data favored EGb761 over placebo for maintaining cognitive performance and improving daily living activities. In these trials of 22–26 weeks duration, EGb761 administered at a dose of 240 mg/day was able to stabilize or slow the decline in cognitive function and behavior [134]. A recent fMRI study on the use of EGb761 (240 mg/day) in elderly with subjective memory impairment indicated increased cognitive flexibility without change in brain activation. The study found no effect for *Ginkgo biloba* on prefrontal dopaminergic function [144]. Rainer et al. [145] found that EGb 761R (240 mg/day) resulted

in a delay in activities of daily living deterioration by 22.3 months when compared to placebo. The cost of treatment with *Ginkgo biloba* extract to achieve treatment success was less than that of cholinesterase inhibitors. *Ginkgo biloba* and donepezil, however, could be used in combination. In this study, subjects aged 50 years or above, with probable AD were treated with EGb 761(R) (240 mg/day), donepezil (5 mg followed by 10 mg/day), or their combination for 22 weeks. The study found no significant difference in the efficiency between EGb 761(R) and donepezil but the combination seemed to be superior to either agent alone [146].

Several mechanisms are thought to account for the effect of *Ginkgo biloba* on cognition. Lowering Aβ-peptide deposition in brain is one goal of anti-AD therapy [147]. In transgenic mouse models of AD, treatment with EGb761 was found to decrease Aβ oligomers [148] and amyloid precursor protein (APP) protein levels [149]. Yao et al. [150] proposed reduction of free cholesterol level as the mechanism underlying inhibition of Aβ-peptide production by EGb761. In their study on aged rats, EGb761 given at 50 mg/kg/day for 28 weeks decreased circulating free cholesterol and both Aβ-peptide and APP protein levels. Colciaghi et al. [151] suggested that EGb761 directs the metabolism of APP towards the α-secretase pathway, the enzyme which regulates the non-amyloidogenic processing of APP. Increased alphaAPPs release was observed in hippocampal and cortical slices incubated with EGb761 and also after treating rats with 80 and 150 mg/kg of EGb761 daily for 5 days. EGb761 might regulate the phenotype of activated microglia, resulting in downregulation of pro-inflammatory cytokines and inducible nitric oxide synthase, and upregulation of anti-inflammatory cytokines [152]. EGb761 also increases Hsp70 expression [153]. Tchantchou et al. [148] showed that EGb761 increases neurogenesis in the hippocampus of a transgenic mouse model of Alzheimer's disease. This effect was observed in both young and old mice. Ma et al. [154] attributed the improvement of spatial memory in mice by effect of bilobalide to increased glucocorticoid receptor expression in the hippocampus. Alterations in brain neurotransmitter levels could also account for the memory enhancing action of *Ginkgo biloba*. Blecharz-Klin et al. [155] reported increased serotonin (5-HT) in hippocampus and norepinephrine in hippocampus and prefrontal cortex of rats given EGb761 50–150 mg/kg/day for 3 months. In another study, EGb 761 at 100 mg/kg/day for 2 weeks increased extracellular dopamine and noradrenaline levels in the prefrontal cortex of awake rats. These effects were mediated by the flavonol glycosides and ginkgolide fractions but not bilobalide [156]. Rats treated with EGb761 100–300 mg/kg/day for 2 weeks exhibited significant elevations in extracellular dopamine and norepinephrine levels in medial prefrontal cortex. When given orally at a dose of 10 mg/kg/day for 2 weeks, the acylated flavonol glycosides quercetin, and kaempferol markedly increased extracellular acetylcholine and dopamine in medial prefrontal cortex [157]. In treating dementia, boosting cholinergic neurotransmission with cholinesterase inhibitors tacrine, donepezil, and rivastigmine results in symptomatic benefit [14]. In their study, Stein et al. [158] found that EGb761 had no effect on basal acetylcholine release in the rat brain. There was no pharmacological interaction between donepezil and EGb761 on the hippocampal cholinergic system, suggesting that both drugs can be taken safely. Free radical mechanisms play an important role in different neurodegenerative diseases [6]. In vitro, exposure of human brain tissue to cobalt 60 irradiation and the subsequent generation of hydroxyl OH or superoxide radicals (O_2) resulted in oxidative protein degradation. This was prevented by the addition of *Ginkgo biloba* (and also *P. ginseng*) extract [159]. EGb761 also results in stabilization of mitochondrial membrane and maintenance of ATP production in PC12 cells exposed to the nitric oxide donor sodium nitroprusside [160]. The effects of *Ginkgo biloba* in PD or AD models are summarized in Table 24.4.

Table 24.4 Neuroprotective effect of *Ginkgo biloba* or its constituents in models of Parkinson's disease and Alzheimer's disease

Model	*Gingko biloba* extract or its constituents	Neuroprotection	Mechanism (s)	Study
APPswe/PS1-ΔE9 double transgenic mouse model of Alzheimer's disease.	Mice fed for 1 month a diet supplemented with EGb761 (100 mg/kg/day).	↑ Cell proliferation in hippocampus (↑ Neurogenesis) ↓ Aβ oligomers	↑ Phosphorylation of cyclic AMP response element binding protein	[148]
Mice transgenic for human APP (Tg2576).	EGb761 supplemented diet (300 mg/kg) for 1 and 16 months	↓ Human APP protein in cortex by 50% (EGb761 for 16 months) (No effect in young mice)	–	[149]
APP/PS1 transgenic mouse model of Alzheimer's disease. Two-month-old APP/PS1	EGb761 (50 mg/kg) daily for 6 months.	↑ Cognitive function ↓ Insoluble Aβ	↓ TNF-α, IL-β, and IL-6 in brain ↑ IL-4, IL-13, and TGFβ ↑ Arginase-1 ↓ iNOS ↑ mRNA levels of macrophage inflammatory protein-1α (MIP-1α) and MCP-1 in brain.	[152]
SH-SY5Y neuroblastoma cells incubated with Aβ (1–42)	EGb761 (100 μg/ml) for 2 h prior to Aβ1-42 oligomer for 24 h	↓ Neurotoxicity (↑ cell viability) ↓ Cell apoptosis-related protein expression.	↑ ER stress activation ↑ Hsp70 expression and subsequent Akt activation.	[153]
PC12 cells expressing APPsw mutation	EGb761	Lessened the decrease of mitochondrial membrane potential in APPsw-bearing PC12 cells and also after treatment with sodium nitroprusside.	↑ Function of mitochondrial respiratory chain. ↓ Caspase-3 activity	[160]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-toxicity in mice	EGb761	↓ Impairment of locomotion ↓ Loss of striatal dopamine ↓ Loss of tyrosine hydroxylase immunostaining in striatum and substantia nigra pars compacta.	↓ Oxidative stress (↓ lipid peroxidation and ↓ superoxide radical production)	[161]
APPswe/PS1-ΔE9 double transgenic mouse model of Alzheimer's disease.	Bilobalide and quercetin	↑ Cell proliferation in hippocampus (↑ Neurogenesis) ↑ Aβ-induced synaptic loss (↑ Synaptogenesis)	↑ Phosphorylation of cyclic AMP response element binding protein ↑ BDNF	[162]

Abbreviations: *BDNF* brain derived neurotrophic factor, *ER* endoplasmic reticulum, *Hsp70* heat shock protein 70, *IL-β* L Interleukin-beta, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-alpha, *COX-2* cyclooxygenase-2, *iNOS* inducible nitric oxide synthase, *MCP-1* monocyte chemoattractant protein-1, *TGFβ* transforming growth factor beta

24.7 Curcumin

Curcumin is a major polyphenolic constituent of the spice *Curcuma longa*. Turmeric is used to add flavor and color to the food [163]. In recent years, curcumin has gained much interest as a potential remedy for AD. Hishikawa et al. [164] described symptomatic improvement in three patients with idiopathic AD following the administration of turmeric. Patients aged 83, 84, and 79 years, respectively, were treated with turmeric at a dose of 764 mg/day (curcumin 100 mg/day) for 12 weeks. The authors reported alleviation of agitated apathy, anxiety, irritability, hallucinations, and delusions. There was also evidence of an improvement in memory. To evaluate the effect of curcumin in persons with mild-to-moderate AD, a double-blind and placebo-controlled randomized trial using curcumin C3 Complex (®) was performed. In this study, 36 subjects with a mean age of 73.5 years received 4 g/day of oral curcumin or placebo, 2 g/day for 24 weeks. The study failed to demonstrate clinical or biochemical evidence of efficacy for curcumin, possibly due to limited bioavailability. Increased blood glucose and lowered hematocrit were observed following treatment with curcumin [165]. Potter et al. [166] suggested that poor oral bioavailability of curcumin and/or starting treatment after the development of substantial neuronal death in dementia might account for the lack of efficacy of curcumin in subjects suffering from Alzheimer's disease. In their study in healthy middle-aged subjects (40–60 years), DiSilvestro et al. [167] administered curcumin in a lapidated form to ensure good absorption. Curcumin 80 mg/day or placebo was given for 4 weeks. Curcumin but not placebo caused lowering of triglycerides and beta amyloid protein concentrations in plasma. There were also increases in plasma myeloperoxidase, nitric oxide, and decreased plasma alanine amino transferase activities. Cox et al. [168] used solid lipid curcumin formulation (Longvida®) to study its effect on cognitive function and mood in 60 healthy adults aged 60–85 years. In this randomized, double-blind, placebo-controlled trial, the authors reported improved performance on sustained attention and working memory tasks one hour after administering 400 mg of Longvida®. Four weeks of treatment with curcumin was associated with improvements in working memory and mood including fatigue and calmness. Curcumin also resulted in reduced total and low-density lipoprotein cholesterol. In another double-blind, placebo-controlled, randomized study on the efficacy of curcumin to prevent cognitive decline, subjects were given 1500 mg/day BiocurcumaxTM or placebo for 12 months. Curcumin but not placebo prevented the decline in cognitive at 6 months [169].

In rodent models of Alzheimer's disease, treatment with curcumin alleviated memory deficits and increased cholinergic neuronal function [170, 171]. APPSw mice fed curcumin exhibited decrements in insoluble Aβ and soluble Aβ plaque burden as well as decreased brain interleukin-1β [172]. Rats given intracerebroventricular injection of Aβ1-42 and treated with curcumin (50–200 mg/kg) for 5 days had their memory improved, possibly due to increased BDNF and phosphorylated ERK in hippocampus [171]. Aβ toxicity in neuronal/glial cultures is reduced by curcumin which decreased microglia and astrocyte activation [170]. It was suggested that curcumin acts by directing the Aβ aggregation pathway towards the formation of soluble oligomers and prefibrillar which are nontoxic aggregates and also by decreasing cell membrane permeabilization and membrane disruptions induced by Aβ aggregates [173, 174]. Liu et al. [170] found that curcumin alleviates neuroinflammation by directly binding to PPARγ and increases the transcriptional activity and protein level of PPARγ. In addition, Reddy et al. [175] showed that curcumin protects against Aβ toxicity by maintaining mitochondrial dynamics, mitochondrial biogenesis as well as synaptic activity.

Curcumin is also likely to benefit PD patients. Using PC12 cells that express the A53T α-synuclein mutation, Liu et al. [176] found that

curcumin protected against cell death by reducing intracellular reactive oxygen species and inhibiting the mitochondrial apoptotic cell death pathway. Mice over-expressing wild type of human α-synuclein had their gait improved by curcumin which resulted in increased phosphorylated α-synuclein at cortical presynaptic terminals [168]. Table 24.5 summarizes the results on the protective effect of curcumin in experimental models of AD and PD.

Table 24.5 Neuroprotective effect of curcumin in models of Parkinson's disease and Alzheimer's disease

Model	Curcumin	Neuroprotection	Mechanism (s)	Study
SH-SY5Y neuroblastoma cells incubated with oligomeric α-synuclein	Curcumin 4 μM	↓ Toxicity of pre-formed oligomeric α-synuclein ↓ Apoptosis Stabilized pre-formed α-synuclein fibrils	↓ Intracellular reactive oxygen species ↓ Caspase-3 activation	[178]
PC12 cells expressing mutant A53T α-synuclein	Curcumin	↓ Cell death	↓ Oxidative stress ↓ Mitochondrial cell death pathway (↓cytochrome c release, ↓ caspase-9, and ↓c aspase-3 activation)	[176]
6-hydroxydopamine rat model of Parkinson's disease.	Curcumin	↓ Loss of dopamine in striatum ↓ Loss of tyrosine hydroxylase-immunoreactive neurons ↓ Number of iron-staining cells.	Iron-chelating activity	[179]
Mice overexpressing wild type of human α-synuclein	Feeding with diet containing 500 ppm curcumin for 5 months	Improved gait impairment	↑ Phosphorylated α-synuclein at cortical presynaptic terminals	[177]
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-toxicity in mice	Long-term (7 weeks) dietary supplementation with curcumin at a concentration of 0.5% or 2.0% (w/w).	↓ Loss of dopaminergic cells in substantia nigra ↓ Loss of dopamine in striatum	↑ Expression of glial cell line-derived neurotrophic factor and transforming growth factor-β1 in striatum	[180]
PINK1 knock down SH-SY5Y neuroblastoma cells treated with paraquat	Curcumin	↓ Cell apoptosis	Preserved mitochondrial function (↑ mitochondrial membrane potential and ↑ maximal respiration)	[181]
Rotenone-treated rats	Curcumin	↑ Motor performance ↓ Loss of tyrosine hydroxylase-immunoreactive cells in substantia nigra	↓ Oxidative damage (↑ glutathione, ↓ reactive oxygen species activity, ↓ malondialdehyde) via activation of the Akt/Nrf2 signaling pathway	[182]

(continued)

Table 24.5 (continued)

Model	Curcumin	Neuroprotection	Mechanism (s)	Study
APPSw mice	Mice fed chow containing curcumin 160 ppm or 5000 ppm for 6 months.	↓ Oxidized proteins and IL-1β (160 ppm or 5000 ppm) in brain. ↓ GFAP (astrocytic marker), ↓ both insoluble and soluble Aβ (160 ppm only)	↓ Oxidative damage	[172]
Aβ (1–42) i.c.v. injection in rat	Curcumin (50, 100, and 200 mg/kg, i.p.) for 5 days	↑ Memory performance	↑ BDNF ↑ Phosphorylated ERK in hippocampus	[171]
APP/PS1 transgenic AD mouse model	Curcumin	↓ Spatial memory deficits ↑ Cholinergic neuronal function	↓ Neuroinflammation (partly PPARγ-mediated)	[170]
Aβ (1–42) toxicity in neuronal/glial cultures	Curcumin (10 μM)	↑ Cholinergic neuronal function ↓ Microglia and astrocyte activation	↓NF-κB signaling pathway ↓ Neuroinflammation	[170]
Aβ toxicity in SHSY5Y cells	Curcumin	↓ Mitochondrial and synaptic impairments Maintained cell viability	Preservation of mitochondrial function.	[175]

Abbreviations: *BDNF* brain derived neurotrophic factor, *NF-κB* nuclear factor kappaB, *ERK* extracellular signal-regulated kinase, *PPARγ* peroxisome proliferator-activated receptor gamma

Conclusions

Data from epidemiological studies suggest that dietary polyphenols could be of value in maintaining cognitive function and in decreasing the risk of progression to AD in the elderly. Benefits from tea and coffee, two widely consumed beverages could also be seen in patients with PD where there is a decrease in the risk for developing the disease. Coffee also improves the cognitive status in these individuals and decreases the risk of dyskinesia associated with dopaminergic replacement therapy. Coffee or caffeine also decreases the risk for developing dementia in late-life. While the administration of ginseng in healthy young subjects has been shown to improve working memory, a possible effect in PD or AD is yet to be determined. The weight of evidence is also in favor for a beneficial effect from *Ginkgo biloba* supplementation on cognitive decline and in preventing dementia. Clinical trials with curcumin suggest that using the herb might have an important role in preventing dementia or its progression. Studies conducted in vitro and in vivo indicated that the abovementioned herbal/dietary supplements and their biologically active constituents studies could reduce neuronal damage and are likely to have a positive impact in reducing neurodegeneration occurring in old age or in the context of disorders such as PD or AD.

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