Preetham Elumalai
Sreeja Lakshmi *Editors*

Functional Foods and Therapeutic Strategies for Neurodegenerative Disorders

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Preetham Elumalai • Sreeja Lakshmi
Editors

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I dedicate this book to my loving late mother without whom I would never be able to succeed in my life, to my late father who instigated me to stay independent and determined, and to all my diligent students whose feedback has always helped me to be a better teacher.

–Preetham Elumalai

Foreword

It is a great pleasure to write the foreword to the book *Functional Foods and Therapeutic Strategies for Neurodegenerative disorders* edited by Dr. Preetham Elumalai and Dr. Sreeja Lakshmi. The book carries importance in the current context where people practice healthy ageing encompassing natural treatment strategies.

Diseases are never ending as far as human life is concerned. Disorders of brain destroy us both physically and mentally. Ageing remains as the major risk factor for many diseases. The highly debated neurodegenerative disorders procure enormous fruitful investigations over the past years, towards a plethora of treatment strategies as these diseases make one's life really challenging owing to their disastrous approach and leaving a high healthcare cost. As age progresses, people suffering from neurodegenerative disorders like Alzheimer's, Parkinson's and Huntington's diseases are unable to bear the side effects as well as the cost of the medications prescribed for the same. As science inclines to natural treatment practices, scientific researches are successfully ahead with exploring natural resources for bioactive compounds that can be applied as efficient tools for treating diseases.

The book is a comprehensive collection of contemporary treatment strategies employing natural bioactive compounds as well as modern diagnostic applications with nanoparticles, biomarkers and in silico techniques towards neurodegenerative disorders. I take this opportunity to appreciate the editors and authors for their

sincere effort to bring this book to enlighten and uplift the knowledge on the management and treatment of neurodegenerative disorders.

K. K. Shailaja Teacher
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Foreword

I am so pleased to have an opportunity to write the foreword for the book *Functional Foods and Therapeutic Strategies for Neurodegenerative Disorders* edited by Dr. Preetham Elumalai and Dr. Sreeja Lakshmi.

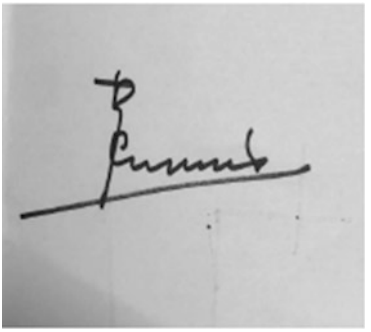
"Old age is the supreme evil, because it deprives us of all pleasures, leaving us only the appetite and it brings with it all sufferings. Nevertheless, we fear death, and we desire old age."- Giacomo Leopardi.

Aging and related health disorders have become an unavoidable phase in the life cycle of every human being. The mechanisms underlying aging, age-linked susceptibility to diseases and the consequential medical treatments for age-related health disorders are emerging as excellent researchable issues among researchers across the world. Neurodegenerative disorders have always been an invaluable research topic, and the most common type of diseases such as Alzheimer's, Parkinson's and Huntington's disease has seen long fruitful years of productive research during the past four decades. Neurodegenerative disorders are most frequently viewed as one of the life-threatening ailments and therefore highly challenging as they bring about heavy burden not only to the patients but to their families and societies at large which attract exorbitant healthcare costs.

We all need healthy brain. Nowadays people understand the importance of healthy aging and scientific practices to contain diseases through diet, lifestyle

factors, exercises, etc. Owing to the high risk of side effects of medicines for neurodegenerative disorders, science is more inclined nowadays to naturopathy which is easily accessible, cost-effective and relatively free from side effects.

In the present book, the authors comprehensively illustrate contemporary treatment protocols in respect of diverse neurodegenerative disorders incorporating bioactive compounds from natural resources as well as advanced interdisciplinary diagnostic practices including nanoparticles, biomarkers and in silico techniques. I sincerely appreciate the painstaking efforts made by the authors in articulating this book which will definitely provide an enchanting reference material for validating the progress attained in the therapy and management of neurodegenerative disorders besides serving as a valuable addition to the existing body of knowledge.



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Preface

Aging is an inevitable phenomenon creating entropy in one's life by deteriorating one's physiological systems and anatomical structures. Senescence is associated with increased risk for a plethora of diseases and some of them end up with loss of homeostasis and finally death. As age progresses many of the biological processes as well as environmental factors conjointly lead to progressive neurodegeneration. Neurodegeneration is an inherent condition characterised by structural and functional impairment of neurons. The most prevalent neurodegenerative disorders—Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic lateral sclerosis (ALS)—are often hired as life threatening as well as a social and economic burden to the healthcare system. Despite the conventional beliefs and strategies, science along with the healthcare system has taken initiatives to step forward with healthy aging for better living of aged people. Past few year's scientific research has explored novel remedies, incorporating diet, lifestyle factors, and bioactive compounds/immunostimulants from natural resources to treat neurodegenerative disorders in the context where therapeutic drugs were found ineffective in reverting or pausing neurodegenerative disorders. Moreover, these natural antidotes are depicted as highlighters that boons the sustainability of healthy brain rendering protection against neurodegeneration.

Our book is anticipated to provide an overview of the current status of research and future perspectives in the field of treating neurodegenerative disorders. The book will integrate different aspects such as advancements in current treatment scenario incorporating prebiotics, phytochemicals, polyphenols and potential marine bioactive compounds towards the treatment strategies along with leading contemporary methods for prophylactic and diagnostic practices, comprising nanoparticles, in silico techniques and biomarkers. We expect that the contents of the book provide potential knowledge regarding future perspectives in the management of neurodegenerative disorders.

Cochin, Kerala, India

Preetham Elumalai
Sreeja Lakshmi

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Preetham Elumalai is an Associate Professor (Biochemistry) in the Department of Marine Biology, Microbiology and Biochemistry at Cochin University of Science and Technology (CUSAT), Cochin, Kerala, and completed his Master's from the University of Madras, Tamil Nadu. He has qualified the National Eligibility Test for Lecturership conducted by ASRB/ICAR/UGC. He received his PhD in Biochemistry and Molecular Immunology from the Institute for Immunology, University of Regensburg, Germany. He did his postdoctoral research in the same University and worked on lectin glycomics.

Dr. Preetham has worked at various universities and has quite a good experience in teaching and demonstrating concepts in Biochemistry and Immunology. His current research practice includes Proteomics and Functional genomic approach for the analysis of pathomechanisms of different aquatic diseases, application of nanotechnology for the regulation of nutrient uptake in fish using nutrigenomic approaches, genetic regulation of gene expression across tissues, time and environments.

He holds editorial positions in national and international journals and is a member of many prestigious societies, including Asian Fisheries Society, European Association of Fish Pathologists, International Veterinary Vaccinology Network, International Complement Society and Society of Neurochemistry. He has widely travelled to more than 20 countries on various teaching and research assignments. He was awarded with the prestigious INSA medal (2017) and MASTS (2019) UK visiting fellowship.

Sreeja Lakshmi holds a Master's degree in Biochemistry from the University of Calicut, Kerala. She pursued her PhD in Biochemistry from Molecular Cell Biology, University of Regensburg, Germany. She did her Postdoctoral Research in the Institute of Tropical Medicine, University of Tuebingen, Germany. Dr. Sreeja is an awardee of HRD Fellowship for Women Scientists by the Department of Health Research and MASTS (Marine Alliance Science & Technology, Scotland) Award for Postdoctoral and Early Career Research Exchanges (PECRE). Currently she is pursuing her Postdoctoral Research in collaboration with Moredun Research Institute (MRI), UK, with International Veterinary Vaccinology Network (IVVN) Fellowship Grant, UK. Her research interests extend through functional attributes of bioactive compounds for therapeutic applications, development of nano and glycovaccines against aquatic diseases, novel treatment strategies against neurodegenerative disorders and protein biochemistry.

Dr. Sreeja has published her works in many peer-reviewed journals and presented at national and international conferences. She holds memberships in International Veterinary Vaccinology Network (IVVN), Society of Biological Chemists, International Complement Society, Indian Academy of Neurosciences and Society for Neurochemistry India.

Part I
Understanding Aging

Chapter 1
Aging and Neurodegeneration: A Preface

Sreeja Lakshmi

Abstract Aging is an irreversible complex phenomenon bringing disorganized status in one's life with threats toward various diseases. While the basic and prime contributor to aging is deteriorative and degenerative biological processes, environmental, genetic, and lifestyle factors stay as co-equal hands. At the stage of aging, one has to encounter a series of disorientations in life and a myriad of diseases, such as diabetes, cancer, cardiovascular diseases, neurodegenerative disorders, and arthritis, to highlight a few. Owing to their high irreversible nature, neurodegenerative disorders are at the frontline among the age-related health disorders, which in all means are accompanied by social and economic burdens. Lack of effective treatment strategies paves more gateways for scientific research to explore the bioactive compounds from various natural resources incorporating healthy diet and lifestyle factors. Even though aging is unique and challenging for each individual, more scientific practices are in need to make the period healthy, which in turn provide a fascinating platform for future research.

Keywords Aging · Neurodegeneration · Alzheimer's disease · Parkinson's disease · Healthy aging

Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
ETC	Electron transport chain
FDA	Food and Drug Association
HD	Huntington's disease

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NMDA	N-methyl-D-aspartate receptor antagonist
PD	Parkinson's disease
ROS	Reactive oxygen species
WHO	World Health Organization

1.1 Introduction

Aging is an inheritable, inevitable complex phenomenon in one's life, which brings an entropy in many ways. Aging is not a stage from which we can escape, but we must definitely encounter it. Even though we struggle to make our aging healthy, the process is highlighted as disease oriented owing to many deteriorative as well as degenerative changes taking place in our body (Rajawat and Bossis 2008). According to the World Health organization, by 2050, the number of people aged 60 years and above is expected to rise to 2 billion, increasing from 900 million in 2015, and the number of people aged above 80 will triple from that of 2015 (WHO Dementia Report. World Health Organization 2019). In parallel, it is also studied that the number of people aged 60 years and older will exceed the number of children below 5 years of age (WHO 2018). Progressive deterioration of bodily functions from cellular to organic level causing complexities with normal physiological phenomena ultimately leads to death. Apart from the biological factors as contributors, aging encompasses a cumulative circumstance elicited from environmental, genetic, and lifestyle elements (Lopez-Otin et al. 2013).

1.2 Major Contributors to Aging

Aging is a period up to which most of the people live. Normal aging brings a chain of biological complications: physical and mental disabilities and diseases, psychological declines, and social deprivations (Hodge et al. 2013; Thompson et al. 2017). Among the string of major and minor contributors to aging, selected few processes enmeshed with aging and related disorders, which persist as cellular and molecular criteria behind aging such as genomic instability and telomere aberrations, epigenetic alterations, loss of proteostasis, cellular senescence, mitochondrial dysfunctions, alterations in intercellular communications, and nutritional irregularities (Lopez-Otin et al. 2013) (Fig. 1.1).

Genomic integrity is attributed to both nuclear and mitochondrial DNA and is disturbed by the accumulation of increased assimilation of damaged DNA caused by any of the factors like DNA strand breaks, mismatches in base pairs, and mutations (Jeppesen et al. 2011). Genetic instability is accompanied by an enhanced release of reactive oxygen species (ROS), which gradually cause inflammatory responses, enhance aging, and unlatch the occurrence of diseases such as cancer and neurodegenerative disorders (Hoeijmakers 2009; Moskalev et al. 2012). Telomere attritions like shortening cause cellular senescence, and it has been identified that the defects in

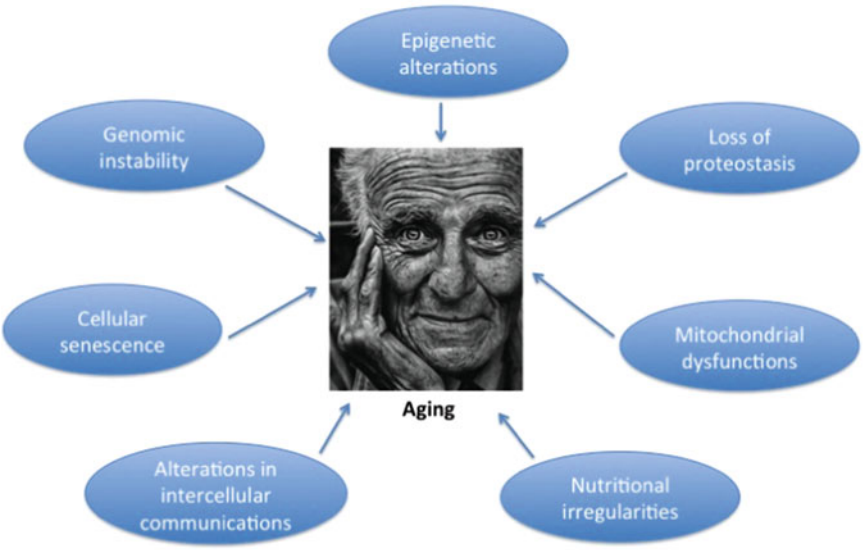

Fig. 1.1 Schematic representation of major hallmarks of aging (modified from Lopez-Otin et al. 2013)

telomere stimulate aging in mice and humans (Blackburn et al. 2006). Aging is also characterized by epigenetic factors like post-translational modifications of histones, DNA methylation, alterations in chromatin remodeling, etc. (Fraga and Esteller 2007; Bradley-Whitman and Lovell 2013), which can be spontaneous or elicited by external or internal causatives. Another key factor that contributes to aging is loss of proteostasis, equipoised by protein synthesis and degradation. Following aging, the accumulation of damaged proteins due to impairment in ubiquitin or lysosomal pathway results in proteotoxicity. The most prevailing neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease (PD) result from the increased deposition of unfolded/misfolded proteins (Tanaka and Matsuda 2014). Accumulation of senescent cells resulting in cellular senescence is yet another contributor to aging (Lopez-Otin et al. 2013). As age progresses, DNA repair capacity of cells declines resulting in the senescence of cells with high levels of damaged DNA (Madabhushi et al. 2014). The close correlation between mitochondrial dysfunction and aging is highly challenging and has been highly studied. Aging results in inefficient working of electron transport chain (ETC) followed by electron leakage and reduced adenosine triphosphate (ATP) production. This causes elevated ROS levels followed by oxidative stress and subsequent inflammatory responses (Johri and Beal 2012). Impaired intercellular communications and consequential inflammatory responses severely affect the nervous and endocrine systems (Salminen et al. 2012). Irregularities in neurohormonal signaling have an adverse impact on the functional properties of the cells resulting in health disorders. Not least of all, healthy aging and individual longevity are dependent on a restricted diet.

Aging associated with nutritional abnormalities is a highly debated, fascinating field of research. It conveys the relevance of diet and lifestyle factors to prevent premature aging and improve metabolic signaling (Houtkooper et al. 2010).

1.3 Aging and Neurodegeneration

As we age, our brains will also age. Aging is not a disease but a potential risk factor for the onset of many diseases. Neurodegeneration is an umbrella term to define the hereditary and sporadic sequence of events determined by the impairment in neuronal functions. Alzheimer's diseases (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) place the top zone of the neurodegenerative disorders (Lakshmi et al. 2018). Even if the abovementioned major hallmarks of aging exist as key players on an even keel, protein abnormalities occur in most of the prevalent neurodegenerative disorders like AD and PD (Fig. 1.2). An overall view of neurodegenerative disorders is often correlated with cognitive disabilities. However, it should also be noted that not all neurodisorders are particularly correlated with the pathological abnormalities related to cognition.

AD, the most prevalent form of dementia, is characterized by the presence of amyloid beta protein (Aβ peptide) and Tau, the major pathological hallmarks, along with defects in presenilin 1 and presenilin 2 (Xia et al. 2018; Bekris et al. 2010). Apart from the above prominent lesions of uncanny accumulation of Aβ peptide and hyperphosphorylated Tau, oxidative stress, mitochondrial dysfunctions, cholinergic

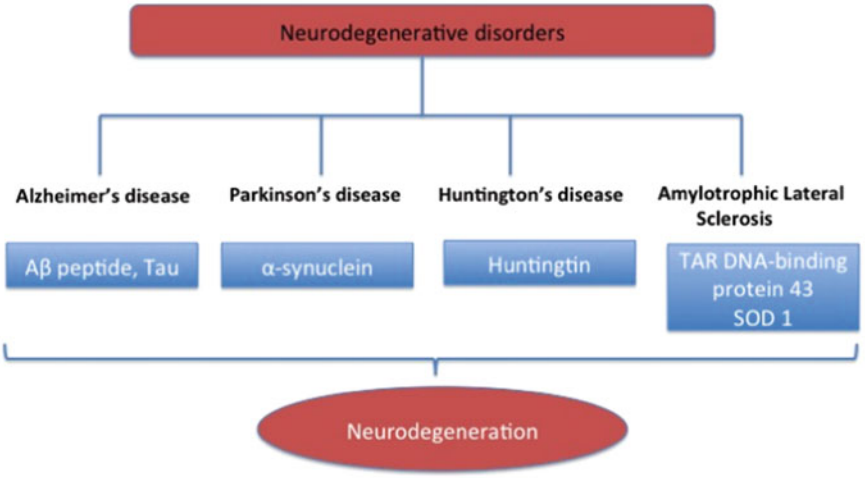

Fig. 1.2 Neurodegenerative disorders. Alzheimer's disease, Parkinson's disease, Huntington's disease, and amylotrophic lateral sclerosis with their respective causative protein aggregates leading to degeneration of neurons

dysfunctions, and inflammatory responses also join the hub of primary causal agents (Lane et al. 2018). Even though we have drugs, such as cholinesterase inhibitors (tacrine, rivastigmine, donepezil, and galantamine) and N-methyl-D-aspartate (NMDA) receptor antagonists (memantine), approved by Food and Drug Administration (FDA) to combat AD, they can only ameliorate symptoms and cannot reverse the underlying disease processes and that also within limited people and limited ability (Lane et al. 2018; Potyk 2005). Since the major hallmarks of aging mentioned previously are in close harmony with AD, future studies can employ any of them to develop therapeutic interventions. Since current studies are more inclined to nondrug approaches as counteragents of AD, lifestyle interventions and treatments with natural bioactive compounds should be incorporated to treat AD. A decline in sleep is often found in old age people, and sleep deterioration is found in most of the AD patients. Studies have shown the interrelationship between sleep impairment and high Aβ and tau proteins' levels (Liguori et al. 2014). On the other side, exercise and caloric restrictions have been reported to alleviate the AD phenotype (Gunn-Moore et al. 2018). Yet, more studies are in need to confirm the interrelationship between exercise and cognitive enrichment activities to reduce the symptoms of AD (Sexton et al. 2016). The incidence of AD is also associated with insulin and lipid changes/cholesterol levels (Arnold et al. 2018; Foley 2010; Hartmann et al. 2007).

PD is characterized by loss of dopamine-producing neurons in substantia nigra leading to Lewi body formation with aggregated α-synuclein (Agim and Cannon 2015). Aging is considered the single largest independent risk factor for the emergence of PD (Gasser 2007). The incidence of aging in humans reaches its heights at the age of 70–79 and declines after 80 (Hirsch et al. 2016). Apart from the basic finding that leads to PD, increased oxidative stress and neuroinflammation, iron and neuromelanin accumulation, α-synuclein deposition, and impaired autophagy, it has been studied that physical activity, ibuprofen, smoking, caffeine, and calcium channel blocking agents offer protection against PD, whereas dairy products and pesticides are considered risk factors (Ascherio and Schwarzschild 2016). Underpinning mechanisms that lead to the initiation and progression of PD will pave the way for novel therapeutic strategies toward disease management.

HD and ALS are characterized by autosomal dominant mutation of the Huntingtin gene, and deposition of TAR DNA-binding protein 43-positive protein inclusions and SOD 1 mutations, respectively, are associated with defects in transportation across endosomes, nucleocytoplasmic transport, decreased axonal transport, defects in oligodendrocyte functions, etc. (Bates et al. 2015; Wobst et al. 2017).

1.4 Concept of Healthy Aging

Increasing life longevity is not only attributed to biomedical interventions but natural remedies to fight the disabilities associated with aging. Incorporation of a healthy brain completes the exact term "fitness" of an individual. A comprehension of management practices including caloric restrictions (Knorre and Severin 2016),

sirtuins (Grabowska et al. 2017), natural bioactive compounds like curcumin (Yang et al. 2013), resveratrol (Baur and Sinclair 2006), quercetin (Dajas 2012), dietary polyphenols (Kelsey et al. 2010), polyunsaturated fatty acids (Luchtman and Song 2013), and antiaging hormones comprising human growth hormone (Khansari and Gustad 1991), insulin-like growth factor (Miller 2005), melatonin, and estrogen (Froy and Miskin 2007) are all preferred on deck to delay the age-associated disorders and promote healthy aging. Neurodegenerative disorders are chronic with emotional disabilities challenging one's self and societal identity. It is certain that we cannot overcome these disorders completely once caught, but we can delay their onset. We have to cross over the traditional casual treatment practices to socioeconomic grounds as determinants to treat the underlying causatives (Petersen et al. 2015). Application of biomedical imaging has been implemented over the past decades using biomarkers, like amyloid imaging probes used in anti-amyloid therapies for AD, which facilitated the early diagnosis of the disease and hence treating at the starting point (Lerner 2013). However, many careful studies are needed to detect the dose to have that efficacy carries utmost importance. Antihypertensive agents were also identified to reduce neurodegeneration in AD (Yasar et al. 2013), but the current scenario compels us to adapt neural stimulatory activities, including yoga, meditation, physical exercises, caring relationships, and being connected with nature, which can positively boost the brain for healthy living (Boyatzis et al. 2006).

1.5 Conclusion

Successful and healthy aging refers to a low risk of diseases, collectively coordinated by physical, mental, and social factors. The conventional thought of aging brings us in a state of age-related stress and depression, increasing our dependency on others, and affects our aging negatively. Science keeps on looking for novel solutions to tackle these disorders by employing earlier detection and subsequent application of prevention strategies. Neurodegeneration is the utmost point of crisis deliberately in need of an integrated approach to find cures, and more scientific and societal practices should be collectively involved and implemented for the much early diagnosis of the diseases.

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Part II
Neurodegenerative Disorders: Underpinning Mechanisms

Chapter 2
Deciphering the Molecular and Genetic Basis of Alzheimer's Disease

Shamprasd Varija Raghu and Avinash Kundadka Kudva

Abstract Alzheimer's disease (AD) is a progressive age-related neurodegenerative disease in humans characterized by a decline in cognitive and personal daily activities. AD is considered a multifactorial disease involving different pathological and molecular phenotypes. Several risk factors like aging, genetic factors, infections, and environmental factors play in the onset of AD in humans. The advances in cellular biology have shed light on understanding the molecular mechanism underlying AD. However, current knowledge on the molecular pathogenesis of AD is complex and involves several hypotheses. Several genes and mutations are hallmarks of the molecular pathology of AD. These new discoveries on molecular pathogenesis and the involvement of genetic factors in AD are crucial because they will help us develop new therapeutic strategies for treating different conditions in AD. In the current chapter, we discuss the molecular mechanism inducing the onset of AD and the different genetic factors involved in it.

Keywords Alzheimer's disease · Cholinergic · Tau · Beta-amyloids · NMDA receptors

Abbreviations

Ach	Acetylcholine
AD	Alzheimer's disease
AP	Aβ plaques
ApoE	Apolipoprotein E
APP	Amyloid Precursor Protein
Aβ	Beta-amyloid

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CAA	Cerebral Amyloid Angiopathy
ChAT	Choline acetyltransferase
EC	Entorhinal cortex
EOAD	Early-onset Alzheimer's disorder
LOAD	Late-onset Alzheimer's disorder
LTD	Long-lasting depression
LTP	Long-term potentiation
PSEN-1	Presenilin 1
PSEN-2	Presenilin 2
VAChT	Vesicular acetylcholine transporter

2.1 Introduction

Alzheimer's disease (AD) is a progressive age-related neurodegenerative disease in humans, and it causes the most common form of dementia (Knopman et al. 2021). AD was described first in 1906 by Alois Alzheimer, and he noticed a presence of amyloid plaques and a massive loss of neurons in his first patient who suffered from memory loss and change in personality before dying. Alois Alzheimer described this condition as a peculiar severe disease process of the cerebral cortex (Alzheimer 1906). However, in recent times, AD is considered a chronic or progressive neurodegenerative syndrome characterized by impaired cognition affecting memory, thinking, learning, and behavior (Knopman et al. 2021). Around 50 million people are currently affected by AD worldwide, and the numbers may increase to 82 million by 2030 and 152 million by 2050 (Alzheimer's Association 2021). The main pathophysiological features in AD include (A) amyloid-β (Aβ) plaques aggregation in the extracellular region of neuronal cells and (B) hyper-phosphorylation of the microtubule-associated protein tau, which leads to neuronal disintegration deposits as neurofibrillary tangles in the intracellular region of neurons (Cubinkova et al. 2018; Lee et al. 2019). In addition, cellular impaired antioxidant systems, bioenergetics, and neurotransmitters also contribute to the onset of AD (Gandhi and Abramov 2012; Guan 2008; Liu et al. 2017). The role of oxidative stress in AD pathogenesis is associated with Aβ and tau proteins. The changes in the functional properties of these proteins are strongly associated with neural damages observed in AD (Liu et al. 2017).

The neuropathological changes of AD can be classified into two steps that provide evidence about the progress of AD disease and different types of symptoms. The first step is positive lesions characterized by the accumulation of neurofibrillary tangles, amyloid plaques, dystrophic neurites, neuropil threads, and other deposits found in the neural tissues. The second step or negative lesion is characterized by considerable atrophy due to neural and synaptic loss. This will lead to neurodegeneration, neuroinflammation, and loss of cholinergic neurons (Serrano-Pozo et al. 2011; Spires-Jones and Hyman 2014; Singh et al. 2016).

The clinical phases in the patients with Alzheimer's disease can be classified into four stages: (A) a pre-clinical stage with mild memory loss and early pathological changes in cortex and hippocampus. This stage can last for several years or more with no functional impairment in the daily activities. This stage will not show any clinical symptoms of AD (Dubois et al. 2016; De-Paula et al. 2012). (B) Mild or early stage of AD characterized by several symptoms that start appearing in patients. These symptoms include loss of concentration and memory, disorientation of place and time, a change in mood, and slow development of depression (Wattmo et al. 2016). (C) Moderate AD stage where disease spreads to cerebral cortex regions, resulting in increased memory loss. This stage also involves loss of impulse control and difficulty in reading, writing, and speaking (Kumar et al. 2021). (D) Severe or late AD stage involving the spread of the disease to the entire cortex area. This further leads to a severe accumulation of neuritic plaques and neurofibrillary tangles in the cortex area. The patients will have severe functional and cognitive impairment and cannot recognize others. The patients will also suffer from difficulties in swallowing and urination, eventually leading to death due to all the above complications (De-Paula et al. 2012; Wattmo et al. 2016).

Aging is the most critical risk factor in AD, and most cases have late-onset that starts mostly after 65 years of age. Aging is an irreversible process involving a reduction in brain volume, a loss of synapses, and enlargement of ventricles in specific areas in the brain. In addition, aging also involves changes in glucose metabolism, cholesterol homeostasis, mitochondrial function, depression, and decline in cognitive ability. Since these changes also appear in normal aging, it is difficult to distinguish them from the early onset of AD. In general, AD is divided into early-onset AD (EOAD) and late-onset AD (LOAD) based on the age at which primary symptoms of AD appear. EOAD is the rare form with around 1–6% cases, and LOAD is more common with age of onset above 65. However, both EOAD and LOAD may occur in people who have a family with a positive history of AD (Guerreiro and Bras 2015; Hou et al. 2019).

In the following sections, major cellular and molecular causes of AD and different genetic factors influencing the early onset of AD pathology are discussed in detail.

2.2 Major Cellular and Molecular Causes of Alzheimer's Disorders

The underlying mechanism of pathological changes in Alzheimer's disease is still unknown. Several hypotheses were proposed as a cause for AD: the amyloid cascade hypothesis, the tau hyperphosphorylation hypothesis, and the neurotransmitter hypothesis. However, at present, none of these hypotheses are completely accepted for explaining AD pathogenesis. In the following sections, the amyloid cascade hypothesis, the tau hyperphosphorylation hypothesis, and the neurotransmitter hypothesis are discussed in detail.

2.2.1 Amyloid Hypothesis

The amyloid hypothesis was first proposed by John Hardy and David Allsop in 1991 (Hardy and Allsop 1991). The beta-amyloid (Aβ) is a transmembrane protein produced by hydrolysis of the Aβ precursor protein (APP) via the amyloidogenic pathway. For decades, it was proposed that abnormal deposition of Aβ plaques (AP) in the central nervous system has a strong correlation with dementia. This led to the concept of the amyloid hypothesis. However, the AP is also getting deposited in normal healthy brains with aging. This raised some doubts in the scientific community whether AP deposition is really responsible for the progressive development of AD. However, currently, the amyloid hypothesis remains the most accepted pathological mechanism for AD.

Aβ is a peptide having higher resistance for proteolytic degradation. It consists of 37–43 amino acids, in which the isoforms 1–40 and 1–42 are the most common. The 1–42 amyloid peptide isoform is hydrophobic and considered to have the greatest toxicity. Structurally, it often acquires the configuration of β-pleated sheet and shows a greater tendency to aggregate and form the core of the AP (Deane et al. 2009; Jucker and Walker 2015). The amyloid hypothesis suggests that the degradation of Aβ is decreased in AD pathological condition leading to the accumulation of Aβ peptides (Aβ40 and Aβ42) in the neurons. Aβ deposited in the hippocampus and basal segment in the form of AP recruits more Aβ peptides to form insoluble aggregates. This leads to mitochondrial damage, unstable homeostasis, and synaptic dysfunction (Lustbader et al. 2004; Hunt and Castillo 2012). At the same time, immune cells like microglia and astrocytes are activated, leading to some inflammatory reactions. This eventually causes neuronal dysfunction and apoptosis, leading to a series of pathological changes of AD (Ferreira and Klein 2011). The soluble Aβ peptides are suggested to be more toxic than Aβ cellulose bodies, and soluble Aβ peptides are proposed to be the initiating factors of developing pathological changes in AD (Ferreira and Klein 2011) (Fig. 2.1).

2.2.2 Tau Hyperphosphorylation Hypothesis

Tau is a microtubule-associated protein and found mainly in the neuronal axons in the brain. They are functionally involved in maintaining microtubule structure, cytoplasmic transport, maintaining synaptic structure, and regulating neuronal signaling (Kimura et al. 2014). In 1988, Claude Wischik isolated tau protein from AP in AD patients' brains, suggesting that tau protein may be the cause of dementia (Wischik et al. 1988). Tau protein kinase 1 activated by Aβ peptides leads to abnormal phosphorylation of tau protein and the development of tau pathology of AD (Vergara et al. 2019).

The tau protein presents six different isoforms in the central nervous system with variation in the binding sites for microtubules. In AD, initially, a phosphorylation

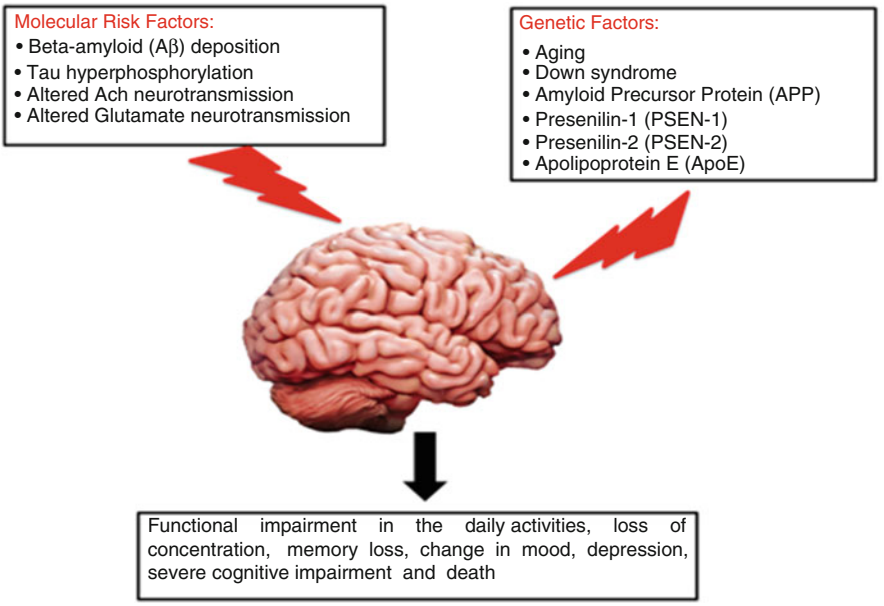

Fig. 2.1 Schematic representation of various molecular and genetic risk factors in the progression of AD and the related symptoms in AD patients

process of tau protein occurs, followed by hyperphosphorylation of tau molecules. The hyperphosphorylated tau protein forms aberrant aggregation with the cytoskeletal proteins. The hyperphosphorylated tau proteins show a lower grade of interaction with microtubules resulting in an increase in the free tau proteins. These extra free tau proteins lead to greater aggregation and fibrilization itself. This leads to subsequent malfunction of axonal transport (Goedert et al. 2006; Kuret et al. 2005; Rafii and Aisen 2009). The changes in the tau proteins and Aβ oligomers are the most important factors responsible for neuronal dysfunction in AD. The neurofibrillary tangles observed initially in the cortex and hippocampus subsequently spread to the amygdala and other cortical areas (Rafii and Aisen 2009), leading to different pathological conditions observed in AD (Fig. 2.1).

2.2.3 Cholinergic Hypothesis

The neurotransmitter acetylcholine (Ach) plays an essential role in cognitive function. The cholinergic hypothesis of AD was proposed based on the significant role of ACh in the cognitive, learning, and memory process. ACh is synthesized in the cholinergic neurons from choline and acetyl-coenzyme A by the choline acetyltransferase (ChAT) enzyme. It is further transported to the synaptic vesicles by vesicular acetylcholine transporter (VAChT). The ACh is involved in several

physiological processes such as memory, attention, sensory information, learning, and other critical functions in the central nervous system. At a molecular level, the cholinergic hypothesis of AD is the first and most studied approach that describes its pathophysiology. It was described 30 years ago as a primary degenerative process in AD capable of selectively damaging cholinergic neurons in different parts of the central nervous system. This cholinergic hypothesis was largely based on the immunohistochemical, neuroimaging, and other analyses revealing a decrease in the density and number of nicotinic acetylcholine receptors in AD patients. A reduction in functional expression of α3, α4, and α7 subunits of nicotinic acetylcholine at the cortex and hippocampus and a decline in the binding ability of α7 nicotinic acetylcholine receptors in the hippocampus and α4 nicotinic acetylcholine receptors in the cortex are observed in AD (Ringman and Cummings 2006; Wu et al. 2010). In AD patients, degeneration of cholinergic neurons was found, causing alternation in cognitive function and memory loss. Aβ is believed to alter cholinergic neurotransmission and cause a reduction in the choline uptake and a release of Ach at the synaptic regions. Various studies have demonstrated that cholinergic synaptic loss and amyloid fibril formation are related to Aβ oligomer neurotoxicity, confirming the interaction between ACh and Aβ (Francis et al. 1999; Ferreira-Vieira et al. 2016). In addition, a reduction in nicotinic and muscarinic Ach receptors is also observed in AD (Ferreira-Vieira et al. 2016) (Fig. 2.1).

The cholinergic and glutamatergic systems significantly interact during neurotransmission. So an alteration in the glutamatergic signaling has been associated with cholinergic disruptions observed in AD. In AD abnormalities of glutamatergic neurotransmission, the initial changes are observed at the entorhinal cortex (EC) followed by neurotransmission defects in the hippocampus, the amygdala, frontal cortex, and parietal cortex (Geerts and Grossberg 2006; Lin et al. 2010). The glutamatergic neurotransmission in the hippocampus mediates synaptic plasticity phenomena such as long-term potentiation (LTP). This facilitates learning and memory consolidation in the brain. The sustained hyperactivation of NMDA glutamate receptors has been associated with excessive depolarization of the postsynaptic membrane promoting the onset of neurodegeneration and cell death in the central nervous system (Beck et al. 2003; Szado et al. 2008). An increase in intracellular calcium as a result of dysfunctional glutamatergic neurotransmission may lead to long-lasting depression (LTD) in the cerebellum. In addition, it also activates nitric oxide synthesis and the generation of free radicals initiating neuronal death (Jung et al. 2009; Ndountse and Chan 2009). Several experiments were carried out to investigate the role of defective glutamatergic neurotransmission in AD. Incubating neurons with glutamate promoted the deposition of filaments similar to neurofibrillary tangles observed in AD. In addition, the neuronal culture exposed to Aβ promotes glutamate-induced neurotoxicity and regulates the expression of NMDA glutamate receptors on the neuronal membrane (Butterfield and Pocernich 2003; Parameshwaran et al. 2008) (Fig. 2.1).

2.3 Genetic Basis of AD

Genetic factors were found to play a major role in the development of AD. Most cases of early onset of AD are inherited in an autosomal dominant pattern. The mutations in the genes for amyloid precursor protein (APP), presenilin 1 (PSEN-1), presenilin 2 (PSEN-2), and apolipoprotein E (ApoE) proteins are associated with AD. Those inheriting mutations to these genes are guaranteed to develop AD if they complete a normal life span. The pathophysiological symptoms tend to develop before age 65 and sometimes as young as age 30. The vast majority of individuals have late-onset AD (Bekris et al. 2010; Goldman et al. 2011) (Fig. 2.1).

People with Down syndrome born with three copies of chromosome 21 (trisomy 21) have an increased risk of developing AD. Chromosome 21 includes the gene that encodes for APP production, and its hydrolysis produces Aβ that accumulates into plaques. An extra chromosome in Down syndrome may increase the production of Aβ fragments in the brain. People with Down syndrome develop AD at an earlier age than people without Down syndrome. By age 40, people with Down syndrome suffering from AD have significant levels of Aβ plaques and tau fibrillar tangles in their brains. According to the report from the National Down Syndrome Society, about 30% of people with Down syndrome have AD dementia by the age of 50, and about 50% of people with Down syndrome have AD dementia by the age of 60 (Lott and Dierssen 2010; Alzheimer's Association 2021) (Fig. 2.1).

In the following section, different genetic risk factors of AD are discussed.

2.3.1 Amyloid Precursor Protein (APP)

APP is a type I transmembrane protein cleaved by α-, β-, and γ-secretase to release Aβ and other proteins. The APP gene encodes APP on chromosome 21. Nearly 30 mutations have been found in the APP gene, and around 25 of them are related to AD. The mutation and functional defect in these genes cause an accumulation of Aβ with elevated concentration. A673T is a protective mutation that acts against the initiation of AD by decreasing the secretion of Aβ, Aβ40, and Aβ42 proteins (Li et al. 2019; Julia and Goate 2017) (Fig. 2.1).

2.3.2 Presenilin-1 (PSEN-1) and Presenilin-2 (PSEN-2)

PSEN1 and PSEN2 genes are autosomal dominant genes located on chromosomes 14 and 1. PSEN-2 and PSEN-1 are homologous with 67% similarity. More than 200 mutations in PSEN1 and around 40 mutations in the PSEN2 gene (Cai et al. 2015; Lanoiselee et al. 2017) are observed. PSEN1 is a core protein that activates the α-secretase complex and plays an important role in Aβ production. Mutations in the

PSEN1 gene increase the ratio of Aβ42/Aβ40 by decreasing Aβ40 secretion. The C410Y or L435F mutations in PSEN1 knock-in mice increased the Aβ42/Aβ40 ratio due to a greater reduction in Aβ40 (Kelleher and Shen 2017). In contrast, PSEN-2 mutations are rare and play a minor role in Aβ production. However, some of the mutations in PSEN-2 cause a significant increase in α-secretase activity with an increased Aβ42 secretion providing a higher Aβ42/Aβ40 ratio and are considered AD pathogenic mutations (Cai et al. 2015; Walker et al. 2005) (Fig. 2.1).

2.3.3 Apolipoprotein E (ApoE)

ApoE protein is a glycoprotein expressed highly in the liver, brain astrocytes, and microglia. It serves as a receptor-mediated endocytosis ligand for lipoprotein particles like cholesterol. Cholesterol is essential for the production of myelin that forms covering around the neurons and helps in normal brain function. The ApoE gene is located on chromosome 19 and has three isoforms: ApoE2, ApoE3, and ApoE4. The ApoE4 allele is a strong risk factor for both early-onset AD and late-onset AD. The ApoE2 and ApoE3 alleles are associated with lower risk and protective (Kim et al. 2009). ApoE4 plays an important role in Aβ deposition as a senile plaque and causes cerebral amyloid angiopathy (CAA). It is considered a marker for AD. In addition, ApoE4 was also associated with vascular damage in the nervous tissues leading to AD pathogenesis (Liu et al. 2013; Giau et al. 2015) (Fig. 2.1).

2.4 Conclusion

AD is currently considered one of the biggest health concerns. During the last decades, extensive studies on AD to understand the molecular pathology, factors influencing the onset of AD, and biomarkers to design therapeutic intervention have been carried out. Many drugs have been designed based on the molecular pathology of AD. However, the molecular pathogenesis of AD is complex, and it is based on different hypotheses. However, none of these hypotheses alone are able to clarify the fundamental aspects of pathology and its molecular regulations. The accumulation of Aβ and cellular mechanism by which it affects cholinergic neurons and following cognitive deficits are still not fully understood. Genetic factors in the form of mutation are also added concern as it is difficult to detect the early onset of AD. Interestingly, the cholinergic hypothesis has served as a basis for the majority of treatment strategies against AD. Most of the drugs were designed as acetylcholinesterase inhibitors, cholinergic precursors, cholinergic receptor agonists, allosteric cholinergic receptor potentiators, and NMDA receptor blockers (Doggrell and Evans 2003). Inhibitors to cholinesterase enzymes, such as galantamine, donepezil, and rivastigmine, and NMDA antagonists, such as memantine, improve memory and alertness. However, it does not prevent the progression of AD. In addition, several

studies have shown that modifications in lifestyles like a healthy diet and good physical exercise can improve brain health and reduce AD progression.

Some of the recent studies were focused on biomarkers of AD such as Aβ and tau peptides. Therefore, further therapies based on these molecules will be a challenging period in the AD treatment strategies. However, many drugs like AN-1792, solanezumab, bapineuzumab, semagacestat, avagacestat, and tarenflurbil targeting Aβ pathways failed to demonstrate their efficacy in the final clinical stages. Several new drugs are currently under investigation. In general, the success of AD treatment depends on its early administration and patient monitoring for disease progression using biomarker diagnosis. Future studies on therapeutic intervention targeting tau pathology and the use of alternative medicinal treatments like Indian Ayurveda or Chinese medicine may potentially influence AD progression. Coming years will be challenging for scientists working on AD as the numbers of people affected with AD and dementia are increasing drastically worldwide.

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Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 3
Tracking Neurodegeneration: Advancement in Experimental Study Models

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Abstract Neurodegeneration is the gradual deterioration and loss of conductive ability of neurons constituting the central and the peripheral nervous system, particularly involving the neurons in the brain and spinal cord. Neurodegenerative conditions are one of the leading causes of disability, affecting millions of people around the world. The risk of the onset of neurodegenerative diseases increases dramatically with age. Although age is considered a dominant factor in the mortality and prevalence rates of many neurodegenerative disorders, the number of cases has increased over the last 25 years. Most neurodegenerative diseases are challenging to diagnose early. On the other hand, reports have stated that early treatment can slow the progression of the disease. Thus, an early diagnosis of the disease would allow the clinician to predict further possible neural damage and subsequent disabilities. Neuro radio imaging tools are traditional methods for monitoring neurodegeneration in clinical settings. Lately, genetic and biochemical tools have also been successfully developed for early diagnosis. In addition, preclinical animal models and human organoids have also been successfully utilized in the neuro drug discovery program and early diagnosis of disease progression. Therefore, in the above-mentioned context, the authors have briefly discussed various strategies for monitoring neurodegeneration and the recent progress made in experimental models.

Keywords Neurodegeneration · Tracking of neurodegeneration · Neurodegenerative disorders · Radio imaging · Genetic test · Preclinical models · Human organoid models

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Abbreviations

6-OHDA	6-Hydroxydopamine
Ach	Acetylcholine
AChT	Acetylcholine transporter
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APOE	Apolipoprotein E
Aβ	Amyloid beta
BBB	Blood–brain barrier
CAA	Cerebral amyloid angiopathy
CB2R	Cannabinoid receptor 2
CBF	Brain blood flow
CNS	Central nervous system
COX	Cyclooxygenase enzyme
DA	Dopamine
ET	Eye tracking
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
GABA	Gamma-aminobutyric acid
HD	Huntington's disease
HIV	Human immunodeficiency virus
iPSCs	Pluripotent stem cells
LTP	Long-term potentiation
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NFTs	Neurofibrillary tangles
PD	Parkinson's disease
PET	Positron emission tomography
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNpc	Substantia nigra pars
SPECT	Single-photon emission computed tomography
TEQ	Therapeutic Engagement Questionnaire
TSPO	Translocator protein

3.1 Introduction

Neurodegeneration is the gradual deterioration with concomitant loss of neuronal ability to communicate across the central and peripheral nervous system. Neurodegeneration is synonymous with diseases such as amyotrophic lateral

sclerosis (ALS), Alzheimer's disease (AD), Huntington's disease (HD), multiple sclerosis (MS), and Parkinson's disease (PD). Neurodegenerative conditions are the most frequent cause of disability and affect millions of people worldwide (Checkoway et al. 2011). According to the US National Institute of Environment and Health Sciences, AD and PD are the most common neurodegenerative diseases globally. Furthermore, the risk of developing a neurodegenerative disease increases considerably with age. Although age is considered to be a predominant factor in mortality and prevalence rates for many neurodegenerative disorders, the number of cases has increased over the past 25 years (GBD 2015 2017). Therefore, the market size of therapies for neurodegenerative diseases is expected to grow by nearly USD 44.90 billion in 2026. This report provides a market analysis based on the findings of AD, PD, MS, HD, and others (Reportlinker 2021).

Neurodegenerative disorders typically cause cognitive or motor dysfunction or both. For example, AD is a common cause of dementia and impairs the individual's quality of life, mental judgment, financial implications, and memory functions. In addition, it is often associated with mood swings in patients (Relja 2004; Sugin et al. 2020). While ALS and PD are progressive degenerative diseases that damage the nerve cells (motor neurons) which control voluntary muscle movement, HD is predominantly an autosomal disease establishing itself by a triad of motor, cognitive, and psychiatric symptoms. Most neurodegenerative diseases are hereditary and progress over several years, ultimately leading to death (Relja 2004; Dugger and Dickson 2017). Unfortunately, it becomes increasingly difficult to treat as the disease progresses. Conditions such as ALS, AD, and PD might be difficult to diagnose early. On the other hand, reports have favored early treatment for slowing down the progress of the disease (Mancuso et al. 2011; Murman 2012; Czaplinski et al. 2006). Subsequently, it has also been believed that the neurodegenerative process is characterized by the loss of the myelin sheath, followed by cell death and behavioral changes; therefore, early diagnosis and treatment would facilitate the arrest of further damage of neurons.

Initially, scientists were not able to accurately correlate the physiopathological pathways with the rate of disease progression. As a result, it was difficult for clinicians to monitor the therapeutic responses and the severity of the disease. However, with recent advances in noninvasive brain imaging techniques, proteomics, and estimation of the biomarkers, a wide range of evidence is available. Now, the researcher can predict the progression of the disease and initiate proper counseling and medications. In the given context, the authors briefly address the monitoring strategies of neurodegeneration and recent advances in the experimental models.

3.1.1 Pathophysiology of Neurodegeneration

As discussed above, neurogenerative diseases have been explained by complex multifactorial pathogenetic mechanisms that vary depending on the clinical state. The exact pathogenicity of neurodegenerative diseases remains largely unknown.

However, the relevant neurochemistry and synaptic transmission levels have been extensively researched. It is important to note that protein abnormalities followed by neuroinflammation that define neurodegenerative diseases might occur prior to the onset of clinical signs (Relja 2004; Dugger and Dickson 2017). Consequently, a thorough knowledge of pathophysiology is necessary to understand the progression of the disease. The common pathophysiology associated with clinical conditions is discussed as follows.

The physiopathology of AD implies cortical atrophy, usually the most important in the medial temporal lobe, which is composed of several important structures related to cognitive function. The impacted brain regions suffer inflammation, granular degeneration, and Hirano bodies. Furthermore, two classical inclusions are considered pathognomonic of the disease: neurofibrillary tangles (NFTs) and amyloid plaques. NFTs are localized aggregates of tau proteins and neurofilaments found in neuronal cell bodies. The distribution and density of NFTs appear to be correlated with clinical conditions. Amyloid plaques are extra neuronal aggregates of Aβ-protein. The plaques, namely neuritic and diffuse, are two kinds that are responsible for the pathology of the disease. The neuritic plaque is an extracellular component of Aβ; the plaque also has a component of dystrophic neuritis that contains tau protein. On the other hand, the diffuse plaques consist mainly of Aβ protein. Interestingly, the histopathology of the brain section of autopsied elderly individuals who are clinically unaffected has also demonstrated the presence of diffuse, neuritic plaques, and NFTs (Pressman et al. 2014).

ALS affects both superior and lower motor neurons and is clinically associated with weakness, muscle atrophy, and spasticity fasciculations. There are instances of atrophy of the precentral gyrus. In addition, gray matter abnormalities, atrophy of the motor cortex, and white matter reduction are also observed in different segmental areas of the central nervous system, with a site predilection to the corticospinal tract and Betz cell degeneration in the motor cortex (Dickson and Weller 2011; Saberi et al. 2015). Other pathology features of ALS include vacuolization, large empty spaces close to neurons, and spongiosis. Recent studies point to the importance of the glial cells and their role in the pathophysiology of ALS (McGeer and McGeer 2002; Boillée et al. 2006a, b; Yamanaka et al. 2008).

HD is a rare, progressive, and degenerative genetic condition caused by a single defective gene on chromosome 4—one of 23 human chromosomes. It is especially identified by the neuronal loss of striatum and cortex (Vonsattel and DiFiglia 1998) but also affects many other nuclei, including the globus pallidus, thalamus, hypothalamus, subthalamic nucleus, substantia nigra, and cerebellum (Petersén et al. 2002, 2005; Kassubek et al. 2004). Additionally, diffusion tensor imaging confirmed white matter pathology in symptomatic patients before and prior to symptoms (Rosas et al. 2006). Recent evidence has suggested that the mutant Huntingtin gene RNA is toxic. On the DNA level, it causes a repeated expansion of somatic GAC in susceptible cells, influencing the progression of the disease (Tabrizi et al. 2020).

PD can be an inherited or a sporadic disease, but they all have neuronal loss in the substantia nigra pars (SNpc) compacta, which provides the dopaminergic

innervation to the striatum (Dickson 2018). The gradual loss of dopamine neurons is a characteristic of normal aging. However, the symptoms of PD are consistent with excessive loss (70–80%) of these neurons. If left untreated, PD progresses over a span of 5–10 years to a rigid and related condition, and patients are unable to fend for themselves. Subsequently, the cross-sections of the PD brainstem demonstrated the loss of the dark pigmentation zone in the SNpc and locus coeruleus. Pigmentation loss was observed to be directly correlated with the death of dopaminergic neurons (DA) containing neuromelanin in the SNpc and noradrenergic neurons of the locus coeruleus (Dickson 2012), which leads to malfunction in other nondopaminergic neurotransmitter systems (Kalia et al. 2013). Degeneration of these systems has been attributed to the various nonmotor symptoms of PD that are refractive to dopamine replacement therapies (Chaudhuri et al. 2006).

Moreover, the roles of neurotransmitters in the pathophysiology of neurogenerative diseases have also been investigated. In addition to clinical conditions, many neurodegenerative disorders have common phenomena in neurotransmitter levels in the central nervous system (Vorobyov and Bobkova 2017). Excitatory amino acids like glutamate are major excitatory neurotransmitters in the human nervous system. Glutamate hyperactivity caused by exogenous or endogenous factors is widely believed to be an etiological factor in chronic neurodegenerative disease (Lewerenz and Maher 2015). Exogenous or endogenous neurotoxic compounds could also activate glutamate receptors, resulting in neurodegeneration. The excitotoxicity of glutamate may also contribute to toxin-induced dopamine cell death, mainly due to the presence of glutamate receptors in substantia nigra (Relja 2004). Therefore, it could be concluded that the understanding of pathophysiology and relevant pathways is essential in the early diagnosis of the disease.

3.1.2 Classification of Neurodegenerative Diseases Based on the Clinical Manifestations

To the best of the authors' knowledge, the present attempt is the first of its kind in classifying the neurodegenerative diseases, based on the functional disability aspects, as follows:

- **Neurodegeneration with physical disability:** Movement disorders, including hyperkinetic, hypokinetic, cerebellar, or dysfunction of the superior and inferior motor neurons (e.g., HD, ALS or Lou Gehrig's disease, PD, MS, spinocerebellar ataxia, spinal muscular atrophy, and motor neuron diseases).
- **Neurodegeneration with mental disability:** Cognitive decline, dementia, and impairment of superior cerebral functions (e.g., AD and other dementias, PD and PD-related disorders, prion disease, and HD).

  Clinical manifestations of neurodegenerative diseases begin either as motor or cognitive dysfunction or early combinations of both.

3.2 Tracking Strategies for Neurogenerative Diseases

The tracking of neurodegeneration at a macroscopic level by identifying the clinical signs and up to the level of cellular pathology is one of the many recent advances achieved in the treatment and management of neurodegenerative diseases. The initial processes of neurodegeneration begin with neuroinflammation, which occurs in the brain and spinal cord regions (Przedborski et al. 2003; Dugger and Dickson 2017). Therefore, early diagnosis of the disease would allow the clinician to predict additional neural damage and subsequent disabilities. Neurodegenerative diseases have a group of pathophysiological events, and these differ with clinical conditions. Therefore, the authors classified the tracking strategies based on the disease progression (Fig. 3.1).

Disease progression could be monitored by imaging anatomical changes and/or quantifying pathophysiologically relevant biomarkers from various body fluids. Recent developments in imaging tools and proteomics analysis techniques would allow researchers to track the disease progression in clinical settings (Mathis et al. 2005; Shimizu et al. 2018). Neuroinflammation refers to the development of several neurodegenerative disorders (Guzman-Martinez et al. 2019), followed by demyelination and neuronal death (Fig. 3.1). Astrocytes and microglia are the dominant immune cells in the CNS (Carson et al. 2006). They are thought to play a critical role in the initial cascade of neuroinflammation by releasing many neurotoxins (Chen et al. 2016). Therefore, comparing the extent of neurochemicals in various body fluids and radio imaging would help understand the rate of progression with clinical correlation support.

3.2.1 Applications of Radio Imaging Tools in the Monitoring of Neurodegenerative Diseases

Recent studies have revealed that nuclear imaging approaches such as positron emission tomography (PET), single-photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI) have been helpful in monitoring the molecular consequences of neuroinflammation. Furthermore, the above-mentioned imaging tools have also been used in investigating changes in the integrity of the blood–brain barrier (BBB) and biomarkers for neuroinflammation, using specific radioligands.

3.2.2 Applying PET to Neurogenerative Disease Tracking

PET is the first nuclear imaging approach aimed at quantifying neuroinflammation in vivo (Jain et al. 2020; Kreisl et al. 2020; Meyer et al. 2020; Zimmer et al. 2014).

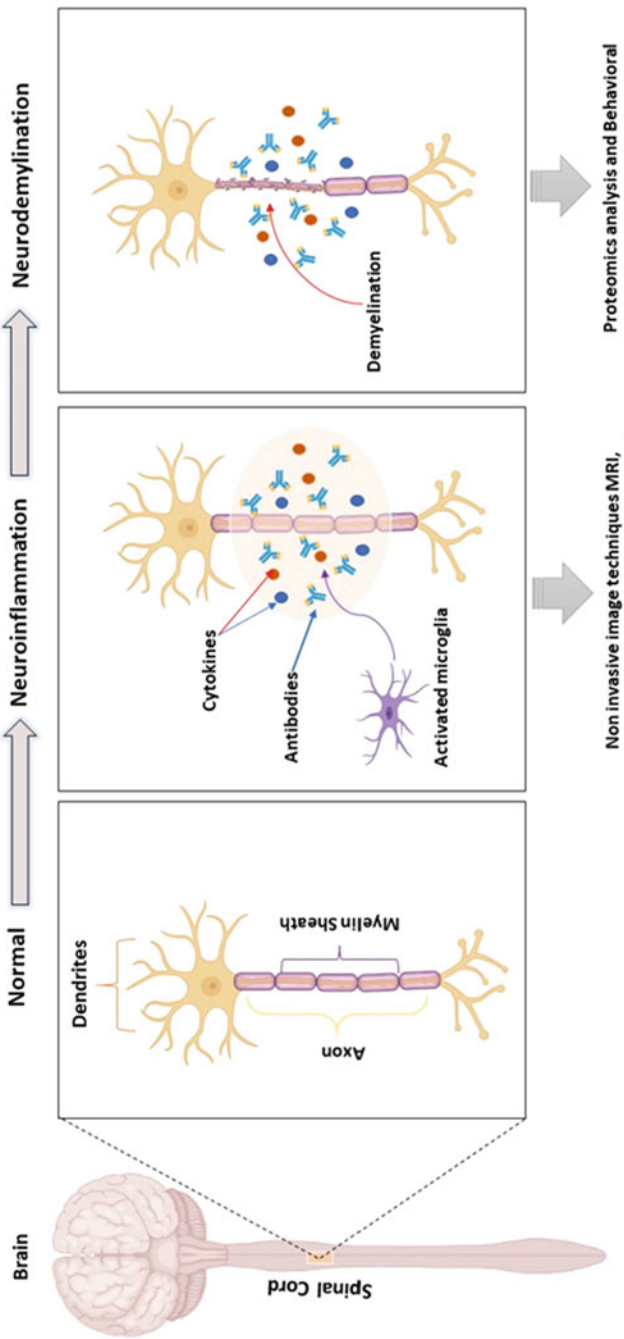

Fig. 3.1 Common neurodegenerative pathways and their applications in disease index tracking

Activation of microglia in the brain and spinal cord region would lead to infiltration of peripheral leukocytes into the CNS, followed by neuroinflammation (Carson et al. 2006; DiSabato et al. 2016). The microglia activation will trigger the release of inflammatory markers such as cytokines and chemokines and the generation of reactive oxidative species to sustain homeostasis (Harry and Kraft 2008; Wang et al. 2015; DiSabato et al. 2016). Increasingly, data from preclinical models and genomic association studies have highlighted the immune system's role in neurodegenerative diseases, which include AD, PD, and MS (Golde 2019). Since neuroinflammation is the primary cascade in neurodegeneration, an initial evaluation of inflammatory markers would enable to predict the neurodegeneration by clinicians and researchers at the early stage of disease progression. In addition, PET has discriminated a minor quantity of inflammatory markers in the brain and spinal cord areas (Carson et al. 2006; DiSabato et al. 2016). The above information would enable investigators to design clinical trials for neurodegenerative therapies. Recent studies have provided considerable evidence of the usefulness of PET studies in monitoring neuroinflammation; however, the clinical application is yet to be initiated. Therefore, additional studies are warranted to apply these strategies toward meaningful treatment.

The foremost advantages of PET in tracking neurodegeneration are that it could detect low concentrations of particular proteins or amino acids in the region of interest using specific radioligands (Kreisl et al. 2020). To detect specific proteins, the specific nontoxic radioligands needed are known as tracers (Fig. 3.2). Tracers are the radiolabeled chemical molecules with higher affinity toward the specific proteins; thus, the injected tracers will bind with the proteins, and the PET scanner would detect the radio signals. The ratio of radioligands between the region of interest and the nonspecific regions would be a surrogate marker of the extent of protein accumulation in the particular region. Changes in the radio signal ratio at baseline and/or after treatment would provide the opportunity to track the impact of the particular treatment on the condition and progression of the diseases (Fig. 3.2). Several PET radiotracers have been developed for candidate biomarkers of neuroinflammation, including 18 kD translocator protein (TSPO), cannabinoid receptor 2 (CB2R), and cyclooxygenase enzymes (Jain et al. 2020).

In clinical trials, TSPO is a presumed biomarker of neuroinflammation, and the elevation of PET ligands shows an increase in TSPO binding, which, in turn, positively correlates with the clinical conditions in AD, as well as during major depressive episodes of the disease (Ching et al. 2012; Dupont et al. 2017; Zhang et al. 2021). The PET ligand for cyclooxygenases enzyme systems such as COX-1 and COX-2, which are components of the cyclooxygenase (Narayanaswami et al. 2018), might be more beneficial in studying the conditions and also to understand whether the given treatment was effective or not.

Furthermore, the markers showed significant differences between the healthy volunteers and patients with neurodegenerative diseases such as AD, MS, HIV-associated cognitive impairment, frontotemporal dementia, chronic traumatic encephalopathy, HD, ALS, epilepsy, corticobasal degeneration, progressive supranuclear palsy, and dementia with Lewy bodies and stroke (Kepe et al. 2013;

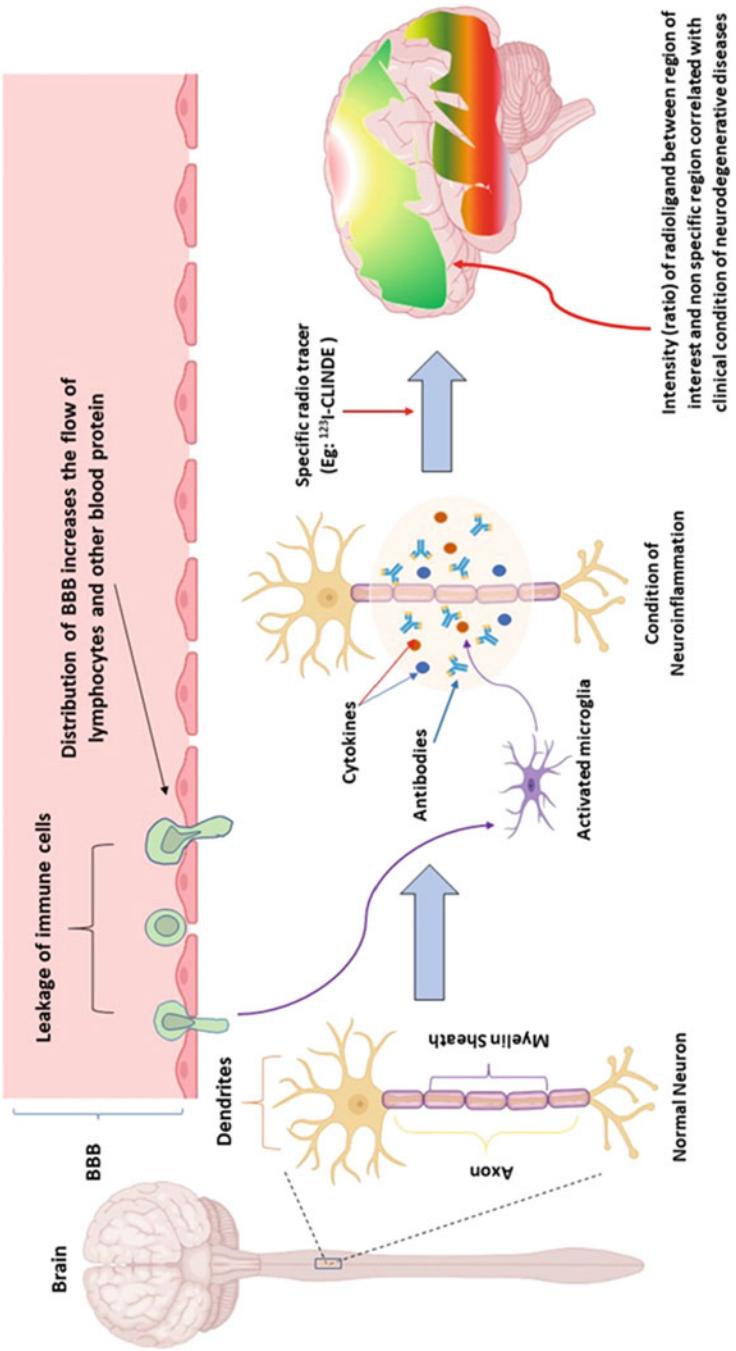

Fig. 3.2 Application of PET/SPET in tracking neurodegenerative disorders

Vera et al. 2017; Beyer et al. 2018; Tiepolt et al. 2019). In addition, the PET acquisition could create three-dimensional images that enable the detailed state of the brain region and spinal cord to be read. However, the main obstacle is the limitation of quantification with the most common neuroinflammatory markers, such as 18 kDa translocator protein (TSPO) due to higher nonspecific binding of the radioligands (Ching et al. 2012).

3.2.3 *Applying Single-Photon Emission Computed Tomography (SPECT) in Neurogenerative Disease Tracking*

The principle of SPECT and PET operation is always common (Fig. 3.2) with various devices. At the same time, PET and SPECT would be distinguished by the type of radiotracers used. PET tracers generally have a shorter half-life, whereas SPECT tracers have a longer half-life, allowing us to take more images (Rahmim and Zaidi 2008; Wahl et al. 2011). Thus, the PET scanner is crucial in the study related to BBB integrity, followed by the infiltration of inflammatory markers involved in the first line cascade of the neuroinflammation process (Breuer et al. 2017; Kreisl et al. 2020). Furthermore, early diagnosis of changes in the BBB integrity in the conditions of stroke and other diseases or injuries would help the physician plan proper treatment and arrest the progress of neuronal damages.

Lorberboym and his colleagues reported (99 m) Tc-diethylenetriamine penta acetic acid as a tracer for BBB integrity assessment during the acute stroke of the middle cerebral artery, occurring between 24 and 48 hrs. The data showed that measuring the degree of BBB disturbance at the initial stage of stroke and edema formation might be used to predict the delayed neurological and functional results (Lorberboym et al. 2003). In another clinical study, Elbert and the team used ^{123}I-CLINDE as a SPECT tracer to study the neuroinflammation in patients after mild traumatic brain injury. ^{123}I-CLINDE, a marker specific to TSPO, is an upregulated protein in the active immune cells. Interestingly, the results showed that neuroinflammation occurred after 1–2 weeks after the injury. Furthermore, it persisted up to 4 months as suspected in the pathogenesis of post-concussion symptoms in patients (Ebert et al. 2019). Thus, the above evidence strongly supported the hypothesis that early diagnosis of neuroinflammation via BBB integrity changes by neuroimaging techniques would be a potential treatment strategy to prevent late neuronal damage and subsequent disability.

In addition, unfortunately, there is no peripheral blood test for PD yet. Therefore, most of the time, PD gets diagnosed only in the advanced stages. Early detection of PD is essential in arresting further damage to the dopaminergic neurons. Nowadays, ^{123}I-ioflupane is widely used as a tracer for the diagnosis of early-stage PD. Many clinical studies have shown the logical relationship of changes in the ^{123}I-ioflupane binding report in left caudate, right caudate, left putamen, and right putamen with

clinical presentation (Pahuja et al. 2019). As discussed earlier in the present chapter, acetylcholine (ACh) is a key neurotransmitter involved in the pathophysiology of PD. Levels of ACh and its vesicular acetylcholine transporters (AChT) would be less in PD patients than in healthy volunteers (Bohnen and Albin 2011). Therefore, monitoring the density of AChT could be a potential diagnostic approach for the early detection of PD. The SPECT radiotracers like ^{123}I-iodobenzovesamicol bind specifically to AChT and reveal the density of acetylcholine containing vesicles. Therefore, changes in the AChT could be correlated with the clinical presentation for early detection of PD. Interestingly, the reduced density of AChT in parietal, occipital lobes and cerebral cortex in PD patients identified by the SPECT radiotracer was also correlated with dementia (Niethammer et al. 2012).

3.2.4 Applying Magnetic Resonance Imaging (MRI) to Neurogenerative Disease Tracking

MRI has taken on an essential and groundbreaking role in diagnostic imaging by enabling the acquisition of high-resolution images without harmful ionizing radiation. The major advantage of MRI is that it works through water molecules within the body and requires no specialized radiotracer. However, the specific proteins or inflammatory markers might not be detected through MRI. Instead, one can detect structural abnormalities such as alterations in brain volume, atrophy, diameter, area, etc. Melzer and his colleagues studied the structural abnormalities of twenty-three nondemented PD patients over the period of one year. The results showed atrophy in temporal and orbitofrontal cortices in the PD group compared with the control group. The significant changes in brain atrophy could be used to monitor dementias in PD patients (Melzer et al. 2015).

Furthermore, the substantia nigra (SN) region is primarily affected due to the after infarction, especially in conditions like stroke or serious injury. SN has dopaminergic neurons of the midbrain that play a vital role in motor and reward function. Therefore, the damage caused by immune cell infiltration due to infarction seriously affects motor function, which in turn leads to motor dysfunction. Linck and his colleagues studied the SN after ipsilateral infarct and the clinical outcomes in 181 participants in their study. After one year of follow-up, the study made a riveting conclusion that the patients with stroke had increased SN R2* in MRI. The elevated SN R2* was related to the increased iron content and clinical outcomes. Therefore, the study concluded that tracking of SN R2* by MRI could be a potential tool for tracking secondary neurodegeneration in stroke patients (Linck et al. 2019).

Astrocytes play a crucial role in cerebral blood flow (CBF). In the course of MS, CBF reduction is known as expected cerebral hypoperfusion. Therefore, changes in CBF would be a potential early marker for the neurodegenerative process. Van Schependom et al. described the comprehensive brain hypoperfusion in the cerebral cortex and cerebellum in MS patients utilizing the arterial spin labeling method

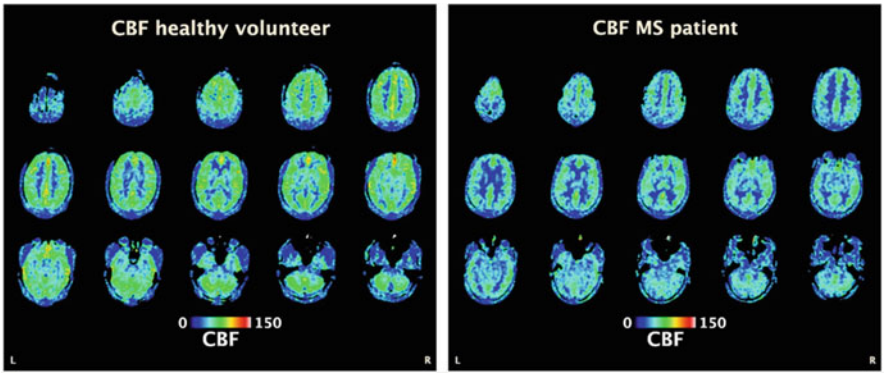

Fig. 3.3 Perfusion-weighted cerebral MRI (labeling of arterial spin) of a healthy volunteer (left) versus recurrent remission MS (right), with CBF map overlay with color code. Cerebral perfusion is generally reduced in multiple sclerosis, compared with healthy volunteers (image adopted in Van Schependom et al. 2019)

without using contrast administration (see Fig. 3.3 for an example from the authors' own records, unpublished data adopted from Van Schependom et al. 2019). The above findings supported the hypothesis that reduced CBF in the early stage of diagnosis would be a potential strategy for prevention from further deterioration.

3.3 Genetic Testing for Neurodegenerative Disease Tracking

Clinical genetic testing techniques on neurodegenerative disorders used for accurate diagnosis provide information on the risk of recurrence and assist family members involved in determining the personal risk and eligibility for counseling or clinical trials.

Genetic detection of neurodegenerative diseases through monogene analysis began in the 1980s when genes were identified for HD, AD, and other neurodegenerative diseases. Recent developments in genetic testing and multigenic panels, whole exome sequencing increases diagnostic efficiency, especially if the specific neurodegenerative state is unknown. At the beginning of the 1990s, genes responsible for AD were identified on chromosomes 21 (APP), 14 (PSEN1), and 1 (PSEN2) (Van Cauwenberghe et al. 2016). These genetic tests have been proposed in families with known or suspected mutations, while recent findings have confirmed the critical role of associations between Lewy's dementia and variants of the APOE, GBA, and SNCA genes (Sanghvi et al. 2020). In addition, another study confirmed that the mutation in a single multigene such as C9orf72, MAPT, and GRN causes 20–50% chances of frontotemporal dementia (FTD) (Olszewska et al. 2016). Unless a specific pathogenic variant of the family is known, genetic testing for FTD is

usually performed using multigene panels (Greaves and Rohrer 2019). Similarly, recent advancements in genome analysis could diagnose other neurodegenerative diseases as well. For example, genes, such as GBA and LRRK2, display the most common genetic variants associated with PD and serve as the basis for the emerging target-based therapies for PD, and it is currently being tested in clinical trials (Sardi et al. 2018).

Unfortunately, there is no diagnostic or radio imaging protocol for the early diagnosis of ALS. It is one of the primary neurodegenerative diseases of the motor neurons, ending in mortality within 3–5 years after the onset of symptoms. However, recent developments in the field of genetic diagnosis would provide the possibility of identifying family inheritance occurs in 5–20% using GWAS. A number of genes have been identified, including C9orf72, SOD1, FUS, and TARDBP, which are directly correlated with the onset of ALS (Peters et al. 2015). In addition, van Rheenen and co-workers recently identified MOBP and SCFD1 as new risk factors associated with ALS (van Rheenen et al. 2016).

HD is a progressive neurodegenerative condition, potentially fatal and incurable, and there is currently no diagnostic test for HD. In early 1983, the polymorphic marker was used to map a gene to a chromosome. The HD gene, now known as HTT, has been correlated with chromosome number 4. The HTT gene was identified and proved to be an extension of CAG trinucleotide replication, which opened the door to direct genetic testing for HD (MacDonald et al. 1993; Roberts et al. 2020).

Together, recent advances in whole-genome analysis and sequencing would make it possible to diagnose neurodegenerative diseases when clinical conditions are unknown. In addition, it also provides an occasion for early diagnosis and clinical advice for the management.

3.4 Advanced Study Models in Neurogenerative Disease Tracking

For the most part, conventional methods for monitoring neurodegenerative disorders include noninvasive imaging techniques and behavioral assessments. However, recent advances in proteomics and in vitro culture would make it possible to predict the rate of neurodegeneration from the patient's peripheral tissue samples. The following are some examples of recent preclinical and clinical models.

3.4.1 In Vitro Human Organoid Models for the Surveillance of Neurogenerative Disorders

Recent studies on iPSC-derived brain organoids using skin samples from AD patients showed that APOE4 exacerbated synapse loss and neurodegeneration

(Zhao et al. 2020). In the above study, human dermal biopsies of healthy volunteers and patients with the genotype APOE ε3/ε3 or ε4/ε4 were used. APOE genotype has been identified by Sanger sequencing using DNA samples from fibroblast lines. Cells were cultured, and induced pluripotent stem cells (iPSCs) were produced by electroporation of three epidermal vectors in fibroblast cells. The IPSC colonies were isolated and extended after 3–4 weeks of cultivation. The author then developed a 3D human brain organoid system to study the pathogenesis of AD. In the study findings, it was observed that APOE4 predominantly worsened the p-tau accumulation. At the same time, AD status has been linked to higher levels of Aβ and p-tau, apoptosis, synaptic loss, and increased formation of stress pellets. It is important to note that APOE4 potentially speeds up apoptosis and the formation of strain granules in the AD state in this 3D organoid model. Therefore, the human iPSC-organoids recapitulate that the APOE4-related model can be a tool for the early identification of degenerative pathways contributing to AD pathogenesis. However, detailed studies in this area are still warranted.

3.4.2 In Vivo Preclinical Models for Monitoring Neurogenerative Disorders Being Investigated

A wide range of animal models that can mimic the human context of the disease is being used to investigate the pathogenesis of the diseases and treatment strategies. The FDA relies on data produced from animal models to evaluate the effectiveness and safety of new drugs. Indeed, animal models have been the gold standard to study the pathophysiology of any disease. In the above context, the authors examined some recent animal models used for monitoring neurodegeneration, and the details have been discussed below.

3.4.2.1 Preclinical Models for AD

Conventional preclinical animals such as mice, rats, monkeys, dogs, and others do not naturally develop AD. However, the studies in animal models showed that several chemicals and physical injuries might develop memory deficits, neuroinflammation, and cerebral amyloid angiopathy (CAA). At the investigative stage, the manifestation of AD in the brain reveals the accumulation of extracellular amyloid plaques, intracellular neurofibrillary tangles, and neuronal loss. Several studies have identified a causal relationship between neocortical NFT and cognitive loss (Giannakopoulos et al. 2009; Nelson et al. 2012; Webster et al. 2014). Interestingly, the triple transgenic 3xTg mouse model displayed learning and memory deficits on the Barnes maze (Stover et al. 2015), and it expresses all disease characteristics of AD. Aβ deposition is progressive, with intracellular immunoreactivity detected in some areas of the brain from 3 to 4 months of age. These mice

develop progressive neuropathology associated with age, including plaques and tangles. The Aβ deposits become apparent in 6 months, and with progressing time, the deposition becomes discernible at the microscopic level. This is complemented by accelerating tau pathology, which becomes profound in 12 months' time. Synaptic dysfunction, including long-term potentiation (LTP) deficits, occurs prior to plaques and tangles (Oddo et al. 2003; Billings et al. 2005).

Interestingly, cognitive impairment occurs within 4 months. Initially, plaques and tangles deposition at the histological level begins to appear prior to the development of memory and learning deficits. Several transgenic mouse models have been developed and/or adapted to closely mimic a specific pathological characteristic of AD. The transgenic mouse model Tg-SwDI shows CAA and accumulation of diffuse and less prominent parenchymal vascular fibrillary plates Aβ, as early as 3 months (Davis et al. 2004). Tg-SwDI mice exhibit neurodegeneration of cholinergic neurons followed by impaired cognition. Furthermore, the APP E693Δ-Tg model expresses the Osaka (APPE693Δ) mutation, resulting in a unique phenotype of enhanced expression of Aβ oligomers and synaptic and cognitive impairment as early as 8 months of age, but no plaque or tau pathology formation (Tomiyama et al. 2010). The above-mentioned animal models are currently being involved in the AD drug discovery program to understand the efficacy of new chemical entities during the drug treatment.

3.4.2.2 Preclinical Models for Huntington's Disease

HD is a life-threatening neurodegenerative disorder and is a consequence arising out of mutation in the interesting transcription gene 15 (IT15), and it is challenging to develop an animal model for HD. Theoretically, in the HD state, nucleocytoplasmic transport is disrupted, and consequently, the drugs that cause restoration could be neuroprotective. Invertebrate models like *Caenorhabditis elegans* and *Drosophila melanogaster* are currently being used in drug discovery programs for high-throughput screening of various classes of novel therapeutics. The *C. elegans* model is known to express expanded polyglutamine repeats in its nervous system, which is comparable to HD in humans. However, none of the animal models show a potential clinical correlation.

3.4.2.3 Preclinical Models for Parkinson's Disease

Chemical-induced PD models show dopaminergic neuron degeneration in substantia nigra pars compacta (SNc) and a subsequent reduction in striatal dopamine content. MPTP-induced PD is a widely accepted animal model for research, which has led to the advancement of our PD knowledge and therapeutic approach (Benazzouz et al. 1993; Bezard et al. 2001; Dovero et al. 2016). Other familiar models for PD have been generated using 6-hydroxydopamine (6-OHDA) by intracerebral administration (stereotaxic approach) (Kin et al. 2019).

Genetic models are a relatively new approach to understand the disease at the molecular scale, and it involves manipulation of genes that are associated with PD. Transgenic animal models have been generated since the identification of human mutations in neurodegenerative conditions and disorders, which result in the abnormal production of PD-related proteins such as α-synuclein, parkin, PINK1, DJ-1, LRRK2, or UCHL1 (Duty and Jenner 2011; Klein and Westenberger 2012). Several types of α-sin transgenic mice have been established, and many behavioral deficits have been observed. These models, however, could not demonstrate profound nigrostriatal degenerations.

3.4.2.4 Preclinical Models for ALS

Due to the complexity of the pathogenicity of ALS, researchers are not able to produce a conventional animal model for ALS. Therefore, modern science focuses on the genetically modified animal model for ALS. The number of genetic animal models that are being added to ALS research is steadily increasing, and such rapid advancements empower the researchers to perform genetic incision of ALS, thereby adding greater clarity to the knowledge base of the disease pathology. Recently, zebrafish models have been used in ALS research to test the hypothesis (Babin et al. 2014). The degeneration of motor neurons in zebrafish after exposure to the industrial plasticizer Bisphenol A was studied (Morrice et al. 2018). Exposure to BPA induces motor neuron degeneration, activates microglia, and senses pathogenic stimuli at the axon terminal prior to cell death, thereby suggesting a retrograde mechanism of degeneration. BPA is an unlikely candidate toxin for ALS disease (Morrice et al. 2018). The most widely used mouse model of ALS is based on the expression of the human SOD1 protein containing the G93A mutation (Philips and Rothstein 2015; Lutz 2018). The mSOD1 model has successfully attempted to demonstrate the putative cellular dysfunction during disease pathogenesis, such as the noncell autonomous nature of ALS (Nagai et al. 2007; Philips and Rothstein 2015). The above model displays rapid worsening of motor neuron architecture leading to paralysis and ultimately resulting in mortality within the first 5 months. Recently, mouse models of TDP-43-Q331K have been developed, each with unique measures of face and construct validity (Lutz 2018). The disease progression in the TDP43-Q331K model is controlled by prion protein gene promoter and displays many ALS-like hallmarks such as progressive motor dysfunction, muscle atrophy, reduced NMJ integrity, and motor neuron degeneration at 10 months of age.

3.4.3 Recent Clinical Studies to Track Neurogenerative Disorders

Apart from the conventional radio imaging techniques, behavioral and biochemical evaluations also showed promising results in the early diagnosis of neurodegenerative diseases. A few of them are discussed below.

In recent days, eye-tracking (ET) studies have been commonly used in clinical configurations to monitor neurodegeneration. The main benefit of ET is its economic and rapid methodology to track changes in behavior. Previously, the Therapeutic Engagement Questionnaire (TEQ) was primarily used in psychological or developmental cognitive studies. However, there is increasing evidence for the application of ET in motion disorders and the measurement of cognitive processes in neurodegeneration. The major drawback is that data is not available for the application of ET studies at more advanced stages of the disease. On the other hand, theoretically, when patients' motor and verbal functions are significantly affected, it is difficult to assess the cognitive functions. Mostly, assessment is not possible. Therefore, ET is a promising tool to track the cognitive defects at the earliest stage of neurodegenerative conditions.

3.5 Ongoing Research Projects Related to the Monitoring of Neurogenerative Disorders

A few ongoing research projects focused on the monitoring of neurodegenerative disorders are as follows:

The project entitled NeuroTRACK (Grant agreement ID: 714388), funded by ERC (European Research Council), is expected to be completed in March 2022. The above project aims to apply the emerging network science tools to evaluate longitudinal, structural, and functional brain connectivity using 3 T magnetic resonance imaging data from patients with frontotemporal lobar degeneration—a devastating, relentlessly progressive, early-onset, neurodegenerative disorder. The project also aims to examine the sporadic and familial cases, including carriers of presymptomatic genetic mutations. The authors hope that the project team would develop tracking tools for diseases like AD and PD, which in turn would lead to early intervention and modification of disease progression (NeuroTRACK 2021).

In another clinical trial, the neurodegenerative tracking protocol for ALS diseases is being evaluated with human volunteers. The primary objective of the study is to assess frontotemporal dementia (FTD) and adult-onset neurodegenerative disorder (ClinicalTrials.gov Identifier: NCT03225144, https://clinicaltrials.gov/ct2/show/study/NCT03225144. Retrieved on July 2021).

Wolf–Hirschhorn syndrome (WHS) is a genetic disorder that affects sporadically anywhere in the body. It is characterized by facial appearance, growth and developmental retardation, intellectual impairment, muscle tone weakness, and seizures.

The clinical trial aims to study the pattern of early neurodegenerative changes in WHS. Preliminary evidence supports the feasibility of this approach and its potential to generate invaluable information about neurodevelopmental and neurodegenerative models within WFS (ClinicalTrials.gov Identifier: NCT02455414, https://clinicaltrials.gov/ct2/show/results/NCT02455414. Retrieved on July 2021).

3.6 Perspectives

Neurodegenerative diseases are those that are characterized by progressive loss of functional neurons terminating in dysfunctions and anatomical alterations associated with the deposition of proteins and neurochemical alterations. However, recent research reports revealed that the neurotransmission mechanisms might be more complex. Therefore, their characterizations might help the researchers to understand the pathogenesis of neurodegeneration in a better manner and design newer pharmacological strategies for the management of neurodegenerative disorders. In addition, the adaptation of genetic tests and calculation of risk scores of diseases with a high prevalence in a healthy population in order to effectively implement essential prevention strategies or to extend early care by initiating drugs administration to those patients devoid of symptoms but are likely to develop the disease due to genetic predilection could be followed.

Conflict of Interest The authors have no conflicting interests.

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Part III
Treating Neurodegenerative Disorders: Natural Remedies

Chapter 4
Pharmacological Application of *Phyllanthus emblica* as Therapeutics in Alzheimer's Disease

Avinash Kundadka Kudva, Manjeshwar Shrinath Baliga, and Shamprasad Varija Raghu

Abstract Today, neurodegenerative disorders such as Alzheimer's disease and dementia are affecting millions of people around the world. Although there are treatments available, the success rate is low in achieving the desired therapeutic benefits. Hence, numerous research groups have been working on finding a novel way to treat these disorders. The traditional system of medicine has been in use for centuries. Herbal drugs comprising various plant-based products have been studied and used for treating neurological disorders including Alzheimer's disease. *Phyllanthus emblica* has been extensively studied for its therapeutic properties that include antioxidant, anti-inflammatory, anti-hyperlipidemic, anti-diabetic, and neuroprotective actions. These pharmacological actions of *Phyllanthus emblica* are corroborated by the evidence collected from preclinical research trials. Hence, it deserves the attention of clinical researchers to develop a viable pharmacotherapeutic strategy. The present chapter elaborated the scientific evidence on the pharmacological role of *Phyllanthus emblica* in conferring protection against Alzheimer's disease.

Keywords Alzheimer's disease · Antioxidant · Oxidative stress · Neuroinflammation · Acetylcholine esterase · *Phyllanthus emblica*

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Abbreviations

AChE	Acetylcholine esterase
AD	Alzheimer's disease
APP	Amyloid precursor protein
Bax	BCL2-associated X
BuChE	Butyrylcholinesterase
CNS	Central nervous system
GABA	Gamma-aminobutyric acid
GSK-3β	Glycogen synthase kinase-3β
NMDA	N-Methyl-D-aspartic acid
pTau	Phosphorylated Tau protein

4.1 Introduction

Phyllanthus emblica Linn or *Emblica officinalis* Gaertn is commonly known as Amla or India gooseberry and is an edible fruit belonging to the family Phyllanthaceae. The plant is indigenous to the Indian subcontinent but also found in other countries like Uzbekistan, South East Asia, China, and Malaysia (Baliga and Dsouza 2011). The fruit is round-shaped, fleshy, smooth or striated, and yellowish-green in color and has a six-celled nut (Baliga and Dsouza 2011). Amla is a rich source of micronutrients and other phytochemicals. The fruit is known to be a rich source of vitamin C and also contains gallic acid, ellagic acid, kaempferol, and ellagitannin (emblicanin A, emblicanin B, punigluconin, and pedunculagin) in sufficient quantities (Baliga and Dsouza 2011; Husain et al. 2019). The fruit of this plant, commonly known as Amla, has been used in Indian cuisine to prepare pickles, murabba, chutneys, and juice concentrates (Baliga and Dsouza 2011).

Every part of *Phyllanthus emblica* is useful due to its medicinal and pharmaceutical properties. The plant has been reported to have antioxidant, antipyretic, analgesic, anti-inflammatory, antidiarrheal, anticancer, adaptogenic, antidiabetic, anti-ulcerogenic, anti-mutagenic, antiatherogenic, nootropic, antimicrobial, nephroprotective, neuroprotective, and immunomodulatory potential (Baliga and Dsouza 2011; Sancheti et al. 2005; Krishnaveni and Mirunalini 2012; Chen et al. 2011; Rajeshkumar et al. 2003; Sultana et al. 2004, 2008; Krishnaveni and Mirunalini 2010; Husain et al. 2019). Besides having beneficial actions in various disorders, *P. emblica* also prevents hyperlipidemia, osteoporosis, and several other ailments (Patel and Goyal 2012).

4.2 Nutritional Value and Phytochemical Constituents

P. emblica is rich in nutrients, and its phytochemical constituents are well characterized. Predominantly, studies indicate that the plant is rich in alkaloids, amino acids, flavonols, tannins, and phenolic compounds. Notably, the fruit is known to be the richest source of vitamin C (478.56 mg/100 mL juice) as compared to other fruits such as lime, apple, grapes, and pomegranates. The approximate composition of *P. emblica* is listed in Table 4.1 (Husain et al. 2019). There are numerous studies where the phytoconstituents present in the plant extract were identified and quantified. A list of the major phytoconstituents is given in Table 4.2 (Variya et al. 2016; Husain et al. 2019). Most notably, the plant is rich in gallic acid, glucogallin, quercetin, chebulinic acid, chebulagic acid, 3-ethylgallic acid, kaempferol, and various phenolic compounds containing mucic acid (Zhang et al. 2000, 2001b, c, 2002, 2003; Habib ur et al. 2007). Also, three norsesquiterpenoids were isolated from the roots, namely phyllaemblicin A, B, and C; phyllaemblic acid; and bisabolene-type sesquiterpenoids (phyllaemblic acid B, phyllaemblic acid C, and phyllaemblicin D with phenolic glycosides, 2-carboxylmethylphenol 1-O-D-glucopyranoside, and 2,6-dimethoxy-4-(2-hydroxyethyl)phenol 1-O-D-glucopyranoside) (Zhang et al. 2000, 2001b; Gaire and Subedi 2014; Variya et al. 2016). Also, six ellagitannins, namely phyllanemblinins A–F, were isolated (Zhang et al. 2001a). Additionally, acrylated apigenin and two acrylated flavanone glycosides were isolated from the leaves (methanolic extract) of *P. emblica* (Zhang et al. 2002; El-Desouky et al. 2008). Also, two new sterols (trihydroxysitosterol and 5,6,7-acetoxysitosterol) were identified by Qi et al. (2013) from the leaves and branches. Also, 5-hydroxymethylfurfural and 5-methyl-2-furyl methyl ketone along with 1,2,3-benzenetriol (pyrogallol) exist in the methanolic extracts of leaves (Balasubramanian et al. 2014). Other phytoconstituents including polyphenols like ellagic acid, 3,5,7,3,4-penta-hydroxy flavone, and (E)-oct-4-ene-1,2,3,4,5,6,7,8-octanol were isolated from the leaves of *P. emblica* (Chugh and Bharti 2014). A

Table 4.1 Composition of *Phyllanthus emblica* L. fruit (100 g serving)

Content	Quantity
Carbohydrates	82.91 g
Protein	6.04 g
Fat	0.51 g
Dietary fiber	2.78 g
Calcium	129 mg
Iron	11 mg
Potassium	2.54 mg
Phosphorus	159 mg
Magnesium	46 mg
Chromium	0.82 mg
Zinc	0.23 mg
Copper	0.22 mg
Nicotinic acid	0.2 mg

Table 4.2 Major phytochemical constituents present in *Phyllanthus emblica* L.

Source	Chemical name
Fruit	Ascorbic acid
	Emblicanin A and B
	Glucogallin
	Chebulagic acid
	Corilagin
	Mucic acid 2-O-gallate
	Coumaric acid
	Caffeic acid
Whole plant	Gallic acid
	Ellagic acid
	Quercetin
Root	Phyllaemblicin A, B, and C
	Phyllaemblic acid
Leaves	Apigenin-7-O-(6′-butyryl-beta-glucopyranoside)
	Luteolin-4′-O-neohesperidoside
	Trihydroxysitosterol

comprehensive analysis of fruit indicates that pulp and seeds are rich in phenolic compounds and tannins, respectively. Quercetin is found only in the pulp. While coumaric acid, myricetin, caffeic acid, and synergic acid are ubiquitously present in the pulp and seed (Nambiar et al. 2015).

4.3 Pharmacological Properties

According to Ayurveda, an Indian traditional medicine system, *P. emblica* has been classified as "*Medhya Rasayana*" meaning agent for cognitive rejuvenation (Malve et al. 2014). Several phytochemicals and semi-synthetic drugs have been tested for the treatment of cerebral multifactorial disorders and neuroinflammatory ailments like Alzheimer's disease (AD) and Parkinson's disease. These are associated with altered pathophysiological conditions like oxidative stress, inflammation, etc. (Kumar 2006; Perry and Howes 2011). Several phytoconstituents are identified to act as an inhibitor of neuroinflammation associated with CNS disorders (Kulkarni et al. 2005; Vasudevan and Parle 2007a). The common risk factors and their effects on CNS are depicted in Fig. 4.1. Here, we discuss the beneficial role of *P. emblica* in ameliorating the neurodegenerative disease.

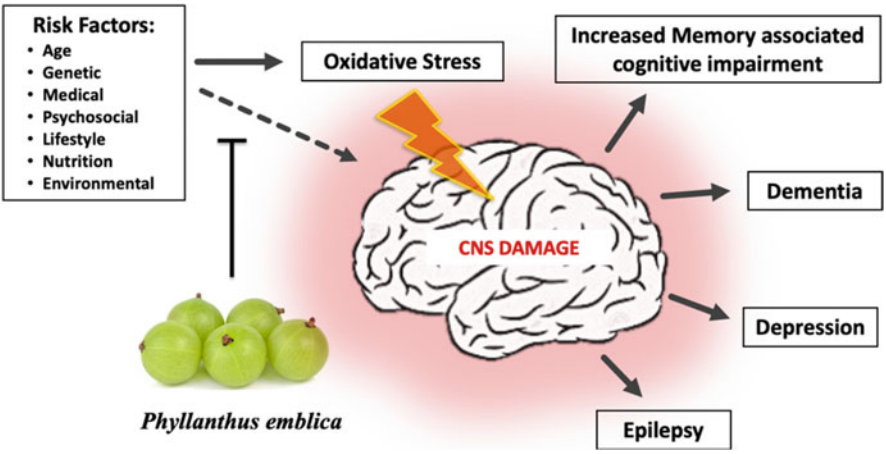

Fig. 4.1 Schematic representation of various risk factors and possible clinical manifestation of Alzheimer's disease

4.3.1 P. Emblica *as an Antioxidant*

Mechanistic studies have shown that feeding *P. emblica* extract enhanced the activity of the various antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase), the phase II detoxifying enzyme, glutathione S-transferase, and antioxidants thiol and glutathione in the blood, with a concomitant decrease in the levels of lipid peroxides (Hari Kumar et al. 2004; Jindal et al. 2009). In addition to this, studies have also shown that *P. emblica* extract was effective in preventing DNA damage caused by genotoxic agents such as radiation (Sharma et al. 2000), lead, aluminum (Dhir et al. 1990), arsenic (Biswas et al. 1999), cesium chloride (Ghosh et al. 1992), nickel (Dhir et al. 1991), chromium (Ram et al. 2003), 3,4-benzo (a)pyrene (Nandi et al. 1997), and 7,12-dimethylbenz(a)anthracene (Banu et al. 2004) and in providing protection against cyclophosphamide-induced suppression of humoral immunity (Haque et al. 2001). In addition to this, studies have also been carried out with the phytochemicals present in *P. emblica*, and research observations suggest that gallic acid (Nair and Nair 2013; Ow and Stupans 2003), geraniin (Kang et al. 2011), corilagin (Li et al. 2018), ellagic acid (Nemavarkar et al. 2004; Ahire et al. 2017; Bhosle et al. 2005; Priyadarsini et al. 2002), quercetin (Nemavarkar et al. 2004; Benkovic et al. 2008, 2009; Devipriya et al. 2008; Mashhadi Akbar Boojar 2020) also possess potent antioxidant properties and a cumulative effect that all these phytochemicals may have triggered to mediate the beneficial effects.

4.3.2 *Neuroprotective Actions of* P. Emblica

Several studies have revealed the beneficial effects of *P. emblica* as an antistress and neuroleptic agent. An earlier study utilizing the *Anwalachurna* made of *P. emblica* led to dose-dependent improvement in memory score at various age groups of both mice and rats (Vasudevan and Parle 2007a). The results using these exteroceptive behavioral models showed a reversal in scopolamine and diazepam-induced amnesia among these animals (Vasudevan and Parle 2007a, b). Other studies from various research groups have also explored the efficacy of *P. emblica* extract against scopolamine-induced amnesia. Based on the outcomes, it can be concluded that it has multifactorial benefits such as antioxidant and anticholinesterase activity that may help in improving and reversing the memory deficits and thus can be used as a remedy for the management of dementia (Perry and Howes 2011; Vasudevan and Parle 2007a, b; Golechha et al. 2012; Vinutha et al. 2007).

Studies have shown that not only did hydroalcoholic extract of *P. emblica* eliminate pentylenetetrazole and kainic acid-induced seizure and status epilepticus, but it also improved cognitive function in rats (Golechha et al. 2010, 2011). Moreover, *P. emblica* showed a dose-dependent inhibition in kainic acid-induced elevated TNF-alpha expression in the brain due to its antioxidant and anti-inflammatory properties (Golechha et al. 2010, 2011). Also, the antiepileptic potency of epigallocatechin-3-gallate and polyphenols was established by Xie et al. against the pentylenetetrazole-induced epilepsy model (Xie et al. 2012).

In addition, acetylcholinesterase (AChE) is considered a vital target in the management of Alzheimer's condition. Treatment with the methanolic extract of the *P. emblica* fruit showed a significant reduction in acetylcholinesterase activity along with improved DPPH scavenging activity with IC_{50} values of <100 μg/mL and < 10 μg/mL, respectively (Mathew and Subramanian 2014). Moreover, treatment of human neuroblastoma cells (SK-N-SH) with aqueous and methanolic extract of the fruit showed improved protection against H_2O_2-induced DNA damage and viability as seen by comet assay (Ramakrishna et al. 2014). More recently, Thenmozhi et al. showed that *P. emblica* when given in $AlCl_3$-intoxicated male Wistar rats, at a dose of 200 mg/kg for 2 months, it showed a significant reduction in acetylcholinesterase activity, amyloid precursor protein (APP), $A\beta_{42}$, β-secretase, and γ-secretase activity in the brain hippocampus and cortex area (Justin Thenmozhi et al. 2016a). Though these rats had deposition of the amyloid protein, they had recovered from the memory-learning and locomotor impairments caused by $AlCl_3$ (Justin Thenmozhi et al. 2016a).

Oxidative stress has been a vital factor in the progression of various ailments and neurological disorders. The use of *P. emblica* extract has been shown to dramatically reduce chronic unpredictable footshock-induced oxidative stress in rats (Bhattacharya et al. 2000). Also, this extract was able to protect against neuroleptic agent haloperidol-induced tardive dyskinesia, thus acting as a prophylactic neuroprotective agent (Bhattachary et al. 2000). An evaluation for its antidepressant activity by Dhingra et al. revealed that mice fed with the *P. emblica* extract had

lowered monoamine oxidase enzyme activity in the brain and inhibited affinity toward α_1- adrenoceptors, GABA-B receptors, serotonin receptors, and dopaminergic D_2-receptor (Dhingra et al. 2012). Also, the antioxidant potential of the *P. emblica* extract seems to protect rats against alcohol-induced brain mitochondrial dysfunction with lowered NO generation and protein carbonylation and improved endogenous antioxidant system and cytochrome C oxidase activity (Reddy et al. 2011). As stress is considered an aggravating factor for altered psychological state and behavior, it is worthwhile to know the key ingredients that can protect against it. Numerous studies revealed that chemical constituents such as flavonoids, tannins, and polyphenols, in particular, emblicanin-A, emblicanin-B, punigluconin, and pedunculagin, have an active pharmacological role in neuroleptic action (Bhattachary et al. 2000; Bhattacharya et al. 2000; Dhingra et al. 2012; Reddy et al. 2011). The use of a hydroalcoholic extract of *P. emblica* has been shown to reduce stress-induced elevated corticosterone levels in mice, thus likely contributing to improved mental health (Arun et al. 2018; Golechha et al. 2010). In another instance, where noise (100 dB noise for 4 h per day for 15 days) was used as an external stressor factor in albino rats, it was shown that treatment with *P. emblica* extract (333 mg/kg) can act as an anti-stressor with improved recovery from noise-induced immobilization and other behavioral alterations (Wankhar et al. 2014). Hence, several studies indicate that *P. emblica* has a nootropic potential and can be used as an adjunct therapy for the treatment of AD-like disorders (Kennedy and Scholey 2006; Hsieh et al. 2000). Also, herbal formulations containing *P. emblica* helped in correcting memory deficits induced by scopolamine and diazepam (Vasudevan and Parle 2007b). Therefore, phytoconstituents of *P. emblica* have a potential role in the management of AD-like conditions (Golechha et al. 2012; Vasudevan and Parle 2007a, b; Vinutha et al. 2007; Husain et al. 2019).

Dementia is a key symptom of neurodegenerative disorders associated with aging. AD has been one of the vital contributing factors leading to dementia, which is emerging as a health risk mainly in the older population. AD is characterized mainly by the deterioration of cognitive functions that are mostly due to the accumulation of extracellular amyloid-beta peptides (neuritic plaques) and intracellular neurofibrillary tangles (Albert et al. 2011; Hussain et al. 2018). Although the exact cause for AD is unknown, it is believed that oxidative stress due to free radicals generation, inflammation, impaired metabolic pathways, hyperlipidemia, and lowered cholinergic neurotransmission are important factors that trigger AD (Husain et al. 2017). Though there are treatments available such as the use of AChE inhibitors (donepezil) and NMDA receptor antagonist (memantine), these only provide symptomatic relief (Parsons et al. 2013; Agatonovic-Kustrin et al. 2018). Thus, there is a large gap and necessity for finding a valid solution to this emerging health problem.

Cholinergic dysfunction is found to be a vital cause of the pathophysiology of AD (Hampel et al. 2019). An in vitro study by Mathew and co-workers has shown that the methanolic extract of *P. emblica* fruit can inhibit AChE enzyme ($IC_{50} < 100$ μg/mL) and confer antioxidant activity as evident from 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity ($IC_{50} < 10$ μg/mL) (Mathew and Subramanian 2014).

Also, another study by Biswas et al., where dry fruit methanolic extract was used, found a significant inhibition in AChE (IC_{50} = 53.88 μg/mL) and butyrylcholinesterase (BuChE) (IC_{50} = 65.12 μg/mL) activities (Biswas et al. 2017). Likewise, in vivo studies in rodents with chemical-induced AD have supported a similar outcome. Thenmozhi and co-workers reported that the active principles from *P. emblica* fruit, mainly tannins, helped in a reversal in levels of AChE activity and amyloid-beta synthesis in various brain regions (Justin Thenmozhi et al. 2016a). Also, it was found that both unripe and ripe fruits of *P. emblica* were helping in increased levels of antioxidant enzymes within the brain, but unripe fruit extract had better potency than ripe fruit. Similarly, Uddin and co-workers observed that feeding *P. emblica* extract provided a concomitant decline in AChE activity followed by a better neurobehavioral performance in rodents, thus signifying a potential role as a treatment for AD (Uddin et al. 2016).

Hyperphosphorylation in the tau protein has been another factor for the pathogenesis of AD (LaFerla et al. 2007). Moreover, disruption in the signal transduction pathways such as Akt/GSK-3β is implicated to play a predominant role progression of AD (Jimenez et al. 2011). A study carried out on $AlCl_3$-induced toxicity and cognitive-deficit rats showed that feeding *P. emblica* extract given at 100 mg/kg for 60 days led to reduced oxidative stress and lower expression of apoptotic markers such as caspases 3 and 9, Bax, cytochrome c, and pTau. Also, alerted expression of glycogen synthase kinase-3β and Akt (phosphorylated) were observed (Justin Thenmozhi et al. 2016b; Singh et al. 2018). Husain and co-workers have recently shown that the increased expression of Nrf2-ARE pathway and NF-κB was a probable cause for oxidative stress and neuroinflammation, thus leading to cognitive disorder induced in rats by high-salt and cholesterol diet (Husain et al. 2018b). However, upon treatment with *P. emblica* extract containing tannin-enriched fraction, a significant improvement in the neurobehavioral parameters was observed (Husain et al. 2018a). Thus, further studies understanding the mechanistic role of *P. emblica* may hold potential in treating AD and associated neurological disorders.

4.4 Conclusion

In recent times, a lot of focus has now been given to find "natural" remedies, and therefore ethnopharmacological research has gained more attention. There is ample evidence and literature available that supports the medicinal property of *P. emblica* for various neurological disorders. Various extraction methods such as aqueous, alcoholic, and dried powder methods have been found their use in many studies. Also, molecular targets have been identified where *P. emblica* has been hypothesized to act, conferring neuroprotective action. However, the molecule/s that solely confer its pharmacological activity has not yet been deciphered. Hence, further research is required to identify specific chemical constituents that can be used for clinical therapy. Therefore, an exhaustive preclinical and clinical study is needed to

validate its use in traditional medicine and develop a sustainable clinical therapy for AD and other neurological disorders.

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Conflict of Interest The authors have no conflicts of interest to declare.

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Chapter 5
Role of the Gut Microbiome and Its Modulation in Neurodegenerative Diseases

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Abstract Neurodegenerative disorders are progressive conditions of the central nervous system (CNS), resulting in increased morbidity and mortality. The gut microbiome is a microecosystem that consists of billions of bacteria and fungi, most of which are of good benefit to the human body's internal milieu via regulating the immune system and controlling the neuronal signals intertwining the gut and the nervous system. Differences and deficiencies in the composition of the gut microbiota have been noted to occur in many chronic neurodegenerative CNS disorders like Alzheimer's disease, Parkinson's disease, frontotemporal dementia, etc. Multiple studies have been done so as to understand the best composition of the gut bacteria, and studies have also been undertaken to understand how we can tweak the gut by introducing probiotics and prebiotic compounds, which benefits the individual in reducing the chances of acquiring a neurodegenerative illness and may also help to control the progression of the diseases in those already afflicted. There are many ways by which the gut microbiome influences the CNS, including immune and hormonal pathways, short-chain fatty acid metabolism, modulation of the gut–brain axis, etc. An imbalance in the microbiome can have massive consequences for the host, resulting in faulty endocrine, immunological, and neuronal signaling that may accelerate the neurodegenerative process, culminating in debilitating diseases. Nutraceutical therapy using probiotics shows immense hope as prophylactic agents or adjunctive treatment strategy in the neurotherapeutics in this regard as it results in the homeostasis of the gut microbiome, which indirectly affects the CNS, resulting in slowing of the neurodegenerative process.

Keywords Neurodegenerative diseases · Probiotics · Gut microbiome · Short-chain fatty acids

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Abbreviations

5-HIAA	5-Hydroxy indole acetic acid
5-HT	5-hydroxytryptamine
Ach	Acetyl choline
AD	Alzheimer's disease
BBB	Blood–brain barrier
CNS	Central nervous system
ENS	Enteric nervous system
GABA	γ-Aminobutyric acid
GIT	Gastrointestinal tract
KP	Kynurenine pathways
PD	Parkinson's disease
SCFAs	Short-chain fatty acids
Spp.	Species

5.1 Introduction

The nervous system forms an important organ system in our body owing to its role as the supreme regulatory center in our body homeostasis. Neurodegenerative disorders are specifically characterized by a long-term, progressive loss of neuronal function attributed to an irreversible localized or generalized loss of neurons and their related tissues and neurotransmitters, thereby culminating in decreased and finally their functions. The commonest neurodegenerative disorders that affect different specific parts of the brain like Alzheimer's disease (AD), Parkinson's disease (PD), etc. are associated with long-term adverse outcomes and a huge physical and emotional burden to the patients, their families, and caregivers. Neurological disorders form the second leading group of illnesses with respect to mortalities. Neurological diseases are the second leading cause of illnesses contributing to long-term morbidity, as shown by the disability-adjusted life-years (DALYs) as well as the years lived with disability (YLDs) (Collaborators 2019). Among different neurological illnesses, the incidence of neurodegenerative disorders increased worldwide owing to an increase in the longevity of humanity as one of their attributable factors. The chronicity of many of the neuropathologies, the common progressive deteriorating nature of neurological illnesses, and a lack of curative treatment for most of these neurodegenerative disorders add up to the heavy load of neurological disorders on health care and its research.

Alzheimer's disease (AD) and Parkinson's disease (PD) are common neurodegenerative diseases. Other common conditions with neurodegeneration include Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and spinocerebellar ataxias (SCAs). Neurodegenerative changes are also found in other neurological conditions like multiple sclerosis (MS),

cerebrovascular accidents (CVAs), or stroke and traumatic brain injury (TBIs). Longevity, advances in medicine and its worldwide increase in access, environmental factors like pollution, pesticide use, change in dietary practices, etc. are a few attributable factors to the steady rise in numbers of patients with neurodegenerative disorders. Recent researches have been emphasizing the relationship between the gut microbiome and brain-health wherein the results clearly point out the altered gut microbiota dynamically altering the physiological milieu and its homeostasis, thus resulting in the pathological conditions of the brain (Chandra et al. 2020). This also emphasizes the beneficial role of prebiotics and probiotics in the prevention and treatment of not only gut disorders but also the neurodegenerative disorders that are typically characterized by their chronicity and lack of therapeutic agents aimed to prevent onset and progression (Peterson 2020).

The enteric nervous system of our gastrointestinal tract has long been considered the "second brain of the human body" owing to its diverse relation with mood regulation, stress, anxiety, eating patterns, etc. There have been obvious associations between neurodevelopmental disorders and gastrointestinal (GI) issues, as seen in autism, Down's syndrome, cerebral palsy (CP), etc. Hepatic encephalopathy, Parkinson's disease, irritable bowel syndrome (IBS), and epilepsy are yet other conditions that connect the digestive system with neurological illnesses (Lynch and Pedersen 2016; Wu et al. 2016).

There is an expansion in the knowledge of the role of the gut microbiome, its qualitative and quantitative variations affecting our neurophysiological well-being, and its disruption contributing to various neuropathologies like neurodegenerative disorders, especially on their onset and progression (Ceppa et al. 2020). The microbial coexistence in the human gut is a symbiotic consortium formed by series of changes that happened during its co-evolution with the host and has time and again proven its beneficial role in normal physiology, including Vitamin K and B synthesis, immunoregulation, and protection from pathogens, digestion, and absorption of nutrients. Our complex human body has as many bacteria as that of our body cells, and the revelation that these microorganisms are metabolically active and can potentially alter our neurological dynamics leads to an uproar in the research interest regarding the gut–microbiota–brain axis and the microbial-derived neurochemicals that are implicated in the biological basis of neurodegenerative disorders.

Diet plays a crucial role in setting up our gut microbiome. Prebiotics are those compounds we eat that stimulate and flourish the growth and activity of the microbial flora. Scientists, medical professionals, and the common folk are aware of the presence of microorganisms that are relevant in health and disease. Microbiologists have isolated thousands of bacteria that have varied responses on the human body, sometimes even resulting in disease and death. In the last 100 years, there has been a revolution in new information acquired, which has advanced our understanding of how gut bacteria positively affects the health of the individual.

5.1.1 Gut Microbiome and the Pre-, Pro-, and Synbiotics

In the early 1800s, during those dark times when death and disease plagued the whole of Europe, it was noted with interest that people in a remote valley in Bulgaria were pretty much unaffected and were in better health. This was largely postulated due to their consumption of fermented milk and yogurt on a daily basis. In 1905, Dr. Stamen Grigorov, a Bulgarian scientist, found the agent causing this fermentation and named it *Lactobacillus bulgaricus*. Interested in this development, Dr. Ellie Metchnikoff, a Nobel Prize winning scientist from Russia, later did research on *L. bulgaricus* and discovered that people in Bulgaria lived much longer than other European countries. He attributed this longevity to Bulgaria's favorite food—yogurt. Yogurt intake resulted in a favorable health advantage conferred to the individual by the gut bacteria, which modified the gut microbiome into one that was less prone to diseases.

Over the years, scientists have come across and categorized products, bacterial and nonbacterial that enhance the gut health of the individual via indirect means. They are probiotics, prebiotics, and synbiotics.

Probiotics are live microbes that are taken in the belief that they have conferred health benefits to the individual. This is mostly by improving or restoring gut flora resulting in favorable metabolic action.

Prebiotics are compounds that ensure that a milieu in the gut is created as such, which results in a thriving growth of good bacteria and fungi, thereby improving the individuals' health.

Synbiotics is a term used for an appropriate mixture of pre- and probiotics, thereby aiming at a synergistic combination of the two. This results in much more balanced gut microbiota.

5.1.2 Gut–Brain Axis

Over the centuries of evolution, over 1000 species of bacteria have colonized 400 m^2 of the human gut, and the gist of this complex symbiosis is still not fully studied (Ley et al. 2006). There are 10^{13} to 10^{14} bacteria in the colon (Bäckhed et al. 2005), and their species mainly comprise Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria (Eckburg et al. 2005). Research shows, without doubt, a definite connection between the gut and the brain through complex neurohormonal pathways called the gut–brain axis. The digestive system has about 100 million neurons, almost half of that in the central nervous system. This system of gut neurons is called the enteric nervous system (ENS) and in turn is mainly mediated by vagus nerve, the Xth cranial nerve.

There are three basic means through which the GIT interacts with the central nervous system (CNS), which is via direct neuronal action, chemical neurohormonal mechanisms, and finally the immune system. Multiple studies have pointed out the

bidirectional dynamic interaction between the brain and the alimentary canal mediated through conglomerate mechanisms involving the intestinal microflora, autonomic nervous system comprising the parasympathetic and sympathetic neuronal axis, endocrine regulation by neuroendocrinal and enteroendocrine pathways, humoral and cytokine-mediated mechanisms, and via other signaling molecules and neuropeptides (O'Mahony et al. 2015).

The enteric nervous system (ENS) is quite often considered as our second brain, and many neurotransmitters like serotonin, dopamine, and GABA are produced in the gut. Serotonin or 5-hydroxy tryptamine (5HT) is referred to as a vital neurotransmitter in the brain, and 90% of it is produced in the GIT. Its importance in neuronal development, homeostasis, and sustenance are irrefutable. The neurochemical homeostasis is maintained by a complex interaction between the neurotransmitters like serotonin, dopamine, γ-aminobutyric acid (GABA), and acetylcholine. Currently, there are many medical compounds that target homeostasis between 5HT, DA, GABA, and noradrenaline so as to attain an improvement in neurological status.

Neurodegenerative diseases are a group of incurable and debilitating neurological diseases, resulting in progressive neuronal death and dysfunction. These cause problems in movement and cognition affecting the patient's ability to move, think, and even breathe. Due to their salutary effects on the gut–brain axis, which has resulted in improved mood, cognition, and general neuronal well-being, pre-, pro-, and synbiotics are being increasingly considered as a therapeutic modality to prevent and treat neurodegenerative disorders.

5.2 Gut Dysbiosis and the Aging Gut

A vast plethora of bacteria form the gut microbiota that reside within the gastrointestinal tract (GIT). A delicate balance between various microbes is maintained, which is important in the normal functioning of the GIT. There stays a homeostatic balance in maintaining the relative microbial population in the gut and is considered that when this homeostasis is deranged, there occurs an imbalance in the usual feedback loop to CNS or the central nervous system that affects the inflammatory modulation in the brain, an increased oxidative stress, and thus an accelerated neuronal degeneration (Westfall et al. 2017). Dysfunction of the gut microbiome is considered to contribute to depression, anxiety, and neurodegenerative disorders. The gastrointestinal hormones also take part in energy homeostasis mediated via gut-related hormones like insulin and glucagon, taking part primarily in glucose metabolism; leptin, and ghrelin are involved with satiety and hunger, whereas glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are involved in growth, repair, and development (Hansotia and Drucker 2005). This highlights the role of insulin, insulin-like peptides, and somatomedins in mitigating neurodegeneration and stresses the GI connection with neurodegenerative disorders.

Aging is associated with alterations in the GI microflora, which culminates in a lack of microbial diversity in the intestines (Hansotia and Drucker 2005). Relative changes in the concentrations of the phyla Bacteroides and Firmicutes are also significant as they form the dominant gut flora (Hansotia and Drucker 2005). Concentrations of pathogenic bacteria would increase at the expense of the symbiotic ones. These changes are known to elicit inflammatory responses that may manifest in sites distant from the GIT, including the CNS.

5.2.1 Gut Microbiome and Pathophysiology of Neurodegenerative Diseases

Many neurodegenerative disorders occur due to an interaction between genetic predisposition, cellular aging, and environmental toxins. In addition to these, current lifestyle choices, especially dietary habits and sedentary lifestyle, can induce physical and mental stress, thereby worsening oxidative stress and the pathophysiological changes associated with it. Microglial activation is known to be modulated by the gastrointestinal microbiota, which is one of the key factors in the etiopathogenesis of many neurodegenerative disorders. Research suggests that GI milieu modification with short-chain fatty acids (SCFAs) producing bacteria is of beneficial value in improving the gut influence on the brain and neurohormonal and immune activation of the CNS.

Neurodegeneration is the common hallmark in chronically progressive conditions like Alzheimer's disease (AD) and Parkinson's disease (PD), which have increased incidence due to longevity. Other neurodegenerative conditions include amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Huntington's disease (HD), and spinocerebellar ataxias (SCAs). Though they have varied manifestations, the basic similar pathology in all found is associated with irregularities being noted in increased oxidative stress, protein folding, and reactive oxygen species (ROS) activation, culminating in cellular inflammation, accelerated neuronal aging, and death. The chronicity of many of the neuropathologies, progressively deteriorating the nature of neurodegenerative disorders, and a lack of curative treatment add up to the extensive search and gut microbiome modification, and modulation of the gut–brain axis offers a solid therapeutic potential wherein the early identification and intervention, especially at high-risk cases, can go a long way in prevention and treatment of neurodegenerative diseases (11) (Fig. 5.1).

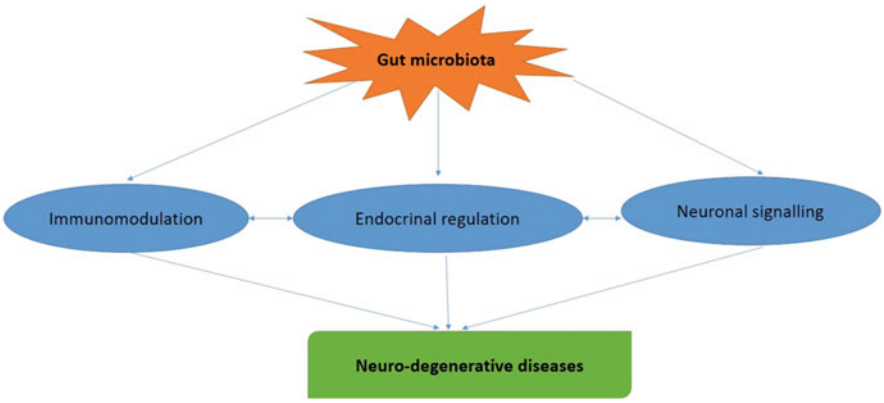

Fig. 5.1 Gut microbiome influencing the neurodegenerative disorders

5.2.2 *Biomolecules in the CNS that Are Targets of Pre/Probiotics*

Nonpathogenic gut microbes secrete multiple neurohormonal mediators implicated in neurological homeostasis. Extensive hormonal and immune cascades connecting the GIT and CNS exist, which co-regulate key processes. A well-balanced microbiome may contribute not just toward good GI health but also to neurological well-being. In this chapter, we would like to discuss the factors affecting the gut–brain axis and a few of the many gut microbiome-altering agents, which may in the future find a use for the early prevention and timely intervention in neurodegenerative diseases. An increase in a specific genus of microbes called *Proteobacteria* spp., a reduction in the numbers of *Bifidobacterium* spp. and species producing butyrate like *Ruminococcus* spp., *Faecalibacterium* spp., etc., and a rise in numbers of the commonly considered pathogenic organisms like *Escherichia* spp., *Enterobacteriaceae* spp., *Bacteroides*, *Clostridium difficile*, etc. can all lead to an activated immune response (Hansotia and Drucker 2005; Westfall et al. 2017). Hence, it is clearly observed that an abundance in the pathogenic bacteria compared to the symbiotic commensals is commonly found and can also modulate neuroinflammation (Table 5.1).

5.2.3 *Gut-Derived Ferulic Acid*

Ferulic acid (FA) is a plant-derived substance found in cereals, vegetables, and fruits. Multiple studies have found this polyphenol, FA, to be a potent inhibitor of ROS pathways and hence has anti-inflammatory properties (Mancuso and Santangelo 2014). FA is well recognized as a free radical scavenger and finds use in various medical conditions, including neurodegenerative conditions. FA can

Table 5.1 Neuromodulatory factors regulated by gut microbiome

Neurotransmitter production
Tryptophan metabolism and kynurenine pathway
Suppression of pathogenic gut bacteria
Immunomodulation and cytokines
Enteric nervous system and vagus mediated
Neurohormonal axis E.g., ghrelin, hypothalamic-pituitary axis
Short-chain fatty acids
Modulation of BDNF (brain-derived neurotrophic factor)

directly stimulate neuronal stem cells, thereby preventing untimely neuronal death, and may also be implicated in neuronal regeneration (Yabe et al. 2010). Current studies have pointed that FA is a key regulator between the activities compassing the commensal gut microbes and the brain (Yu 2011). Apart from the aforementioned natural sources of FA, certain commensal bacteria in the gut, namely *L. fermentum* and *B. animalis*, can synthesize FA because they possess the ferulic acid esterase enzyme (Tomaro-Duchesneau et al. 2012). Hence the supplementation of the diet with these probiotics results in achieving a protective level of ferulic acid, thereby protecting against neurological ailments, including chronic debilitating conditions associated with AD, PD, traumatic brain injury, stroke, etc., through its antioxidant and antiapoptotic action (Cheng et al. 2016; Durairajan et al. 2008; Mori et al. 2013; Srinivasan et al. 2007; Yan et al. 2013; Zhang et al. 2015).

5.2.4 Short-Chain Fatty Acids (SCFAs)

Colonic bacteria produce a good number of major metabolites that exert influence via the gut–brain axis. Soluble fibers such as fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) undergo fermentation, thereby producing a wide variety of various metabolites and have a significant role in the modulation of gut bacteria, thereby exerting an influence on the brain (Kasubuchi et al. 2015). GOS and FOS are prebiotics that provide a favorable milieu for the population of the gut with the beneficial bacteria (den Besten et al. 2013). Other prebiotic SCFAs like acetate (Fushimi et al. 2006), propionate (Todesco et al. 1991), and butyrate (Brahe et al. 2013; Gao et al. 2009) are formed in the gut in ample quantities by fermentation of fiber facilitated by a large number of nonpathogenic bacteria. These commensal bacteria mainly include *Bacteroides* spp., *Bifidobacterium* spp., *Propionibacterium* spp., *Eubacterium* spp., *Lactobacillus* spp., *Clostridium* spp., *Roseburia* spp., and *Prevotella* spp. (Tagliabue and Elli 2013; Verbeke et al. 2015; Westfall et al. 2017). The gut microbes belonging to phyla of Firmicutes, particularly of the genera called *Roseburia*, *Eubacterium*, etc., and Lachnospiraceae including *Clostridia* spp. also produce butyrate actively, whereas species like *Bifidobacteria* generate lactate and acetate (Pérez-Reytor et al. 2021). The role of SCFAs in the well-being of intestinal

health has long been known, and further studies have shown light to how they influence the normal physiology of other organ systems.

SCFAs are beneficial to the host by multiple means, including the regulation of metabolic activities. From the gut, it is often transported to the central circulation with specific benefits in lipid, glucose, and cholesterol metabolisms (Pérez-Reytor et al. 2021). They are well-known potent anti-inflammatory agents. SCFAs are considered to affect the synthesis of many neurotransmitters and the expression of receptors to major neurotransmitters like nicotinic and GABA receptors (Lacassagne and Kessler 2000). The SCFAs, propionate, and butyrate are known to be depleted in the vagal nerve (Lal et al. 2001; Sjögren et al. 2002) in the common neurodegenerative disorders like AD and PD (Merrill et al. 2006). Improving SCFA concentration in the gut via means of supplementation of the necessary pre- and probiotics (FOS, GOS, Bifidobacteria, Lactobacilli, etc.) can be a step forward in tackling difficult-to-treat neurodegenerative disorders.

5.2.5 Microbiota-Modulated Ghrelin

Ghrelin is a peptide molecule produced mainly in the stomach, which stimulates appetite and growth hormone secretion. Ghrelin is an important neurohormone involved in neuromodulation and elicits an important role in metabolism, hunger, energy homeostasis, and neuroinflammation (Merrill et al. 2006). The role of the hunger hormone in neurodegeneration is of great emphasis as it has been shown to exert neuroprotective effects in conditions like Alzheimer's and Parkinsonism (Merrill et al. 2006). An experimental research study wherein rats were given pre/probiotic agents as nutritional supplementing agents depicts an obvious relationship between the hormone ghrelin and the GI microbiome in rats (Zhang et al. 2009). The ghrelin secreting abilities of gut bacteria give hope that a ghrelin-specific pre/probiotic mixture is of promising therapeutic potential in the future for the treatment of neurodegenerative conditions.

5.2.6 Serotonin, Tryptophan, and Kynurenine Pathway (KP)

Serotonin is a key neuromodulatory player in the GIT. Its effects include affecting GI secretion, bowel movement or peristalsis, dilation of blood vessels or vasodilation, and perception of pain and nausea. It exerts these multiple effects via a large variety of 5-HT receptors. Tryptophan absorbed from the GIT enters systemic circulation, then crosses the blood–brain barrier (BBB), and then initiates the production of serotonin, thereby demonstrating its action in the CNS. This emphasizes the importance of intestinal metabolism of the amino acid tryptophan and its importance for the serotonin-mediated signaling pathways in the brain (Zhang et al. 2009). It is via the KP that catabolism of tryptophan takes place. Irregularities involving the

serotonin and tryptophan metabolism influence the incidence of neurodegenerative diseases like Alzheimer's disease, memory disturbances, dementia, Huntington's disease, etc. (Fukumoto et al. 2003; Verbeke et al. 2015).

The type of commensal bacteria in the GI microbiome influences the metabolism of tryptophan and thereby elicits a significant effect of the KP (Reigstad et al. 2015). A few experimental studies done using rats demonstrated that administration of *B. infantis* reduced 5-HIAA (5-hydroxy indole acetic acid) levels (Desbonnet et al. 2008). 5-HIAA is an important product of serotonin metabolism. It is also regarded as a reliable marker that is found abundant in the frontal cortex along with increased plasma tryptophan levels too (Westfall et al. 2017; Xu et al. 2018; Yahfoufi et al. 2020). When 90% of 5HT is produced in the GIT, the influence of the gut microbiota on its breakdown seems highly relevant. Hence, it stands to reason that administering probiotics as part of the treatment strategy is beneficial in regulating KP dynamics and would be of major benefits when given as part of the neurotherapeutics as prophylaxis and to mitigate the progression in patients with neurodegenerative disorders (Thomas et al. 2012).

5.3 Neurometabolites and the GIT

Neurometabolites are compounds that include "neurotransmitters that act directly on CNS signaling cascades and through other biochemical effectors that have direct or indirect implications on CNS activities" (Westfall et al. 2017). Deficiencies of neurotransmitters have historically been noted to result in neurodegenerative disorders. Nonpathogenic commensals like *Lactobacillus* and *Bifidobacterium* strains produce significant amounts of GABA, which is an inhibitory neurotransmitter. Alterations in neurotransmitter levels are found to be associated with behavioral changes and can also manifest as an increase in dystonia and spontaneous motor activity due to increased levels of neurotransmitters like dopamine (DA), noradrenaline (NA), and serotonin in the striatum of the basal ganglia, which may be beneficial in neurodegenerative movement disorders like PD. Various probiotics have been shown to directly secrete neurotransmitters into the bloodstream, which crosses the BBB and increases the neurotransmitter concentration in the synaptic clefts and vesicles (Barrett et al. 2012).

5.4 Conclusion

The gut microbiome is a very important part of the gut–brain axis, which modulates the functional interaction between these two very important organ systems. An imbalance in the microbiome can have far-reaching consequences resulting in faulty endocrine, immunological, and neuronal signaling that may accelerate the age-dependent neurodegenerative process, which can culminate in debilitating

diseases. Biological therapy using probiotics shows immense potential as therapeutic or prophylactic agents against neurodegenerative disease as it results in the homeostasis of the gut microbiome, which indirectly affects the CNS, resulting in slowing of the neurodegenerative process. However, presently, our knowledge of gut bacteria and the CNS is still in its infancy. Larger studies incorporating subjects suffering from neurodegenerative diseases and the effect of pre/pro/synbiotics in them should be taken up so that new avenues can open up in the treatment of these prognostically bleak diseases.

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Chapter 6
Recent Advances in Application of Dietary Polyphenols to Treat Age-Related Neurological Disorders

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Abstract One of the main causes of synaptic damage and impaired neural transmission in numerous neurodegenerative disorders is the progressive buildup of misfolded amyloid proteins in intracellular and extracellular areas. Effective therapies for these illnesses are still unavailable, although they are nevertheless the subject of intense research. Despite decades of study, just a few synthetic chemicals, small molecules, and medicines have been found to prevent amyloid protein aggregation and reduce their neurotoxic effects. Antioxidants, particularly those derived from food, have been proposed as potential medicines for the prevention and treatment of Alzheimer's disease in recent years. Polyphenols are known to lower the risk of neurological disorders such as Alzheimer's disease (AD), stroke, multiple sclerosis (MS), Parkinson's disease (PD), and Huntington's disease (HD) through reducing oxidative stress. Polyphenols have the ability to address the genesis of neurological diseases by attenuating their complicated physiology by simultaneously regulating several therapeutic targets. Several research published in recent years aimed to verify sensitive and reliable translational techniques for mechanistically characterizing brain bioavailable polyphenols as disease-modifying drugs to help prevent the onset of Alzheimer's disease and other neurodegenerative disorders. Several research groups from around the globe with expert knowledge in Alzheimer's disease, plant biology, nutritional sciences, and botanical sciences have published high-quality study results that have finally provided the necessary evidence that polyphenols and their metabolites, which can be found in a variety of foods, can help to prevent Alzheimer's disease. The studies mentioned in this review article back up the findings of recent research that have had a significant influence on neurodegenerative disorders by giving crucial information on polyphenols' protective functions. Despite the fact that current polyphenol research has had minimal influence on clinical practice, there is significant evidence and testable hypothesis that they can contribute to therapeutic advancements and therapeutic discovery in the field of age-related neurological diseases.

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Keywords Polyphenols · Neurodegenerative disease · Alzheimer's disease · Multiple sclerosis · Parkinson's disease

Abbreviations

AD	Alzheimer's disease
BDPP	Bioactive dietary polyphenol preparation
EGCG	Epigallocatechin-3 gallate
GPx	Glutathione peroxidase
GR	Glutathione reductase
HD	Huntington's disease
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MCI	Mild cognitive impairment
MS	Multiple sclerosis
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PD	Parkinson's disease
Syn	α-Synuclein

6.1 Introduction

Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, and other neurodegenerative illnesses are only a few examples. Because their instances are sporadic, determining the cause of these diseases and preventing them are difficult. Many neurological disorders and age-related degenerative processes have been linked to oxidative stress. Neurodegenerative disorders have a complicated etiology involving a complex combination of hereditary and environmental variables. The bulk of these instances are of an environmental or sporadic nature. The most prevalent neurodegenerative illnesses are Alzheimer's disease (AD) and Parkinson's disease (PD). They are complex, progressive, age-related, and genetically and environmentally affected. Despite the fact that they are public health issues that have been extensively researched, there are no viable remedies. Currently, treatments are only symptomatic and aimed at improving patients' quality of life (Przedborski et al. 2003). Furthermore, there are no diagnostic techniques for early diagnosis of these illnesses, which share several clinical characteristics, particularly at the start. Specific proteins have been linked to the diseases, but it is unknown when and how they lose their function and become abnormal and toxic. Several routes of cellular malfunction have been put forward to explain the disease toxicity; however, the pathological significance of the proteins implicated is still debated. Personalized therapies and targeted medicines are by far the most efficient treatment methods. Alternative pharmacological therapies and natural compounds,

particularly those linked with the Mediterranean diet, such as polyphenols, have sparked fresh attention in the recent decade. Polyphenols' surprising advantages and broad range of characteristics imply that further research is needed to have a better understanding of their mechanism of action and to employ them in further successful treatments (Bagetta et al. 2020).

Because it uses so much oxygen for energy and has so few antioxidant defense enzymes, the brain is particularly sensitive to oxidative damage, especially as it ages. Furthermore, the membranes of brain cells contain very high levels of polyunsaturated fatty acids (PUFAs). Neurons are particularly prone to toxic chemicals and to damage caused by ischemia/stroke, seizures, and other excitotoxic injuries among the many kinds of brain cells (Milatovic et al. 2009). Lipid peroxidation (oxidative damage to lipids) is linked to a gradual loss of membrane integrity, a decrease in mitochondrial membrane potential, and an increase in plasma membrane permeability to Ca^{2+}. Carbonyl and nitrosylated derivatives are formed when proteins are damaged by oxidation. In addition, ROS damage to DNA causes nuclear condensation and changes in gene expression. Different reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl and peroxyl radicals, are generated in cells under normal and pathological circumstances, according to research (Valls et al. 2005). Oxidative damage to DNA, proteins, and lipids occurs when the rate of ROS production surpasses the capability of antioxidant defense. Oxidative stress has been linked to neuronal cell damage in the central nervous system (CNS) in a variety of disease conditions. The term "nitrosative stress" has lately been coined to describe cellular damage caused by reactive nitrogen species (RNS), which would include nitric oxide (NO) and its derivative products such as peroxynitrite and nitroxyl anion (Klatt and Lamas 2000). Oxidative and nitrosative stresses are both involved in the pathology of many neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD).

In in vitro and in vivo models of neurodegeneration, many antioxidant substances produced from natural products (nutraceuticals) have shown neuroprotective effects: (1) flavonoid polyphenols, such as epigallocatechin 3-gallate (EGCG) and quercetin; (2) non-flavonoid polyphenols, such as curcumin and resveratrol; (3) phenolic acids or phenolic diterpenes, such as rosmarinic acid or carnosic acid; and (4) organosulfur compounds, such as isothiocyanate, L-sulforaphane, and thiosulfinate (allicin) (Kelsey et al. 2010). They directly scavenge free radicals or indirectly boost endogenous antioxidant defenses by activating the nuclear factor erythroid-derived 2-related factor 2 (Nrf2) transcription factor pathways, for example. The intrinsic free radical scavenging activities of these nutraceutical antioxidants suggest that they may have potential utility in mitigating neuronal oxidative stress and neurodegeneration as shown in the Fig. 6.1. Other mechanisms of action of these compounds include modulation of signal transduction cascades or effect on gene expression (Tuñón et al. 2009). The following sections will be discussing the mechanism of action of polyphenols to help in treating neurodegenerative disorders.

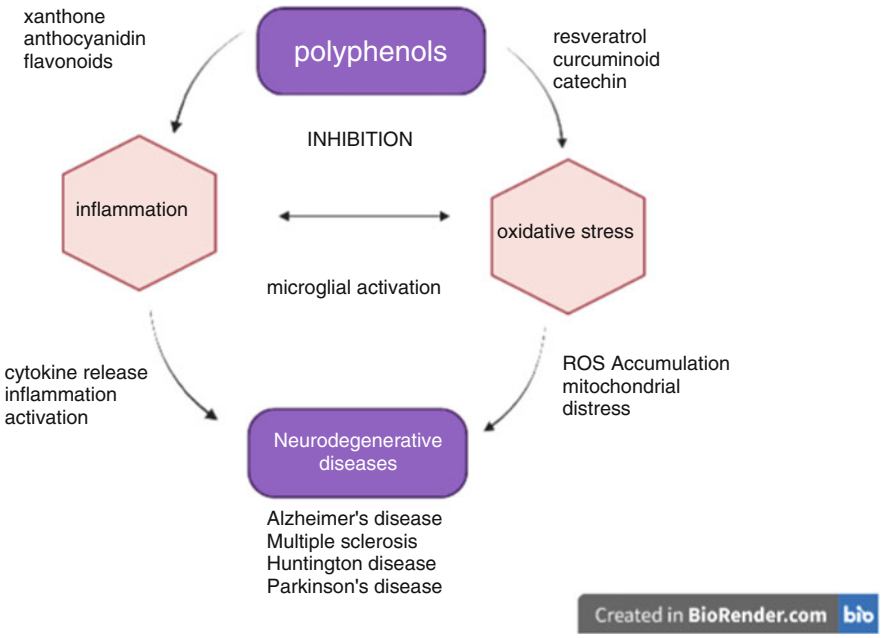

Fig. 6.1 Role of polyphenols in neurodegenerative disease treatment

6.2 Introduction to Neurodegenerative Disorders

6.2.1 Alzheimer's Disease (AD)

Alzheimer's disease is marked by a progressive decrease of cognitive function, memory loss, and behavioral abnormalities. A synaptic deficiency in the neocortex and limbic system, neuronal loss, white matter loss, astrogliosis, microglial cell proliferation, and oxidative stress are all common symptoms of the illness. The appearance of intracellular flame-shaped neurofibrillary tangles with extracellular plaques in the brain is a pathological marker of Alzheimer's disease (Stutzmann 2007). The Tau protein, in a hyperphosphorylated state, is the most common cause of tangle formation in the perinuclear cytoplasm. The plaques are caused by the accumulation of amyloid-β-peptide (Aβ) in a filamentous form throughout time. The neuritic plaques range in size from 10 to more than 120 μm in diameter. The procedures for determining the pathology's diagnostics have been standardized. The number and degree of compactness of neuritis amyloid plaques and neurofibrillary tangles are discussed. As a result of their location and development, AD aggregates can be categorized as positive or negative lesions. Amyloid plaques and neurofibrillary tangles, neuropil threads, and dystrophic neurites, all of which are fundamentally generated by hyperphosphorylated Tau, are typical positive lesions. Neurons and neuropil threads are lost in the negative lesions (Selkoe 1991).

6.2.2 Parkinson's Disease (PD)

Motor symptoms such as bradykinesia, stiffness, resting tremor, and instability are common in people with Parkinson's disease. Because there is no conclusive test for the diagnosis of Parkinson's disease, the emergence of these clinical symptoms is critical for the illness's early treatment. The loss of dopaminergic neurons in the *Substantia nigra pars compacta* and the deposition of intraneuronal proteinaceous clumps, primarily formed of α-synuclein (Syn), known as Lewy bodies and Lewy neurites, are hallmarks of Parkinson's disease (Taylor and Saintcyr 1995). Syn was also discovered in the Lewy body form of Alzheimer's disease and multiple system atrophy pathological inclusions. Syn inclusions are also seen in other neurodegenerative illnesses known as synucleinopathies, including Down's syndrome, progressive autonomic failure, and familial and sporadic Alzheimer's disease. Shahmoradian and colleagues have shown that Lewy bodies are produced not only by Syn deposits but also by clusters of lipid vesicles. These significant findings show a link between Syn–lipid interaction and neurodegeneration (Halliday and McCann 2010).

6.2.3 Multiple Sclerosis (MS)

MS is a systemic illness that affects the central nervous system's gray and white matter. Inflammatory and degenerative mechanisms occur simultaneously, and the balance between them determines whether the illness is relapsing–remitting or progressive. It is unclear if basic progressive MS is a distinct disease entity or just a disease phenotype without high-grade inflammatory processes. The same dilemma applies to very active MS patients with tumefactive lesions, and the question is if there is a molecular key that defines whether an immune response is overwhelming or subtle (Stadelmann 2011). The existence of localized white matter lesions, characterized by initial demyelination with partial preservation of axons and reactive astrocytic scar formation, was originally used to describe its pathophysiology. Although, in comparison to full demyelination, axons are relatively well maintained inside the lesions, they are nonetheless damaged, and axonal loss has been proven to be a key predictor of persistent neurological impairment in MS patients. When patients have clinical relapses and remissions in the early stages of the disease, inflammatory demyelination causes localized plaques to develop, which are mostly seen in the white matter. Additional pathology is found in later stages of the disease, especially in individuals with secondary or main progressive disease, such as extensive demyelination in the cerebral and cerebellar cortex, as well as diffuse degenerative alterations throughout the white and gray matter (Lassmann 2018). Patients with long-term severe illness eventually have significant brain and spinal cord atrophy, as well as widespread tissue loss and cerebral ventricle dilation. While the illness begins with inflammatory-driven localized demyelinating lesions that

develop around tiny drainage veins in the white matter, it eventually progresses to widespread neurodegeneration that affects the whole CNS (Lassmann et al. 2012).

6.2.4 *Huntington's Disease (HD)*

Huntington's disease (HD) is a neurological illness that causes mobility problems, cognitive decline, and mental symptoms. A (CAG)n trinucleotide repeat expansion near the 5′ end of a gene that codes for the huntingtin protein causes it as listed in the Table 6.1 (van der Burg et al. 2009). The repetitions induce illness by providing a new detrimental function on huntingtin, which is translated into an abnormally enlarged polyglutamine tract. The pathophysiology of HD is characterized by the neuronal loss that is selective, with the striatum and deeper layers of the cerebral cortex suffering the most. The development of intraneuronal inclusions or aggregates is linked to the illness. With a clinical course of >15–20 years, HD causes catastrophic brain atrophy and death (Vonsattel and DiFiglia 1998). The striatal medium spiny neurons (MSNs) of the brain appear to be particularly susceptible in HD, while other brain areas may also be impacted. GABAergic MSNs are found in the striatum and project to the substantia nigra (striatonigral) and globus pallidus (striatopallidal). Even though the specific causes for this selective susceptibility and loss of striatal MSNs are unknown, it has been observed that HD patients lose around 88% of their striatal neurons as compared to healthy persons (Holley et al. 2018).

6.3 Introduction to Polyphenols

Polyphenols are substances having at least two hydroxyl groups linked to one or more phenyl rings. Thousands of polyphenolic compounds exist in plants, each with several alternative double bonds, hydroxyl groups, and more than one phenyl ring.

Table 6.1 Age-associated neurodegenerative diseases and their associated protein

Disease.	Affected region	Protein aggregated	Deposition	References
Alzheimer's disease	Cerebral cortex, hippocampus	Amyloid-beta, tau	Intracellular tangles and extracellular plaques	Selkoe (1991)
Huntington's disease	Striatum, cerebral cortex	Huntingtin	Intracellular nuclear inclusions	Ghosh and Tabrizi (2018)
Parkinson's disease	Substantia nigra	Alpha-synuclein	Intracellular cytoplasmic inclusions	Taylor and Saintcyr (1995)
Multiple sclerosis	CNS: Gray and white matter	Amyloid precursor, tau	Cerebrospinal fluid lesions and plaques	Stadelmann (2011)

Table 6.2 Major polyphenols and their characteristics (Han et al. 2007)

Polyphenols	Dietary form	Chemical nature	Source of availability
Flavanols	Catechin, epicatechin EGCG	Methyl sulfate conjugates; EGCG in unconjugated forms	Teas, apple pears, chocolate
Curcuminoid	Curcumin	Desmethoxycurcumin, bismethoxycurcumin	Turmeric
Anthocyanidins	Cyanidin, malvidin	Glucosides	Red, blue, purple berries
Stilbene	Resveratrol	Glucuronides, sulfate conjugates	Purple grape, redwine, peanut
Dihydrochalcone	Aspalathin	2,3,4,4-pentahydroxy-3-C-β-d-glucopyranosyl dihydrochalcone	Legumes
Xanthones	Xanthones, mangiferin, and isomangiferin	Tricyclic aromatic system	Honeybush tea

Anthocyanins, flavanols, flavones, isoflavones, stilbenes, and lignans are only a few examples of plant-derived polyphenols (Scalbert et al. 2005). Polyphenols are secondary metabolites that are considered to protect plants from radiation, infections, and other stresses. In edible plant components such as fruit, leaf, stem, root, and seed, over 500 polyphenolic chemicals have been discovered of which, major are listed in the Table 6.2. Preclinical, clinical, and epidemiological research shows that a plant-based diet, with a concentration on fruits and vegetables, decreases the risk of neurodegenerative disorders substantially. Polyphenolic chemicals found in edible plants are a vital class of micronutrients that contribute to the health advantages of a diet rich in fruits and vegetables (Dai and Mumper 2010). Polyphenols have a lot of antioxidant characteristics, including the capacity to scavenge free radicals and boost the production of antioxidant enzymes, according to a lot of data. Polyphenols have also been demonstrated to reduce the production of pro-inflammatory cytokines and inhibit the inflammatory cascade. Dietary polyphenols' antioxidant and anti-inflammatory properties lower tissue damage risk and contribute to health benefits in age-related chronic illnesses (Oliviero et al. 2018). Polyphenols have been shown to affect cellular signaling mechanisms and signal transduction pathways associated with oxidative stress, redox modulation, the inflammatory cascade, cell proliferation and migration, and a variety of other processes linked to the pathogenesis and progression of chronic diseases (Kang et al. 2019). Several important dietary polyphenols have been studied in preclinical in vitro and in vivo models of chronic illnesses during the last decade. Green tea catechins and blacktea theaflavins (found in green tea and black tea), curcuminoids (found in the curry spice turmeric), anthocyanins (found primarily in berries), and resveratrol (found in red wine, grapes, and peanuts) have all been studied for their therapeutic potential in the treatment of age-related chronic diseases (Hano and Tungmunnithum 2020).

6.3.1 Tea Polyphenols

After water, tea, made from the leaves of the *Camellia sinensis* plant, is the most frequently consumed beverage on the planet. The main difference between white, green, oolong, and black teas is the degree of processing throughout the production process, which largely consists of drying and fermenting. The most significant polyphenols in tea are catechins and theaflavins, whose amounts vary according to the degree of oxidative fermentation (Xing et al. 2019). Catechins are found in the greatest concentrations in nonfermented white and green teas, which dimerize to create theaflavins when oxidized. Black tea, which is made by fermenting tea leaves, is high in theaflavins, which are orange-red pigments that give it its color. Oolong tea is a hybrid of green and black teas that have been partially fermented (Kamat et al. 2008). Along with other catechins and theaflavins, epigallocatechin-3 gallate (EGCG), the most famous and well-known tea polyphenol, has been widely investigated in preclinical models of chronic illness and clinical trials. EGCG and other tea polyphenols have been found to have potent antioxidant and anti-inflammatory properties, as well as modulatory action for a variety of disease progression signaling pathways (Williams et al. 2004).

6.3.2 Curcuminoids

Turmeric is a rhizome (*Curcuma longa*) that grows in tropical South Asia and is also known as "Indian saffron." Turmeric's therapeutic properties are owed to the intensely yellow curcuminoid polyphenols curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin, which have shown therapeutic potential in a variety of age-related chronic ailments, including autoimmune, cardiovascular, cancer, and neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (Sharma et al. 2005). Curcumin, the main curcuminoid found in turmeric, is one of the most researched dietary compounds, with strong antioxidant and anti-inflammatory effects as well as the capacity to influence a variety of signaling pathways (Tønnesen and Karlsen 1985).

6.3.3 Anthocyanins

Anthocyanins are a category of water-soluble pigments found in a variety of flowers, fruits, and vegetables. They are present in large amounts in several popular berry fruits of the genus *Vaccinium*, as well as many other genera such as bilberry, blackberry, blueberry, cranberry, lingonberry, mulberry, raspberry, and strawberry, as well as cherries and currants, and are responsible for the blue and purple color of berries. Pomegranate (*Punica granatum*), a "wonder fruit," and chocolate both

contain anthocyanins (Francis 1982). Among the most frequent dietary anthocyanins are cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin, to name a few. Many epidemiological studies have highlighted the health advantages of anthocyanin-rich dietary components, whose potent antioxidant, anti-inflammatory, and therapeutic activities have recently been studied (Farzaei et al. 2018).

6.3.4 Resveratrol

Resveratrol, a stilbene polyphenol found in several dietary and nondietary plant sources, is classified as a phytoalexin, synthesized in response to bacterial and fungal attack. Resveratrol is also produced in response to several environmental stresses, including ultraviolet (UV) radiation (Tian and Liu 2020). Some of the major dietary sources of resveratrol are berries, grapes, red wine, and peanuts. Resveratrol is a widely studied molecule and is most well known as the agent responsible for the "French paradox": a phenomenon whereby the consumption of red wine is suggested as the reason why the French suffer from a comparatively low incidence of cardiovascular disease despite having a diet rich in saturated fats (Darvesh et al. 2017). Resveratrol is a strong antioxidant and anti-inflammatory drug that has been proven to extend longevity in preclinical and clinical trials in a variety of oxidative stress and inflammation-related illnesses, including cancer, cardiovascular disease, infections, and neurological diseases (Das and Das 2007).

6.3.5 Dihydrochalcones

The plant Rooibos (*Aspalathus linearis*) belongs to the legume family and is only found in a tiny area of South Africa's Western Cape. Its dried and fermented leaves and twigs are used to produce herbal tea, which has been popular in the area for generations and is now exported and enjoyed worldwide (Street and Prinsloo 2013). The dihydrochalcones aspalathin (2,3,4,4-pentahydroxy-3-C-β-d-glucopyranosyl dihydrochalcone) and its structural homolog nothofagin are responsible for rooibos' antioxidant action (differing only in that it lacks the A ring catechol group) (Snijman et al. 2009). Using the thiobarbituric acid reactive substances (TBARS) test, Inanami et al. investigated the effect of long-term (>20 months) administration of rooibos extract on lipid peroxidation in the rat brain. Increased lipid peroxidation is significantly linked to AD and aging in humans, as previously mentioned. Lipid peroxides were found to be substantially greater in the frontal and occipital cortex, the hippocampus, and the cerebellum of control groups of 24-month-old rats compared to juveniles in a research study by Inanami and coworkers (aged 5 weeks). Surprisingly, the signal intensities for age-related markers in the frontal cortex, hippocampus, and cerebellum of rooibos-treated rats matched those of 5-week-old rats in an additional MRI study by the same authors, but the same areas in untreated 24-month-

old rats showed significant age and lipid peroxidation-related elevation of these markers (Darvesh et al. 2010). As a result, these researchers conclude that age-related accumulation of lipid peroxides in the brain, which was closely correlated with morphological changes seen on MRI, revealed that chronic rooibos administration prevented age-related accumulation of lipid peroxides in several regions of the rat brain (Inanami et al. 1995).

6.3.6 Xanthones

The genus *Cyclopia* includes a number of shrubs native to the Cape Fynbos area of South Africa, the most common of which is *Cyclopia intermedia,* often known as the honeybush shrub. Honeybush herbal tea, which is extensively drunk in South Africa, has been made from this plant for decades (McKay and Blumberg 2007). The honeybush has yielded many polyphenols, including the xanthones mangiferin and isomangiferin, as well as the flavanones hesperidin and eriocitrin. Mangiferin has been the most extensively investigated of these substances in paradigms relating to the underlying pathophysiological processes of Alzheimer's disease. Mangiferin protected mouse brain against oxidative damage caused by 12-O-tetradecanoylphorbol-13-acetate (TPA), according to Sánchez et al. In addition, when compared to TPA controls, mangiferin provided significant (22%) protection against DNA fragmentation in the brain and reduced lipid peroxidation in brain homogenates by 39% (Matkowski et al. 2013). Gottlieb et al. found that in the presence of submicromolar quantities of mangiferin, neuronal cell death caused by glutamate in in vitro cell cultures was reduced. Receptor-mediated calcium influx was reduced, oxidative stress was reduced, and apoptosis was significantly reduced in these cultures (Gottlieb et al. 2006).

6.4 Polyphenols and Neurodegeneration

6.4.1 Neuroprotective Activity

NDs are a diverse set of diseases defined by post-mitotic neuronal cell malfunction and/or gradual loss in the central or peripheral nervous systems. Cognitive decline, dementia, motor irregularities, sleep problems, and behavioral and psychiatric disorders are the most common clinical manifestations of neurodegeneration. The buildup of aberrant, misfolded, and aggregated proteins; mitochondrial dysfunction; inflammation; oxidative stress accumulation; improper neural transport; impairment of the autophagic process; and change in proteasome activity are all prevalent cellular and molecular processes in NDs. NDs are incurable, progressive, and highly debilitating illnesses that pose a major burden in terms of human suffering and healthcare expenditures. The challenge for physicians and researchers is to develop

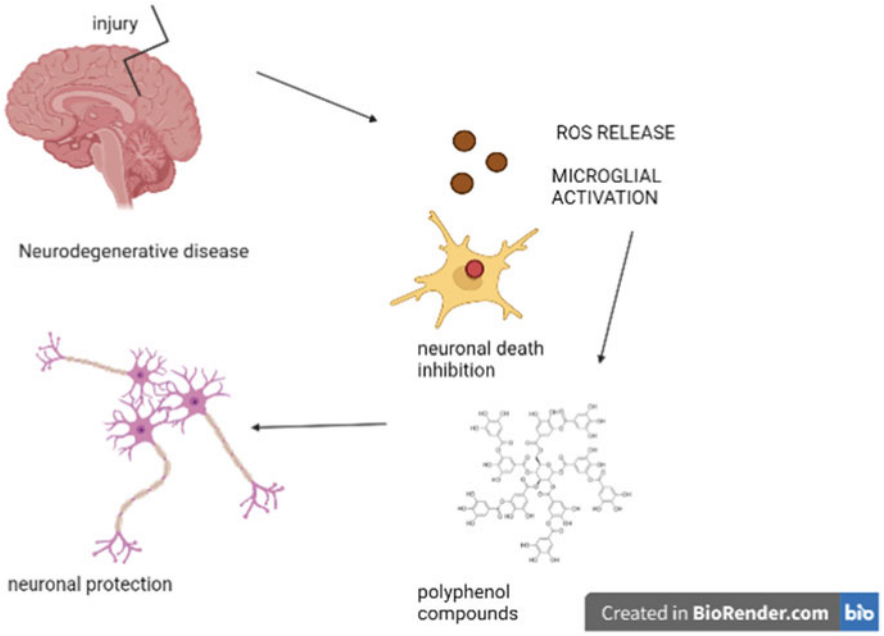

Fig. 6.2 Neuroprotective mechanism of dietary polyphenols

new medications to slow down neurodegeneration and enhance their patients' quality of life due to a lack of effective treatments. Increased cellular oxidative stress plays a significant part in the main etiologies of NDs, in addition to risk factors such as genetics and environmental influences (Tsuji 2002). Polyphenol consumption in the diet has been shown to reduce cellular oxidative stress and is a viable method for ND prevention. Polyphenols inhibit free radical damage by interacting with the hypoxia-inducible factor 1-alpha (HIF-1α) pathway, modifying production of protective genes against oxidative stress, regulating ROS via engaging with oxidative pathways, and scavenging metal ions. Metal ions (Fe^{2+}, ZN^{2+}, Cu^{2+}) accumulate in certain brain areas of ND patients, causing oxidative stress, which can be chelated by a wide range of polyphenols as shown in the Fig. 6.2. These chemicals, for example, appear to work by complexing transition divalent metal ions, resulting in a decrease in the quantity of iron and its accumulation (Shoval et al. 2008).

Polyphenols' anti-inflammatory properties have been linked to a reduction in the risk of neurodegeneration. These chemicals can regulate the expression of pro-inflammatory genes such nitric oxide production, lipoxygenase, cyclooxygenase, chemokines, and numerous cytokines, mostly through NFkB and MAPK signaling. Curcumin has been found to suppress NFkB signaling, which results in chemokine and Aβ aggregate production being reduced (Korkina et al. 2011). Similarly, resveratrol can reduce A-mediated microglial inflammation (a hallmark of Alzheimer's disease) through a mechanism that involves the Toll-like receptor

4 (TLR4)/NF-kB/signal transducers and activators of transcription (STAT) signaling cascade, and EGCG can prevent amyloidogenesis by inhibiting neuroinflammatory cytokines released by astrocytes (Rahimifard et al. 2017).

Other processes can be attributed to polyphenol compounds' neuroprotective effects, one of which is the decrease in amyloid-beta (Aβ) aggregates and/or fibril development (a hallmark of AD). Curcumin, resveratrol, and EGCG have been shown to have a positive impact on direct disruption of β-pleated sheets in several in vitro experiments (Udhayakumar 2020). Polyphenols, in particular, appear to bind to diverse surface regions of the β-sheet structure, causing a variety of consequences, including the formation of short, nontoxic oligomers.

Polyphenols interact with various pathways engaged directly or indirectly in the neurodegenerative process, in addition to the processes mentioned above. They are particularly important in signaling pathways involved in survival, cell proliferation, apoptosis, and autophagy. Polyphenols impact cellular function in this way by changing the phosphorylation state and expression levels of proteins involved in the phosphoinositide 3-kinase (PI3K), Akt/protein kinase B (Akt/PKB), tyrosine kinases, and protein kinase C (PKC) signaling pathways. Protein kinase A (PKA), PKC, topoisomerase, mitochondrial ATPase, and the benzodiazepine binding sites of type A gamma-aminobutyric acid (GABA-A) receptors are all possible targets for flavonoids. Several protein kinases have also been found to be inhibited by resveratrol, and EGCG is shown to interfere with the PKC signaling pathway. Several studies have highlighted the importance of the PKC signaling route in regulating critical molecular processes involved in associative memory storage, as well as how a defect in the PKC signaling pathway plays a key role in the pathophysiology of NDs (Ajami et al. 2017).

The structure–activity connection of polyphenolic compounds is another important factor to consider when addressing their neuroprotective effects. The structure–activity connections linking the physiological actions of antioxidants, such as polyphenols, to their compositions and structures have received a lot of attention. Lu and colleagues investigated the structure–activity connection for various gallic acid derivatives' antioxidant activity in an in vitro liposome system, as well as their neuroprotective efficacy against 6-hydroxydopamine-induced stress in human neuroblastoma cells. The study found that polyphenolic chemicals' ability to scavenge free radicals and their hydrophobic characteristics, which allow them to easily penetrate cell membranes and reach their targets, are both critical for their neuroprotective impact against oxidative damage. More recently, an interesting research study used H_2O_2-scavenging activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging activity tests to evaluate the antioxidant potential of certain polyphenolic compounds (Youdim and Mandel 2012). The findings revealed that the H_2O_2-scavenging activity of phenolic molecules is strongly influenced by the chemical structure of the molecule, as well as the type, number, and position of the active group (OH or NH_2) and the kind, number, and position of the substituted group. The combination of an amino group and an electron donor at the ortho or para position has a detrimental influence on the scavenging activity of polyphenolic compounds, according to the study. Similar to the number of active groups, the

DPPH-scavenging activity is proportional to the number of active groups; hence, more active compounds contain more than one active group (Ingale and Kasture 2014).

In vivo studies on numerous animal models have also revealed the neuroprotective benefits of curcumin, resveratrol, and EGCG. Mansouri et al. looked into the neuroprotective benefits of intraperitoneally administered curcumin against homocysteine-induced neuronal damage in a rat model of Parkinson's disease. The findings revealed that homocysteine had neurotoxic effects on dopaminergic neurons in the substantia nigra and curcumin therapy decreased apoptosis and alleviated behavior symptoms. In a *Drosophila melanogaster* model of HD, similar results were obtained (Ataie et al. 2010). Curcumin therapy dramatically reduces neuronal motor dysfunction by reducing cell death, according to researchers. Ishrat and colleagues established the efficiency of curcumin treatment in preventing cognitive deterioration in a rat model of sporadic dementia of the Alzheimer's type. The hippocampus and cerebral cortex of treated rats showed a substantial improvement in cognitive impairments, as well as a reduction in glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) levels, and an increase in choline acetyltransferase (ChAT) activity. According to findings from a study done in a mouse model of HD, resveratrol therapy improved motor coordination and learning while also increasing the expression of mitochondrial-encoded electron transport chain genes. In a rat model of Alzheimer's disease, resveratrol has been shown to protect the brain against A-induced damage (Ishrat et al. 2009). Research on bioactive dietary polyphenol preparations found that resveratrol's neuroprotective benefits were linked to a decrease in inducible nitric oxide synthase (iNOS) levels and lipid peroxidation, as well as an increase in heme oxygenase-1 synthesis (HO-1). Karuppagounder and colleagues published similar findings, demonstrating that resveratrol can reduce plaque development in a brain-specific area in a mouse model of Alzheimer's disease. This positive impact on A was linked to a reduction in brain glutathione and a rise in cysteine, which is a precursor to glutathione.

Xu et al. have investigated the neuroprotective effects of EGCG in a mouse model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD. By decreasing oxidative stress and modulating the iron-export protein ferroportin in the substantia nigra, EGCG alleviated MPTP-induced neurotoxicity. In a mouse model of Alzheimer's disease, the effects of EGCG on neuroinflammation and amyloidogenesis were investigated (Xu et al. 2020). The researchers discovered that EGCG therapy reduced memory impairment and neuronal cell apoptosis caused by lipopolysaccharide (LPS), as well as enhanced the production of tumor necrosis factor-alpha (TNF-1α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6). He and colleagues used an AD animal model to show that EGCG protects against neurodegeneration by lowering the levels of the amyloid precursor protein (APP) and Aβ in the hippocampus of afflicted mice. Overall, the data support the idea that dietary polyphenols, which primarily protect against Aβ plaque development, oxidative stress, and inflammation, can help postpone or prevent neurodegeneration (Rehman et al. 2021).

6.5 Advances in Application of Dietary Polyphenols to Treat Age-Related Neurological Disorders

6.5.1 Polyphenol Combination Therapy as a Novel Technique for Delaying the Onset of Alzheimer's Disease in People with Mild Cognitive Impairment

MCI is described as a syndrome characterized by subjective and/or objective evidence of cognitive impairment but no (or minimal) evidence of functional decline. Amnestic MCI (memory impairment) and nonamnestic MCI (nonmemory impairment) are two subtypes of MCI (affecting other cognitive domains only). Individuals with amnestic MCI are at a high risk of acquiring incident AD, and the majority of them have a high amyloid burden in their brains. Because people with MCI are by definition functioning well, finding therapies to prevent their development to dementia (secondary prevention of dementia) would be a significant public health benefit. There is presently no FDA-approved medication for secondary prevention of MCI, and trials of current Alzheimer's treatments have failed horribly. MCI is a risk group, not a disease state, and many people with MCI do not develop dementia or Alzheimer's disease; these people are unlikely to have a brain disease. Given these findings, it is especially critical that an intervention be reasonably safe and noninvasive (Lissek and Suchan 2021). The majority of existing interventions in this sector are cognitive or behavioral, with only a few medication trials, the most advanced of which being intranasal insulin, which is being investigated based on ideas that are similar to ours. MCI patients usually possess metabolic risk factors such as insulin resistance and prediabetes, putting them at an increased risk of cognitive deterioration (Biessels et al. 2014).

BDPP (bioactive dietary polyphenol preparation) is a novel nutraceutical combination that combines three bioactive and commercially available polyphenol products (Concord grape juice, grape seed extract, and resveratrol) to provide three selective, bioactive, polyphenol-rich dietary preparations to simultaneously target multiple AD pathogenic targets, as well as metabolic syndrome markers (primarily through resveratrol action). Each of the BDPP components has its own mode of action against AD pathogenic pathways, as we will go over in more detail below. As a result, when compared to individual BDPP components, BDPP application provides a more comprehensive coverage of AD pathogenic targets, making it a more effective method for treating individuals with early AD and prediabetes. They discovered that BDPP addresses amyloid burden, synaptic plasticity, and cognition in animal models of Alzheimer's disease and metabolic syndrome in current investigations. Those with prediabetes who have MCI are at a higher risk of developing Alzheimer's disease. This nutraceutical's benefits include a low risk of side effects, oral administration, and a refreshing lack of intellectual property difficulties, all of which help keep costs low. This is especially important as we move closer to a time when we may be able to target prodromal and preclinical Alzheimer's disease for secondary prevention. Because secondary prevention entails treating a large

population, it would be especially beneficial if a secondary preventive intervention was also economical, which is doubtful with the currently investigated treatments (for example, passive immunotherapy and beta-secretase inhibition) (Zhao et al. 2020).

6.5.2 Polyphenols as Mitochondrial Medicine in Neurons

Wine polyphenols have been shown to lower oxidative stress and boost the production of antioxidant enzymes such as catalase, superoxide dismutase, glutathione reductase, and glutathione peroxidase. Resveratrol increases antiapoptotic Bcl-2 protein expression while decreasing Bax protein expression. Resveratrol also acted as a mitochondrial antioxidant by increasing the levels of the antioxidants thioredoxin-2 (TRX2) and X-chromosome-linked inhibitor of apoptosis protein (Tsai et al. 2017). Another study discovered that resveratrol enhanced Bcl-2 expression, which prevented neuronal death. Resveratrol reduced mitochondria-mediated apoptosis and regulated oxidative stress in PC12 cells by downregulating Bax and upregulating Bcl-2. Similarly, lutein has been found to protect mice from ischemia damage by increasing Bcl-2 levels while decreasing Cox-2 and pancreatic ER kinase (PERK). Baicalein also inhibited mitochondrial apoptosis by reducing Bax and tBid expression and elevating Bcl-2-like proteins in the cytoplasm. Ferulic acid, a phenolic acid, inhibits mitochondrial apoptosis by inhibiting Bax and tBid expression and elevating Bcl-2-like proteins (Rodrigo et al. 2013). Flavones like chrysin, apigenin, and luteolin upregulate important ERK/Nrf2 pathway transcription factors including glutamate cysteine ligase catalytic (GCLC) and glutamate-cysteine ligase, modifier subunit (GCLM) to resist oxidative stress. The levels of glutathione peroxidase (GPx) were altered by red wine polyphenols, resulting in oxidative stress resistance. In hypoxia studies, the phenolic antioxidant 3,3′, 5,5′-tetra-t-butyl-biphenyl-4,4′-diol regulated both HIF-1α and GPx expression levels. Hesperidin carsonic acid, a key rosemary polyphenol, inhibits ROS, MAPKs, caspase-3, and COX-2 in neurons during hypoxic stress, resulting in significant anti-inflammatory effects. JNK inhibition is used as a mitochondrial treatment in not only stroke but also in Alzheimer's disease, as JNK activation contributes to tau hyperphosphorylation and A pathogenesis in the AD brain. Curcumin and resveratrol showed neuroprotection in astrocytes via increasing the activity of NAD(P)H quinone oxidoreductase (NQO1) via the Nrf2 pathway. Likewise, structurally modified isomers of resveratrol increased NQO1 activity, suggesting that antioxidant effects via the NRf2 pathway are possible. Endophilin-B1, otherwise known as SH3GLB1, which is essential for mitochondrial morphology and has a role in apoptosis, was regulated by ECG. Superoxide dismutase (SOD) and glutathione peroxidase (GPX1), two mitochondrial antioxidant enzymes, were also upregulated by EGCG (Bhullar and Rupasinghe 2013).

In a mouse model of hypoxic-ischemic (HI) brain damage, flavonoid-enriched fraction (AF4) isolates obtained from the peel of "Northern Spy" apples were shown

to suppress the expression of IL-1, TNF-, and IL-6. Phloridzin, another apple polyphenol, was shown to increase the expression of SOD1 and SOD2 genes, thus safeguarding mitochondria against oxidative stress. Polyphenols are essential mitochondrial therapies because they modulate apoptosis, antioxidant activity, signal transmission, and inflammation in mitochondrial biochemistry (Al-Gubory et al. 2010).

6.5.3 *Nanotechnology as a Novel Polyphenol Delivery Method*

The most well-studied nanoparticle-mediated polyphenol delivery techniques use biodegradable and biocompatible polymers to encapsulate polyphenolic compounds in nanostructures such as NSs, NCs, SLNs, CDs, LSs, and MCs. Oral, intravenous, intraperitoneal, and transdermal delivery are all options for delivering these nanoparticles. NSs are spherical particles with sizes ranging from 10 to 200 nm that have a hydrophobic component in the center and hydrophilic chains arranged on the surface. They protect the medication against enzymatic and chemical breakdown and might be amorphous or crystalline in form. Polyglycolic acid (PGA), polylactic acid (PLA), poly-lactic-co-glycolic acid (PLGA), polyethylene glycol (PEG), polycaprolactone (PCL), and chitosan (CS) are the most commonly used polymers for the preparation of nanoparticles because they have homogeneous solid matrices in which the polymer chains are organized in a frozen status phase-separated from the core of the solution. The medication is essentially dissolved, entrapped, encapsulated, or chemically attached to the polymer matrix (Siddiqui et al. 2009). Nanocapsules (NCs) have a diameter of 10–1000 nm. They are made up of a core–shell construction in which the medication is put in a cavity that is encased in a polymer membrane or coating. The medication can be in liquid, solid, or molecular dispersion form in the cavity of nanocapsules. In addition, active chemicals can be transported on nanovector surfaces or ingested through the polymeric membrane.

Solid lipid nanoparticles (SLNs) are nanoparticles that range in size from 50 to 1000 nanometers. They are one of the innovative possible colloidal carrier systems made up of lipid emulsions (oil in water) with a solid core lipid covered with aqueous surfactants in place of the liquid lipid. These nanoparticles have a number of benefits, including great biocompatibility, bioavailability, physical stability, and tolerability, as well as the capacity to preserve integrated labile medicines from degradation. Another benefit of using SLNs is the relative simplicity with which large-scale manufacturing may be accomplished. Nonetheless, particle development, an unpredictable gelation propensity, and unanticipated polymeric transition dynamics are all frequent drawbacks of these nanoparticles (Faridi Esfanjani and Jafari 2016).

Cyclodextrins (CDs) are a collection of structurally similar natural compounds that are formed when cellulose is digested by bacteria. CDs are toroidal or cone-shaped rather than perfect cylinders due to the limitation of unrestricted rotation around the bonds linking the glucopyranose units. The hydroxyl functions are directed to the cone's exterior, and the center cavity is covered with the glucose residues' skeletal carbons and ethereal oxygens. Alpha, beta, and gamma CDs are natural CDs that contain six, seven, and eight glucopyranose units, respectively. There have also been reports of CDs having 9 to 13 glucopyranose units. The CDs, particularly cyclodextrin, have a low water solubility. Many other types of modified CDs have been created, but only those made using water-soluble CD derivatives have been employed commercially in the pharmaceutical industry. The hydroxypropyl (HP), methyl (M), and sulfobutylether (SBE) substituents are present in these potent CD derivatives. CDs have been employed as complexing agents to boost the bioavailability and stability of weakly water-soluble medicines by incorporating them into the CD cavity via van der Waals forces, hydrophobic interactions, or hydrogen bonds (Nonaka et al. 2008).

The most well-studied nanosized carriers for targeted drug delivery are liposomes (LSs). LSs are phospholipid vesicles with particle sizes ranging from 30 nm to several micrometers that are made up of one or more concentric lipid bilayers surrounding aqueous gaps. They self-assemble by hydrating lipid powder in an aqueous solution including cholesterols, sphingolipids, glycolipids, membrane proteins, and nontoxic surfactants. Liposomal vesicles can encapsulate a wide spectrum of medicines due to their capacity to entrap both lipophilic and hydrophilic molecules; hydrophobic molecules are imprisoned in the lipid bilayer membrane, while hydrophilic molecules can be imbedded in the aqueous gaps. The ability to self-assemble and biocompatibility are two benefits of liposomal delivery methods. Furthermore, medication molecules are protected against early inactivation, degradation, and dilution in the circulation when they are encapsulated within liposomes (Squillaro et al. 2018).

Micelles (MCs) are self-assembled, nanoscale colloidal particles with diameters ranging from 5 to 100 nm. They are made up of amphiphilic copolymers that self-assemble in aquatic environments at specific concentrations and temperatures. When the surfactant concentration exceeds the critical micelle concentration (CMC), which is defined as "the minimum surfactant concentration necessary for the self-aggregation process," MCs develop. The hydrophilic parts of the surfactants pointing outward toward the solvent create the shells, while the hydrophobic pieces of the surfactants pointing toward the micelle's center or interior constitute the core (Ghaywat et al. 2021).

The use of biodegradable and biocompatible polymers allows for a more rational design of novel nanostructures capable of encapsulating polyphenols that can cross the BBB, overcoming the limits of traditional administration methods. Curcumin is the most researched therapeutic option in this situation, owing to promising results in animal models of neurodegenerative disorders. However, curcumin's efficiency has so far been restricted by its poor water solubility, low gastrointestinal adsorption, and fast metabolism. Crossing the BBB with poly-lactic-co-glycolic acid (PLGA)

nanospheres containing curcumin may be the best method. Curcumin–PLGA nanoparticles have been shown to interfere with A aggregation and enhance the brain self-repair process, boosting neural stem cell proliferation and neuronal differentiation in recent studies. Similarly, curcumin-loaded liposomes can effectively prevent the production of Aβ fibrils in vitro and their deposition in the brain. MD and central oxidative stress appear to be helped by curcumin–solid lipid nanoparticles. Curcumin and piperine co-loaded glycerol mono-oleate nanoparticles can also inhibit Syn aggregation, decreasing oxidative stress and apoptosis. Curcumin was also considered for administration to the central nervous system using nanoemulsions intranasally. Nanoemulsions of curcumin (added in the oil phase) may efficiently traverse the mucosa in the presence of CS without causing cytotoxicity (Rafiee et al. 2019).

Resveratrol is another potential possibility. It is well-known for its propensity to cause APP degradation and the removal of A. Resveratrol, on the other hand, has a variable bioavailability due to its fast and extensive metabolism. PEG-PCL and PLGA nanoparticles containing resveratrol provide a regulated release profile of the medication, which is necessary for maintaining its plasmatic level and antioxidant action. The oil-in-water nanoemulsion is a promising technique. With the addition of Vitamin E and other surfactants, this formulation can effectively reach the brain via the nasal route. Curcumin and resveratrol (1:1 weight ratio) are also co-encapsulated in mucoadhesive nanoemulsions, which shield the active ingredients from degradation and retain their antioxidant effects. In vivo quantification of the two polyphenols in the animal brain revealed an increase in their amounts after 6 hours. Unfortunately, these systems have not yet been tested in clinical trials, but the data gathered thus far encourages the development of novel treatment methods (Ratheesh et al. 2017).

6.6 Conclusion

Despite mounting evidence of dietary polyphenols' positive benefits in the prevention and treatment of NDs and brain tumors, their limited bioavailability is a major roadblock to their widespread use in clinical practice. Clinical hurdles researchers must overcome include a lack of clinical studies based on the use of polyphenols for the treatment of NDs and brain tumors, as well as a failure to replicate in vivo the therapeutic benefits seen in in vitro models. Neurodegenerative disorders are becoming a major issue for modern society as people live longer. In fact, as the population ages, neurodegenerative diseases will have a greater influence on medical and socioeconomic circumstances in industrialized countries. As a result, it is critical to developing techniques that help persons with dementia avoid cognitive deterioration and enhance their quality of life (Bhullar and Rupasinghe 2013).

Polyphenols are bioactive chemicals found in foods and drinks that have the ability to influence the metabolic process, therefore improving health and avoiding cognitive, motor, and sensory loss as people age. They also protect cells from stress

damage by modulating several cellular signaling pathways. Understanding the molecular processes by which polyphenols work is therefore critical for using them as dietary supplements to prevent neurodegenerative diseases. Polyphenols also protect mitochondria by activating prosurvival cell signaling, which protects them against pathogenic events. Polyphenols boost antioxidant enzymes including catalase and superoxide dismutase (SOD1, SOD2), as well as prosurvival pathways like Bcl-2 and PERK. The survival of neurons is also aided by the downregulation of Bad/Bax, c-jun, JNK, COX2, AP-1, and caspase-3.

Following advancements in the synthesis and characterization of novel materials, nanotechnologies have become widely employed in everyday life. Innovative nanotechnology-based technologies for improved drug delivery and cell targeting are expected to make significant advances in the medical and pharmacology areas.

Polyphenol research in the future should strive toward the clinical acceptability of health claims derived from preclinical in vitro and animal model studies. As a result, future research should focus on human clinical trials of various strong polyphenols and their combinations. Polyphenols must also be examined for risk assessment and safety evaluation in order to detect any negative effects. Polyphenols' pharmacological importance for humans will be determined by their clinical research results (Kumar 2015).

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Chapter 7
Prophylaxis Through Marine-Derived Bioactive Compounds Toward Neurodegenerative Disorders

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Abstract Of the estimated 8.7 million species on our planet Earth, 1.4–1.6 million are marine species. A good percentage of this is yet to be discovered and described. Neurodegenerative diseases are one of the dreadful diseases as there is no known course of action to countermand or undo the progressive degeneration of neurons, and hence these diseases are considered to be incurable. There are many limitations in the present scenario of our health facilities. Biological barriers like blood–brain barriers obstruct the effective drug delivery to the brain, reducing the potential benefit of the medication administered. Only a lower concentration of the drug reaches the central nervous system by the intravenous or oral route of administration, which poses a great challenge. Marine drugs have neuroprotective action for neurodegenerative diseases. This chapter deals with the prophylaxis through marine-derived bioactive compounds toward neurodegenerative disorders.

Keywords Neurodegenerative disorders · Central nervous system · Parkinson's disease · Alzheimer's disease · Marine-derived bioactive drugs

Abbreviations

AD	Alzheimer's disease
AXT	Astaxanthin
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system

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COMT	Catechol-O-methyltransferase
DA neurons	Dopaminergic neurons
DNA	Deoxyribonucleic acid
ELSD	Evaporative light scattering detection
FX	Fucoxanthin
GFC	Gel filtration chromatography
GSH	Glutathione
HPLC	High-performance liquid chromatography
IEX	Ion-exchange chromatography.
MS	Mass spectrometry.
NDD	Neurodegenerative disorder
PD	Parkinson's disease
ROS	Reactive oxygen species
SNc	Substantia nigra of pars compacta

7.1 Introduction

Ocean comprises about 90% of the world's living biomass, which almost makes up to half of the total global biodiversity. The last 20 years paved the way for the discovery of new drugs with novel pharmacological targets. Marine molecules have a large spectrum of action due to the wide diversity of marine habitats. Marine-derived bioactive compounds are potentially used for the treatment of cancer, hypertension, diabetes, and even crucial neurodegenerative diseases (Catanesi et al. 2021).

Neurodegeneration is the degeneration of the function and structure of neurons, which leads to neural damage and even death of the cells (Yildiz-Unal et al. 2015; Przedborski et al. 2003). The prefix "neuro" refers to the neurons or the nerve cells, and degeneration indicates the process of loss of structure or dysfunction of cells or tissues or organs (Gao and Hong 2008). It mainly affects the central nervous system (CNS). The neurodegenerative disease is a type of disease that causes the damage or death of cells of CNS, which can be acute or chronic neurodegeneration. The neurons get rapidly damaged, which extends to the death due to any sudden head injury, strokes, cerebral hemorrhage, and so on in acute neurodegeneration, while in chronic degeneration it starts gradually but later it gets intensified and turns to be irreversible damage in discrete parts of CNS (Jellinger 2010). Huntington's disease, Parkinson's disease, and Alzheimer's disease are examples of chronic neurodegenerative disorders.

The drugs from marine invertebrates are developed mainly for the modulation of CNS channels and inhibition of enzymes, which is the major reason for the elevated number of neurodegenerative diseases that adversely affect the elderly population. The synthetic form of peptide ω-conotoxin MVIIA found in the snail's venom *Conus magus* is the selective blocker of N-type calcium channels Prialt (ziconotide), which has an antinociceptive activity (Hong-Shuo et al. 2020).

The examples of some compounds are the following:

- Xestosaprols and tasiamide B, the beta-secretase 1 inhibitors from Indonesian marine sponges, genus *Xestospongia* and cyanobacteria.
- Linckosides, the neurotrophic-like agents from Okinawan starfish *Linckia laevigata*.
- Dysideamine, neuronal growth inducers obtained from Indonesian marine sponge, genus *Dysidea*.
- Ircinialactams from Australian sponges, family Irciinidae for glycine receptor modulators.

7.2 Neurodegenerative Disorders

Neurodegenerative disorders (NDDs) are disorders that are seen to progress with age in which neurons are affected both in structure and their action and may also lead to their decease (Palop et al. 2006). People suffering from NDDs show a large change in their neurological functioning due to the variation in the action of neurons and thus chronic intoxication by unusual proteins that the brain is unable to control. These disorders vary in their pathophysiology, which affect a person's memory and intellectual activities, thus affecting a person's ability to do basic things like movement, respiration, and talking. There are various NDDs like Alzheimer's disease, Parkinson's disease, anxiety, stroke, multiple sclerosis, Huntington's disease, traumatic brain injury, and amyotrophic lateral sclerosis (Jellinger 2010).

It has been seen that oxidative stress is also associated with NDDs, which is caused by the imbalance of antioxidants and prooxidants, thus creating reactive compounds of oxygen and nitrogen (ROS and RNS) (Masters and Bush 2004). Neuroinflammation is also seen with NDDs in which it triggers the microglial cells, negotiator of the inborn responses in CNS, hence making pro-inflammatory and neurotoxic factors such as nitric oxide, prostaglandin (PGE2), ROS, interleukin (IL) 1beta, and tumor necrosis factor (TNF) alpha, which can stimulate neurodegeneration. Synaptic loss is a frequent pathological sure sign of many NDDs leading to the destruction of subsets of neurons (Lull and Block 2010). There is another biological activity, excitotoxicity, which is also involved in the pathogenesis of this NDD in which there is death of neurons due to long-lasting activation of glutamate receptors by excitatory amino acid (excitotoxin) in the central nervous system of a person (Nurshafika Mohd Sairazi et al. 2020), having a significant role in neurodegeneration. It is seen that transition metals are significant in many biological activities. Changes in their balance lead to an increase in the production of free radicals, which is in turn supported by Fe, Cu catalysts. Mostly, metal-mediated oxidative stress is connected to mitochondrial dysfunction. When the quantity of Fe in the brain increases, related with different NDDs, it has chances to enlarge the number of free radicals through the Fenton reaction (Double et al. 1998). Multitarget-directed ligands (MTDLs), gene transfer therapy, medicinal chemistry-based treatment, nanotechnology, and stem therapy have shown good results in the treatment of NDDs (Baldi et al. 2003).

Even though some chemical remedies are available for NDDs but have many prominent side effects, researchers are on analysis for the usage of natural bioactive compounds to treat those NDD conditions by using their antioxidant and anti-inflammatory function.

7.2.1 Alzheimer's Disease

It is an NDD that progresses with age, and presently available drugs can only make improvement to the condition and not completely cure the disease, that too in a limited number of cases and for a certain period (Russo et al. 2015a, b). In recent years due to increased healthcare facilities, resulting in increased average life expectancy, Alzheimer's is becoming a leading cause of death. The condition of the disease worsens with time, and the person suffers from memory loss, which is the usual cause of dementia, inability to effectively communicate and do daily activities, many aspects like memory, thinking, language, etc. As it is a developing disease condition, which gradually aggravates over time and affects the region of the brain that is involved in learning, the person initially finds light memory loss but at later stages cannot even respond to the surroundings.

Several hypotheses have been put forward for AD, such as amyloid cascade hypothesis, Cholinergic hypothesis, and glutamatergic hypothesis (Hardy and Selkoe 2002). In this condition, two unusual structures called tangles and plaques are the major causes that destruct the nerve cells, in which the plaques are protein bits called beta-amyloid that fills the gaps in between nerves cells and tangles are twisted fibers of a different protein called tau, which builds up within the cell. In Alzheimer's disease, nucleic acid oxidation can result in the compound called 8-hydroxydeoxyguanosine in mitochondrial and nuclear DNA (Mullaart et al. 1990). Most exceptional biochemical changes in the brain of AD patients are the decrease of acetylcholine levels in the hippocampus and cortex (Syad et al. 2012). It has been found that three genes with scarce variation cause this AD, and several genes are contributing factors for this disease. As the advancements in studies are taking place, genetic profiling is assumed to become a potent risk evaluation tool (Kang et al. 2013).

Now the diagnosis is often so late that the disease has critically caused serious brain destruction. Despite the fact that the presence of amyloid plaques is the main feature of the disease, the occurrence of only this cannot be used as a diagnosis. As there are many instances that there is the presence of plaques in the brain but no symptom of Alzheimer's disease, amyloid imaging cannot be recommended as a routine method for diagnosis in doubtful cases.

For the cure of neurodegenerative disorder, components promoting neuronal growth are anticipated to show results. Dysideamine from marine sponge *Dysidea* promotes neurite outgrowth in experimental animals. Lembehyne A, isolated from sponges *Haliclona* spp., promoted neurogenesis (Fig. 7.1; Table 7.1).

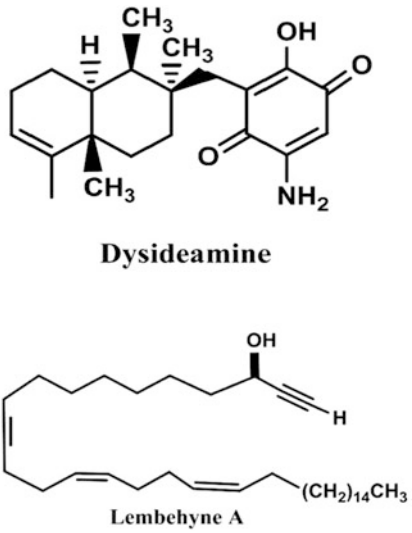

Fig. 7.1 Dysideamine and lembehyne A. Potent marine drugs affecting neuronal growth and synaptic functions (adapted with permission from Paula B. Andrade from bioactive marine drugs and marine biomaterials for brain disease 2014)

Table 7.1 Marine bioactive compounds for protection against Alzheimer's disease (adapted and modified with permission from Elumalai 2018)

Sl no.	Compound	Source	Mode of action	Reference
1	Bryostatin	Bugulaneritina	Modulate neuronal synapses under synaptic dysfunctions Improve cognition and activities of daily living in moderate-to-severe AD Enhance spatial learning, memory improvement, reduction of Aβ peptide, and recovery of neurotrophic activity	Russo and Kisialiou et al. (2015a, b) Blanco et al. (2017) Farlow et al. (2017)
2	Chitosan	Crab/shrimp	Reduction of Aβ level by BACE-1 inhibition activity; potential AchEI Role in drug delivery in AD by chitosan nanofiber	Tan et al. (2013) Pangestuti and Kim (2010)
3	Gracilins	Marine sponge (*Spongionella gracilis*)	Inhibit BACE-1 Reduce tau phosphorylation Antioxidant and anti-inflammatory properties	Leirós et al. (2015) Rateb et al. (2009) Nirmal et al. (2008)
4	Fucoidan	Brown seaweed *Fucus vesiculosus*	Inhibit neuronal death by deactivating caspase-9 and caspase-3 Anti-inflammatory	Cowan et al. (2001)
5	Tramiprosate	Seaweed	Prevent formation of Aβ fibrils Activator of sirtuin 1	Gervais et al. (2007)

7.2.2 Parkinson's Disease

Nerves are the basic building block of our nervous system, which connect and coordinate our bodily activities. They communicate with each and every part of our body by electrical and chemical means. Messages are passed from eyes, ears, skin, muscles, etc. to the central nervous system (brain and spinal cord) by nerves. Our body movements, which are possible by the contraction and relaxation of our muscles, are also coordinated by these nerves wherein the central nervous system gives the commands. So, the degeneration of these nerves may affect our bodily activities. Most of these diseases are genetic. It is a serious life-threatening disease as it has no cure. Treatments may help in improving the symptoms, such as relieving pain and increasing mobility.

Parkinson's is one such common neurodegenerative disease caused due to the destruction of neurons in the midbrain. The victim may suffer from the symptoms like shaking and problems with movement, which mainly occurs among the elderly peoples. Decrease of dopamine content in the striatum along with the loss of dopaminergic (DA) neurons in the substantia nigra of pars compacta (SNc) located in the midbrain causes the aggregation of α-synuclein formation in the brain. The slow movements and limbs' rigidity of the affected person are due to the demise of dopaminergic neurons in SNc that causes inhibition of thalamic activity, thereby reducing the excitatory input to the motor cortex. High cost of treatment, care, and rehabilitation for the disease created high socioeconomic consequences in the society. Levodopa, rasagiline (dopamine agonist), and catechol-O-methyltransferase (COMT) inhibitors in early treatment, which increase the bioavailability of dopamine in CNS by reducing peripheral dopamine metabolism, were some of the anti-Parkinson drugs. These drugs could relieve motor or nonmotor symptoms, but they barely had neuroprotective effects. The concomitant effects of these drugs include nausea, vomiting, insomnia, and nightmare due to blockage of aromatic amino acid decarboxylase. Long-term use may lead to on–off phenomenon that is a controller between mobility and immobility in the patients. So, there was a necessity to discover drugs with the least side effects and neuroprotective effects.

The marine world has countless resources. Moreover, aquatic organisms, including plants and animals, contribute diverse resources for new drugs. Sponges, sea cucumber, corals, algae, fungi, bacteria, and even archaea provide natural products against PD activities. Some of the examples are as follows:

Archaea: Mannosylglycerate from thermophilic bacteria acts by the inhibition of α-synuclein aggregation. These hyperthermophilic bacteria found in hot environments produce this compatible solute MG, which also increases the folding of α-synuclein, and thereby it acts as a well-qualified candidate for PD.

Bacteria: Different species of bacteria produce a variety of compounds that form a rich source for drug development and diverse biological activities.

- NP7, which is an antioxidant from *Streptomyces*, acts by inhibiting H_2O_2-induced neurotoxicity in neurons and glial cells. It is described as a developed neuroprotective agent against oxidative stress in Parkinson's victims.

- Piloquinones A and piloquinones B from marine-derived *Streptomyces* species CNQ 027 act by inhibition of MAO-A (monoamine oxidase A) or MAO-B (monoamine oxidase B).

Coral: 11-dehydrosinulariolid derived from the *Simularia flexibili* species of soft corals acts by activation of PI3-K/Akt, p-CREB, and Nrf2/HO-1 pathways. PI3-K/Akt pathway is regulated by protecting DA neurons against 6-OHDA-mediated damage. Thus, it represents a promising candidate against Parkinson's disease.

7.3 Classification of Marine-Derived Bioactive Compounds Used in Prophylaxis of Neurodegenerative Disorders

There are numerous kinds of neurodegenerative diseases. They are believed to overcome all the diseases to be the most common reason for people's death. So, finding ways to tackle those is vital for all of humankind. There are various pathways through which these disorders occur and affect our nervous system. All of these disorders work differently to weaken our system. One kind of disorder may also be making changes through not only one way but through numerous ways. So, finding methods and creating links between different systems that contribute to the occurrence of the disease is primary for knowing the disease. After knowing more about the condition, we know more about the requirements of remedial drugs and compounds. We also take up various procedures for diagnosing these conditions as we have seen they are multifaceted. As we continue to make advances in scientific research, we are face to face with the demand of the compounds. This is why scientists keep looking for newer and better sources, which have lesser side effects, which are more readily available, easier to isolate, and abundantly found in our surroundings (Lakshmi et al. 2018).

Marine organisms are the oldest forms originated. They have survived the harshest of environments. This is why they are found to synthesize a large group of metabolites, which are now under newfound discussion among researchers. People are always eager to find novel ways to tackle diseases as they also continue to evolve. Newer compounds are now looked at with hope. Nearly 10,000 compounds of biological use are found through marine organisms, which are mostly at the bottom of the marine food chain (Blunt et al. 2017).

Classifying drugs aids us to group them accordingly. It makes it easier to study and to use. Different sources give us different drugs, or different sources may also provide similar kinds of resources. Different disorders need differing practices. Major groups include bacterioplankton, marine microalgae and macroalgae, sponges, marine tunicates, and marine arthropods.

Classes of compounds being used in treating Alzheimer's disease are many. Groups like fucoidan, homotannin, gracillins, manzamines, etc. are being extensively used (Lakshmi et al. 2018). In Parkinson's disease, groups like fucoidan,

astaxanthin, and xyloketal B, among others, are being administered to patients (Blunt et al. 2017).

Compounds of bryostatin, xanthin, and dehydrosinulariolide, as well as numerous others, have been observed to be beneficial for people suffering from degeneration of nervous tissue or/and cells (Catanesi et al. 2021).

Some of the larger groups of compounds include the following:

- Alkaloids: They are cyclic compounds having Nitrogen in their ring. They have antioxidant effects. Also, they inhibit inflammation by enzyme inaction. Newer compounds like racemosin A and racemosin B have been seen to be effective against neural degenerating diseases (De Souza et al. 2009).
- Phlorotannins: They have many uses biologically, like anticancer (Lee et al. 2012), antiallergic (Sugiura et al. 2009), anti-microbial, etc. They are also divided into six groups. They have a role in neuroprotection via many pathways (Myung et al. 2005). Dieckol worked against inflammation of nervous tissue. DPHC also protected against degenerative. The antioxidant property of phlorotannin is seen because of its aggressive nature toward compounds having nitrogen or oxygen or due to metal chelation (Li and Kim 2011).
- Terpenes: They are closely related to isopentyl pyrophosphate or IPP. Sargachromenol found in *Sargassum* helps in nerve growth. It has a nerve growth factor. Sargaquinoic acid also has an effect on nerve cells favorably and protects neural cells and the system (Klegeris et al. 2007). Caulerpenyne works through many different mechanisms against neurodegenerative disorders (Manev et al. 2011). Many other terpenes are also being looked at as potential drugs that are under study.
- Sterols: They can be in free form or conjugated with fatty acids. Fecosterol was more dominant in Chlorophyta and Phaeophyta. Rhodophyta has more amount of cholesterol. Phaeophyta has been more effective as it has more fucoxanthin.
- Polysaccharides: Many kinds of long-chained compounds from this group are being used in therapy. Fucoidan and carrageen are more in discussion. Carrageen has been found to have anti-inflammatory, antitumor, and other functions. It has neuroprotective effects due to the sulfate group present in the compounds (Xu et al. 2012). Fucoidan too has many effects. Anti-inflammatory and anticoagulant properties are also among its features.
- Pigments: Pigments along with their primary role of trapping sunlight have other benefits too. Fucoxanthin was able to reverse nerve damage in cases of low oxygen (Ikeda et al. 2003). It also has anti-inflammatory effects. Astaxanthin stabilizes free radicals in glial cells and terminates chain reactions. Pheophytin A related to chlorophyll is a strong neurodifferentiating compound. C-Phycocyanin also has neuroprotective effects (Romay et al. 2003). Mytiloxanthins found in tunicates and shellfishes act as scavengers of free radicals. They also eliminate singlet oxygen.
- Floridoside from Rhodophyta suppressed inflammation of microglial cells and can be used as a therapeutic drug.
- Other groups.

Macrolides such as bryostatin and 11-dehydrosinulariolide show great results in mice and rat models. They are being put to use. They show antiapoptotic and anti-inflammatory effects.

Gambierol is an ether. It has action on neuropathic pain (Kiuru et al. 2014).

Hyaluronic acid and chondroitin sulfate from sharks and other fishes have benefits in spinal cord injury and oxidative stresses.

7.4 Production of Marine-Derived Bioactive Compounds

Marine-derived bioactive compounds pave a new way for the cure of neurodegenerative diseases caused due to the progressive degradation of nerve cells. The main diseases under this category are Alzheimer's and Parkinson's disease. The abrupt development in the production of marine bioactive drugs holds a probability of developing a healthier society.

The marine environment turns out to be an enriched source of diverse and complex compounds, which display various fascinating biological effects. Neuroprotection involves intricate methods that are correlated. Therefore, compounds applying neuroprotective effects through various pathways would exhibit a feasible approach to the treatment of neurodegenerative diseases. In fact, much research had already furnished hopeful results in the neuroprotective effects of several compounds extracted from various macroalgae species (Barbosa et al. 2014).

Alzheimer's disease-targeted marine-derived bioactive compounds are bryostatin (*Bugula neritina*), chitosan (crab/shrimp), gracillins (marine sponge: *Spongionella gracilis*), bastadins (marine sponge: *Lanthella basta pallas)*, galantamine (marine Amaryllidaceae), manzamine (Okinawan sponge), tramiprosate (seaweed), fucoidan (brown seaweed: *Fucus vesiculosus)*, and pholotannins (brown algae) (Lakshmi et al. 2018).

Parkinson's disease-targeted marine-derived bioactive compounds are mannosylglycerate (archae); NP7, piloquinone A, and piloquinone B (bacteria); neoechinulin A, xyloketal, secalonic acid, 6-hydroxy-N-acetyl-β-oxotryptamine, 3-methylorsellinic acid, 8-methoxy-3,5-dimethylisochroman-6-ol, candidusin A, 4-dehydroxycandidusin A, and diketopiperazine mactanamide (fungi); astaxanthin (algae); fucoxanthin (edible brown algae); gracillins, tetrahydroaplysulphurin-1,24-hydroperoxy-24-vinylcholesterol, and iotrochotazine (sponges); 11-dehydrosinulariolide (coral); staurosporine (prosobranch mollusk: flatworm and ascidians); and glucocerebrosides (*Cucumaria frondosa*) (Huang et al. 2019a, b).

Production of marine-derived bioactive peptides can be done through the following methods:

- Microwave-assisted extraction: The technique makes use of electromagnetic radiation of the frequency of 300 MHz–300 GHz to heat up and separate the compound in need.

- Chemical hydrolysis: This process undergoes by breaking peptide bond by acid or alkali.
- Enzyme hydrolysis: This breaks the peptide bond by selected proteolytic enzymes (Wang et al. 2017).

7.5 Extraction and Purification of Marine-Derived Bioactive Compounds

7.5.1 Extraction

There are different methods by which the bioactive compounds derived from marine sources can be extracted from their matrix. Some of the important techniques include microwave-assisted extraction and enzyme hydrolysis, which are discussed below.

7.5.1.1 Microwave-Assisted Extraction

Microwave-assisted extraction techniques are used as one of the most important methods for the extraction of various biologically active compounds from a wide variety of natural sources. This technique uses electromagnetic radiations of frequency ranging from 300 MHz to 300 GHz to heat up solvents that are in contact with the sample required to be extracted from the sample matrix. This technique is through inter- and intramolecular friction, along with the movement and collision of very large number of charged ions, which causes the rapid heating of the system and results in the breakdown of cell wall and membrane.

This technique is useful for degrading organisms like algae of which the cells are surrounded by dynamic complex and carbohydrate-rich cell walls. Here, the compounds are extracted more selectively and quickly. The use of less energy and solvent volume results in reduced costs and also makes it more environmentally friendly than any other extraction process (Michel et al. 2011).

7.5.1.2 Chemical Hydrolysis

Chemical hydrolysis of marine-derived bioactive lipids are achieved through cleaving peptide bonds with an acid or alkaline. This method is inexpensive and quite simple to conduct. Through acid hydrolysis, the structure and functional properties of peptides are changed. Acid hydrolysis is chosen over other pretreatment methods because of its low cost and effectiveness. The most common type of dilute acid used here is sulfuric acid (H_2SO_4). Acid hydrolysis requires high temperature, and also the hydrolase enzyme contains a huge amount of salt; in addition to this, acid hydrolysis could destroy the tryptophan, which is an essential amino acid. Chemical

hydrolysis causes the easy hydrolysis of peptide bonds and obtains a high yield of peptides. The method is considered to be insecure and environmentally unfriendly, thus making it mainly used only for industrial productions (Lee and Jeffries 2011).

7.5.2 Purification

Peptides usually contain 3–20 amino acid residues. The bioactivity of amino acids is due to the amino acid composition and sequences, which makes each amino acid unique. Some of the best methods used for the purification of marine-derived bioactive compounds like the peptides are membrane filtration systems, gel or size exclusion chromatography, ion-exchange column chromatography, and reversed-phase high-performance liquid chromatography and are discussed below.

7.5.2.1 Membrane Filtration

Advancement in material science and membrane manufacturing technology has taken the membrane technique to a higher level for the separation of natural products. Membrane filtration can be used at different levels. Ultrafiltration with a high-molecular-weight cutoff could be used for the separation of macropeptides and nonhydrolyzed proteins. Membrane filtration can operate at normal temperature, and no chemical reactions are involved in this process. This technique provides a large number of separations compared to other chromatographic separations. However, membrane filtration is restricted to desalination due to the poor selectivity of the filtration membrane. In the present-day scenarios, researchers use membrane filtration as the first purification step. Membrane filtration technology has shown its promising application in the separation of bioactive products. One of the main problems in using the membrane separation technique is fouling, which could shorten the membrane life and increase the cost (Vandanjon et al. 2007).

7.5.2.2 Gel Filtration Chromatography

Gel filtration chromatography (GFC), also called size exclusion chromatography, has been used for more than 40 years of duration for the separation, desalting, and molecular weight estimation of peptides and proteins. It is one of the simplest separation techniques and separates molecules on the basis of the size difference. The mechanism involves filtering molecules on the basis of their sizes; the smaller molecules enter the pores of the gel and travel a longer distance, while larger molecules show much shorter retention times. Since the molecules do not bind to the chromatography medium, the composition of the buffer does not directly affect the resolution. An important advantage of GFC is that elution conditions can be altered or changed to adjust with the type of sample as well as the requirements for

further purification, analysis, or storage without altering the separation. GFC can be used in case if the biomolecules are very much sensitive to changes in pH, concentrations of metal ions or cofactors, and harsh environmental conditions. GFC has high selectivity and high resolution in its techniques, which is an important step in purification. The main limitations of this process are the loading amount being seldom compared to the membrane filtration and collecting sample costing a lot of time. In addition, the resolution is influenced by many factors such as particle size, particle uniformity, bed height, column packing quality, flow rate, sample concentration, and volume. The range of molecular weight over which a GFC medium can separate its molecule is referred to as the selectivity of the medium. Modern GFC media covers a molecular weight range of $100^{-8} \times 10^{7}$ Da. Different GFC media have different properties; for example, Superdexa GFC media is designed for high resolution, short run time, and higher recovery rate. Even though using GFC is cumbersome, time-consuming, and costly, its high selectivity and high resolution make it applicable to various separation and purification fields (Jai Ganesh et al. 2011).

7.5.2.3 Ion-Exchange Chromatography

In the present time, the utilization of ion-exchange chromatographic (IEX) techniques for the separation, detection, and structural determination of proteins, peptides, and small nucleotides has marked its place in the field of science. IEX media consist of charged functional groups that bind to molecules with an opposite charge; bounded molecules are eluted from the medium by the process of displacement via the application of an increasing concentration of a similarly charged molecule. IEX is used for collecting the target proteins or bulk impurities from large volumes. It is also used as an intermediate purification step or as a final step for high-resolution purification. Based on some factors like pH or conductivity of the sample, the target may adsorb while the contaminant is unretained; this condition is referred to as positive chromatography, while the reverse is referred to as negative chromatography. Extensive range of IEX media and a suitable IEX medium are chosen based on the target, sample, and resolution that are required for the process. The IEX media includes Capto, Macro Cap, Mini Beads, Mono Beads, Sephadex, Sepharose, and SOURCE. Each media has its own special conditions at which they work such as pH, buffer, and capacity, and, thus, it is used for purifying different types of samples. An important limitation of the IEX technique is that it is highly costly, complex, and not well-suited for biomolecules that are sensitive to pH, metal ions, and other factors (Hsieh et al. 2008).

7.5.2.4 High-Performance Liquid Chromatography

HPLC exists as the most used technique for the separation, identification, and purification of bioactive peptides. The main advantages of this technology are the

ease of operation, high resolution, and high sensitivity, and it gives the elution spectra in a short interval of time as compared to the GFC and IEX, which needs a time period of about 20–30 hours. At present, HPLC is combined with qualitative equipment such as mass spectrometry (MS) and liquid chromatography followed by tandem mass spectrometric detection. The main limitations of HPLC are that the chromatographic columns are expensive, elution composition contains an organic solvent, and it is environmentally unfriendly. Even though this method is very precise and robust, it is very expensive and time-consuming one. Researchers have developed a new, rapid, specific, and cost- and time-effective method, like the high-performance liquid chromatography with evaporative light scattering detection (HPLC-ELSD), which can be used as an investigation tool for purification and quantitative measurements (Dolashka et al. 2011).

7.6 Mechanism and Potential Therapeutic Targets of Marine-Derived Bioactive Compounds

Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease are examples of neurodegenerative illnesses that are defined by a continuous and particular depletion of cells in the selective exposed neuronal populace with respect to the central nervous system (Jellinger 2010).

Biological factors such as neuroinflammation, oxidative stress, mitochondrial malfunction, apoptosis, and excitotoxicity have all been linked to the onset and pathogenesis of neurodegenerative disorders.

Oxidative stress takes part in the pathogenesis of many neurodegenerative disorders. DNA repair system degradation, mitochondrial malfunction, and cellular damage originate from a contrast of inadequate antioxidant protection capacity and reactive oxygen species (ROS) generation, which leads to oxidative stress. These will hasten the succession of neurodegenerative disease and the development of neurodegeneration (Singh et al. 2019).

Furthermore, neuroinflammation has been suggested to act in the pathogenesis of neurodegenerative illnesses (Chen et al. 2016a, b). Neuroinflammation is defined as an inflammatory procedure in the central nervous system, which requires both the innate and adaptive immune systems and is linked to neurodegeneration.

Excitotoxicity, a biological phenomenon, may also play a role in the etiology of neurodegenerative disorders. It is characterized as a pathogenic process in which glutamate receptors in the CNS are over or continuously activated by excitotoxins or excitatory amino acids resulting in neuronal death (Salińska et al. 2006).

Because neurodegeneration is connected with complicated pathogenic causes, addressing numerous modes of action is a viable technique for the interruption and treatment of neurodegenerative illnesses. There are various potential treatment targets for neurodegenerative illnesses that could be researched upon.

As a result of their anti-inflammatory, antiapoptotic, and antioxidant capabilities, marine medications can provide protection. ROS and oxidative stress have been linked to the beginning of neurodegeneration, as previously stated. Despite the fact that cells have antioxidant defenses, neurons become sensitive to oxidative stress over time. The intensity of the effects is proportional to the seriousness of the damage. Use of antioxidants looks to be one of the most promising therapeutic options, even if the success of their delivery in real patients is debatable (Fadaka et al. 2019).

Natural marine carotenoids, for example, have shown to be effective as a dietary supplement, yielding promising outcomes. Reactive species and free radicals are scavenged in part by carotenoids. Wu et al. found that oral delivery of superoxide dismutase and glutathione peroxidase to rats results in a retrieval of superoxide dismutase and glutathione peroxidase activity as well as a rise in GSH levels. AXT (a xanthophyll) can reduce rat brain aging by upregulating brain-derived neurotrophic factor (BDNF); mature BDNF is important for memory development and storage (Chen et al. 2016a, b) and is downregulated in the brains of Alzheimer's patients and other neuropathologies. Furthermore, it has the potential to suppress microglia-supported inflammatory responses, resulting in tissue damage reduction.

Other research looked at the neuroprotective outcomes of ketamine on rat brain ischemia-reperfusion injury. Because of its antioxidant capabilities, pretreatment with AXT before producing ischemia injury was successful (Lu et al. 2010). Human health has also benefited from the protective benefits. FX and AXT share similar characteristics, including anti-inflammatory and antioxidant effects (Galasso et al. 2018).

Microglial cells as pro-inflammatory mediators and a subgroup of enzymes like cyclooxygenase-1 and -2, nitro oxygenase, and other cytokines are all involved in neuroinflammation, which is a common feature of CNS disorders. Anti-inflammatory effects have been discovered in several biocompounds secluded from the *Sinularia* genus of soft corals. *Xyloketal* B, a neuroprotective compound discovered from the mangrove fungus *Xyloketal* sp., is another specimen (Huang et al. 2012).

These are only a few examples of chemicals with neuroprotective properties: they help to prevent inflammation, oxidative stress, and cell death. Each chemical lays down certain properties and has the ability to activate different pathways. Because of their diversity, it is impossible to characterize or classify the chemicals comprehensively.

7.7 Modern Advancement in Neural Regeneration

Marine organisms synthesize a surplus number of bioactive proteins, which are widely used for studies due to their drug actions and potential to cure neurodegenerative disorders. The risk of neurodegeneration increases with longevity. The advancement in modern technologies helped to diagnose disorders related to the

Table 7.2 List of some of the marine-derived compounds for the treatment of neural disorders

Marine bioactive compounds	Source	Uses	Reference
α-Conotoxin Vc1.1	*Conus victoriae* Reeve	Treatment of neuropathic pain	Essack (2012)
χ-Conotoxin MrIA/B	*Conus marmoreus* L.	Treatment of neuropathic pain	Essack (2012)
Conantokin-G	*Conus geographus* L.	Treatment of epilepsy	Essack (2012)
ω-Conotoxin CVID (phase II trials in Bruguière)	*Conus catus* Hwass	Treatment of neuropathic pain	Mortari (2007)
Contulakin-G	*Conus geographus*	Neuropathic and chronic inflammatory pain treatments	Mortari et al. (2007)
3-(2,4-Dimethoxybenzylidene)-anabaseine (DMXBA)	Synthetic derivative produced from the alkaloid anabaseine (nemertines)	Treatment of schizophrenia [6] and Alzheimer's disease (AD)	Mayer et al. (2010) Kem (1997)

central nervous system. Modern research aims at developing potential marine-derived compounds in the treatment of neural disorders focusing mainly on CNS and stimulating neurogenesis. Conotoxin peptides (Table 7.2) are recently used in neuroscience due to their ability to bind with receptors and help in understanding drug-binding site interactions (Essack et al. 2012).

Various research studies have been conducted from the year 2000 and are still continuing, mainly aiming at preventing neurodegeneration.

7.8 Challenges Faced in Neural Regeneration

Complete cures associated with neurological disorders are not possible as of now due to the repeated damage caused by the primary injuries leading to inflammation and turd cell communication and gene exhibition, edema, and hemorrhage. This acts as the causative agent for the destruction of neurons as well as glial cells and the connection between neurons.

Due to the presence of neuron growth inhibitors in the nervous system, the regenerative capacity is beyond our hope. Astrocytes produce glial scars, which retard or prevent neural growth. It also triggers the release of several associated extracellular inhibitors of neural regeneration like chondroitin sulfate proteoglycans (Yiu and He 2006). Likewise, the role of regenerative proteins present in CNS is also damaged after the injury. To overcome such limitations, new inventories in the field of neurology and neuroregenerative drugs paved the way for the treatment of neuron depletion. Modern approaches include the following:

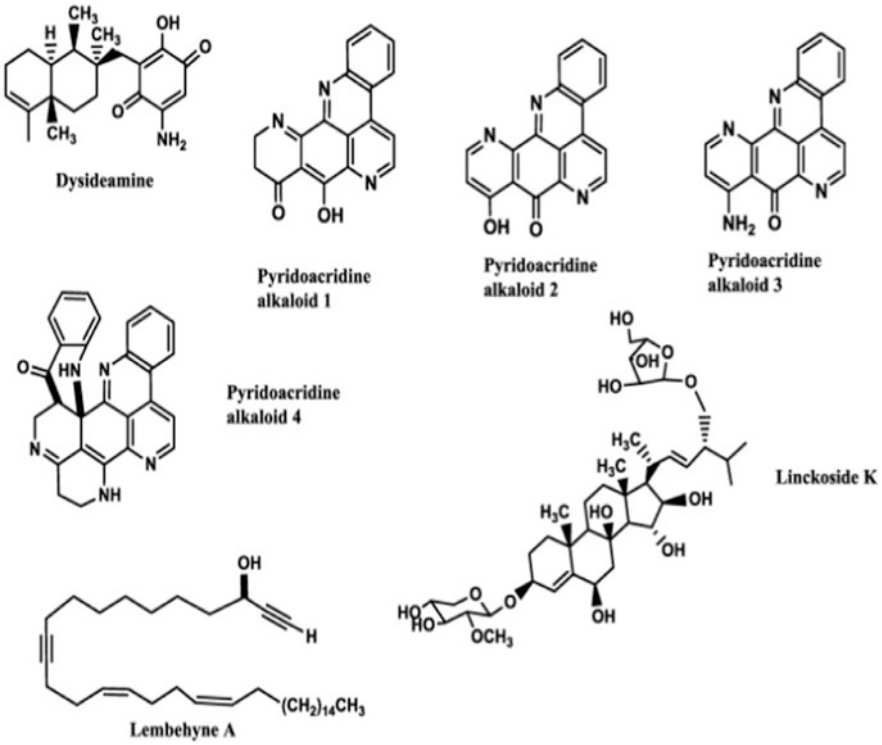

Fig. 7.2 Bioactive marine drug with neural regenerative properties. (Adapted with permission from Paula B. Andrade (2014): Bioactive Marine Drugs and Marine Biomaterials for Brain Diseases 12: 2543)

- Stem cell therapy.
- Administration with neutral trophic factors.
- Neuron growth inhibition removal.
- Use of transmission factors for signaling.
- Regulating immune response.

Regardless of the benefits of transplantation as a reassuring method, the rejection in host cells leading to neuronal cell death is also challenging.

Neuron growth-enhancing compounds (Fig. 7.2) give us hope and are nowadays widely used in neurodegenerative diseases. Dysideamine (extracted from a marine sponge, *Dysidea* sp. 05C33) belonging to the category of sesquiterpene aminoquinone was reported with neuron regenerative potential in neuroblastoma Neuro 2A cells of mouse (Suna et al. 2009). Neurogenesis was reported in pyridoacridine alkaloids (Fig. 7.2) discovered from *Biemna fortis*, a marine sponge (Aoki et al. 2003).

7.9 Conclusion

The search for neuroprotective drugs is still a pressing matter, and natural products obtained from marine invertebrates are excellent candidates for drug development programs (Grosso et al. 2014). Marine compounds and their derivatives have been one of the main research areas for over a decade. One of the reasons is undoubtedly the wide range of molecules and secondary metabolites obtainable and their variety due to the different drastic environments of the oceans and the almost infinite number of organisms that populate them. Among the different pharmaceutical activities on which the research focuses, there are certainly the antioxidant and anti-inflammatory properties, applicable to the treatment of neurodegenerative diseases. One of the examples is represented by the carotenoids FX and AXT, which are used as food supplements and potential pharmacological treatments for PD and AD after years of research. Along with pharmaceutical research practices, the active compounds are utilized for the design of drugs, e.g., 9-methylfascaplysin from fascaplysin. It shows that marine natural products may constitute a promising "library" of natural compounds to design new treatments, adjuvant to gold standard therapies, improving the efficiency of conventional drugs and exerting synergistic or additive positive effects for neurodegenerative diseases (Catanesi et al. 2021).

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Chapter 8
Recent Advancements in Omega Fatty Acids to Treat Neurodegeneration

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Abstract For decades, the predominance of neurocognitive and mental diseases has been increasing, and new treatment techniques are urgently needed due to the current pharmacological treatments' dismal therapeutic efficacy and safety profile. Nutrition is now widely acknowledged as a critical factor in the management and cure of neuropsychiatric illnesses. Both unsaturated fats (EPA and DHA) have vital roles in nerve cell function, neurotransmission and also in immune and inflammatory responses in neurocognitive disease. Much etiological research has laid the foundation for the current interventional clinical studies. The importance of these omega fatty acids in the most common neurodegenerative illnesses, as well as their impact on the human brain, is the objective of this review.

Keywords EPA · DHA · Omega fatty acids · Alzheimer's disease · Parkinson's disease · Schizophrenia · Neurological disorders

Abbreviations

AD	Alzheimer's disease
ALA	Alpha linolenic acid
ARA	Arachidonic acid
BBB	Blood–brain barrier
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid

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LNA α-Linolenic acid
LPS Lipopolysaccharide
PD Parkinson's disease
PUFA Polyunsaturated fatty acids

8.1 Introduction

Many studies have been published worldwide on increasing omega fats consumption to treat neurodegeneration (Denis et al. 2015). Polyunsaturated fatty acids (PUFAs) are considered as a type of unsaturated fats that helps on neurological and physiological developments of an individual. The omega fatty acids, which include the n-3 and n-6 groups, seem to be the key components in unsaturated fats. The brain is highly rich in arachidonic acid and docosahexaenoic acids, which are the precursors and essential sources of omega-3 and -6 families (Bazinet and Layé 2014). Polyunsaturated fatty acids are categorized into two: ω-3 and ω-6. Linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid are omega-3 fatty acids, whereas omega-6 fatty acids are linoleic acid and arachidonic acid. Most abundant PUFAs in the frontal cortex are DHA and ARA. DHA brings cellular features and physiological functions together, such as membrane shape, permeability and mobility, neurotransmitter release, gene function, maturation, neurogenesis, and neuronal development. Both n-3 and n-6 fats are regarded particularly as essential for individuals because they cannot be manufactured from scratch. They play an important role in a range of processes, such as muscle action and the expansion of cells. Forest fish (particularly tuna, sardines, salmon, and cod), pasture-raised eggs, flax, hemp, and walnut are the best sources of these fatty acids. Oily fishes are rich sources of DHA and EPA. PUFAs foster membrane fluidity by disrupting the orderly packed phospholipid bilayer of biological membranes (Avallone et al. 2019).

Although research on the therapeutic impacts of unsaturated fats has been conducted, there has been little discussion of their neurodegenerative qualities. This review focuses on neurodegenerative characteristics of omega fatty acids as well as recent breakthroughs in this field. Dementia, parkinsonism, schizophrenia, and depression are among the most frequent neurodegenerative disorders.

8.2 PUFA and Brain

The cerebral cortex is sensitive to variations in dietary consumption because it contains significant quantities of n-3 and n-6 fats, particularly docosahexaenoic acid and arachidonic acid (Marteinsdottir et al. 1998). Unsaturated fatty acids (PUFAs) and n-3 and n-6 series are necessary molecules that people must get through their diet (Fig. 8.1). In omega-3 PUFAs, there is alpha-linolenic acid,

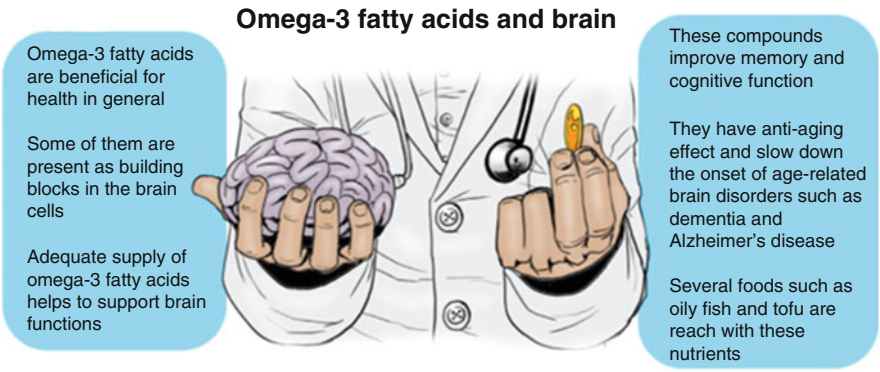

Fig. 8.1 Effect of important fatty acids on brain

eicosapentaenoic acid, and docosahexaenoic acid, whereas omega-6 PUFAs include linoleic acid and arachidonic acid (Michael-Titus 2009). Isolated linoleic acid and alpha-linolenic acid from PUFA are considered essential fatty acids and are discovered to be the progenitors of two distinct PUFA series: omega-6 and omega-3 (Dyall and Michael-Titus 2008).

PUFAs of neuronal membrane structural components, have an impact on cellular function and membrane characteristics, serve as a source of lipid-derived messengers. For cerebral growth and efficient visual function, a sufficient omega-3 PUFA supply is required. An increase in n-3 PUFA consumption and eicosapentaenoic and docosahexaenoic acids evidenced that it benefits neurological disorders and neurodegenerative conditions (Dyall and Michael-Titus 2008). According to data analyzed from multiple organizations, the concentration of particular brain lipids is largely dependent on dietary consumption. Preclinical studies show that omega-3 polyunsaturated fatty acids (n-3 PUFAs) improve cognition, promote neuroprotection (and possibly neurorestoration), reduce neuroinflammation, and alter neuronal function, whereas high-fat diets have the opposite effect (Kerdiles et al. 2017).

Calon and Ouellet's animal studies have proven the role of plasma nonesterified DHA diffusion throughout the brain barrier to provide the cerebrum (Chen and Bazinet 2015) through false readiness (Kerdiles et al. 2017; Ouellet et al. 2009). BBB protects cerebral tissue from both endogenous and exogenous molecules and lipids that are bioavailable for cerebral tissue. Long-chain polyunsaturated fats (PUFAs) included in the diet, like DHA and EPA, also have a direct influence on the brain. The key property of free diffusion into the brain is the chemical structure of fatty acids, which has a nanoparticle size, a few possible hydrophobic interactions, and lipid-soluble moieties (Kerdiles et al. 2017).

8.2.1 Metabolism

Metabolism of unsaturated fatty acids proceeds via a sequence of processes involving continuous dehydration and deformation, with the same enzymes taking part in the series (Fig. 8.2). The first rate-limiting step in omega-3 PUFA metabolism is ALA desaturation, which is followed by elongation. D5-desaturase catalyzes a second desaturation reaction, which produces EPA. The EPA is then extended once more. The pathway is then completed by a final desaturation by D4-desaturase, resulting in the production of the terminal PUFA, DHA (Dyall and Michael-Titus 2008).

In neurophysiology and psychotherapy, omega-3 deficiency has been linked to several diseases, including mental illness, chronic depression, psychosis, and hyperactivity disorder, as well as neurodegenerative diseases such as dementia,

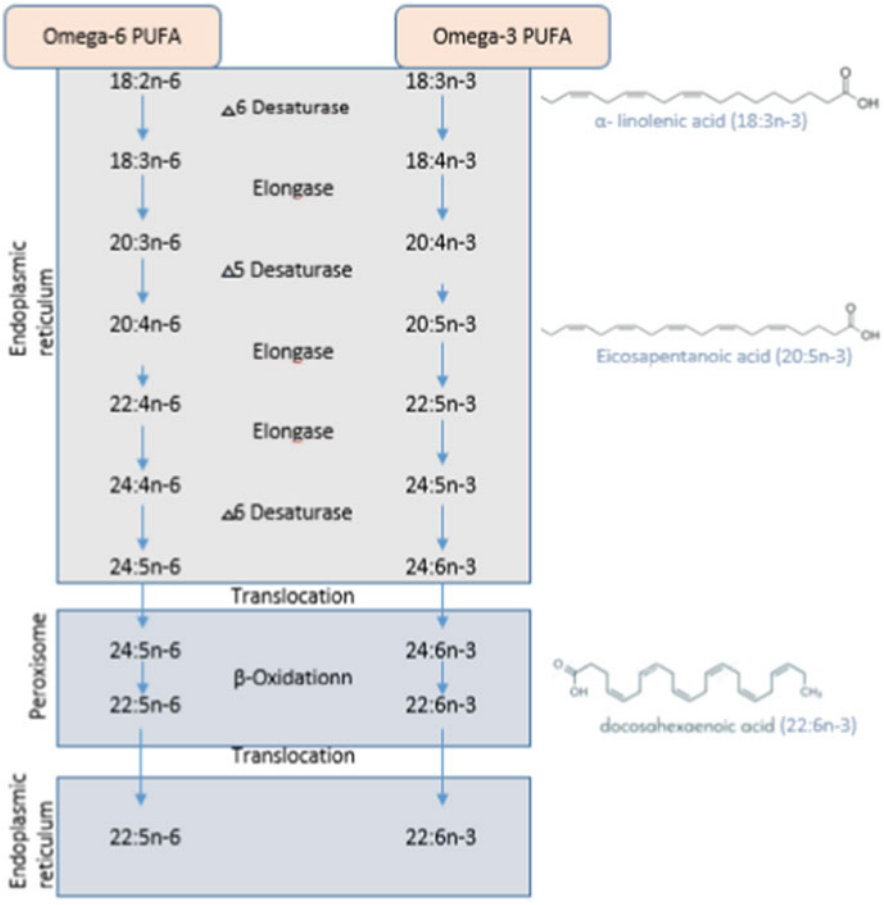

Fig. 8.2 A summary of the biosynthesis pathway for n-3 and n-6 PUFAs (Dyall and Michael-Titus 2008)

Parkinson's, and Huntington's disease (Michael-Titus 2009). In humans, sex differences in conversion efficiency have been discovered; the conversion rate is less than 5%, indicating that women convert more efficiently than men (Burdge 2006), who convert LNA to EPA and DHA. The increased DHA synthesis during pregnancy is important for fetal supply control by estrogen (Dyall and Michael-Titus 2008; Michael-Titus 2009).

8.2.2 Microglia as an N-3 PUFA Target

Microglia are myeloid glial cells that keep the brain in a stable equilibrium gender, maturity level, and area manner (Hanisch and Kettenmann 2007). Microglia are densely packed and syncing cells that are involved in everything from brain growth to pathological changes via treated and nontreated responses (Leow-Dyke et al. 2012). DHA has the ability to normalize the abnormalities caused by lipopolysaccharide (LPS) in microglia (Chen and Bazinet 2015). Pathologic conditions or abnormal signals activate microglia, causing them to discharge inflammatory factors and divert their phagocytic activity to the removal of potentially harmful factors (Leow-Dyke et al. 2012; Layé et al. 2018).

Omega-3 acids (polyunsaturated fatty acids) are potent effectors of microglial activity. The first proof came from the study of cultured cells, which was published in 2007. Moon and colleagues investigated the effect of EPA in LPS-stimulated glial cells (BV2 cell line) for the expression of proinflammatory mediators as well as their inflammatory responses (Moon et al. 2007). Eicosapentaenoic acid also inhibited the production of inflammatory cytokines. This was followed by a study in which native courts of rat microglia were treated with lipopolysaccharides and omega-3 PUFAs at the same time (Liuzzi et al. 2007). The authors tested the LPS-mediated activation of matrix metalloproteinase in the relative importance of a combination of EPA and DHA. Adding omega-3 polyunsaturated fatty acids to lipopolysaccharides on glial cells reduced MMP9 expression levels in a dosages way (Liuzzi et al. 2007).

Ever since, 16 scientific publications using numerous cultured cell models have agreed on omega-3's anti-inflammatory effects on immune cells (Nadjar et al. 2017). New critical functions for omega-3 in such cell types, such as microglial phenotypic expression modification, migratory, phagocytic, apoptosis, or lipid tissue accumulation, along with some of the molecular basis were also explored. To summarize, regardless of the inflammatory challenge, all research studies revealed a dosage reduction throughout the development of pro-inflammatory factors when they were given omega-3 PUFAs (De Smedt-Peyrusse et al. 2008).

Several dietary recommendations with universally accepted ratios of linolenic acid/alpha-linolenic acid nearer to 4–5 and 500 mg/day of eicosapentaenoic acid and docosahexaenoic acid capable of fulfilling our body's omega-3 polysaccharides need to safeguard against the risk of heart disease (Burdge 2004; Lucas et al. 2009). DHA levels in mice and human brains are comparable: between 12.3% and 15.9% in rat and mouse frontal lobe and 14.1% and 15.9% in human postmortem prefrontal cortex (Layé et al. 2018).

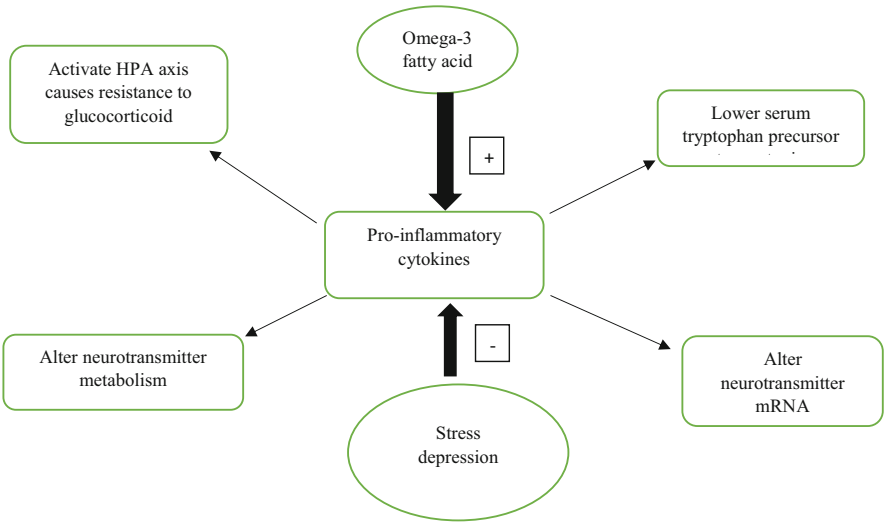

Fig. 8.3 The influence of omega-3 fats on pro-inflammatory cytokines

8.2.3 N-3 Anti-Inflammatory Effects within Central Nervous System

Swelling in the brain through reaction to pathogens aids in the maintenance of organ homeostasis. The activation of microglial cells, which are brain stem macrophages, causes brain swelling (Aloisi 2001). When activated, bodies create inflammatory and noncytokines. Anti-inflammatory cytokine production has become highly toxic, resulting in brain loss involved in brain diseases. Limiting inflammation is critical, and identifying mediators provides new objectives in brain damage management and cure (Layé et al. 2018). A diverse range of studies indicates the effect of omega fatty acids or their products as indicators for limiting neuroinflammation (Fig. 8.3) (Calder 2006).

8.3 The Presence of Polyunsaturated Fatty Acids in Various Forms of Neurodegeneration

The presence of polyunsaturated fats in neurodegeneration has been widely studied by many researchers. In the following section, the forms that are very common are discussed, and additional diseases are discussed in detail at the end of this chapter.

8.3.1 Alzheimer's Disease and Normal Brain Aging

Enhanced oxidative damage, mitochondrial dysfunction, respiration changes, and DNA damage are widely characterized as normal brain aging (Canugovi et al. 2013). A decrease in brain volume and weight and changes in membrane lipid content are all correlated with maturity level systemic alterations inside the central nervous system (Svennerholm et al. 1997). The most common brain condition is the type of dementia in the senior citizens, affecting cognitive function, mood, and behavior in 4.4% above the age of 65 (Selkoe et al. 2012). Pathological features of Alzheimer's disease include neurofibrils composed primarily of hyperphosphorylated tau proteins and sensile amyloid-protein-containing plaques (Lloret et al. 2015). AD's pathogenesis and etiology are poorly understood, and a recent study discovered that only a few clinical trials for AD therapies have been conducted, with no successful compounds reported thus far (Cummings et al. 2014). This highlights the need for novel approaches to treating this disease.

The research done worldwide indicates a direct link between omega-3 fats and cognitive behavior. Many studies have also pointed out the positive omega-3 fat effects on cognitive outcomes and on the life of survivors at their early stages of AD and mild cognitive impairment (La Rosa et al. 2018). Neutral results have also been reported by some interventional studies. Zhang et al. (2016) reported that omega-3 PUFAs found in fishery products are linked to AD patients by decreasing their risk of cognitive decline. A study done in healthy subjects by Nunes et al. (2017) also comes with a similar outcome, in which both the ratios of omega-6/omega-3 and the n-3 index were linked to cognitive function and also in hippocampal and total brain volume.

The biological plausibility of n-3 PUFAs' therapeutic benefits in dementia is supported by experimental research, that is, anti-free radical actions, increased neuroplasticity, and other mechanisms with a more direct link to the disease that manifests itself in memory loss, including anti-inflammation, amyloid, tau proteins, and proof of these effects (Belkouch et al. 2016). Docosahexaenoic acid has the capacity to be more neuroprotective than EPA because DHA depletion is strongly linked to cortical dysfunction as well as hippocampal atrophy (Hashimoto and Hossain 2011). DHA also increases dendritic density while decreasing the levels of amyloid-beta and tau protein.

Aside from these processes, n-3 PUFAs may have an indirect influence on the brain impairment. Dementia and Alzheimer's are both linked to coronary heart disease in the brain and microinfarcts, for example, and it has been demonstrated with n-3 unsaturated fatty acids that it can improve cognitive abilities through lowering cardiovascular morbidity (Luo et al. 2018). Wide variation in the research plan, working area, time intervals between measurements and carry intervals, variants of PUFA administrations, distinct diagnostic markers for fat condition, variety of cognitive situations, and different (and partly low) tolerance in omega-3 in AD are all reasons that could describe the various studies on omega-3 and dementia. As far

as AD is concerned, this is of special importance as the disease begins years before clinical symptoms appear (Kulzow et al. 2016).

8.3.2 Parkinson's Disease

It is the most prevalent form that comes next to AD, and its pervasiveness increases with age. PD is caused mostly by progressive destruction of dopamine-producing nerve cells in the substantia nigra compacta (SNpc), which is essential for the dopamine, a neurotransmitter involved with movement and control process. Symptoms of PD include tremor, dementia, anxiety, muscular rigidity, akinesia, bradykinesia, postural insecurity, and depression in advanced cases. Quasi symptoms of Parkinson's disease, such as sleep disturbances, mood disorders, somatic system failures, and cognitive impairment, are also important aspects of the disease's clinical manifestation. Scientists believe that environmental factors, such as exposure to farming chemicals, like pesticides and herbicides; UV rays and ionizing radiations; and viruses, bacteria, and heavy metals can also trigger the development of PD. The cause of Parkinson's disease is complicated as a result of a large communication of nature and nurture. This disease is diagnosed when 50–80% of the neurons seem to be mortally wounded, and there is no therapy to solve the issue. There is a growing need to establish therapies that can slow the progression of neurodegeneration or stop their progression (Li and Song 2020). Because of their anti-inflammatory, antioxidative, neuroprotective, and metabolic properties, omega-3 fats have been found in the management of these neurological conditions in studies. Several studies, however, suggest that a balanced diet in unsaturated fatty acids may actually improve the management of such diseases.

In mice with Parkinson's disease, the impact caused by omega-3 fats has been studied. Several studies have pulled off excellent results from the investigation done on the intake of omega-3 PUFA and Parkinson's. However, immunological and preclinical research has revealed that consumption of decreased intake of omega-3 PUFAs relates to a higher risk for PD, and its high intake associates with the low risk for PD. Dietary supplements of PUFUs are also found to slow down the progression or reduction in the complication of the disease. More controlled trials are required to look into the effects of fats on patients with Parkinson's disease.

Omega fatty acids nourish the myelin sheath, which provides insulation for neurons. As PD is associated with neuroinflammation, a suitable therapy is required that could both increase dopamine and reduce neuroinflammation. Therapeutic levels of omega-3 might be considered as a remedy to postpone the PD process and improve PD symptoms (Avallone et al. 2019).

PUFAs have the specific potential for crossing blood–brain barrier that most drugs are deprived of. They are also known to elevate dopamine levels and reduce neuroinflammation in the brain (Li and Song 2020).

PUFAs enter the bloodstream before passing the blood–brain barrier and combining to the cell wall. The DHA of serum is bound to proteins, primarily albumin.

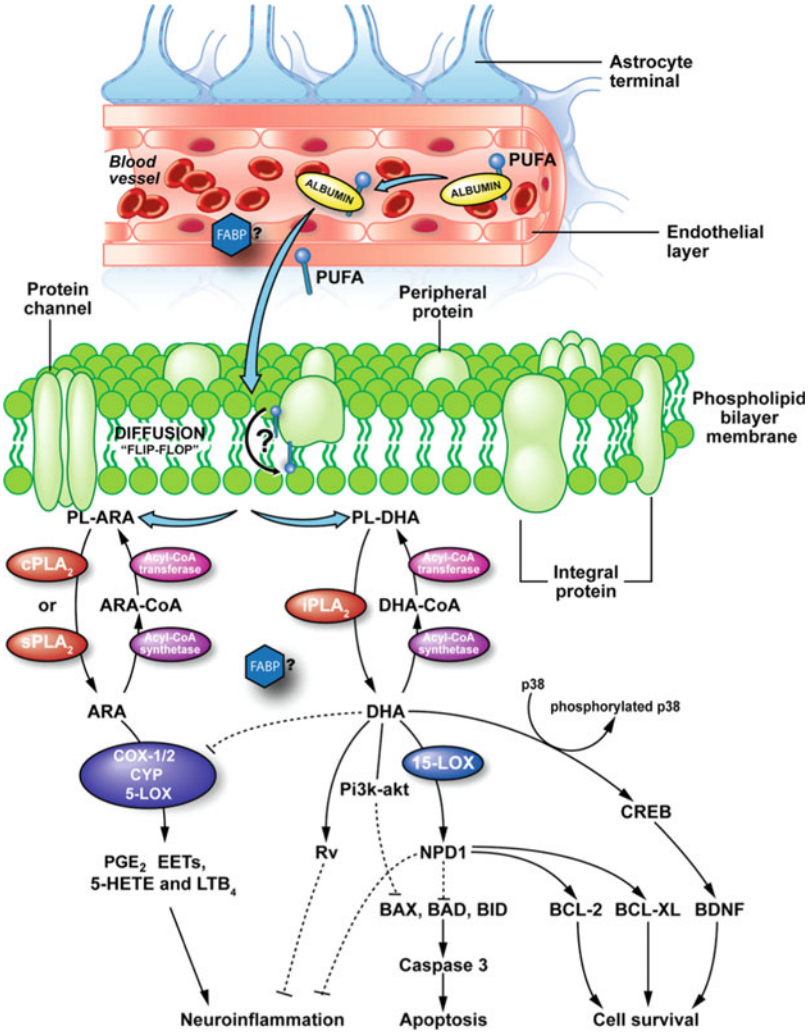

Fig. 8.4 Effect of PUFAs in PD (Bousquet et al. 2011)

The docosahexaenoic acid in the lipid bilayers improves cellular function and assists membrane receptors, channels, and function of other proteins. The cytosolic compartment is where from PUFAs are released (Fig. 8.4).

The findings of the Université Laval research team could help prevent the disease and slow down its progression. Within the experiment, the researchers noticed that once mice have been fed with a diet rich in omega-3, they were found to be resistant toward MPTP (a toxic compound), which leads to brain damage similar to Parkinson's. Bosquet (Bousquet et al. 2011) showed the effectiveness of this

compound in targeting and destroying the dopamine-producing neurons of the brain. This revealed the importance of n-3 fats in brain functioning.

According to research, dietary supplements have a major impact on balancing metabolism and cerebral swelling of Parkinson's disease. Some other studies found that combining n-3 fats with vitamin E has a positive impact on PD. Studies also suggest a possibility to manage genes involved in brain inflammation and body metabolism of PD survivors. Inflammatory responses associated with Parkinson's disease complicates the studies. Omega-3 fats have an important effect in the prevention of cell damage.

Canadian research demonstrates the effectiveness of omega-3 in preventing Parkinson's disease. In this research, one group of mice received omega-3 supplements for 10 months, while the other group served as a control before being injected with a chemical that causes Parkinson's disease. The control group was observed with a significant drop in dopamine levels, whereas the group that received omega-3 supplementation did not (Avallone et al. 2019).

Without hesitation, much research should be conducted throughout this framework of Parkinson's disease, associated with the diagnosis with fats, such as eicosapentaenoic acid, which appears to have a stronger anti-inflammatory oxidative role in the treatment of depressive episodes and neurological disorders.

8.3.3 Schizophrenia

Schizophrenia is a chronic nervous breakdown that affects 20 million people worldwide. Schizophrenia typically manifests itself in men in their early twenties and women in their twenties to thirties. Children could also be affected by schizophrenia, but this is not common. According to the National Institute of Mental Health, 0.25–0.64% of US adults are affected with schizophrenia. Initial symptoms of schizophrenia include isolation from friends and family, lack of concentration, sleep problems, poor academic performance, and lack of motivation (Frith and Johnstone 2003). Common symptoms include distortions of reality, often experiencing delusions or hallucinations, disorganized speech, distortions in thinking, perception, and sense of self behavior, lack of motivation, trouble with logical thinking, abnormal movements, irritability or depressed mood, confused thinking and speech, nonsensical behavior unable to begin or complete actions, lack of interest in living or interacting socially, emotionally flat, talking in a boring, disconnected way, lack of pleasure or interest in life, a decrease in performance, difficulty sleeping, and a challenge to memorize, express their ideas, or solve problems. Schizophrenia damages individuals' thinking ability, emotion management, and decision-making ability (Hodgins et al. 2013).

It is a complicated, long-term medical condition. However, early diagnosis and treatment of schizophrenia could be able to manage symptoms and diminish recurrence. Symptoms of schizophrenia are usually visible in early adulthood and must persevere for at least 6 months for a diagnosis to be made. Several studies have found

that a variety of genes and environmental influences, as well as their interactions, play a role in symptom causation. People with a family history of schizophrenia have a higher probability of developing this disorder. Environmental factors such as viral diseases, environmental toxins such as marijuana, stressors, and nutritional problems prior to birth all have an impact on the disease. Schizophrenia can occur in children up to the age of five, but it is uncommon. Puberty-related brain changes may trigger schizophrenia in people who are predisposed due to genetics, environmental exposures, or the types of brain differences. Researchers discovered differences in brain structure and function in people who have schizophrenia. They may be unable to modulate chemical messengers such as neurotransmitters that affect the cell in ways that in turn affect emotion and behavior. Schizophrenia is more often visible when the body undergoes physiological and hormonal changes. It can also arise when there is an imbalance of dopamine or serotonin in the brain (Hsu et al. 2020).

n-3 fat intakes have been indicated among studies to be efficacious in decreasing the symptoms caused by schizophrenia. Abnormal metabolism of PUFA was found to be one of the many reasons involved in the development of this disorder (Frith and Johnstone 2003). Uncontrolled metabolism of PUFA disturbs neural development, expands the inflammatory responses, and finally leads to abnormal neurotransmission. Adolescents supplemented with omega-3 fatty acid showed improved psychotic symptoms (Lange 2020).

According to studies, the absorption of n-3 fats is lowered in schizophrenics, and the administration of fatty acids was shown to be efficient besides symptom relief (Bozzatello et al. 2016). The concentration of omega fats within the central nervous system is lower in schizophrenia (Fig. 8.5). In rodents, dopamine concentration decreased as a result of low polyunsaturated fats. The link between schizophrenia and omega-3 fats first came to the light in the late 1980s (Ohara 2007).

Because lipids account for 50–60% points of the mature nervous system, they play a key role in brain mental functioning. Abnormally elevated metabolism in the brain impairs brain function. Nutrients can affect the formation of lipids in the brain, and thus dietary management, such as n-3 fatty acid supplementation, would prevent the development of mental diseases (Hodgins et al. 2013). Omega-3 s, such as DHA, have the ability to restore the structural capacity of cell membranes and neurotransmission receptors (Fig. 8.6) (Hsu et al. 2020). A unique idea that includes omega-3 PUFAs in addition to different therapies aimed from the beginning of the disease might be successful in reducing the severity of symptoms as well as recurrence.

8.3.4 Depression

Large clinical and internal medicine studies have discovered a strong relationship between n-3 polyunsaturated status and symptoms of depression (Grosso et al. 2016). Decreased omega-3 index and limited fish consumption have indeed been linked to a high risk of severe mental illness (Sanchez-Villegas et al. 2018). As a result, in multiple clinical trials, enhancing the n-3 index lowers the incidence and

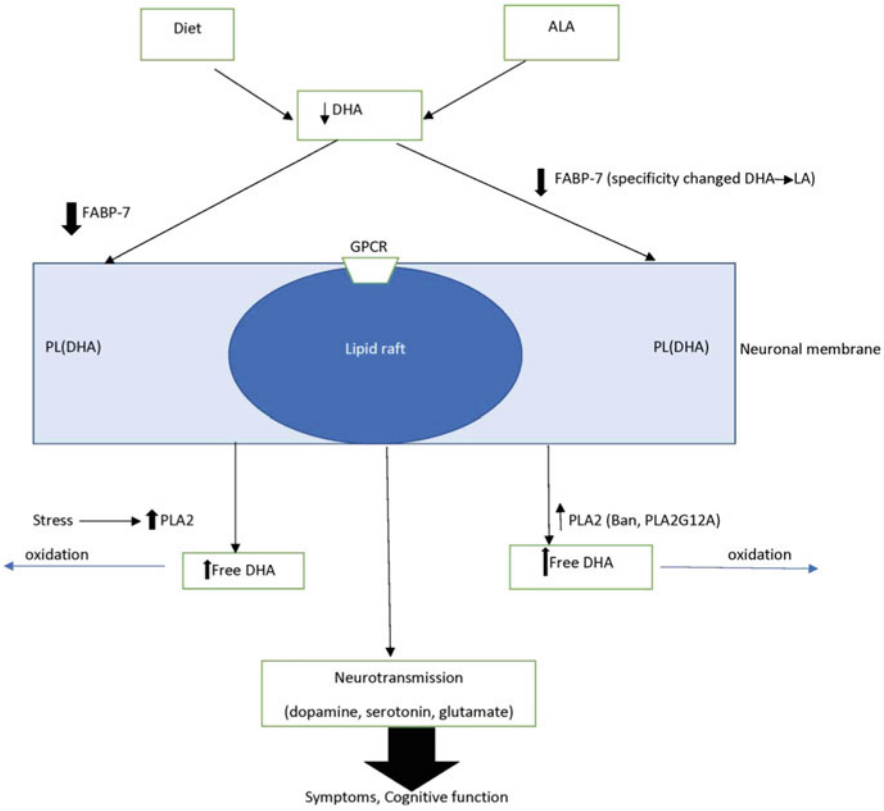

Fig. 8.5 Causes of deficiency of PUFA (with special reference to DHA) in schizophrenia

severity of depression symptoms. One study found that every 1% rise in the omega-3 index reduced the risk of developing depression by 28% (Reeves et al. 2017). Depressed people have increased omega-6/omega-3 ratios compared with nondepressed people (Swenne et al. 2011).

Despite the fact that some of the research yielded mixed findings, certain other studies verified antidepressant capacity of eicosapentaenoic acid and docosahexaenoic acid (Yang et al. 2018). Therapeutic benefits have been observed at daily dose scales from 1 to 3.5 g per day. Although each was suggested as one of the most powerful intermediaries of n-3 PUFAs' potential benefits in depression, it is unclear whether their antidepressant efficacy differs (Su et al. 2018). The amount of DHA in blood phospholipids distinguished responders from nonresponders in the latest research, but not the amount of EPA (Gananca et al. 2017).

The effectiveness of omega-3 fats in particular subgroups, such as kids, teenagers, senior citizens with fundamental co-morbidities, or in associated disorders like bipolar illness, has only been studied in part. The majority of existing research suggests the beneficial effect of omega-3 in these patient populations (Berger et al.

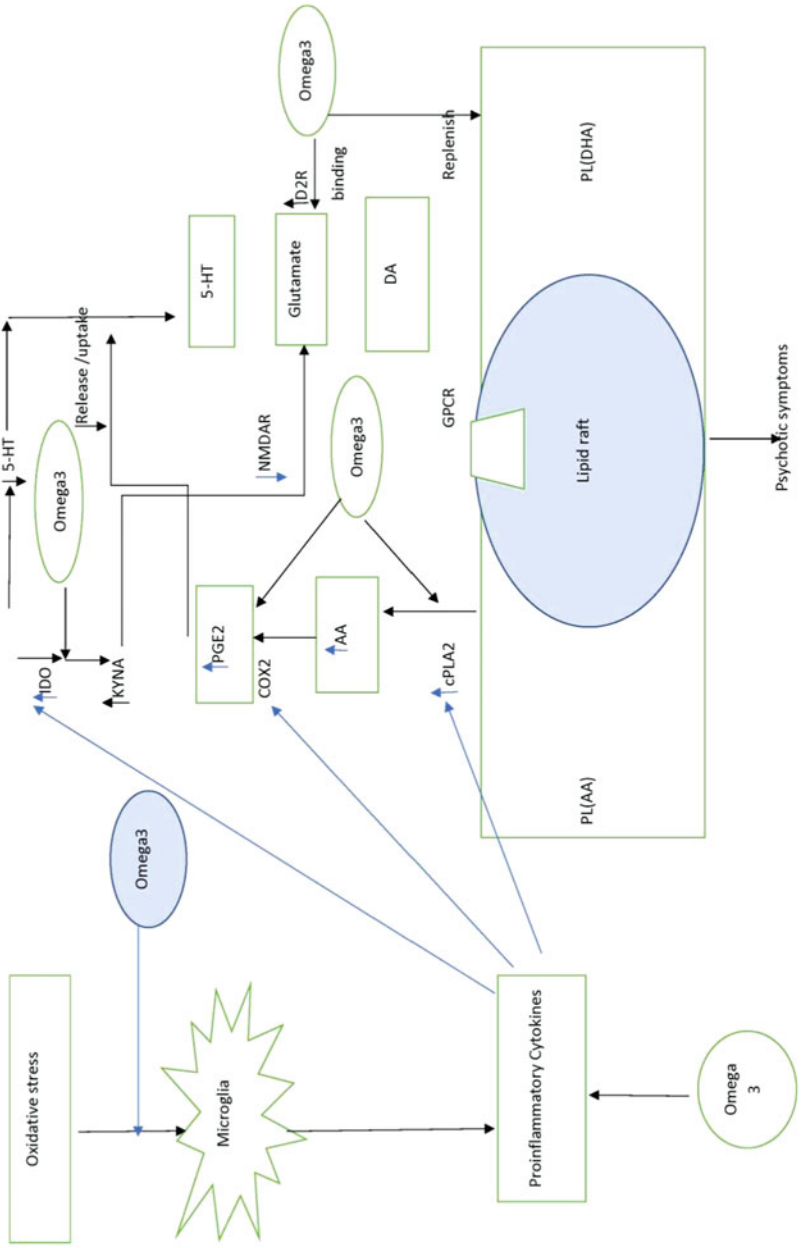

Fig. 8.6 This diagram depicts the mechanisms by which omega-3 fatty acids improve neurotransmitter levels in schizophrenics (Hsu et al. 2020)

2017). It is unclear how n-3 fats work as antidepressants. Multiple unspecific modes of action appear to be in work at the moment. Stabilization of G-protein signaling is one such mechanism that has been proposed. Other processes include alterations in telomeres and regulation of mediators in inflammation (Czysz and Rasenick 2013).

PUFAs have been observed in a number of other neurodegenerative diseases, including multiple sclerosis, Huntington's disease, attention deficit hyperactivity disorder (ADHD), Friedreich's ataxia, ischemic stroke, and others. Mitochondrial failure is a common early pathophysiological event in Alzheimer's disease and other neurodegenerative diseases. Most research studies indicate the positive effect of omega-3 fats in neurodegenerative diseases, but it is partially understood. Various research studies are currently being explored on polyunsaturated fatty acids (PUFAs). Clinical studies on PUFAs have to be done in much more efficacious manner (Eckert et al. 2013). Mitochondria, prostaglandins, and leukotrienes are the main focus of n-3 fats in neurodegenerative diseases.

Though there is some limited evidence that PUFA supplementation may be beneficial, with little proof. There is a need for more elevated research. Studies also come up with scientific evidences that PUFA supplementation aids in the treatment of ADHD symptoms in kids and teenagers (LaChance et al. 2016).

8.4 Conclusion

It is reasonable to believe that there is a link between dietary factors and neuropsychiatric diseases. Several observational datas have discovered a strong link between low omega-3 fatty acid concentrations and a high intensity of a variety of neuropsychiatric diseases. As a result, numerous interventional studies examining the effectiveness of omega-3 fatty acid intake associated with various psychiatric disorders have been conducted. These findings are backed up by a massive amount of research, which provides a stable foundation. At the moment, omega-3 fatty acids are found to be more helpful in protracted prevention than in acute episode treatment. The quantification of omega-3 fatty acids in blood for diagnoses, risk evaluation, and medicinal prescription is a low-cost and widely available procedure. Although the use of omega-3 fatty acids as medicinal alternatives in the treatment of neuropsychiatric illnesses in its early stages, its medicinal prospects, excellent safety characteristics, simplicity, and low cost call for further research.

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Conflict of Interest The authors have no conflict of interests.

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Chapter 9
Role of Alkoxyglycerol to Pause Tau-Induced Alzheimer's Disease

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Abstract Alzheimer's disease (AD) is a common form of brain disorder and proper dysfunctionality which affect our thinking, learning, decision-making ability, results into inability to perform tasks efficiently. Dementia was studied and brought into light long since, from the pieces of evidences by Greek philosophers. But AD which is a common form of dementia appeared in 1906 in a 50-year-old woman suffering from common mental disorders. With passing time, the percentage increase in number of cases of AD and lack of proper diagnosis and treatment made it one of the most researched chronic mental disorder within scientific community. A lot of scientific studies and research have revealed that the hyperphosphorylated microtubule associated tau protein can be the cause of many neurodegenerative diseases in which tau mutations may be the cause for the hyperphosphorylated tau formation which effect neurons harshly results into neurodegeneration in brain. Many drugs are invented and used for the AD but they are not found to be much suitable cure for the disorder due to cost benefits and availability at right time everywhere. Later on, it is found that the AD and related dementia is the decline of plasmalogens, so a key glycerophospholipid may help in normal neuron function. With dietary supplementation of alkoxyglycerols (AKG) selective levels of plasmalogens can be restored and augmented in human body. AKG are ether lipids which is abundantly found in shark liver oil. Hence, AKG extracted from liver oil prove to be the suitable cure for AD and other types of neurodegenerative diseases. The researches are going on which is paving the way for invention of more effective and suitable cures.

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Keywords Alzheimer's · Alkoxyglycerols · Dementia · Glycerophospholipid · Phosphorylated tau · Plasmalogens

Abbreviations

A β	β-Amyloid
AD	Alzheimer's disease
AKG	Alkyl glycerol and /or alkoxy glycerol
Alkyl-Gro	1-*O*-alkylglycerols
ARTAG	Aging-related tau astrogliopathy
BBB	Blood Brain Barrier
CAA	Cerebral amyloid angiopathy
CNS	Central nervous System
CSF	Cerebrospinal Fluid
DHA	Docosahexaenoic acid
FDA	Food and Drug Administration
NFTs	Neurofibrillary tangles
PART	Primary age-related tauopathy
PHFs	Paired helical filaments
PlsEtns	ethanolamine plasmalogens
PNS	Peripheral Nervous System
ROS	reactive oxygen species
SLO	shark liver oil

9.1 Introduction

Alzheimer's disease is a common form of dementia which is the chronic and persistent mental disorder. The initial symptom of disease is like the mild memory loss and having inability to carry on conversation and environment respond (Mucke 2009; Goedert and Spillantini 2006). In scientific studies it has been found that risk of having AD and other types of dementia increases with increasing age. Increasing age can not only be the reason for the risk of having AD and other types of dementia. Studying the risk factor by comparing individuals of 65 to 85 years old revealed that individuals of 65 years old and above have a double chance of AD risk while individuals of 85 years and above only have a one-third chance of having AD (Keller 2006; Bartzokis 2004; Bell et al. 2019) (Fig. 9.1). The possible cause of AD can be genetic mutation. Other than genetic mutation, environmental impact and lifestyle factors can also be the possible cause of AD. The risk of having AD because of any of the above factors may vary from person to person (Purandare et al. 2006; Bush 2003; Drachman 2014). Tau mutation can cause neurodegeneration through tau hyperphosphorylation that results into formation of neurofibrillary tangle in brain. There is self-assembly of PHF/SF in AD brain due to tau mutation. Thus, a promising therapeutic treatment of AD can be offered by inhibition of abnormal hypophosphorylation of tau (Kandimalla

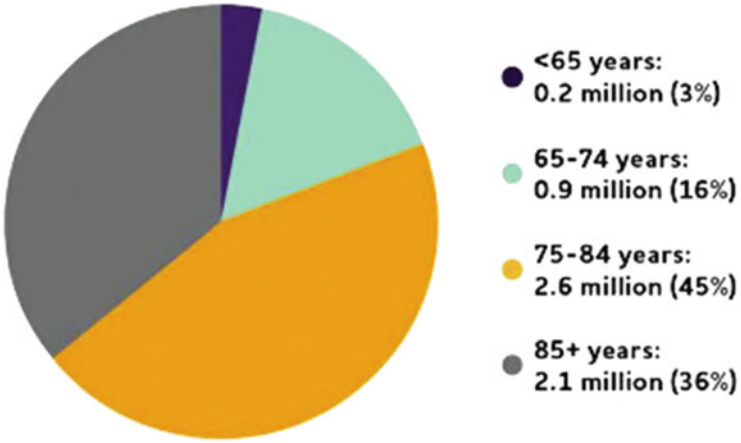

Fig. 9.1 Diagrammatic representation of ages of people with AD. (Adapted from Alzheimer's Association (2019))

et al. 2018). Hence there is a subsequent surge in healthcare costs due to unprecedented increase in number of people having this disorder. Clinically it is found that only four drugs are available for the treatment of AD symptoms, but they are unable to modify the disease. Consequently, having suitable cure is urgent demand of the time (Hung and Fu 2017). One emerging cure is alkoxyglycerol extracted from shark liver oil, found to have potential to cure AD (Poleschuk et al. 2020) In this chapter, various aspects related to AD will be summarised.

9.2 Dementia

Dementia is a group of symptoms which consistently occur together or a syndrome which shows a persistent nature and a combination of different states which are characterised by deterioration of activities of brain such as obliviousness, loss of capability for discernment along with reduced potential in social interactions and capacity to think. The people with dementia face impacts physically, psychologically, socially and economically, which significantly affects not only on their personal life but also on their related sectors of life including their walk of life, families, household and society at a large extent (Masika et al. 2020). Worldwide with nearly 60% people living in economically developing countries, 50 million people often suffers from dementia. In general population among the people aged 60 and higher, the people suffering with dementia at a specific period of time comes in between 5 and 8%. The total number of people who are suffering from dementia is estimated to come across about 82 million by 2030 and by 2050 it will be 152 million (Masika et al. 2020).

Dementia is acknowledged by World Health Organization as something which have gained a good priority in public health and endorsed a global agenda on public

health response in 2017–2025 to dementia which furnishes a blueprint for action in addressing dementia as a public health property, by establishing dementia friendly initiatives, which includes detection, therapy, consideration, research and innovation (Masika et al. 2020).

9.2.1 Alzheimer's Disease

Alzheimer's disease acts as seed or foundation to instigate chronic or persistent dementia. It is considered as a neurodegenerative disease and is also an important protein conformation disease, which is predominantly engendered by the adherent polymerisation and processing of commonly soluble neuronal proteins (Tiwari et al. 2019). When misfolded, soluble neuronal protein attains altered conformation, which happens due to external factors, genetic mutation, ageing and aggregate, and leads to abnormality in neural functionalities. The discovery of AD titled as a neurodegenerative disease has been accredited to Alois Alzheimer, who was a German neurologist. He examined an old woman aged 50 years, who was named as Auguste Deter. She was suffering from loss of memory, difficulty in speaking especially in areas of language, confusions and hallucinations. Her autopsy has revealed the existence of divulged tangles and plaques in the cerebral cortex, which has persuaded him that this has gone yonder the tangles of dementia. This discovery of Sir Alois Alzheimer has paved way to further research which has disclosed certain facts like the existence of neuritic Amyloid β plaques in people who are suffering from this particular disease (Tiwari et al. 2019).

The symptoms of AD and related changes in the brain are usually noticed after 20 years of an individual in dementia patients. All these symptoms will happen when nerve cells in various sites of brain which are involved in various activities like learning, thinking and memory are destroyed. The advancement of Alzheimer's disease from brain that are concealed to the individual affected starts with problems in memory and finally leads to physical disability, which is termed as the Alzheimer's disease continuum. This continuum has been broadly classified into three broad phases termed as (1) pre-clinical, (2) mild cognitive impairment and finally (3) dementia from AD (Dubois et al. 2016) (Fig. 9.2).

The pathogenesis of AD has been assigned to intracellular neurofibrillary tangles made with tau-protein which is hyperphosphorylated and found at human brain's cortical- limbic areas. The aggregates of amyloid beta plaques are found as extra-cellular aggregate matters. Upon identifying and interpreting the important causative factors and mechanism of pathogenesis of AD, it seems to be essential to come across the fields such as pathogenesis, different mechanisms, diagnosis and finally various ways that help to develop best therapeutics.

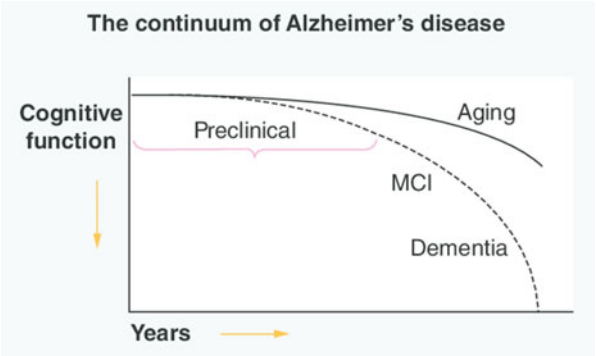

Fig. 9.2 The continuum of AD (Adapted from Sultan et al. 2018)

9.2.2 Neurological and Pathological Changes in AD

AD is a neurodegenerative disease. It results from a person's conditions like his/her genetic makeup, their age, enlightenment and society. Many postulates are there that set down the bedrock to attain the idea behind the examination of AD, from the most primitives like the cholinergic hypothesis. This cholinergic hypothesis is according to certain facts like AD patients usually exhibit abatement in action of acetyl cholinesterase and choline acetyltransferase in the cerebral cortex of their brain in comparison to the ordinary human brain. The post-mortem brain tissue from patients who had suffered from AD has established the sunken neurotransmitter pathway activity, which reveals the loss of cholinergic neurotransmission which mainly hand out to the cognitive impairment found in people with AD (McGirr et al. 2020).

The macroscopic features of AD include a brain often with at least moderate cortical atrophy that is usually marked in brain's limbic lobe structures. The temporal and frontal cortices usually have atrophy of the gyri, with inflated sulcal spaces, while the somatosensory cortices and primary motor are usually found undisturbed. Finally, due to this, there is usually an inflammation of the temporal and frontal horns of the lateral ventricle, and the brain weight is found to be reduced in most affected individuals (Fig. 9.3) (DeTure and Dickson 2019).

The area of study towards perceptiveness of Alzheimer's disease pathogenesis is wide. The reported histopathological characteristics of AD revealed that they are extracellular aggregates of neurofibrillary tangles which seem to be intracellular aggregations. Aβ plaques are composed of hyperphosphorylated microtubules associated tau (Tiwari et al. 2019). The development of Aβ plaques is initiated in temporal region, then at basal portion and finally at neocortex region's orbitofrontal portion. In later stages it progresses throughout the neocortex, amygdale, hippocampus and diencephalon. In serious cases Aβ progresses throughout the mesencephalon, cerebellar cortex and lower brain stem (Tiwari et al. 2019).

The important neuropathological hallmarks of Alzheimer disease (AD) consist of positive bruises such as cerebral amyloid angiopathy, amyloid plaques, and

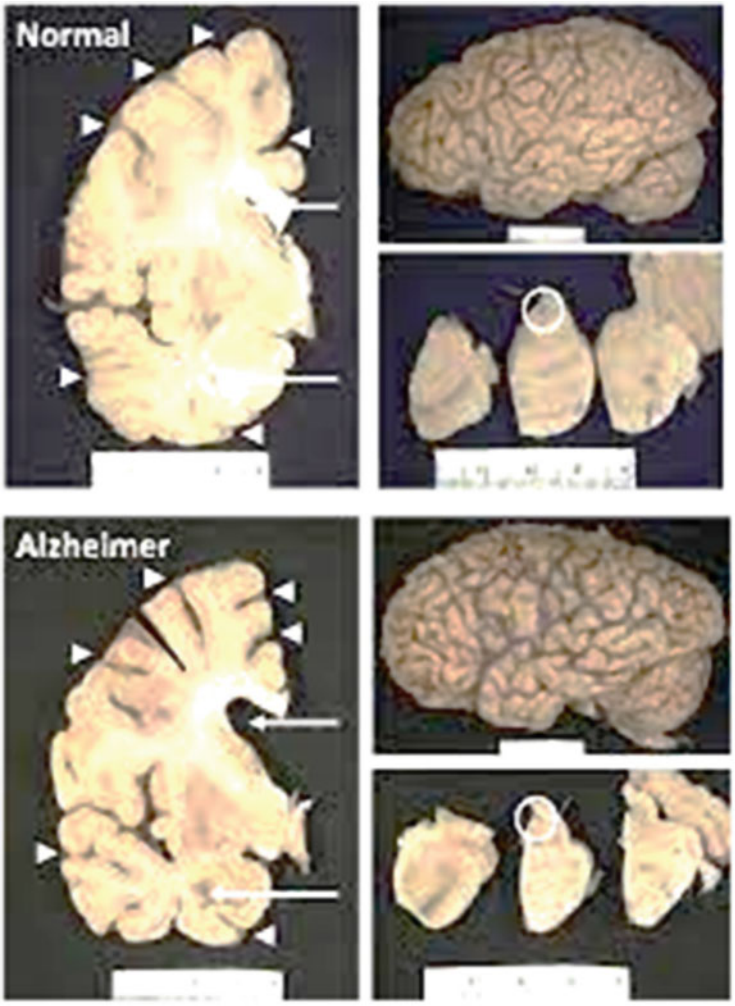

Fig. 9.3 Gross Anatomy of Alzheimer's Brain. (Adapted from DeTure and Dickson 2019)

neurofibrillary tangles and the negative bruises such as synaptic and neuronal damage (Serrano-Pozo et al. 2011).

Synapse and neuronal loss pave a great way for the tangle formation (Gómez-Isla et al. 1997; Arnold et al. 1991). This neurodegenerative process is distinguished by primordial impairment of synapses with retreating deterioration of axons which finally results in the atrophy of the perikaryon and dendritic tree. The synaptic loss at the limbic system and neocortex strongly correlate the cognitive deterioration seen in patients suffering from AD. The trans-synaptic delivery of Aβ promotes neurodegeneration characterised by synapsis loss. The Aβ oligomers secreted by

cultured neurons have the potential to damage spines and interfere with activity of regulated cytoskeleton associated protein distribution (Serrano-Pozo et al. 2011).

The pathology of AD is evidently simplistic and deceitfully multiplex. It is outwardly facile since it is composed of only two main bruises each and everything with a dominant singular protein, derived from well-circumscribed metabolic pathways. Both of these bruises occur with age and are the results of normal cellular metabolism from embryonic life to senescence (Castellani et al. 2006).

9.3 Physiological Function of Tau

As a microtubule allied protein, tau stabilises neuronal tubulins in turn stimulating axonal outgrowth. This intrinsically unfurled protein is soluble and has chance for aggregation (Wang and Mandelkow 2016). Tau protein regulates axonal transport, myelination, microtubule dynamics, neuronal excitability, iron equipoise, neurogenesis, learning and memory motor function, glucose metabolism, and DNA conservation (Fig. 9.4) (Kent et al. 2020). Sporadically tau protein happens to undergo alteration, mainly through phosphorylation that leads to certain pathological situations which are toxic to neuron. This results in several neurological disorders collectively called tauopathies, among which Alzheimer's disease is most common (Avila et al. 2004) (Fig. 9.5).

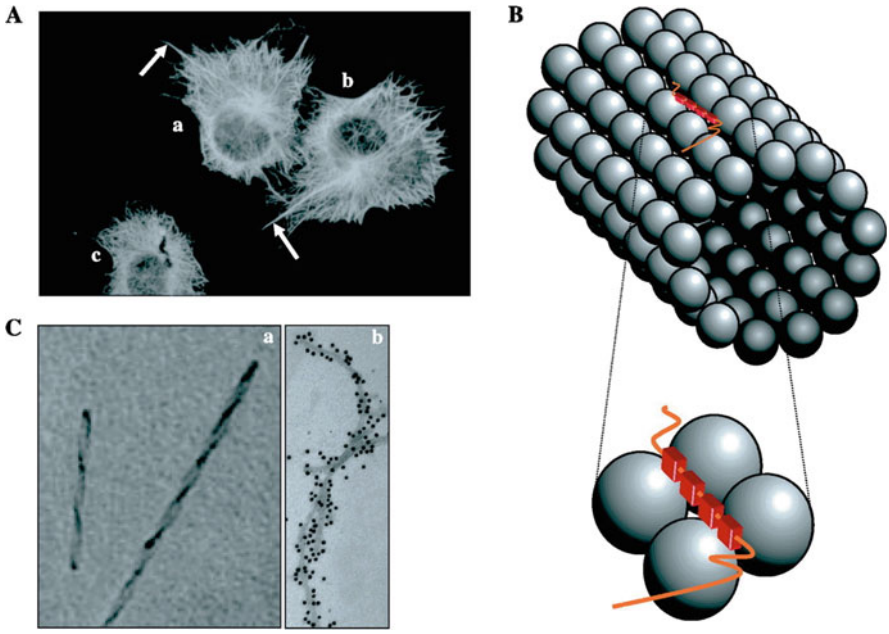

Fig. 9.4 Schematic representation of tau protein. (Adapted from Avila et al. 2004)

9.3.1 Role of tau in Neurodegenerative Diseases

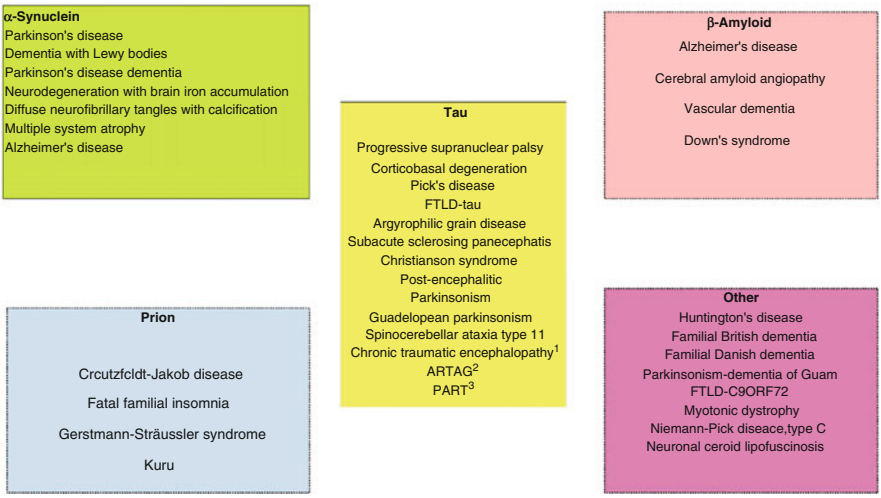

Fig. 9.5 Diagrammatic representation of tau pathology expressive in various neuropathological conditions where the middle box dictates the principal pathological feature of tau. The criss-crossing panels depict tau incorporation succeeded by disease associated auxiliary proteins.1. Chronic traumatic encephalopathy includes dementia pugilistica and traumatic brain injury; 2. ARTAG (Aging-Related Tau Astrogliopathy) that embraces Globular Glial Tauopathy; 3. PART (Primary Age-Related Tauopathy) and it involves clinically asymptomatic cases and tangle-predominant dementia; 4. FTLD, fronto-temporal lobar degeneration. (Adapted from Avila et al. 2004)

9.3.2 Tau Phosphorylation

Of the post-translational modification undergone by tau, phosphorylation is the most popular. A total of 85 putative phosphorylation sites are observed in tau, including 5 tyrosine residues, 35 threonines, and 45 serine which comprise 6, 41, and 53% of the phosphorylable remnants, respectively, on tau. These 85 acknowledged sites are categorised into two major categories: sites that are modified by kinases directed by proline such as tau protein kinase I (glycogen synthase kinase 3, GSK3), MAP kinase (p38), JNK, tau protein kinase II (cdk5), and other stress kinases like cdc2. The latter group is sites that can be modified by kinase directed by non-proline, like protein kinase A & C (PKA and PKC), calmodulin (CaM) kinase II, MARK kinases (23, 49, 65, 86, 117, 146, 199, 251), or CKII which alter residues abreast of acidic residues principally in exons 2 and 3 (49) (Fig. 9.6) (Guo et al. 2017).

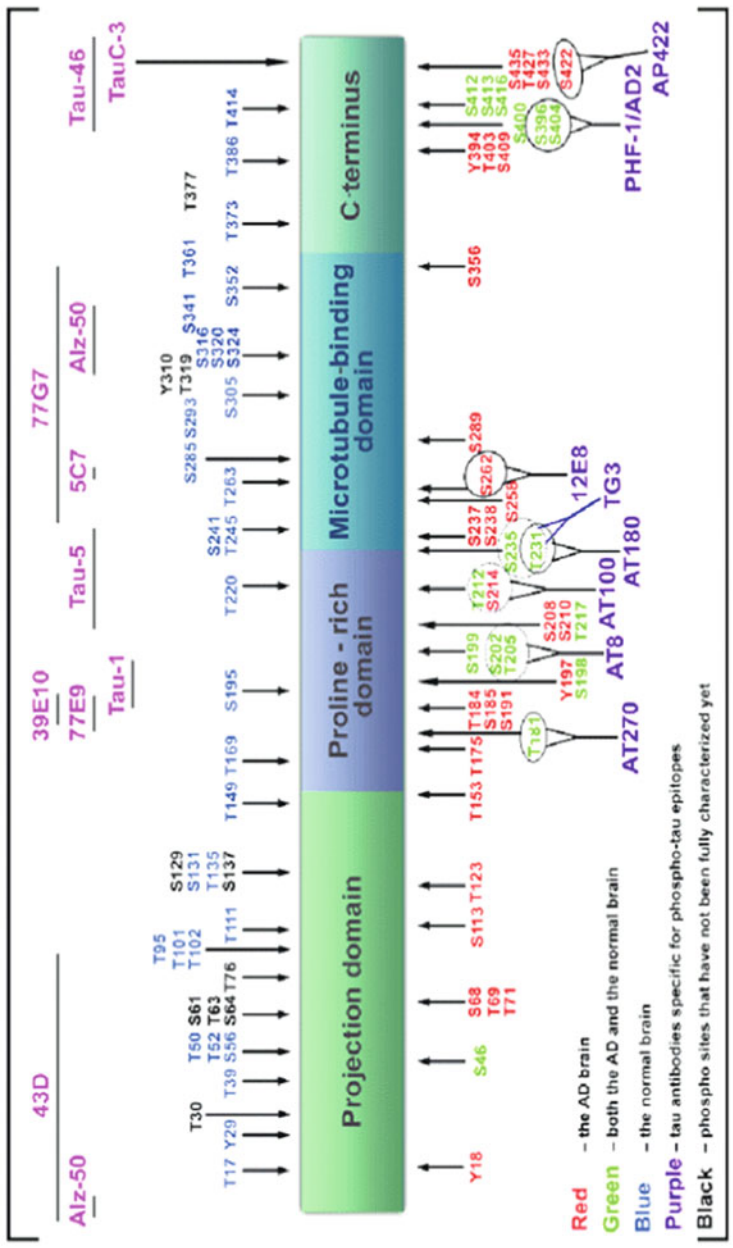

Fig. 9.6 Putative phosphorylation sites on tau (Adapted from Šimić et al. 2016)

9.3.3 Tau Toxicity in Neurodegenerative Disease

Hyperphosphorylation and formation of aggregates could be the leading cause of cytotoxicity mediated by tau in neurodegenerative diseases. In AD, it has been found that neurofibrillary lesions lead to an abate number of survivors, suggesting that degenerative processes may be involved in the development of extracellular tangles. In contrast, they are found in neurons having intracellular NFT. Accordingly, intracellular insertion occurs prior to apoptosis, the ligature of tau on extracellular matrix constituent like sGAGs(235) induces extracellular NFT to cell lysis. Thus tau aggregates appearing as sticky structure are toxic that could cohere and bereave off from cell (Avila et al. 2004).

9.3.4 Human tau Gene Expression

With 16 exons on chromosome 17q21, tau in humans is enciphered at MAPT, a microtubule associated protein tau gene (Wang and Mandelkow 2016). Tau protein has six isoforms of 37–46 kDa in the human nervous system resulting from differential splicing of the mRNA transcript of 6 kb. In the cerebral cortex, the 3R and 4R isoforms of tau are found in approximately identical quantities in healthy adults (Guo et al. 2017). By omitting exon 10, tau will have 3 microtubule-binding domains (3R), while from including it, tau will have 4 microtubule-binding domains (4R) (Fig. 9.7). Additionally by regulating exons 2 and 3, tau can incorporate (2 N or 1 N) or exclude (0 N) insertion of amino-terminals. Exons 4A, 6, and 8 can be translated in the PNS, producing larger tau proteins. Human tau expression changes with developmental stage, as foetal brains express only 0N3R tau, while adults express all isoforms (Kent et al. 2020).

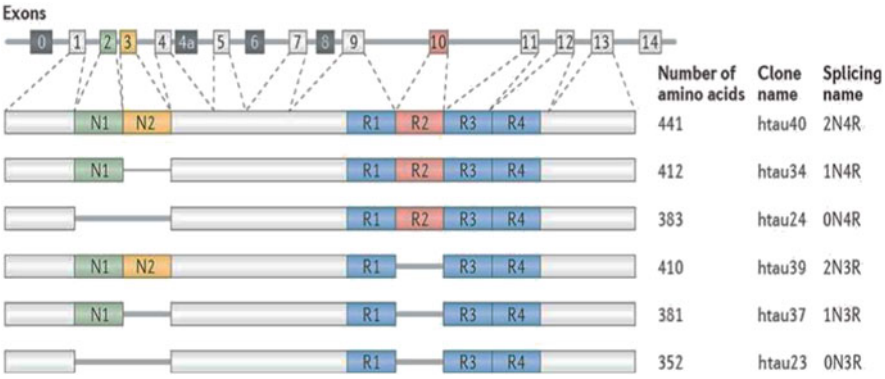

Fig. 9.7 Diagrammatic representation of the splice isoforms of tau in the human brain and MAPT gene. (Adapted from Wang and Mandelkow 2016)

9.3.5 *Immune Histochemical Localisation of tau in AD*

From various studies, it is established that tau is predominantly a neuronal protein yet its existence in distinct kind of glia cells is described to create neural disease. Tau can be modulated by phosphorylation in neural cells which are associated with the plasma membrane or as previously mentioned they can also be bound with microtubules. Evidence indicate the existence of nuclear antigen in proliferating cell that reacts with many tau antibodies. Furthermore, tau seems to be phosphorylated in the cytosol prior to transport into the nucleus (Avila et al. 2004).

In the developing neurons, the distribution of tau is affected by the phosphorylation. It is prominent in proline-rich region present in the somatodendritic region whereas in the distal part of the axon they are dephosphorylated. Phosphorylated tau in the distal axonal region seems to have carboxy-terminal domain (Avila et al. 2004).

9.3.6 *Pathological Alteration of tau in AD*

According to Mandelkow and Mandelkow (1998), these are the properties of tau that change in several ways during AD.

- Abnormal 'hyperphosphorylation' has been shown in AD at many sites such as 13, 14, and 26. Some of the enhancement is also seen in the tissue of foetal and mitotic cells and it prompts the theory where 'mitotic signals' gained by evolved neurons trigger tau hyperphosphorylation and, ultimately, apoptotic death. Most of the aberrant phosphorylation plots are Thr-Pro or Ser-Pro patterns, which shows why antibodies react with such phosphorylated epitopes mainly produced against AD-tau and are currently utilised to diagnose Alzheimer's brain tissue or to develop cell models. This research discovered that anomalous tau phosphorylation occurs before to aggregation.
- Microtubule binding is also lost in AD-tau, which is likely due to hyperphosphorylation at locations that release tau from microtubules (such as S262 or S214). This could explain the absence of microtubules, which would result in the breakdown of intracellular traffic, causing axons to die back.
- Tau is not being directed into the proper compartment in Alzheimer's disease, as evidenced by its reallocation from an axonal to a somatodendritic pattern. Various causes, such as increasing mRNA levels and tau synthesis, could lead the cell to become overburdened, causing the sorting system to malfunction.
- Tau agglomeration is primarily studied because of its unique solubility and unexplained behaviour. The aggregates are known as 'paired helical filaments (PHFs)' because of their two-stranded appearance. Their widths range from 10 to 20 nm, with 80 nm crossover repeats. PHFs clump together to form 'neuropil threads' or 'neurofibrillary tangles'. PHF accumulates in an ineffective amount in

in vitro, although it can be enlarged through oxidation, which triggers tau dimerisation, and interaction with polyanions like RNA or heparin.
- Proteolysis along with ubiquitination are the two post-translational modifications of AD-tau that are most likely cellular endeavour to reduce the abnormal protein (via the proteasome or the calpain pathway); although a few proteolytic fragments are detected early in the process, this could also lead to the nucleation of PHFs.
- Crosslinking and oxidative impairment elicit glycation and build up tangles.
- The amount of tau in the CSF has increased from 200 pg ml^{-1} to 600 pg ml^{-1}, indicating that withering neurons 34 are to blame. This feature has the potential to be used as a screening tool for early diagnostic test.

9.4 Alkoxy Glycerol

9.4.1 Definition

Alkoxy glycerol are naturally present ether lipids that present in haematopoietic or blood forming organ like bone marrow, liver and spleen. They are also found in cow's milk and various organs of human.

9.4.2 Chemical Composition

The principal alkoxyglycerol consists of batyl (octadecyl),chimyl (hexadecyl),and selachyl (octadecyl) ethers. Hallgren and Larsson (1962) conclude that glycerol ethers present as a form of diesters inside the tissue and also as alkyl acyl phosphatides (Fig. 9.8). 1-*O*-(2-methoxyalkyl) form of glycerol. Alkylglycerols were separated from the phospholipids and neutral lipids of milk of human, cow and sheep, human's colostrum, and also in the blood component like red bone marrow of human, uterine carcinoma, red blood cells, and blood's plasma.

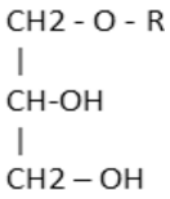

Fig. 9.8 Chemical Structure of Alkyloxy glycerol

Table 9.1 Percentage of AKG in the liver oil of shark and human milk and its bone marrow

Alkylglycerols	Milk (Human)	Liver oil (Greenland shark)	Bone marrow (Human)
14:0		2.0	
15		0.7	
16:0	23.9	9.1	29.4
16:1	Trace	10.8	
17	3.6	3.6	7.6
18:0	22.8	2.8	24.6
18:1	33.8	59.4	16.7
18:2	1.4	1.6	
18:3			
19	2.4	1.5	6.1
20:0	1.6		2.9
20:1	2.3	6.2	3.2
22:0	0.7		0.7
22:1	3.4	2.2	5.1
24	2.1		

(Fully adapted from Iannitti and Palmieri 2010)

9.4.3 Sources

AKG is found naturally in the milk of human, cow and sheep, and majorly found in shark liver oil (Table 9.1). The concentration of glycerol ethers in unsubstituted form is 10 times more in human milk when compared to cow's milk and it is double when compared to sheep's milk. The principal constituents of 2-methoxy-substituted glycerol ethers are the glycerol ethers having the long hydrocarbon chains of 16 and 18 carbon atoms. In some shark species or rat fish (elasmobranch fishes) liver oil is the major marine sources of alkyloxy glycerol (Iannitti and Palmieri 2010)

9.4.4 AKG and Blood Brain Barrier

In 1913, a researcher Edwin Goldman inserted a dye into the animal brain directly and concluded that injected dye into the brain was unable to spread and finally suggested that some types of barrier are halting the dye and discovered the blood brain barrier (BBB) (Iannitti and Palmieri 2010). Blood brain barrier is a major difficult path for delivery of the drugs into central nervous system (CNS) of human brain. It consists of several barriers in parallel and consists of two vascular BBB, namely the capillary bed and the barrier of blood-cerebrospinal fluid that consist mainly of the choroid plexus (Neuwelt et al. 2008). A single layer of cells in the brain paved together by close-fitting junction and formed BBB and this layer also prevents or controls the plasma seepage into the CNS. The BBB plays various roles such as: it blocks the entry of circulating substances present nearby CNS; it facilitates the

movement of critical substances necessary for the CNS function; and it also facilitates the movement of critical substances from the blood to the CNS and vice versa. So, it maintains the immune environments, nutritive and homeostatic of the CNS, and it controls the interchange of some informational components between blood and the CNS (Banks 2009).

Iannitti and Palmieri (2010) concluded about the opening of BBB and showed that intracarotid short-chain AKG shows low toxic and effective strategy against temporary BBB opening. It help to overcome a problem associated with the narrow admittance of various drugs such as cytotoxic drug to the brain. It has been noticed that AKG also transfers the methotrexate to the brain and lots of instruments and techniques have been illustrated for increasing barrier permeability by controlling the AKG movement. It has been proved that there are no toxic effects of BBB opening at therapeutics level. So, a new therapeutic procedure is represented by intracarotid AKG that helps in overcoming the narrow access of therapeutic agents and various drugs to the CNS.

9.4.5 Health Beneficiaries of Alkyl Glycerols from Shark Liver Oil

Various types of glycerols are present in the cells and fluid of body as bioactive ether lipids and are also known as Natural 1-*O*-alkylglycerols or alkyl-Gro. Alkyl-Gro are the precursors of phospholipids ether and contribute in the structure and function of specific cells membrane such as white blood cells (WBC) and macrophages. These compounds can also be extracted from milk and bone marrow lipids of human (Hallgren and Larsson 1962). High levels of alkylglycerol are associated with the liver oil in some species of shark and combination of these compounds is differed by the length and unsaturation of chain in the alkyl-glycerol group (Bordier et al. 1996a, b).

Some research shows that alkyl-Gro stimulates antibody production and haematopoiesis and found in the milk and other body fluids (Linman et al. 1959). It has been noticed that newborn offspring gets beneficial effects during oral treatment of pregnant animals. Oral treatment of shark liver oil (SLO) in the pregnant sows raise the particular immunoglobulins in the serum of sows used for treatment (Mitre et al. 2005). Oral supplement of SLO and natural purified alkyl-Gro shows the anti-tumour effects. The development of grafted tumour can be reduced and compaction of the number of pulmonary metastases can be done by the supplement of these two components (Pedrono et al. 2004; Deniau et al. 2010).

9.5 Relation of Plasmalogens, AKG and Alzheimer's Diseases

Plasmalogens are a type of glycerol-phospholipids that contain an alkenyl chain mainly in as O-16:0, O-18:0 or O-18:1 and an acyl chain present at the position of sn1 and the sn2, respectively, and primarily an ethanolamine head or choline group at the position of sn3 (Paul et al. 2021). These specific phospholipids are necessary components of cell's plasma membranes and are considered as endogenous form of antioxidant phospholipids (Lee 1998). They play vital roles in additional cellular process such as regulation of cholesterol, transport of cholesterol regulator and efflux of high-density lipoprotein-mediated cholesterol (Paul et al. 2021). All mammalian tissues contain plasmalogens but concentration is highly variable between tissues (Table 9.2). The concentration of plasmalogens is moderately high in brain, skeletal muscle, kidney and some immune cells types and low in liver.

In human, various diseases are associated with the deficiency of plasmalogens concentration in the organs. Due to some genetic disorders, broad deficit of plasmalogens can be detected in specific peroxisomal disorders because it affects the plasmalogens biosynthesis pathway (Steinberg et al. 2006). In some complex diseases, such as coronary artery disease, Alzheimer's disease, obesity and type 2 diabetes, reduced concentration of plasmalogens has been reported (Paul et al. 2021).

9.5.1 Plasmalogens and Alzheimer's Disease

On the basis of previous two decades' research, it has been concluded that there is a direct link of plasmalogens insufficiency with AD. It has been confirmed that concentration of ethanolamine plasmalogens (PlsEtns) decreased in the post-mortem samples of brain as well as in cerebrospinal fluid, plasma, RBCs and serum of patients diagnosed with AD (Rothhaar et al. 2012; Molina et al. 1998). The concentration of PlsEtns reduced during examination of the AD patient's brain (Onodera et al. 2015; Wood et al. 2010). It has been showed that high decrease in the concentration of PlsEtns was detected on the site of neurodegeneration in the brain such as temporal cortex, hippocampus and frontal cortex of AD brain.

Stress leads by the oxidation in the cells can lead to loss of PlsEtns in the AD brain and lastly leads to degradation of plasmalogen by reactive oxygen species (ROS) (Braverman and Moser 2012). It is the presence of vinyl ether bond by which plasmalogens become more vulnerable to oxidative stress (Mangold and Weber 1987). It shows that plasmalogens protect the other lipids from oxidative destruction and they act as scavengers (Reiss et al. 1997). Plasmalogens show antioxidant effects towards diverse range of ROS. The reduction of plasmalogens enhance the oxidative and membrane damage during AD (Su et al. 2019).

Table 9.2 Plasmalogen content in different human tissues

Tissue	PlsCho (%total PL)[a]	PlsEtn (%total PL)[a]	PlsCho (% GPCho)	Plasmalogen (%total PL)[a]	PlsEtn	Reference
Brain	0.8–0.9	20	1	22	58	Heymans et al. (1983), Panganamala et al. (1971)
Heart	11	15	26	–	53	
Kidney	4.7	14	5		46	
Skeletal muscle	6.5	14	19		48	
Liver	3.4	4.7	3		8	Han et al. (2001)
Grey matter						
Cerebellum					78	
Frontal cortex				54	57	
Temporal cortex					56	
Parietal cortex				51	58	
White matter						
Cerebellum					78	
Frontal cortex				76	84	
Temporal cortex					83	
Parietal cortex				100	81	
Neutrophils			3.6		68	Chabot et al. (1990)
Eosinophils			4		72	Ojima-Uchiyama et al. (1988)
Erythrocytes		20				Farquhar and Ahrens (1963)

[a]Overall phospholipid level comprises cardiolipin, PlsEtn, GPEtn, GPCho, GPIns, PlsCho, sphingomyelin, and GPser. (Fully adapted from Braverman and Moser 2012)

The level of antioxidant properties of plasmalogens is reduced significantly during oxidative stress and neuroinflammation (Katafuchi et al. 2012). It has been advised that there is correlation loop between ROS production, β-amyloid accumulation, neuroinflammation and plasmalogen deficiency (Su et al. 2019). The dilapidation of PlsEtns is mediated by the enzyme plasmalogen-selective phospholipase A2 which discharges arachidonic acid or DHA from glycerol. It leads to the loss of PlsEtns in the brain and is stimulated by ceramide which is produced during the inflammatory conditions (Farooqui and Horrocks 2001; Latorre et al. 2003). The

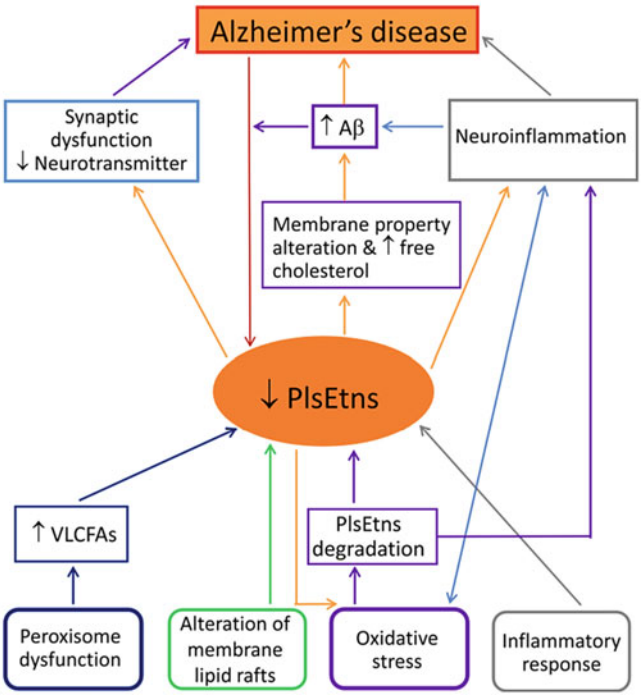

Fig. 9.9 Mechanism associated with ethanolamine plasmalogens insufficiency and Alzheimer's disease. (Fully adapted form Su et al. 2019)

synthesis of thromboxanes is done by DHA and it is also the precursor of leukotrienes and prostaglandins (Su et al. 2019). Metabolites such as maresins, docosatrienes, resolvins, and neuroproteins involved in anti-inflammatory process are derived from DHA. Arachidonic acid along with these derivatives control the pro- and anti-inflammatory processes (Su et al. 2019) (Fig. 9.9).

9.5.2 Plasmalogens and AKG

As per the study done by Destaillats et al. (2010), it has been found that some compounds from the group of alkyl glycerol and/or alkoxy glycerol enhance the plasmalogens in a mammal to a great extent than the endogenous level of plasmalogens in healthy mammal. These AKGs increased the levels of endogenous plasmalogens by avoiding the rate limiting peroxisomal stage of plasmalogen synthesis through several enzyme-catalysed reactions on the endoplasmic reticulum (Paul et al. 2021). They can be derived from natural biomass such as microorganism, animal by-products and natural products. Mostly these compounds are extracted

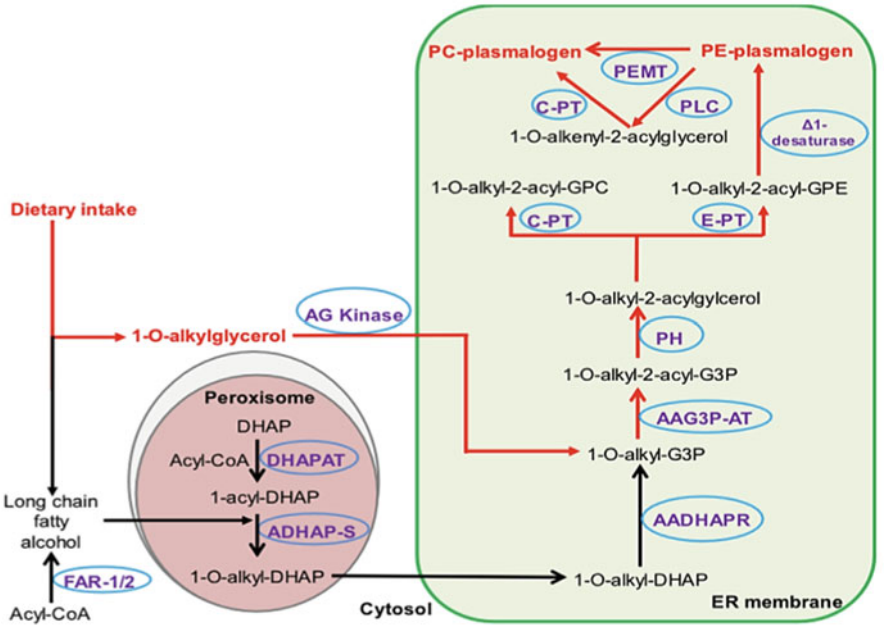

Fig. 9.10 Endogenous plasmalogen content can be modulated by AKGs. Red pathway indicating the bypass of rate- limiting peroxisomal biosynthetic steps by AKGs. Enzymes are shown in violet and metabolites are shown in red and black. (Fully adapted from Paul et al. 2021)

from marine oil such as fish oils and/ or egg lecithins. The major source of AKGs is shark liver oil (Destaillats et al. 2010).

Based on the research, it has been found that in initial stage AKGs metabolised into alkyl ether phospholipids and then change into alkenyl ether phospholipids or plasmalogens (Fig. 9.10) (Paul et al. 2021). So, increase in the concentration of alkyl ether phospholipids following AKG supplementation is a primary indicator of the integration of AKGs into the biosynthetic pathway of plasmalogens (Paul et al. 2021). AKG supplementation increases the level of alkyl-phosphatidylethanolamine (PE(O)) and alkyl-phosphatidylcholine (PC(O)) in liver, adipose tissue and skeletal muscle and typically reaching at peak after two-four weeks of AKG supplement treatment in mice. The level of alkenylphosphatidylethanolamine (PE(P)) and alkenylphosphatidylcholine (PC(P)) gradually increased in plasma of mice during the first 2 weeks of AKG treatment and the level of PE(P) progressively increased in the adipose tissue during 4 weeks treatment of AKG supplement (Paul et al. 2021). The increases in the level of PE (P) and PC (P) in the liver were most projecting after 12 weeks of duration during the AKG treatment (Paul et al. 2021). Additionally, the AKG supplement enhances the concentration of multiple plasma and adipose plasmalogen species that contain different alkenyl chains. It also increases the level of multiple hepatic plasmalogens but in smaller concentrations (Paul et al. 2021).

9.5.3 A Possible Therapy for AD: Plasmalogens

The promising result has been noticed by several researchers for preventing AD by applying plasmalogens replacement therapy in animals (Su et al. 2019) that intraperitoneal administration of purified form of plasmalogens in the hippocampus of adult male mice reduces the neuroinflammation by inducing lipopolysaccharide (Katafuchi et al. 2012). In the hippocampus accumulation of β-amyloid protein can be eliminated by plasmalogen treatment and it is mainly correlated with the decline in the concentration of plasmalogen content in the hippocampus (Katafuchi et al. 2012). Nishimukai et al. (2003) concluded that concentration of plasmalogen increases by the factor of 3 in blood plasma, and in the liver, it is increased by 25% after feeding a phospholipids (10 wt.%) containing test diet to rats for 7 days. Wood et al. (2011) showed that reduced level of PlsEtns can be restored in plasma and brain of adult mice by oral supplementation of plasmalogens precursor PPI-1011 dose @ 100 mg/kg/day and this restoration of plasmalogen is related with a triggered re-myelination of neuronal cells.

9.6 Conclusion

In the twenty-first century, development in bio science is touching the sky but still after lots of research we could not get the full treatment of Alzheimer's diseases. It takes millions of lives each year. Because the age-related complete treatment of this disease is very hard and with ageing our cells functioning degrade. In AD concentration of plasmalogens and some protein such as tau protein get fluctuated. But we can avoid this situation by using some natural compounds such as AKG. AKG is the best natural source that is obtained from shark liver oils majorly. It affects the concentration of plasmalogens and on the basis of research it has been proved that it helps in the curing process of AD. But the availability and purification of AKG affect a large number of shark's population. It affects the ocean ecosystem seriously. So, the main focus of scientist should be on production of AKG by some other organism such as microorganism like bacteria, fungi, etc. with the help of gene modification. Researchers should get the way of producing AKG synthetically so that there is no burden on our ecosystem. Even AKG also plays a vital role in other diseases such as cancer, opening of blood brain barrier and activating the immune system. AKG does not show any toxic effect on the opening of blood brain barrier. So, it has great importance for human life as it can avoid several diseases.

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Part IV
Perspectives with Developmental Strategies

Chapter 10
Application and Efficacy of Nanoparticle-Based Therapy Among Neurodegenerative Diseases

M. Vijay Kumar and Kartik Bhairu Khot

Abstract Human body is well known for its central nervous system (CNS) where physiological iteration in the form of any neurodegenerative disease dismays its normal function, voluntarily or involuntarily. The presence of complex tightly packed endothelial cells of blood–brain barrier (BBB) prevents the entry of most of the biologically active molecule into the brain, affirming us the dilemma present and the challenges posed in its treatment strategy. Thus, the current conceptualization delves into the concept of various nanotechnological approaches, their method of preparation and application with a broad view of neurodegenerative diseases, and application of nanotechnology as a therapeutic strategy with prime focus on pharmaceutically engineered dimension of 1–100 nm nanoparticles conferring vivid features alongside multifunctional properties of the special ability to cross BBB.

Keywords Nanoparticle · Neurodegenerative diseases

Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
CAG	Cytosine, adenine, guanine
CMC	Critical micellar concentration
CNS	Central nervous system
DDS	Drug delivery system
HD	Huntington's disease

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Lt	Lactoferrin
Ltf	Lactoferrin receptor
LUVs	Large unilamellar vesicles
MLVs	Multilamellar vesicles
MWNTs	Multiwalled nanotubes
NDs	Neurodegenerative diseases
o/w	Oil in water
PAA	Polyacrylic acid
PD	Parkinson's disease
PEG	Polyethylene glycol
PVP	Polyvinylpyrrolidone
QDs	Quantum dots
RES	Reticuloendothelial system
ROS	Reactive oxygen species
SLNs	Solid lipid nanoparticles
SOD	Superoxide dismutase
SUVs	Single unilamellar vesicles
SWNTs	Single-walled nanotubes
UV	Ultraviolet

10.1 Introduction to Nanotechnology

Nanotechnology as an allied science inherits the term nano from Latin parlance meaning "dwarf," implicating that the unit size of nano in meter is one thousand-million-meter, i.e., 1 nm $= 10^{-9}$ m. Nanotechnology imbibes an expanded version in the conventional mode of drug delivery system, flaring its application in the area of CNS and tumor targeted delivery with leading edge in gene delivery too (Modi et al. 2010). Thus, nanotechnology as a branch of science deals with the matter of nanoscale length size (Bhatia 2016).

Nanostructures play an important role in the field of pharmacy by eliminating the difficulties associated with dosage forms (Orive et al. 2003; Tiwari et al. 2012). The nanocarrier as a potential medication was recently highlighted as an advanced drug delivery system (DDS) in the field of nanotechnology. The unique physicochemical property and smaller size of nanocarrier provide more surface area, allowing it to pass the impermeable membrane. Therefore, among several strategies being adopted to improve the therapeutic efficacy of pharmaceutical dosage forms, the drug delivery system (DDS) is of primary concerns. DDS as a novel mode in pharmaceutical sciences is capable of providing defense against the rapid clearance of drugs and improving the concentration of drugs at a minimal dose. This is only feasible if there is a variation in the therapeutic or harmful impact of a medicine due to a difference in dose or concentration. Another type of drug delivery system is a cell- or tissue-specific strategy, in which the drug is bound to a carrier that can deliver into

the desired location. In short, although the **marketed** formulation has some effect on reducing the need for effective ND therapy, it has also created a wall of drawbacks including poor distribution, rapid first pass effect, limited efficacy, unfavorable side effects, and nonselectivity in delivering the dosage form to the required site (Kumar et al. 2020a, b; Bhatia 2016). Hence, the goal of drug distribution to the desired place followed by a treatment of neurodegenerative diseases (ND) is of critical concern at present times.

10.2 Classification of Nanoparticles

1. One-dimensional nanoparticles: Thin film or monolayer commonly applicable in the field of solar cell panels, optical fiber, optical devices, and other information storage systems.
2. Two-dimensional nanoparticles: Carbon nanotubes.
3. Three-dimensional nanoparticles: Dendrimer, quantum dots, and fullerenes (carbon 60) (Pal et al. 2011).

10.3 Types of Pharmaceutical Nanosystem

Carbon-based structures: Carbon nanotubes are organized in the shape of a graphite sheet. These buckyball-filled cylindrical structures of nanotubes are hexagonal carbon atoms with a diameter of 1 nm and a length of 1–100 nm (Bhatia 2016). Carbon nanotubes penetrate the cellular membrane by endocytosis mechanism showing their drug targeting capability; hence, they can be used in the development of various pharmaceutical dosage forms with the motive of treating the disease condition (Barron and Khan 2008). Based on their structure and size, carbon nanotubes are divided into two types: single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). With respect to above-configured types, C_{60} (fullerenes) is another carbon-based structure included in it. Fullerenes are carbon-based hollow cylindrical cage structures, also called buckyballs, and differ by arrangement of graphite cylinder. The size, surface, and geometrical properties of fullerenes are some of the key properties suggesting them as a drug carrier. The diameter of SWNTs and C_{60} fullerenes is in the range of 1–2 nm, whereas the diameter of MWNTs ranges from several nanometers to 10 nanometers having a distance of 0.36 nm between the layers of MWCNTs. The length of these carbon-based nanostructures varies from 1 μm to several micrometers (Reilly 2007). They are concentric forms of nanotubes used as stable drug carriers. They are prepared by laser ablation, chemical vapor deposition, electric arc discharge, or combustion technique.

Quantum dots:(QDs) are semiconducting nanocrystals with a diameter of 2–10 nm that have an inorganic semiconductor core (CdSe) and an aqueous

organic-based shell (e.g., ZnS) covering it, which increases optical properties and emits fluorescence when exposed to light. QDs have a cap that increases their solubility in an aqueous buffer. The color produced by a QD is determined by its core, whereas the outside aqueous shell is required for biomolecule conjugation. Biomolecules that can be conjugated in QDs are the targeted biomarkers essential for the targeted delivery of various therapeutic moiety. QDs' property varies with respect to their size ranging from 2 to 10 nm in radius. QDs are used for intracellular tracking as a nanotheranostic drug delivery system because of their emission, fluorescence, photostability, and UV excitation properties (Bailey et al. 2004; Iga et al. 2007).

10.3.1 Nanoshells

Nanoshells are modified forms of nanoparticles that are formed by a combination of a silica core with a metallic outer coating. The core to shell ratio can be changed to alter their qualities. It can be customized in terms of size, shape, and morphology. Nanoshells are used to create various morphological arrangements, which begin with the use of morphological core particles such as rings, wires, rods, tubes, cubes, and other morphological core particles coated with a thin shell. Nanoshells are utilized for chemical stability, luminescence enhancement, biosensors, and other applications (Bhatia 2016).

10.3.2 Polymeric Nanoparticles

Polymeric nanoparticles are biodegradable biocompatible nanoparticles made up of natural polymer or polymer from synthetic origin. The potential application of these nanoparticles is in tissue engineering, drug delivery strategy, and gene delivery, which is completely based on the biodegradable and biocompatibility of polymer (Guterres et al. 2007). Various advanced natural polymers consist of synthetic polyester like poly(D,L-lactide) or polycyanoacrylate and related polymers like poly(lactide-co-glycolide) PLA or poly(lactid acid) (Parveen et al. 2012). In addition to the above polymers, various natural polymers like gelatin, chitosan, sodium alginate, and albumin are also used to prepare nanoparticles. On the basis of their fabrication method, polymeric nanoparticles are also categorized as nanocapsules or nanospheres. Nanospheres have a matrix core where active moiety gets dispersed and even gets adsorbed to the surface of nanospheres. Nanocapsules have an inner core where active molecules get incorporated and also adsorbed at the surface. Polymeric nanoparticles can attain particles of less size than 100 nm, which is one of the important criteria for brain targeted delivery. Polymeric nanoparticles have lesser circulation time as they get opsonized by the reticuloendothelial system (RES), which is increased by coating them with hydrophilic nonionic polymer like

polyethylene glycol (PEG) that increases their circulation time. This approach improves the circulation time but lacks target specificity, which is improved by conjugation of protein, ligand, or antibodies that can attach to the targeted receptor (Parveen et al. 2012; El-Say and El-Sawy 2017).

10.3.3 Liposomes

Liposomes are novel vesicular drug delivery systems that are fabricated by thin-film hydration techniques. There are several methods by which liposomes can be prepared; among those methods, thin-film hydration is the common one. Liposomes consist of a lipid bilayer encircling the aqueous core. These are the first-generation nanoparticles having a similar characteristic property with the cellular membrane. Phospholipid and cholesterol are the two important components of liposomes where phospholipids form a lipid bilayer while coming in contact with the aqueous solution, and cholesterol increases the stability of the composed bilayer. Liposomes are available in different size ranges; based on their classification, liposomes are categorized as single unilamellar liposomes/vesicles (SUVs), large unilamellar liposomes/vesicles (LUVs), and multilamellar liposomes/vesicles (MLVs). The size of liposomes varies from 100 nm to several micrometers. SUVs are considered as the nanocarrier as their size ranges from 50 to 150 nm (Akbarzadeh et al. 2013). Liposomes are versatile in nature; they can entrap a wide range of molecules irrespective of their properties. Liposomes can entrap both lipophilic and hydrophilic molecules in a single vesicle reducing the risk of nonspecific effects with improved bioavailability. Stealth liposomes are similar to PEGylated polymeric nanoparticles, increase the circulation time of liposomal formulation, and can be fabricated into ligand-modified PEGylated liposomes for targeted delivery (Cascione et al. 2020).

10.3.4 Lipid Nanoparticles

Lipid nanoparticles are also called solid lipid nanoparticles (SLNs). SLNs were used as an alternate therapy to liposomes (nanovesicles), polymeric nanoparticles, and polymeric emulsions. Solid lipids, such as pure triglycerides, complicated glyceride mixes, or even waxes are used in the preparation of SLNs. These lipids are solid at room and body temperature that gives them a prominent name as solid lipid nanoparticles. Surfactant stabilizes SLNs, which increases their characteristics when compared to other nanocarriers. SLNs are biocompatible and biodegradable nanoparticles that can be targeted to the specific site of the body for the treatment of various disease conditions. Targeted delivery in SLNs is achieved by conjugating a ligand on their surface, which is even called stealth SLNs. Stealth SLNs are used to improve the circulation time of nanoparticles and to attain targeted delivery (Parveen et al. 2012).

10.3.5 Polymeric Micelles

Polymeric micelles are the micelles of block copolymer consisting of hydrophilic and hydrophobic monomer units. Preparation of micelles depends upon the amount of block copolymer. An increase in the concentration of block copolymer above the critical aggregation concentration (CAC) or critical micellar concentration (CMC) in an aqueous medium results in the escape of hydrophobic monomer unit aggregate from the aqueous phase to form micellar core structures (Xu et al. 2013). The hydrophobic monomer units of polymeric micelles are a copolymer of lactic acid and glycolic acid, hydrophobic poly(amino acid), and poly(caprolactone), while hydrophilic monomer units are polyethylene glycol (PEG), which is most widely used. Polyvinylpyrrolidone (PVP) and polyacrylic acid (PAA) are used as PEG alternatives. Polymeric micelles have a long circulation time because of their small size and hydrophilic shell, which minimizes their RES uptake. The hydrophilic portion of micelles composed of PEG can be used as a conjugating portion for the binding of specific ligand or targeting molecule. Chemical binding or conjugation of specific ligands increases the concentration of drugs in the organ with the disease state. Polymeric micelles increase the solubility of the poorly soluble drug, which increases the systemic availability of the drug and increases the penetrability of the drug across the biological membrane (Parveen et al. 2012).

10.3.6 Ceramic Nanoparticles

Ceramic nanoparticles contain inorganic materials that are used to encapsulate biomacromolecules for the delivery of the drugs across the membrane. Ceramic nanoparticles protect the biomacromolecules from the altered pH of biological fluids; hence, specific enzymes and other pH-sensitive macromolecules can be loaded on them. Silica, alumina, and titania are the commonly used inorganic materials for the preparation of ceramic nanoparticles as they are inert in nature. Ceramic nanoparticles are smaller in size, and hence it is more effective in evading the RES uptake. The surface of ceramic nanoparticles is conjugated with a specific ligand or antibody in order to have a targeted approach (Parveen et al. 2012).

Apart from the above-mentioned nanoformulations, there are other nanoparticles such as metal nanoparticles and magnetic nanoparticles that have played a significant role in the field of pharmacy. A schematic diagram representing some of the nanoparticles is illustrated in Fig. 10.1.

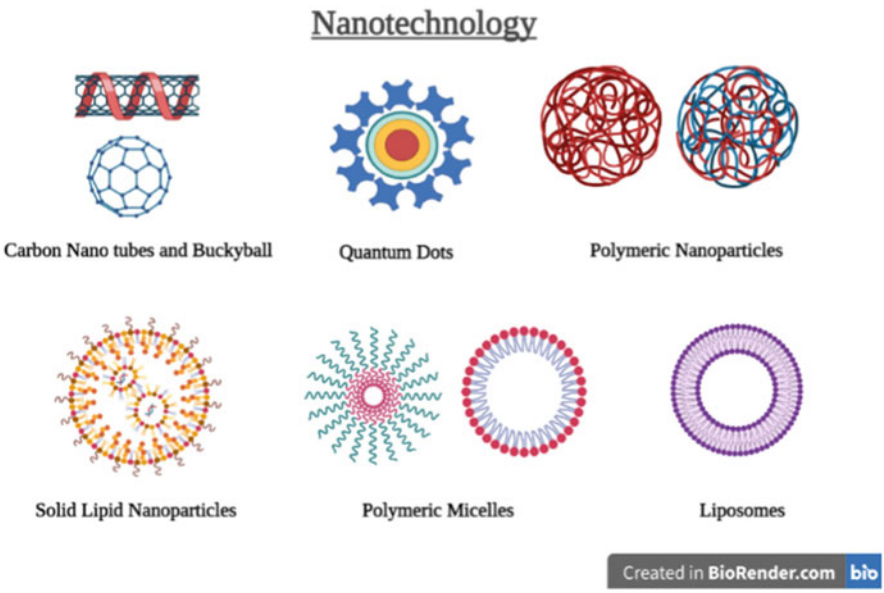

Fig. 10.1 A diagrammatic representation of nanoparticles

10.4 Method of Preparation of Nanoparticles

Depending upon their types, nanoparticles are prepared by various methods. To reduce the size and to confer compatibility according to the biological environment, several polymers are used. Polymers are biocompatible, biodegradable, and nonimmunogenic; hence, based on the types of polymer and drug to be loaded, nanoparticles are prepared.

10.4.1 Solvent Evaporation Method

It is the most extensively used technique for synthesizing nanoparticles. The method follows two steps: first, emulsification of polymeric solution in aqueous phase; and second, removal of organic solvent via evaporation. In this method, hydrophobic medicaments and polymers are dissolved in an organic solvent. The prepared organic solution is emulsified in an aqueous phase containing emulsifying agents or surfactants to form oil in water (o/w) type of emulsion. Emulsion so formed is kept under continuous stirring to evaporate the organic solvent under reduced pressure to form a stable emulsion. The amount and type of stabilizer, polymer concentration, and homogenizer speed were found to influence the size range of nanoparticles. To create nanorange particle sizes, ultrasonication or high-speed homogenization are frequently used. The prepared nanoparticles are centrifuged

and then washed with distilled water to eliminate the remaining stabilizers or free drugs before being lyophilized for storage (Bhatia 2016; Pal et al. 2011).

10.4.2 Diffusion Method

This method is also called as solvent diffusion method. The approach behind this method follows solvent evaporation. In this method, the minimum concentration of water-miscible and water-immiscible solvent act as an oil phase. Interfacial turbulence is created during the spontaneous diffusion of solvents between the two phases, which can lead to the foundation of smaller particles. The concentration of water-miscible solvent can be increased to achieve smaller particle sizes. Drugs that are hydrophobic or hydrophilic can be tested using this method. A multiple w/o/w emulsion with the drug dispersed in the internal aqueous phase is required for hydrophilic drugs (Bhatia 2016; Pal et al. 2011).

10.4.3 Emulsion Diffusion Method

This is yet another popular approach for making nanoparticles. In this method, polymer is mixed in a partially water-miscible solvent and saturated with water. This saturated polymeric solution is emulsified in an aqueous solution containing a stabilizer where diffusion of solvent occurs within the exterior phase. The process of diffusion is based on the oil-to-polymer ratio. Finally, the solvent is removed by evaporation or filtration. There are a few benefits associated with this approach: high encapsulation efficiency (usually 70%), no requirement for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, and a restricted size distribution (Bhatia 2016; Pal et al. 2011).

10.4.4 Precipitation Method

Precipitating preformed polymer in an organic solution and diffusing the organic solvent into the aqueous media are the steps in this approach. Diffusion of organic solvents can occur with or without the addition of a surfactant. Polymers, drugs, and some of the lipophilic surfactants are dissolved in a semi-polar solvent like ethanol or acetone. After they have completely dissolved, the solution is poured or injected into an aqueous solution containing a stabilizer while being stirred magnetically. The rapid solvent diffusion produces nanoparticles almost instantly. The solvent is then withdrawn under reduced pressure from the suspensions. The size of the particles is determined by the amount of organic phase added to the aqueous phase. It was also discovered that as the mixing rate of the two phases rises, both particle size and drug

entrapment decrease. This approach is better for medications that are not very soluble. The particle size, percentage yield, and drug release of nanoparticles can be controlled by the optimization of several factors (Bhatia 2016; Pal et al. 2011).

10.5 Neurodegenerative Disease

Neurodegenerative disease is a clinical condition of the central nervous system (CNS) where the normal functioning of neuronal cells gets affected. The pathologies of neurodegenerative disease are different in the case of Alzheimer's, Parkinson's, Huntington's disease, amyotrophic lateral sclerosis, and frontotemporal dementia, while the symptoms associated with it are memory loss, cognitive impairments, behavioral impairments, and adverse events like inability to speak, move, and breathe. The pathological events in neurodegenerative disease include genetic mutation, deposition of abnormal protein, and formation of neurotoxic molecules, all leading to apoptosis. The exact mechanism of neurodegenerative disease is unknown, but above all, pathological events are the hallmark of neurodegenerative disease (Spuch and Navarro 2011; Kumar et al. 2020a, b; Cano et al. 2020).

10.5.1 Common Neurodegenerative Disease

10.5.1.1 Alzheimer's Disease

Alzheimer's disease (AD) is the principal cause of dementia, especially affecting the elderly population. The exact mechanism of AD is unknown, but it is believed that the deposition of senile plaques and neurofibrillary tangles in the extracellular and intracellular region of the brain is the pathological hallmark of AD. Senile plaques are the extracellular amyloid-beta originated from amyloid precursor protein (APP), and neurofibrillary tangles are the hyper-phosphorylated form of tau protein. Deposition of proteins triggers the immune cells to produce pro-inflammatory factors that mainly affects the mitochondria of neuronal cells to produce more amount of reactive oxygen species, which results in oxidative stress and finally neuronal death (Kumar et al. 2015).

10.5.1.2 Parkinson's Disease

Parkinson's disease is a chronic neurodegenerative disease and the second most affecting ailment after Alzheimer's. It is one of the commonly occurring disease, especially in elderly populations. PD is caused by the degeneration of dopaminergic neurons at the substantia nigra pars compacta (SNPC) region of the brain (Kumar et al. 2020a, b). The death of dopaminergic neurons decreases the level of dopamine,

which is the essential neurotransmitter required to transmit signals within the brain cells for normal motor functions. Reduction in dopamine levels induces impaired motor and behavioral functions like stiffness of limbs, tremors, slow movements, difficulty in movements, balance, and coordination. Similar to Alzheimer's, the exact cause for the disease progression is unknown, but it is believed that deposition of Lewy bodies containing α-synuclein protein is the pathological hallmark of PD. α-Synuclein is a protein localized in the presynaptic terminals of the neurons. Increased accumulation of α-synuclein causes toxicity, leading to neuronal dysfunction. Another molecular mechanism revealed the progression of the disease, where cell–cell transmission of α-synuclein increases the onset of disease. Cellular transmission of α-synuclein synergizes the pathological events called synuclopathy that invades all the normal neuronal cells of the brain. This in turn degenerates the neurons of nigrostriatal bundles leading to deficiency in dopamine production (Kumar et al. 2020a, b; Singh and Devasahayam 2020).

10.5.1.3 Huntington's Disease

Huntington's disease (HD) is an autosomal neurodegenerative disease (ND) characterized by defects in motor, behavioral, and cognitive functions. It is an inherited genetic disease of the striatum caused by the expansion of CAG trinucleotide gene expression of the huntingtin gene. The repeated cytosine, adenine, guanine (CAG) trinucleotide sequences of the huntingtin gene are 17–20 in the normal population, which vary in HD-affected patients where the repeated CAG trinucleotide is 40 and more. During the pathogenesis of HD, an increase in the number of polynucleotide sequence above the threshold converts the protein α-helix to the β-folded chain. These chains will coagulate together, representing the structural form of amyloid complexes within the neuronal cells. Aggregation of polyglutamine is initiated by lysis of N-terminal huntingtin protein by caspases, calpains, and other endoproteases, thus exposing the mutant N-terminal fragments that are aggressive against the surrounding substrates. The neurodegeneration cascade events are initiated after the translocation of aggregated polynucleotide within the neuronal cells. The cleaved part of the polynucleotide can cross the neuronal nuclear membrane where the actual phase of polynucleotide disease is triggered, resulting in neuronal death (Jimenez-Sanchez et al. 2017; Illarioshkin et al. 2018).

10.5.1.4 Amyotrophic Lateral Sclerosis (ALS)

ALS is a progressive ND characterized by the death of upper motor neurons of the brain and lower motor neurons of the brain stem and spinal cord, leading to paralysis. ALS occurs by the mutation of genes in a motor neuron by environmental factors or by hereditary aspects. There are several gene mutations that have been identified; Cu/Zn superoxide dismutase 1 gene (SOD1), TAR DNA-binding protein 43 (TDP43), fused in sarcoma (FUS)/translocated in sarcoma, and ubiquitin 2 are

some of them. Mutation of the SOD1 gene is the common form in ALS, which was identified in 20% of ALS cases. SOD has three isoforms encoded in the human gene: the cytoplasmic Cu/Zn SOD (SOD1), the mitochondrial Mn SOD (SOD2), and the extracellular Cu/Zn SOD (SOD3). These isoforms are the product of different genes having different cellular localization, but to catalyze some reactions they require metals. During cellular respiration, anionic radical is released in the form of H_2O_2, a toxic reactive oxygen species (ROS), which is further removed by the catalase enzyme to release water and oxygen. Hence, SOD provides antioxidant defense toward the ROS species. The etiopathological events initiated during the progression of ALS follow several mechanisms, which lead to cellular dysfunction. Mutant SOD present in the cytoplasm is also present in mitochondria that alter the normal physiological functions of mitochondria imitating it to release ROS, which triggers apoptosis. Apart from that several cascades of events like neuro-inflammation, glutamate excitotoxicity, protein, and neurofilament aggregations affect the normal function of neuronal cells, leading to degenerations of neuronal cells (Bonafede and Mariotti 2017; Ralli et al. 2019).

10.6 Application of Nanoparticles in ND

Neurodegenerative disease is a chronic condition that still has not got proper treatment because of the drawbacks that are related to the complex structure of the brain and its barrier. Different nanoformulations have extensive physicochemical properties that comply with the natural physiology of the brain and its barrier to boost the penetration rate of therapeutic drugs, which has shown certain improvement in the treatment of disease conditions. Some of them have been elaborated here with their main strategy against the disease condition.

In Alzheimer's disease, plaques get accumulated in the extracellular synaptic gaps of neurocortex region. Clioquinol, chemically derived as 5-chloro-7-iodo-8-hydroxyquinoline, is a quinolone derivative that can dissolve plaques in synaptic gaps. Clioquinol is capable of dissolving plaques in the Alzheimer's disease model of transgenic mice. To treat Alzheimer's disease, nanomaterials can be employed as a carrier to carry clioquinol over the BBB. For example, clioquinol can be encapsulated in n-butyl-cyanoacrylate nanoparticles and delivered over the BBB (Huang et al. 1999; Roney et al. 2005). Donepezil is a cholinesterase inhibitor that is generally unable to penetrate the BBB and is used to treat Alzheimer's disease. Bhavna et al. used PLGA nanoparticles ranging in size from 83.24 nm to 96.10 nm as a nanocarrier to deliver donepezil to the brain. Their findings showed that the nanoparticles were successfully administered into the brain with burst release at first, followed by steady release (Bhavna et al. 2014).

Several novel nanoapproaches have been employed in targeting the brain for the treatment of ND; the method of nanoparticles with ligand conjugated on their surface is one of them. Hernando et al. studied the efficiency of an encapsulated glial cell-derived neurotrophic factor, employing a transactivator of transcription peptide

coupled to a lipid carrier. Wen et al. conjugated odorranalectin to PLGA nanoparticles to increase the transport across the nasal route. In each of these investigations, the intranasal route was utilized to bypass BBB (Hernando et al. 2018; Wen et al. 2011).

Lactoferrin (Lf) is a protein substance that belongs to the transferrin family. It is one of the iron-binding proteins of molecular weight 80 kDa. Lf receptors are extensively expressed on epithelial cells of the respiratory tract and endothelial cells of BBB and neurons. In neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, these LfR are overexpressed in capillaries and neurons of the brain. Bi et al. used an intranasal method to deliver lactoferrin-modified PEG-PLGA nanoparticles of rotigotine to the target brain for the treatment of Parkinson's disease (Bi et al. 2016).

Carbon nanotubes have been identified as a promising therapeutic strategy in the treatment of PD, AD, and other neurodegenerative diseases where it has been used as a wireless biosensor. Carbon nanotubes placed in a carbon nanochip can track changes in dopamine levels and maintain them at a constant level (Dugan et al. 2001).

Free radicals are produced in the neuronal cells of brain inducing oxidation, and it remains in the brain due to low antioxidant activity; this results in the death of neurons. In ND-like HD, the lack of antioxidant activity in the brain can lead to neuronal damage that significantly leads to its death. As a result, antioxidants can be employed as treatments in Huntington's disease to prevent oxidative stress. Fullerenols are hydrophilic compounds derived from fullerenes that can take free radicals from the exposed environment of the cell. Even fullerenols are referred to as radical sponges because of their capability of removing free radicals. Because of their effective antioxidant properties and hollow spherical shapes, they have been used as carriers in the treatment of HD (Grebowski et al. 2013) Fullerenols' antioxidant properties have been repeatedly established. According to Jin et al., fullerenols were found to be effective in inhibiting glutamate receptors in an antagonistic manner and can thus be employed for neuroprotective purposes (Jin et al. 2000).

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Chapter 11
In Silico Techniques: Powerful Tool for the Development of Therapeutics

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Abstract Drug discovery process develops drugs from candidate molecules to use in clinical practice. Generally, the discovery of successful therapeutics is an expensive, time-consuming process and require huge manpower. In this connection, the computer-aided drug design (CADD) came to the attention of researchers because of the reduced process time, effort, and cost. The CADD started in the nineteenth century and now it became a crucial part of therapeutic development. In this regard, many approaches have evolved in the CADD for the discovery of novel therapeutics. Development of therapeutics involve identifying a specific therapeutic target protein of the disease and discovering a therapeutic ligand molecule. Screening of drug candidate compounds against therapeutic targets is crucial in CADD, which is possible by ligand-based virtual screening (LS-VS) or structure-based virtual screening (SB-VS). There are many interconnected approaches involved in CADD; they are homology modeling, ADME analysis, molecular docking, molecular dynamics simulation, and free energy calculations. Neurodegenerative diseases (NDD) are the most devastating diseases of the old-aged population. The discovery of novel therapeutics to NDDs is highly complicated because of nervous system complexity, lack of methods to study therapeutic action in the central nervous system (CNS), and unavailable methods to confirm therapeutic drug molecules could cross the blood-brain barrier (BBB). In this regard, in silico CADD addressed the above difficulties in the discovery of therapeutics for NDDs using various mathematical models. Most of the successful drug designs for NDDs are using in silico methods and are validated by in vivo and in vitro studies to make them available in the market in a short period with less cost. Currently, in silico techniques are effectively used for the development of therapeutics.

Keywords In silico · Computer-aided drug discovery · ADME analysis · Molecular docking · Molecular dynamic simulation · Therapeutics · Neurodegenerative diseases

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Abbreviations

2D	2 Dimensional
3D	3 Dimensional
AChE	Acetylcholine esterase
AD	Alzheimer's disease
ADMET	Absorption Diffusion Metabolism Excretion Toxicity
ALS	Amyotrophic lateral sclerosis
BACE1	β-site amyloid precursor protein cleaving enzyme 1
BBB	Blood brain barrier
CADD	Computer-aided drug design
EM	Electron microscopy
GSK3β	Glycogen synthase kinase 3β
HD	Huntington's disease
IUPAC	International Union of Pure and Applied Chemistry
LB-VS	Ligand-Based Virtual Screening
MD	Molecular dynamics
NDD	Neurodegenerative diseases
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
PD	Parkinson's disease
QSAR	Quantitative Structure-Activity Relationship
SAR	Structure-activity relation
SB-VS	Structure-Based Virtual Screening
TB-VS	Target-Based Virtual Screening
USD	United States dollar

11.1 Introduction

The development of a therapeutic agent is identifying and developing a drug that potentially modifies disease pathology. It comprises the transformation of a compound to a product approved to market by authorities (Rang and Hill 2013). The drug discovery process is high cost and the laborious process requires an average time of 10–15 years and a price of 0.8–2 billion USD (Am Ende and Am Ende 2019). In this scenario, computer-aided drug development seeks more attention from pharmacologists to reduce the processing time, cost, and efforts. Computer-aided drug design (CADD) applies computational tools in the drug development process (Talele et al. 2010). Concerning the commonly used biological terms in vivo and in vitro computer-aided experiments are termed 'in silico'. Many licensed drugs are optimized using CADD, such as dorzolamide, aliskiren, captopril, nolatrexed, and oseltamivir (Talele et al. 2010). Neurodegenerative diseases (NDDs) are associated with age and are complicated by the death of neurons. Complexity in the nervous

system makes a hurdle to neuronal drug development. So, in silico drug development is a promising approach in NDDs. This chapter provides ideas about CADD and its different approaches describing the importance of CADD in neurodegenerative diseases.

11.2 History of In Silico Techniques

There are objections among researchers about who coined the term "in silico." However, the earliest published journals by Sieburg (1990) and Danchin et al. (1991) used the word "in silico" (Ekins et al. 2007). In silico drug development and its related disciplines might start with the idea of structure-activity relation (SAR) in the nineteenth century. From the year 1869, scientists started focusing on the structure of a chemical to relate its function, a universal explanation for drug action (Albert 1971). Later, the "Lipoid theory of cellular depression" was proposed by Meyer (1899) and Overton (1901) to explain the relationship between physicochemical property and activity of a chemical. This theory says that the higher the partition coefficient, the more the depressant activity. Corwin Hansch, the founding father of drug design, co-related the lipophilicity and electronic properties of drugs with their pharmacokinetic and pharmacodynamic events (Ekins et al. 2007). Crum Brown and Fraser raise the concept of connectivity of atoms present in the 2-dimensional (2D) structure of a chemical are essential for its pharmacological activity (Albert 1971). The biological relations of optically active substances leads to changing a chemical structure from 2D to 3 dimensional (3D) (Cushny 1926). Later in the middle of the twentieth century, more research focused on the conformational changes in its bioactivity (Burgen 1981).

Langley first coined the concept of the receptor in 1878. In the late nineteenth and early twentieth century, the receptor term was developed as the target of drug action by Alfred Clark and Paul Ehrlich (Ariens 1979; Parascandola 1980). Albert (1971) co-relates the concept of a receptor as enzymes with their substrates. Quantitative relations between the structure and activity of receptors and drugs generated a surplus of information beyond the human handling ability. Ekins et al. (2007) reviewed in the 1950s, Hansch started using machine and statistical calculations to make the quantitative relationship between the structures and activity in drug design; progress to the development of QSAR (Quantitative Structure-Activity Relationship) model in drug discovery. Later, a vast data repository was needed and insisted the researchers combine it with computer science. Computer graphics and modeling in the 1980s and 1990s developed the drug discovery path in a drastic progression. Chemistry-biology-informatics triad seeded a new path to CADD. The evolution of different types of computer-based database systems, 3D structure builders, and a diverse set of virtual screening techniques popularized the CADD methods. Liu et al. (2021) reviewed the computational approach for the de novo drug design and expecting more sophisticated artificial intelligence (AI) approaches to accelerate therapeutic drug development.

11.3 Computer-Aided Drug Design (CADD)

Finding therapeutic drug candidates is an integrated interdisciplinary approach using advanced techniques (Harvey 1995). Rational drug design combines the modern concepts of Medicinal chemistry, an interdisciplinary science. The International Union of Pure and Applied Chemistry (IUPAC) defined Medicinal chemistry as a chemistry-based discipline that refers to the biological, medical, and pharmaceutical sciences. Also, computational resources combine chemical information to form a new discipline, chemo-informatics. The influence of computational tools and resources in drug development enlightens CADD. Bajorath (2015) divided the in silico drug discovery approach into three categories.

- Designing, developing, and maintaining computational tools to operate, arrange, scrutinize, and accumulate the drug discovery data.
- Implement methods to identify and prioritize the therapeutic targets of disease.
- Execute developmental strategies for drug candidates.

Here we are explaining more about the possible computational approaches to develop drug candidates. Figure 11.1 explains the different approaches to CADD. Each area of CADD covers different tools and is interconnected to each other while designing a drug.

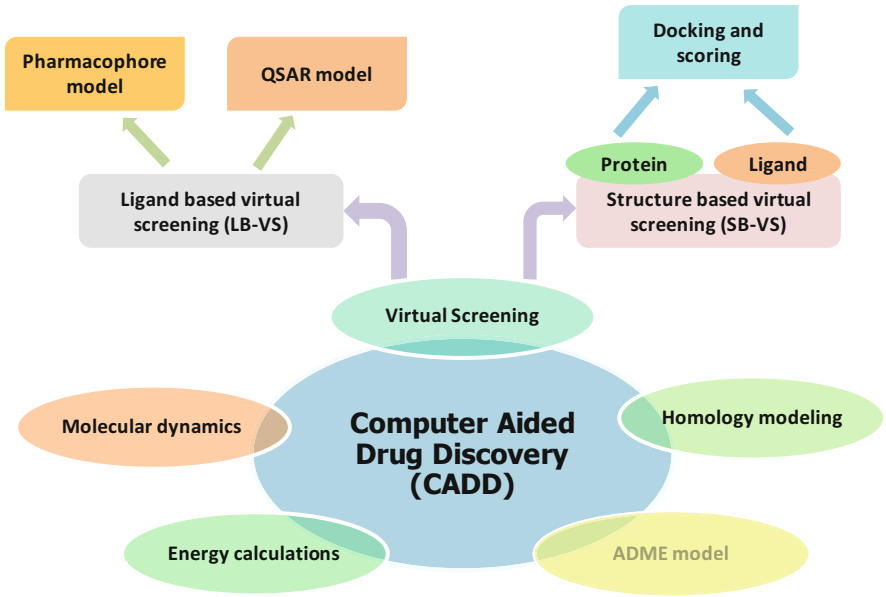

Fig. 11.1 Different approaches in computer-aided drug discovery

11.4 Virtual Screening (VS)

It is an in silico method to select bioactive drug candidates against the selected therapeutic target from the available compound library using computational techniques (Kühne 2006). Horvath (1997) proposed it, and later it became a popular technique to screen a sizeable virtual database in terms of cost and time-effective (Surabhi and Singh 2018). This screening method works like a funnel, in which an extensive database is an upper portion, and almost appropriate compounds after the screening would be selected for the in vitro or in vivo studies. The virtual screening method can be divided into—ligand-based virtual screening (LB-VS) and structure-based virtual screening (SB-VS).

11.4.1 Ligand-Based Virtual Screening (LB-VS)

When the 3D structure of the protein of interest is lacking, the LB-VS method, a neighborhood behavior search is using to find out the biological activity of therapeutic candidate molecules from their structural and physicochemical characters. Here details of selected ligands are considered to find an active therapeutic target (Reddy et al. 2007). Prathipati et al. (2007) is assuming that structurally alike compounds show similar biological activity. There are two essential elements involved in LB-VS, an efficient similarity search and scoring method. Also, screening of many potential ligands should be with reasonable accuracy and speed (Hamza et al. 2012). Drug-like molecules used to screen in LB-VS can be chemically synthesized or derived from the chemical databases. Chemical databases contain information about the chemicals, including structures, chemical and physical properties, spectral information, uses, and other details. Important chemical databases and their details are listed in Table 11.1 LB-VS uses the pharmacophore model and QSAR model to predict the properties of therapeutic molecules and to select compounds with similar activity against the protein of interest.

(a) **Pharmacophore model:** Ehrlich (1909) developed the concept of pharmacophore, which refers to the molecular structure with a frame of atoms. Later, this concept changed as the 3D arrangement of a chemical that can recognize protein or therapeutic targets causes structural changes and biological activity (Gund 1977). The pharmacophore model's screening aims to find compounds with varying scaffolds and analogous 3D structures of functional groups of therapeutic target protein (Vuorinen and Schuster 2015). Usually, H-bond donor and acceptor or their combinations, groups with formal charges, aromatic rings, hydrophobic centers, and often aromatic groups of compounds are considered to develop a pharmacophore model (McGregor et al. 2007). By searching databases, developed model can be improve its progressive analogs against 3D structure of therapeutic target protein (Taft et al. 2008). Pharmacophore modeling is a successful computational tool used to screen

Table 11.1 Details of commonly used databases in CADD

Database	Details	Size	Reference	Url
Freely available and widely used chemical databases				
BIAdb	Database for Benzylisoquinoline Alkaloids	846 unique compounds	Singla et al. (2010)	http://crdd.osdd.net/raghava/biadb/
Binding DB	Public, web-accessible database for small, drug-like molecules	353,949 compounds	Gilson et al. (2016)	http://www.bindingdb.org/
ChEMBL	Manually curated database of bioactive molecules	2,086,898 distinct compounds	Mendez et al. (2019)	https://www.ebi.ac.uk/chembl/
ChemDB	Commercially available Chemical database	Nearly 5M small molecules	Chen et al. (2007)	http://cdb.ics.uci.edu/
ChemSpider	Free chemical structure database	Over 107 million structures	Pence and Williams (2010)	http://www.chemspider.com/
DrugBank	Database of drug data with comprehensive drug targets	500,000 drugs & drug products	Wishart et al. (2018)	https://go.drugbank.com/
HMDB	Database of small molecule metabolites found in the human body	115,398 metabolite	Wishart et al. (2018)	https://hmdb.ca/
KEGG compound	Collection of small molecules, biopolymers, and other chemical substances	–	Kanehisa et al. (2021)	https://www.genome.jp/kegg/compound/
NCI	Public web server for search chemical structures	250,250 open structures	Voigt et al. (2001)	https://cactus.nci.nih.gov/
NPACT	Curated database of Plant derived natural compounds that exhibit anti-cancerous activity	1574 entries	Mangal et al. (2013)	http://crdd.osdd.net/raghava/npact/
PDBe	Dictionary of chemical components	34,290 ligands	Velankar et al. (2016)	https://www.ebi.ac.uk/pdbe-srv/pdbechem/
PubChem	Open chemistry database at the National Institutes of Health (NIH).	109,908,881 compounds	Kim et al. (2021)	https://pubchem.ncbi.nlm.nih.gov/
SMPDB	Interactive, visual database of small molecules of human	30,000 small molecules	Jewison et al. (2014)	https://smpdb.ca/
SuperDrug	Resource for approved/marketed drugs	4600 active pharmaceutical ingredients	Siramshetty et al. (2018)	http://cheminfo.charite.de/superdrug2/

(continued)

Table 11.1 (continued)

Database	Details	Size	Reference	Url
Supernatural	Natural product database	325,508 natural compounds	Banerjee et al. (2015)	http://bioinf-applied.charite.de/supernatural_new/index.php
Zinc	Free database of commercially available compounds	230 million purchasable compounds	Sterling and Irwin (2015)	http://zinc.docking.org/
Widely used protein/protein-ligand complex databases				
MOAD	Subset of the protein data bank (PDB)	38,702 protein-ligand Structures	Smith et al. (2019)	http://www.bindingmoad.org/
RCSB-PDB	1st open access digital data resource	178,229 biological macromolecular structures	Berman et al. (2002)	https://www.rcsb.org/
PDB-Bind	Binding affinity database of all type of biomolecular complexes	21,382 biomolecular complexes	Liu et al. (2015)	http://www.pdbbind.org.cn/
ProPairs	Data set of crystal structures of protein complexes	5642 protein complexes	Krull et al. (2015)	http://propairs.github.io
PDSP K_i database	Provides information on the abilities of drugs to interact with an expanding number of molecular targets	–	Roth et al. (2000)	https://pdsp.unc.edu/databases/kidb.php
NRLiSt BDB	Database dedicated to the nuclear receptor (NR) ligands and structures pharmacological profiles	48 members of superfamily related transcription factors	Lagarde et al. (2014)	http://nrlist.drugdesign.fr/
SCORPIO	Online repository of protein-ligand complexes which have been structurally resolved and thermodynamically characterized	29 different proteins, 176 ligands, and 90 unique protein-ligand complexes	Ladbury and Chowdhry (1996)	http://scorpio.biophysics.ismb.lon.ac.uk/scorpio.html
M-CSA	Database of enzyme reaction mechanisms.	Contains 964 hand-curated entries	Ribeiro et al. (2018)	https://www.ebi.ac.uk/thornton-srv/m-csa/

Detailed information about the listed tools can be found at http://www.click2drug.org/

drug candidates for a novel bioactive compound (Langer and Wolber 2004). There are several computational programs available to perform automatic pharmacophore development. Available programs for pharmacophore model are Pharmer (http://pharmer.sourceforge.net.), PharmaGist (http://bioinfo3d.cs.

tau.ac.il/PharmaGist/), Boomer (https://www.boomer.org/), and ZINCPharmer (http://zincpharmer.csb.pitt.edu/).

(b) **QSAR model:** This model of LB-VS is an efficient technique, significantly correlate chemical structures and their physicochemical characteristics or category and biological/toxicological properties by building mathematical models (Cherkasov et al. 2014). But QSAR model went through a few transformations, including changes from 1D to nD dimensional models of molecular descriptors and diverse methods to find the relation between structure and biological activity of chemicals (Polanski 2009). QSAR model is used for hit identification and hit to lead optimization of drug molecules from massive databases to obtain drug candidates of desired biological properties. It is a laborious, time, and cost-effective computational screening technique to identify drug compounds before synthesizing and testing in the laboratory (Cherkasov et al. 2014). QSAR toolbox (https://qsartoolbox.org/), CORAL-QSAR/QSPR (http://www.insilico.eu/coral/), QSAR-Co (https://sites.google.com/view/qsar-co), EasyQSAR (http://easyqsar.blogspot.com/), QSAR4U (http://www.qsar4u.com/), and LQTA-QASR (https://lqta.iqm.unicamp.br/portugues/siteLQTA/LQTAgrid.html) are some of the QSAR modeling tools.

11.4.2 Structure-Based Virtual Screening (SB-VS)

Structure-based virtual screening (SB-VS) or target-based virtual screening (TB-VS) predicts the best 3D interaction poses between the protein and ligand to form a complex (Maia et al. 2020). Thus, a suitable drug target or therapeutic target of a disease and its structural information could be identified and validated. There are online databases such as TTD (Therapeutic Target Database) (http://db.idrblab.net/ttd/) to identify and confirm a valid therapeutic target of the disease. The therapeutic target of disease will be a protein or enzyme associated with that disease, and a drug can make a desired therapeutic effect on the disease progression. 3D structure of a therapeutic target (receptor protein) can be retrieved from different protein databases available (Table 11.1). Structural and computational biologists generate protein structures using X-ray crystallography, nuclear magnetic resonance (NMR), cryo-electron microscopy (EM), and homology modeling (Anderson 2003). If desired 3D structure of the therapeutic target protein is unavailable, homology modeling technique is used to construct it. Possible active ligands against a target protein can be retrieved from chemical databases; they can be drug molecules, phytochemicals, peptides, or synthesized chemicals. Identifying the active site of the therapeutic target, predicting the best pose of a therapeutic target -ligand complex, search algorithm, and scoring are the critical steps of SB-VS. Figure 11.2 is the representation of the SB-VS method of drug screening.

(a) **Identification of the ligand-binding/active site**: Finding all the available binding sites of a therapeutic target molecule is the first step of SB-VS. It also gives

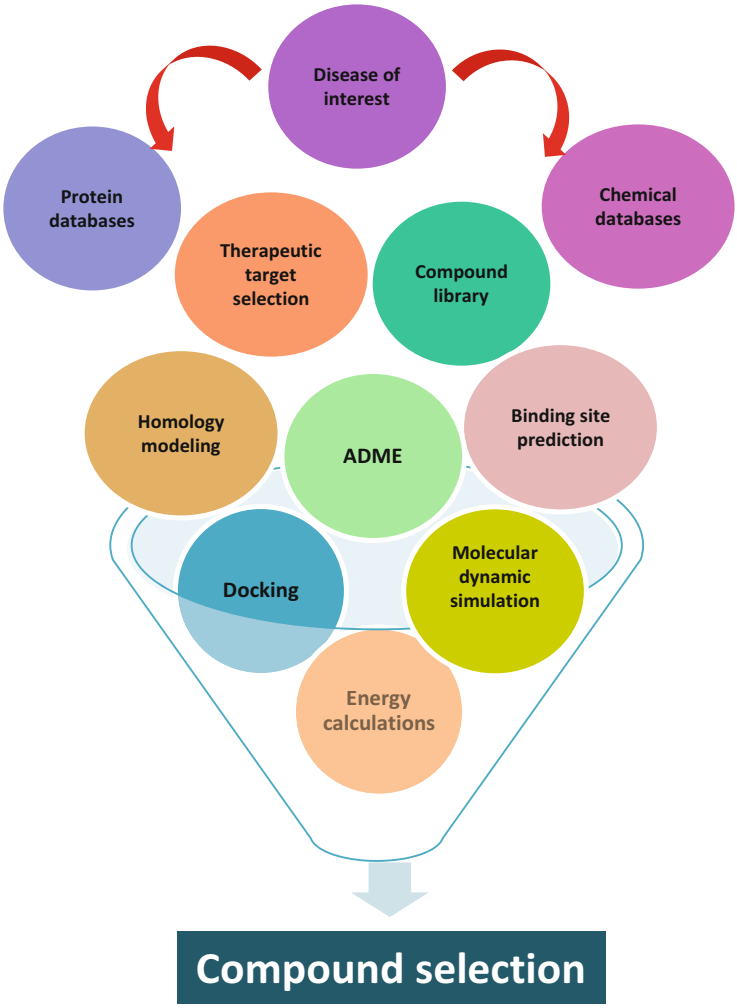

Fig. 11.2 Representation of the SB-VS method of drug screening

the idea of druggability, ligandability, and bindability. Furthermore, accurate prediction of the binding site enables us to know about the properties of the binding site pockets such as volume, details of residues and their hydrophobicity, hydrogen (H) bonding, energy potential, solvent accessibility, desolvation energy, and residue propensity (Yuan et al. 2013). Commonly used binding site prediction tools are provided in Table 11.2.

(b) **Tools for drug discovery:** It can be found at http://www.click2drug.org/, https://www.vls3d.com/

Table 11.2 Details of the tools used in CADD

Program/server	Description	Url
QSAR model programs		
3D-QSAR	Simple and advanced web-based tools for developing QSAR models	https://www.3d-qsar.com/
ChemSAR	Web server building a Structure-Activity/Property Relationship (SAR/SPR) model	http://chemsar.scbdd.com/
CQSAR	Dual databases of over 21,000 QSAR models	http://www.biobyte.com/index.html
SeeSAR	Program for interactive, visual compound promotion and optimization	https://www.biosolveit.de/SeeSAR/
QSARPro	Advanced software suite for QSAR modeling and activity prediction	https://www.vlifesciences.com/products/QSARPro/Product_QSARpro.php
Binding site prediction tools		
MED-SuMo	Macromolecules surface similarities detection at PDB scale	http://medit-pharma.com/index.php
TRAPP	Analysis of transient binding pockets and druggability indices in proteins	https://trapp.h-its.org/
CAVER	Software tool for analysis and visualization of tunnels and channels in protein structures	https://caver.cz/index.php?sid=100
fpocket	Fast open-source protein pocket (cavity) detection	http://fpocket.sourceforge.net/
GHECOM	Program for finding multi-scale pockets on protein surfaces	https://pdbj.org/ghecom/
metaPocket 2.0	Server to identify pockets on protein surface to predict ligand-binding sites.	https://projects.biotec.tu-dresden.de/metapocket/index.php
CASTp	Identification and measurements of surface accessible pockets	http://sts.bioe.uic.edu/castp/index.html?2r7g
SiteMAP	Program for binding site identification	https://www.schrodinger.com/
Molecular docking tools		
FlexPepDock	High-resolution peptide-protein docking	http://flexpepdock.furmanlab.cs.huji.ac.il/
Autodock	Automated docking tools	http://autodock.scripps.edu/
DOCK	Anchor-and-Grow based docking program	http://dock.compbio.ucsf.edu/
DockingServer	Web-based, easy to use interface that handles all aspects of molecular docking from ligand and protein setup	https://www.dockingserver.com/web
GEMDOCK	Program for computing a ligand conformation and orientation relative to the active site	http://gemdock.life.nctu.edu.tw/dock/
GlamDock	Monte-Carlo with minimization (basin hopping) search	http://www.chil2.de/Glamdock.html
Glide	Exhaustive search based docking program	https://www.schrodinger.com/products/glide

(continued)

Table 11.2 (continued)

Program/server	Description	Url
GOLD	Program for virtual screening, lead optimization, and identifying the correct binding mode of active molecules	https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/
iGEMDOCK	Graphic environment for docking, virtual screening, and post-screening analysis.	http://gemdock.life.nctu.edu.tw/dock/igemdock.php
ParDOCK	Automated server for protein ligand docking	http://www.scfbio-iitd.res.in/dock/pardock.jsp
PATCHDOCK	Web server for structure prediction of protein-protein and protein-small molecule complexes	http://bioinfo3d.cs.tau.ac.il/PatchDock/php.php
SCIGRESS	Multiplatform molecular design, modeling and dynamics software suite	https://www.fqs.pl/en/chemistry/products/scigress
SwissDock	Web service to predict the molecular interactions	http://www.swissdock.ch/
YASARA	Molecular graphics, modeling and simulation program	http://www.yasara.org/
Homology modeling tools		
Modeller	Software for producing homology models of protein tertiary structures	https://salilab.org/modeller/
I-TASSER	Used for protein structure prediction and structure-based function	https://zhanglab.dcmb.med.umich.edu/I-TASSER/
MPACK	Integrated protein modeling suite	http://curie.utmb.edu/mpack/
ProModel	Package for modeling proteins	https://www.vlifesciences.com/products/VLifeMDS/Protein_Modeller.php
Prime	Fully integrated protein structure prediction program, providing graphical interface, sequence alignment, secondary structure prediction, homology modeling, protein refinement, loop-prediction, and side-chain prediction.	https://www.schrodinger.com/
CABS	Versatile coarse-grained tool for protein modeling	http://biocomp.chem.uw.edu.pl/tools/cabs
RaptorX	Protein structure prediction program	http://raptorx.uchicago.edu/download/
SWISS-MODEL	Automated protein structure homology-modeling server	https://swissmodel.expasy.org/
Robetta	A protein structure prediction service	http://www.robetta.org/
Phyre2	Automated 3D model building using profile-profile matching and secondary structure	http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index
ADMET analysis tools		
ADMET Predictor	ADMET property prediction and QSAR model-building application	https://www.simulations-plus.com/software/admetpredictor/medchem-studio/
QikProp	ADME predictions of drug candidates	https://www.schrodinger.com/

(continued)

Table 11.2 (continued)

Program/server	Description	Url
GastroPlus	Simulates the oral absorption, pharmacokinetics, and pharmacodynamics for drugs in human and preclinical species.	https://www.simulations-plus.com/software/gastroplus/
pkCSM-pharmacokinetics	Predicting small-molecule pharmacokinetic properties using graph-based signatures	http://biosig.unimelb.edu.au/pkcsm/
DDDPlus	Models and stimulates the in-vitro dissolution of active pharmaceutical ingredients	https://www.simulations-plus.com/software/dddplus/
MolScore-Drugs	Expert system to identify and prioritize drug candidates	http://www.pharmainformatic.com/html/molscore-drugs.html
SwissADME	Compute physicochemical descriptors as well as to predict ADME parameters	http://www.swissadme.ch/
PreADMET	Web-based application for predicting ADME data	https://preadmet.bmdrc.kr/
Toxicity Checker	Webserver for searching substructures commonly found in toxic and promiscuous ligands	https://mcule.com/apps/toxicity-checker/
Tools for molecular dynamic simulation		
Amber	Biomolecular simulation programs	http://ambermd.org/tutorials/MD.php
Gromacs	Package for performing standard MD simulations, energy minimizations, NMR refinement	http://www.gromacs.org/
Desmond	High speed molecular dynamics simulations of biological system	https://www.deshawresearch.com/resources_desmond.html
NAMD	Molecular dynamics package for simulation of large biomolecular systems	http://www.ks.uiuc.edu/Research/namd/
ProtoMol	Object-oriented component-based framework for molecular dynamics simulations.	https://sourceforge.net/projects/protomol/
CHARMM	Program for macromolecular simulations	https://www.charmm.org/
OpenMM	Program to run modern molecular simulations	https://simtk.org/projects/openmm
MOIL	A suite of molecular dynamics programs	http://clsbweb.oden.utexas.edu/moil.html
Tools for energy calculation		
Hyde	To assess binding affinities and contributions to binding of a complex, with a visual feedback at atomic level	https://www.biosolveit.de/products/#HYDE
BAPPL-Z server	Binding affinity prediction of a protein-ligand complex containing zinc	http://www.scfbio-iitd.res.in/software/drugdesign/backup/bapplz1.jsp
BAPPL server	Binding affinity prediction of protein-ligand	http://www.scfbio-iitd.res.in/software/drugdesign/bappl.jsp

(continued)

Table 11.2 (continued)

Program/server	Description	Url
PreDDICTA	Predict DNA-Drug Interaction strength	http://www.scfbio-iitd.res.in/software/drugdesign/preddicta.jsp
Binding Free Energy Estimator	Tool for automate generation and post-analysis of accurate binding free energy calculation	https://www.ks.uiuc.edu/Research/vmd/plugins/bfeestimator/
VeraChem	Software for computer-aided drug discovery and molecular design	https://www.verachem.com/
AMBER MM-PBSA.py	Free energy calculator	http://ambermd.org/tutorials/FreeEnergy.php

(c) **Molecular docking:** Aims to guess the best binding pose and affinity of a ligand within the active site of the studying therapeutic target molecule (Guedes et al. 2014). Three main connected goals of molecular docking are virtual screening, guess the binding pose, and estimation of binding affinity (Jain and Nicholls 2008). It screen a large set of chemical compounds by ranking the ligands according to the binding mode and score (Kolb and Irwin 2009). Molecular docking predicts the best orientation of ligand within the binding site of receptor protein to become a stable complex. The binding affinity score determines the quality of the binding pose after docking. Molecular docking is a widely used tool for developing drugs because of reduced time and cost. The search algorithm and the score function are the important docking components (Guedes et al. 2014). Details of well-known molecular docking tools are given in Table 11.2.

(d) **Search algorithm and scoring:** Search algorithm systematically finds orientation and confirmations of ligand at the binding site of the protein. It investigates the different positions of ligands in their translational and rotational degree of freedom in the protein's binding site (Maia et al. 2020). Molecular docking software estimates the non-covalent interaction forces between a ligand and the therapeutic target protein using mathematical scoring functions. The mathematical scoring function is the most crucial part of docking of SB-VS and is used for predicting the binding affinity (Maia et al. 2020; Huang et al. 2010). There are three types of scoring functions—force field-based, empirical, and knowledge-based (Huang et al. 2010; Wang et al. 2003). Force field scoring functions are classical force field and energy sum of the therapeutic target-ligand intermolecular interactions (Guedes et al. 2014). The interactions can be van der Waals force, electrostatic bond, stretching/bending/torsional force interactions (Ferreira et al. 2015). The empirical scoring function usually reproduces experimental binding affinity data (Guedes et al. 2014). There will be a high accuracy correlation between the binding free energy to the sum of non-related variables such as H bonding, ionic and apolar interactions, desolvation, and entropic effects of a set of protein-ligand complexes with known binding affinity. It uses a training set to perform multiple linear regression analyses to create a

statistical model. The knowledge-based function of scoring is on the statistical analysis of the 3D structure of interacting atom pairs of a protein-ligand complex. Molecular interactions considered the pairwise energy potential of the protein-ligand complex to obtain a general function. The frequency of pairwise potential of different molecular interactions are divided and weighed according to the frequency of occurrence. The output score will be the sum of these different types of interactions (Ferreira et al. 2015).

11.5 Homology Modeling

Homology modeling is the construction of a therapeutic target protein of interest with atomic-resolution from its amino acid sequence or 3D structure of a homologous protein (Muhammed and Aki-Yalcin 2019). Protein 3D structure prediction or modeling is very important in drug discovery research if a high-resolution protein structure is unavailable. There are two basic concepts behind homology modeling. The first one, amino acid sequence is the unit of a protein; and the second, protein structure is conserved. Suppose there is no available homologous template structure, the de novo structure prediction method could be used to synthesize 3D protein structure, mostly limited to small protein synthesis compared to the comparative model (Jauch et al. 2007). Protein 3D model construction mainly involves 4 steps—(1) template selection, (2) sequence alignment, (3) building model, and (4) evaluation of the quality of the model (Bordoli et al. 2009). In modeling, template for the target protein will be evolutionarily related, experimentally solved protein structures. If the template is identical or analogous to the target sequence, the 3D structure developed would show high accuracy. According to the target protein, the alignment can be possible through the sequence alignment method and manual adjustment (Bordoli et al. 2009). Widely available alignment methods are Clustal W (https://www.genome.jp/tools-bin/clustalw), MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/), and T-coffee (https://www.ebi.ac.uk/Tools/msa/tcoffee/). After alignment, a 3D model will be created on the basis of sequence alignment. Tools or applications for the 3D homology modeling are listed in Table 11.2. Finally, the quality of the 3D structure will be evaluated. According to the sequence similarity, quality of templates, and environmental factors, the generated model will be different. The quality of the protein model can be evaluated by measuring bond length, torsion angle, and rotational bond. A robust analysis method to determine protein structure quality is the Ramachandran plot (Muhammed and Aki-Yalcin 2019). Application of homology modeling is virtual screening, mutagenesis experiments, or logically studying the sequence variation (Kopp and Schwede 2004; Hillisch et al. 2004).

11.6 ADMET

Natural and synthetic chemicals or compounds are primarily available in chemical libraries or databases. Before experimenting with binding assays with target molecules in a short duration, high-throughput screening of these chemicals is essential. The ADMET analysis process is take less time, cost-effective and effort-reducing screening technique. It aims to predict the performance of a compound inside the body when it became a drug by considering all its kinetic characters (Yamashita and Hashida 2004). It covers the pharmacokinetic property of a compound to determine whether the drug candidate molecule gets to the desired therapeutic target molecule in the body and the drug candidate molecule's time duration stays in the bloodstream. ADMET predictions are based on data size, lipophilicity, and functional groups of the compound (Young 2009). Reliable screening filters in ADMET analysis are factors such as absorption, distribution, metabolism, excretion, and toxicity. A study confirms that 40% of the drug molecule fail in clinical trials due to low ADMET properties (Maltarollo et al. 2015).

ADMET analysis also helps to assume the different properties of a drug candidate such as oral bioavailability (oral absorption), blood-brain-barrier (BBB) permeability, intestinal permeability, central nervous system activity, hepatic metabolism, and its drug-likeliness. Drug-likeliness is a qualitative or quantitative indication based on the ADMET properties, determining whether a drug candidate would be an effective drug. Well-known criteria for drug-likeliness is Lipinski's rule of five (Lipinski et al. 2012). Orally bioactive compounds should meet the following criteria: (1) H bond donors ≤ 5, (2) H bond acceptors ≤ 10, (3) molecular weight ≤ 500, and (4) calculated log $P \leq 5$. Many software and servers are available to determine the ADMET property of compounds, including their drug-likeliness (Table 11.2).

11.7 Molecular Dynamics Simulation (MD Simulation)

MD simulation is a time-dependent dynamic behavior of atoms or molecules of a microscopic system. In 1977, MD simulation was first reported in a protein containing 9.2 ps trajectory in vacuum (McCammon et al. 1977). The increased computing power makes to run simulations of much larger protein surrounded by water and ions with more or less 100 ns period (Karplus and Kuriyan 2005). The principle behind the interaction and movement of atoms or molecules in a system is Newton's law of physics. The system's overall energy is calculated by estimating the forces between atoms with the help of a force field (ff) (De Vivo et al. 2016). Output trajectories of MD simulation specify positions, velocities, and forces between particles of the studied system within a period of time. MD trajectories are the source of data set from which various properties of the system can be calculated. Overall binding free energy, kinetic measures, and other quantities compared with experimental observations are used to interpret the stability of a trajectory. MD

simulation estimates the biomolecular processes such as conformational changes, protein folding, and position of atoms or molecules within femtosecond intervals. These observations can predict mutation, phosphorylation, protonation, and the addition or removal of ligand (Hollingsworth and Dror 2018).

In drug discovery, MD simulation predicts how the protein in the system interacts with the atoms of the ligand molecule over time. The discovery of lead compounds from the chemical library uses molecular docking for virtual screening. However, this widely used technique lacks information about the flexibility of target protein. MD simulation obtains multiple target confirmation and gives information about the flexibility of target protein (Liu et al. 2018). Moreover, it gives the data on conformational changes and stability of protein upon ligand binding. The combination of MD simulation and binding free energy calculations is an improved strategy in virtual screening since it can get high accuracy in the data of protein target-ligand interactions (Alonso et al. 2006). So, this is a widely used method to study the drug-target interactions by combining the MD simulation and binding free energy (Yang et al. 2011; Zheng et al. 2016). Commonly used MD simulation tools are listed in Table 11.2.

11.8 Free Energy Calculations

The binding free energy of the therapeutic target-drug complex is accurately predict how strongly a potential drug candidate binds to its target. It is relatively calculating the protein-ligand binding affinity using statistical mechanics to the trajectory of MD simulation (Cournia et al. 2017). The pharmaceutical companies and academic laboratories are using computational tools to calculate binding free energy in the lead optimization during the drug discovery. These computational tools are faster and cheaper (Christ and Fox 2014). The ligand forms a reversible non-covalent bond with the target protein at equilibrium in a neutralized solvent system. According to Gilson et al. (1997), the free energy difference between two endpoints can be calculated by many microstates (solvent, complex, target, or ligand) samples, considering the partition function (Z) and free energy of each microstate.

$$\begin{aligned} \mathrm{RT}\ \ln(K\mathrm{D}) = \Delta G0\mathrm{Bind} = -\mathrm{RT}\ \ln\left[\frac{\mathrm{Zcomplex}}{\mathrm{Zsolvent}}\right] \\ Z\mathrm{state} = \sum_i^{\mathrm{state}} \exp\left[-\frac{Ei}{k\mathrm{B}T}\right] \end{aligned} \tag{11.1}$$

Equation (11.1) is the calculation method for the binding free energy when the energy of each state is known. To reduce the number of samplings, a simplified Eq. (11.1) was derived.

$$\begin{aligned}\Delta\Delta G0A, B &= \Delta G0B, \text{Bind} - \Delta G0A, \text{Bind} \\ &= \text{RT } \ln \left[\frac{ZB\text{Complex}ZA\text{Solvent}}{ZA\text{Complex}ZB\text{Solvent}}\right]. \\ &= \Delta G0A, B\text{Complex} - \Delta G0A, B\text{Solvent}\end{aligned} \tag{11.2}$$

Equation (11.2) eliminates the error in the sampling. Free energy calculations can use a variety of schemes. They are free energy perturbation, Bennett acceptance ratio (BAR), and the multistate BAR (MBAR) variation (Zwanzig 1955; Bennett 1976; Shirts et al. 2003; Shirts and Chodera 2008). Even though all these implementations are related to each other, MBAR variation is the most widely used one (Luzhkov 2010). Abel et al. (2017) were hopeful that the future clinical drug discovery process would be enabled by advanced quantitative prediction of free energy calculation, quantum chemistry, and simulation technology. Many free energy calculating tools are available for the drug discovery (Table 11.2).

11.9 Role of CADD in Neurodegenerative Diseases (NDD)

Neurodegenerative diseases (NDD) are devastating disease conditions that result in the brain's neurons degeneration (Salman et al. 2021). Main NDDs are Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). The development of therapeutics in neuroscience is the leading pharmaceutical subject area, but insufficient drug exposure to the whole brain area, mainly in the CNS, leaves many of the neuronal disorders untreated (Di and Kerns 2015). Vatansever et al. (2021) suggest multiple factors lower the success rate of CNS drugs, including insufficient data on the pathophysiology of CNS disease conditions, poor target selection/engagement, lack of efficacy in early stages of disease development, and drug BBB permeability. These factors increased the average drug development time of 18 years against NDD (Mohs and Greig 2017). CADD can help to screen the chemical compounds with a potential role in CNS exposure according to their physicochemical properties. Molecular descriptors that influence the drug accessibility to the brain are H bond donors and acceptors, polar surface area (PSA), rotatable bonds, and molecular weight (MW) (Zheng et al. 2016). Compared to other disease drug discovery methods, neuronal targeted drug development needs more attention to BBB permeability (Yuan et al. 2018).

BBB is a semi-permeable membrane that isolates the CNS from circulating blood. A variety of factors influence the transferring ability of compounds through the BBB; that is, passive permeation, the influence of transporter molecules, and binding plasma proteins (Yuan et al. 2018). Screening of compounds permeable through BBB using the in vitro model is still challenging (Bicker et al. 2014). The use of in

silico methods to screen a large number of compounds permeable to BBB is comparatively easy within a short time period. There are many in silico models available to predict the BBB permeability of compounds. They are two types—quantitative and qualitative. The quantitative BBB permeability prediction model predicts the ratio of the concentration of a candidate molecule in the brain to the blood (LogBB) or the ratio of permeability surface area product (LogPS). The qualitative BBB permeability prediction model provides a binary classification to predict whether the compound is permeable or not (Yuan et al. 2018). For the BBB penetration predictive model, there are many learning approaches available based on molecular weight, H-bond donors and acceptors, acidic and basic atom numbers, water accessible volume, van den Waals volume, ionization potential, rotatable bonds, topological polar surface area, hydrophilicity and lipophilicity (Vatansever et al. 2021). The CADD methods influence recent researches on the NDDs drug development. Its therapeutics development process for NDDs schematic workflow or methodology is given in Fig. 11.3.

There are many studies on developing therapeutic agents against the most crucial NDDs, Alzheimer's disease (AD). It has been suggested that the acetylcholine esterase (AchE), N-methyl-D-aspartate (NMDA) receptor, muscarinic and Nicotinic Ach receptor, glycogen synthase kinase 3β (GSK3β), and β-site amyloid precursor protein cleaving enzyme 1 (BACE1) are the main therapeutic target of AD (Sehgal et al. 2018). Parkinson's disease (PD) is another neurodegenerative disease that causes motor disability in the old-age human population (Beitz 2014). The main therapeutic target proteins of PD are LRRK2, α-synuclein, and PARK2. Another important lethal, adult-onset NDD is amyotrophic lateral sclerosis (ALS). It affects the neuronal cells in the body characterized by loss of upper and lower motor neurons and leads to loss of muscle control (McGown and Stopford 2018). SOD1, caspase 3, and Protein Kinase CK-1 are the therapeutic targets of ALS (Sehgal et al. 2018). Vesicular monoamine transporter (VMAT2) protein and huntingtin (Htt) protein are the therapeutic target of interest for its drug discovery research. Huntington's disease (HD) is an inherited NDD that causes progressive neuronal degeneration in the brain. HD patients show problems in their psychiatric, movement, and thinking abilities. There are studies on the therapeutic strategies of different NDDs using CADD methods; some of them are described in Table 11.3. The development of effective drugs using in silico strategies is vital in modern drug discovery.

11.10 Conclusion

In silico technique-based drug discovery is less time, less effort, and cost-effective. There are different approaches for the CADD like virtual screening, homology modeling, ADME model, molecular dynamics, and energy calculation. CADD-based therapeutic development shows a similar effect in the in vitro and in vivo

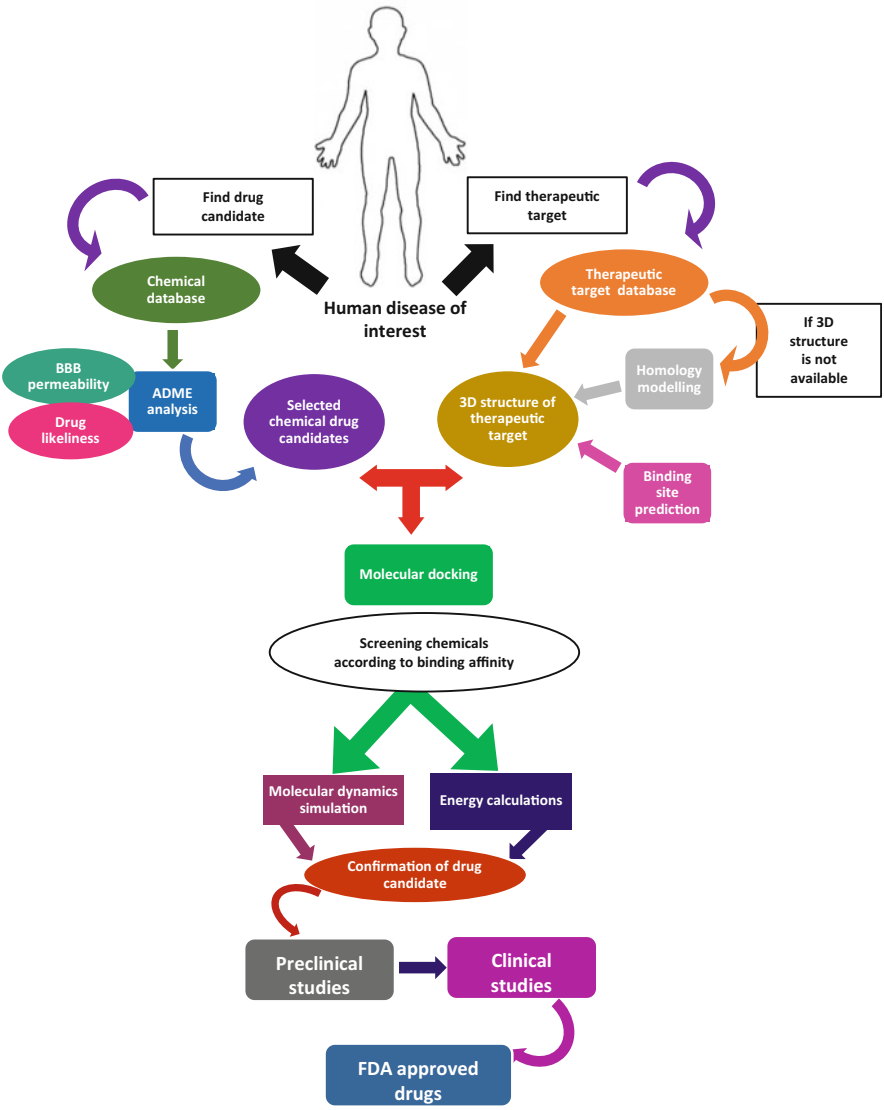

Fig. 11.3 In silico methodology followed in the neurodegenerative disease drug design

Table 11.3 Different types of NDDs and their drug discovery studies

Disease	Therapeutic target	Drug candidate	Type of study	Reference
Alzheimer's disease	AchE	Pyridopyrimidine	In silico In vitro	Basiri et al. (2013)
		4-Hydroxycoumarin		Samadi et al. (2012)
	NMDA	Memantine	In silico In vitro	Limapichat et al. (2013)
	GSK3β	Derivatives Of Pyrimidine Thiazolidinedione	In silico In vitro	Hanger et al. (2009)
	BACE1	Triptofordin B1	In silico	Huang et al. (2014)
		Quercetin And Myricetin		Butini et al. (2013)
	Monoamine oxidase B (MAO-B), N-methyl-D-aspartate (NMDA), β-secretase, Butyrylcholinesterase (BChE), β-amyloid, Acetylcholine esterase (AChE), γ-secretase	Dibenzylidene Ketone Derivatives, A1k1 and A2k2	In silico In vitro	Bashir et al. (2019)
	Cholinesterase and Aβ	Nordihydroguaiaretic Acid	In silico In vitro	Razavi et al. (2013)
Parkinson's disease	Leucine-rich repeat kinase 2 (LRRK2)	Pyrroloquinoline	In silico In vitro	Kim et al. (2010) Cheng et al. (2021)
	α-synuclein	Pyrroloquinoline	In silico In vitro	Kobayashi et al. (2006) Abel et al. (2017)
	DOPA decarboxylase (DDC)	Carbidopa and Trihydroxybenzylhydrazine	In silico In vitro	Daidone et al. (2012)
Huntington's disease	Vesicular Monoamine Transporter (VMAT2) protein	Tetrabenazine	FDA approved treatment	Yero and Rey (2008)
	Ku70	Hepta-Histidine (7H)	In silico, In vitro, In vivo	Imamura et al. (2016)
	Htt protein	Np_1	In silico	Kohli et al. (2021)
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase type 1	2,3, 5, 4-Tetrahydrostilbene Hesperidin	In silico	Anzai et al. (2016)
	Caspase 3	Curcumin And Rosmarinic Acid	In silico	Khan et al. (2015)
	Protein Kinase CK-1	CK-1 Δ And N-Benzothiozolyl-2-Phenyl Acetamide	In silico	Salado et al. (2014)

studies. CADD methods are its full-fledged state to supports the classical drug discovery process and improve more in the future.

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Chapter 12
Biomarkers: Potential Perspectives in Detection, Diagnosis, and Prognosis of Neurodegenerative Disorders

H. P. Chethana, Gauthami Hemachandra, and Arshdeep Sidhu

Abstract The cornerstone for diagnosis and treatment of neurodegenerative diseases is the ability to diagnose the disease before the onset of irreversible cellular damage and intervene with suitable therapy to stop or slow down the disease progression. Neurodegenerative diseases are mostly polygenic in origin and present with heterogeneous symptoms in the clinic. Therefore, specific and sensitive biomarkers are required for the early diagnosis and management of these diseases. The four most common neurodegenerative diseases by prevalence are Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease. For familial forms of these diseases, characterized by the early age of onset, the genetic biomarkers are highly reliable for risk assessment, diagnosis, and prognosis. For sporadic forms of the diseases, with typically a late age of onset, the symptoms develop gradually over decades with little or no manifest symptoms at the beginning of the disease. At present, no cure exists for any of the neurodegenerative diseases; therefore, prevention and management of disease through reliable biomarkers for each type and substage of the disease is necessary. The current biomarkers for sporadic form of the diseases—genetic biomarkers, biochemical biomarkers, and neuroimaging-based biomarkers—provide limited information on disease prognosis and progression. However, the field of biomarkers for neurodegenerative diseases is in a state of flux; the traditional approach for a unique biomarker for each disease condition is being replaced by a panel of biomarkers that report on the systemic health of the patient and not just the nervous system.

Keywords Biomarkers · Neurodegenerative diseases · Alzheimer's disease · Parkinson's disease · Amyotrophic lateral sclerosis · Huntington's disease

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Abbreviations

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
C9ORF72	Chromosome 9 open reading frame 72
CSF	Cerebrospinal fluid
CT	Computerized tomography
DPET	Dual photon emission tomography
DTI	Diffusion tensor imaging
ELISA	Enzyme-linked immunosorbent assay
FDG	^{18}F-6-fluoro-2-deoxyglucose
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma
HD	Huntington's disease
Htt	Huntingtin protein
MRI	Magnetic resonance imaging
PD	Parkinson's disease
PET	Positron emission tomography
PMCA	Protein-misfolding cyclic amplification
PNCSs	Phrenic nerve conduction studies
RT-QuIC	Real-time quaking induced conversion
SNIP	Sniff nasal inspiratory pressure
SOD1	Superoxide dismutase 1
SPECT	Single-photon emission computerized tomography
TDP-43	Tar DNA binding protein of 43 kDa
Tw Pdi	Twitch trans-diaphragmatic pressure
α-Syn	α-Synuclein

12.1 Introduction

Etiologically neurodegenerative diseases are of two types: the sporadic form and the familial form. The majority of the neurodegenerative diseases are sporadic in origin and are strongly associated with increased age. The typical age of onset, i.e., when the definitive symptoms manifest, is more than 60 years. Neurodegenerative diseases are caused by loss of neuronal function; therefore by the time symptoms manifest, a critical number of neurons have already died. The lost neurons cannot be regenerated with the present scientific know-how, making the cure impossible. Thus, the best strategy to alleviate the burden of neurodegenerative diseases on healthcare systems is to find preventive measures. Even for preventive measures, we should be able to diagnose presymptomatic individuals. This can only be achieved by discovering or developing biomarkers for early diagnosis for specific neurodegenerative diseases

(DeKosky and Marek 2003). The power of early diagnosis and appropriate preventive measures is highlighted by the observation that a 5-year delay in the onset of Alzheimer's disease can reduce the disease risk by 50%, while a delay by 10 years likely prevents it completely (Brookmeyer et al. 1998).

Based on prevalence, the common neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Fig. 12.1). All these diseases share many common clinical and molecular features. Clinically, late age of onset, motor-neuron dysfunction, and dementia are common symptoms, while at molecular level, amyloid deposits of intrinsically disordered proteins and progressive spread coupled with the loss of function are the main features. Also, all neurodegenerative diseases show high patient-to-patient variability. The diagnostic and pathological hallmark of all these diseases is the presence of protein aggregates and the loss of specific neurons (Table 12.1). The exact molecular mechanisms in each neurodegenerative disease however are distinct, which necessitates the development of a specific set of biomarkers for each condition.

12.2 Biomarkers

Biological markers or biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working 2001).

The ideal biomarker should have high specificity and sensitivity for the disease and the substages of the disease; it should be noninvasive and affordable. Depending on the use of biomarkers, they can be diagnostic, predictive, prognostic, and pharmacodynamic. Diagnostic biomarkers help determine the presence of a disease and identify the stage of a pathological condition. Predictive biomarkers evaluate the effectiveness of a given therapy. Prognostic biomarkers provide an overall outcome for the patient, regardless of the therapy. Pharmacodynamic biomarkers indicate the effect of the drug on the patient and the efficacy of the treatment. All clinical biomarkers need to be validated in diagnosed pathological cases. For neurodegenerative diseases especially, the biomarkers should be able to distinguish between the four common diseases and specific types of dementia, which shows highly overlapping clinical symptoms.

Biomarkers for neurodegenerative diseases are broadly classified into three categories: genetic, biochemical, and imaging-based. Combinations of all these biomarkers are used to diagnose and stratify susceptible individuals. Stratification identifies and classifies susceptible individuals or patients in groups of relative risk of developing a disease (DeKosky and Marek 2003).

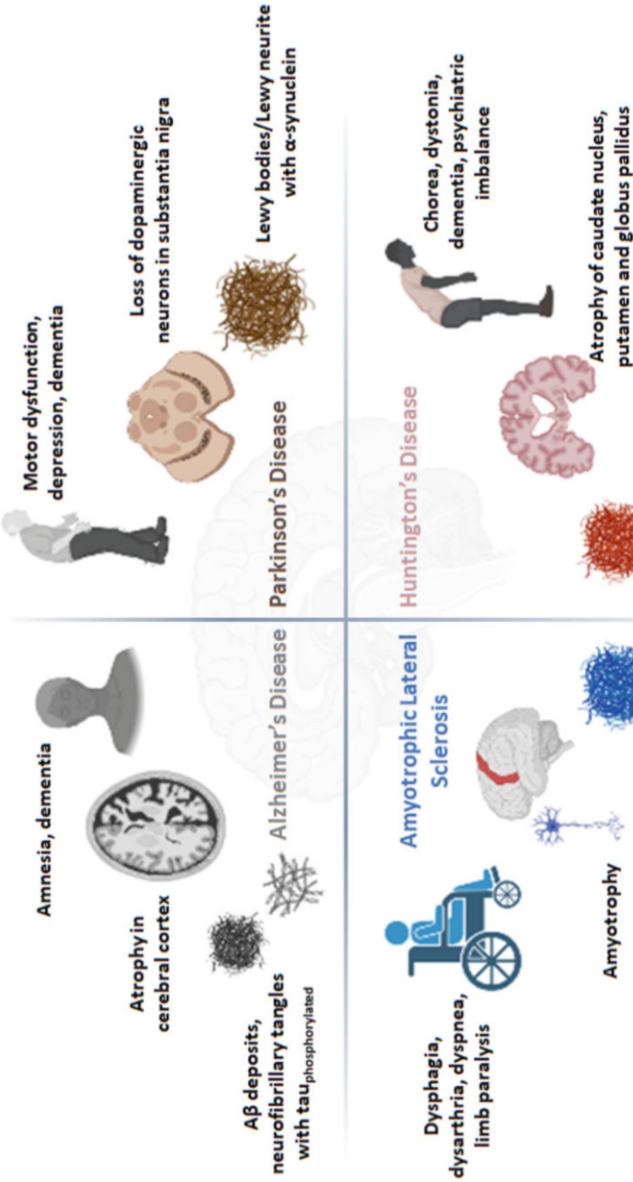

Fig. 12.1 Common neurodegenerative diseases with clinical symptoms at the individual level, changes at the tissue level, and amyloid deposits at the molecular level

Table 12.1 List of common neurodegenerative diseases with associated proteins and neurons

Disease	Key proteins	Affected neurons
AD	Amyloid β, p-tau, presenilin	Cerebral cortex and hippocampus
PD	α-Synuclein, LRRK2, parkin	Substantia nigra pars compacta
ALS	FUS, TDP-43, C9ORF72	Upper and lower motor neurons
HD	Huntingtin	Caudate nucleus, putamen, and globus pallidus

Table 12.2 Genes commonly mutated in familial forms of neurodegenerative diseases

Disease	Genes
AD	APP, Presenilin-1, Presenilin-2, ApoE (polymorphism)
PD	SNCA, LRRK2, GBA, PINK1, UCHL-1
ALS	TDP-43, FUS, C9ORF72, SOD1, ALS
HD	HTT

12.2.1 Genetic Biomarkers

Nucleic acid-based biomarkers—DNA and RNA—form the genetic biomarkers. Mutations and polymorphisms in genes that lead to familial forms of neurodegeneration are the primary biomarkers for the respective diseases for risk assessment, diagnosis, and prognosis. Examples of genes mutated in the familial form of neurodegenerative disease are listed in Table 12.2. The updated list of mutations—missense/nonsense, splicing, regulatory, small deletions, small insertions, small indels, gross deletions, gross insertions/duplications, complex rearrangements, and repeat variations—in these genes can be accessed through The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php). Any of the validated mutations can be developed into a genetic assay to be used as a biomarker. DNA-based biomarkers allow early risk assessment of the susceptible individual, can predict the early vs. late onset of the condition, and can predict the course of the disease (prognosis). For familial forms of neurodegeneration, DNA biomarkers can be tested anytime from childhood to early adulthood in individuals with a family history of the disease, as the mutations or polymorphisms are present in the genomic DNA.

In recent years, RNA-based biomarkers like mRNAs, miRNAs, long noncoding RNAs, etc. are under intense investigation for each neurodegenerative disease. RNA biomarkers provide information about the dynamics of gene expression and regulation. Since a majority of the neurodegenerative diseases are sporadic in nature, the loss of gene function arises at an extragenetic level. Sporadic DNA mutations can also lead to loss of gene function, but their effect is restricted to the neurons in which they arise, as neurons do not divide. Hence, sporadic DNA mutations are seldom a cause of sporadic neurodegenerative diseases. An exception to this hypothesis is if the neurodegenerative diseases spread in a prion-like manner, which implies that misfolded proteins from one neuron can nucleate misfolding of other proteins in the neighboring cells. Prion-like spread of neurodegenerative diseases involves the

transport of misfolded proteins from one cell and uptake into another neuron (Goedert et al. 2010).

12.2.2 Biochemical Biomarkers

Cerebrospinal fluid (CSF) circulates through the brain ventricles and drains into venous blood via the arachnoid villi of venous sinuses. CSF is an ultrafiltrate of plasma with a minimal concentration of blood metabolites and proteins. Therefore, biomolecules and metabolites of CSF are representative of neural homeostasis. Metabolites from intra-neural and extra-neural biochemical processes are removed by the circulating CSF. Regeneration of bulk of CSF takes place during deep sleep. Lack of sound sleep results in the accumulation of metabolites in the cerebral interstitial fluid that drains into CSF. Emerging evidence shows that elderly people with chronic sleep disturbances are more likely to develop neurodegenerative diseases (Lucey et al. 2018; Shokri-Kojori et al. 2018; Ju et al. 2017; Kang et al. 2009). Therefore, CSF, blood, and urine can yield potential biochemical biomarkers to map the presence and progression of neurodegenerative diseases. The accessibility and noninvasiveness of the body fluid follow the sequence: urine > blood > CSF. However, the concentration of the metabolites, in respective body fluids, is in the reverse order. Aggregated α-synuclein, Aβ peptides, tau, etc. are detectable in the CSF from presymptomatic individuals, but due to the highly invasive nature of CSF collection, it cannot be performed routinely for mass screening and follow-ups, whereas low concentration of the metabolites in blood and urine poses technical challenges for detection. The sensitivity of CSF as a source of biomarkers is due to its direct contact with the brain tissue, while the blood is separated by the blood–brain barrier and the urine is separated by a dual barrier of blood–brain and glomerular filtration. Each degree of separation from the brain tissue reduces the types and quantity of potential biomarkers (Table 12.3).

Absolute measurement of a single biomarker is prone to biological variations due to unknown factors like other health conditions and medications. Therefore, a ratio of two or more biomarkers normalizes the measurements and improves the accuracy. A pair of biomarkers that represent the total vs. altered biomarker status is an efficient method for diagnosis and staging, e.g., measurement of $A\beta_{42}/A\beta_{4o}$ and $tau_{total}/tau_{phosphorylated}$. CSF and blood biomarkers are measured by standard techniques like ELISA (enzyme-linked immunosorbent assay), electrochemiluminescence, xMAP® technology, etc. (Parnetti et al. 2019). The aggregated forms of proteins in CSF/blood sample are detected by using the prion-like behavior of the aggregated forms. They act as seeds for aggregation in assays like protein-misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) (Paciotti et al. 2018).

Table 12.3 Selected biochemical biomarkers used for neurodegenerative diseases

Disease	Biomarker	Information	Type
AD	CSF, plasma: phosphorylated-tau	AD pathology	Diagnostic, predictive
	CSF, plasma: $A\beta_{1-42}$	AD pathology	Diagnostic
	Glycosylated acetylcholinesterase	Acetylcholine signaling	Diagnostic
	Glycosylated butyrylcholinesterase	Acetylcholine signaling	Diagnostic
	Aggregated β-amyloid peptides	AD pathology	Diagnostic
	ε-ApoE	Lipid metabolism	Prognostic
PD	Lewy bodies	PD pathology	Diagnostic
	Oligomeric α-Syn	PD pathology	Diagnostic, predictive
	Altered dopamine transporter	Dopamine signaling	Diagnostic, prognostic
	β-Glucocerebrosidase	Lysosomal function	Predictive
	β-Hexosaminidase	Lysosomal function	Predictive
	Aggregated α-synuclein	PD pathology	Diagnostic, prognostic
	Lewy neurites	PD pathology	Diagnostic
ALS	Aggregated FUS	ALS pathology	Diagnostic, prognostic
	Aggregated TDP-43	ALS pathology	Diagnostic, prognostic
	8-Hydroxy-2′-deoxyguanosine	Oxidative stress	Predictive
	3-Nitrotyrosine	Oxidative stress	Predictive
	4-Hydroxy-2,3-nonenal	Oxidative stress	Predictive
	8-Oxodeoxyguanosine	Oxidative stress	Predictive
	^{15}-F(2 t)-isoprostane	Oxidative stress	Predictive
HD	CSF: Htt	Neuron damage	Prognostic
	Aggregated Huntingtin protein	HD pathology	Diagnostic

12.2.3 Imaging-Based Biomarkers

The least invasive and the most direct way of learning about and assessing brain function is through neuroimaging techniques available in the clinic and laboratories. The imaging techniques used in biomarker discovery and to study neurodegeneration include the following:

- Single-photon emission computerized tomography (SPECT).
- Positron emission tomography (PET)/dual photon emission tomography (DPET).
- Magnetic resonance imaging (MRI)/computerized tomography (CT).

Neuroimaging is a powerful method as it provides quantitative and qualitative measurements in real-time and can be used to follow the disease progression (Table 12.4). PET and SPECT imaging is based on the detection of photons emitted from the target tissue labeled with specific radiotracers. Radiotracers are radioactive

Table 12.4 Some of the neuroimaging biomarkers used for neurodegenerative diseases

Disease	Biomarker (Technique)	Target	Use
AD	Amyloid plaques (MRI)	Cerebral cortex	To detect the presence of amyloids
	Brain volume (MRI)	Whole brain or specific regions	To detect brain atrophy and mild cognitive impairment
	^{18}F-fluorodeoxyglucose (PET)	Glucose metabolism	To assess neuron health
	^{11}C-PiB (PET)	Amyloid-β	To detect and follow aggregation of amyloid-β
	^{11}C6-OH-BTA-1 (PET)	Amyloid-β	To detect and follow aggregation of amyloid-β
	^{18}F MK-6240 (PET)	Tau protein	To detect and follow aggregation of tau
PD	^{18}F-Dopa (PET)	Mass of dopamine neuron	To diagnose hemi-, pre-, and symptomatic PD
	^{11}C- DTBZ (PET)	Endocytic vesicles with monoamines	To measure the number of vesicles with dopamine
	^{123}I-β-CIT (SPECT)	Dopamine transporter ligands	To measure dopamine levels in the striatum
ALS	Diffusion tensor imaging (MRI)	White matter	To diagnose early ALS
HD	Brain volume (MRI)	Whole brain or specific regions	To detect brain atrophy
	^{11}C-β-CIT (PET)	Presynaptic dopaminergic neuron	To map disease progression
	^{11}C-DTBZ (PET)	Presynaptic dopaminergic neuron	To map disease progression
	^{11}C-SCH22390 (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{11}C-raclopride (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{11}C-FLB457 (PET)	Postsynaptic dopaminergic neuron	To map disease progression
	^{18}F-FDG (PET)	Glucose metabolism	To assess neuron health
	^{15}O-H_2O (PET)	Blood flow	To assess brain function

molecules that bind specific receptors or enzymes. Both the techniques have nanomolar sensitivity, with positron-emitting radioactive isotopes (^{15}O, ^{11}C, ^{18}F, ^{76}Br) in PET and γ-emitting isotopes (^{123}I, ^{99m}Tc) in SPECT imaging (Brooks 2005). The ability to replace natural isotopes with positron-emitting isotopes—^{11}C, ^{15}O, ^{13}N, and ^{18}F for hydrogen—allows to develop specific labels for virtually all the biomolecules in the body (Phelps and Mazziotta 1985).

Radiotracers against specific receptors or enzymes measure activity in all the neurons that use them. For example, radiotracers like ^{18}F-6-fluoro-2-deoxyglucose (FDG) labels all the neurons undergoing glucose metabolism. Healthy neurons with normal metabolism show higher FDG signals and appear as bright spots on images,

while dead or dying cells show reduced signals and appear as less bright spots on the image. The ability to visualize live cells and neuron function—through custom radiotracers—allows to study, diagnose, and determine the disease progression.

Magnetic resonance imaging (MRI) is based on the stimulation and relaxation of the proton spin in the water molecules. Typically, the subject is placed in an external magnetic field that aligns all the proton spins in the direction of the field. Next, a series of radiofrequency pulses are passed through the tissue under examination, and the protons absorb the radio energy that changes their spin direction. At the end of the pulse, the proton spin realigns with the external magnetic field while releasing energy. MRI detectors detect the released energy and build a composite image from multiple stimulation–relaxation cycles. MRI allows label-free imaging of the soft tissues of the body (higher water content provides more protons, hence a higher signal-to-noise ratio). Brain MRIs are sensitive to distinguish gray and white matter tissue, in addition to the various other parts like cerebellum, amygdala, hypothalamus, thalamus, striatum, etc. (Rosas et al. 2003). MRI scans are very efficient in determining the volume of the whole brain as well as of specific regions. Thus, MRI is routinely used as a diagnostic biomarker for various brain conditions including neurodegenerative diseases. Computerized tomography (CT) scan is based on X-ray images and complements the information from an MRI scan. All the neuroimaging techniques require specialized infrastructure and trained manpower; thus, they are not universally affordable.

12.3 Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of neurodegenerative disease. Loss of memory—amnesia—and consistent decline in cognitive functions—dementia—over a period of up to 20 years are the primary clinical symptoms for AD. The progressive degeneration of the cerebral cortex and hippocampus region leads to loss of cholinergic neurons resulting in the manifestation of AD. Postmortem brain autopsy that shows the presence of neurofibrillary tangles and/or amyloid plaques positive for phosphorylated tau protein and amyloid β (Aβ), respectively, confirms the diagnosis. AD pathology is broadly categorized as amyloid-β positive or negative ($A\beta^{+}/A\beta^{-}$) depending on the presence or absence of the Aβ deposits (Masters et al. 1985; Grundke-Iqbal et al. 1986; Braak et al. 2006). The clinical diagnosis, with 70–80% accuracy, based solely on the clinical symptoms—behavior and neuropathology—is poor and necessitates for better diagnosis to improve disease management and cure. An optimal diagnosis regimen should cover various stages of disease development and progression.

Molecular diagnosis is based on the molecular mechanisms of the disease. Since the main component of the AD amyloid plaques is the $A\beta_{1-42}$ form of amyloid-β along with tau protein (Miller et al. 1993; Masters et al. 1985; Grundke-Iqbal et al. 1986), the first ELISA-based diagnostic assays for AD measured the CSF level of total-tau, phosphorylated-tau, and $A\beta_{1-42}$. The diagnostic profile in these assays

included an increased level of tau proteins (total + phosphorylated) and decreased levels of $A\beta_{1-42.}$ (Andreasen et al. 1999; Vandermeeren et al. 1993; Vanmechelen et al. 2000). Reduced levels of CSF $A\beta_{1-42}$ correlate with increased $A\beta_{1-42}$ amyloid deposit in the brain, as corroborated by PET imaging (Fagan et al. 2006). A decrease in CSF $A\beta_{1-42}$ levels can be measured before amyloid aggregates can be imaged by PET, showing that the accumulation of $A\beta_{1-42}$ in the brain is a precursor for aggregation and subsequent clinical symptoms of dementia. The CSF ratio of $A\beta_{42}/A\beta_{4o}$, rather than $A\beta_{1-42}$ levels alone, normalizes the background variations and improves the specificity of the diagnosis as $A\beta_{40}$ levels do not change in AD (Palmqvist et al. 2016).

CSF levels of total and phosphorylated tau proteins also directly correlate with the progression of AD. The ratio of $tau_{total}/tau_{phosphorylated}$ is a reliable indicator of the severity of AD. Tau can be phosphorylated at multiple sites—81, 199, 231, 235, 396, 404—each of which can be used as a biomarker (Blennow and Zetterberg 2018). Many other proteins like neurogranin, neuron-specific enolase, visinin-like protein, and neurofilament light chain protein also show elevated levels in AD patients but their association with specific molecular pathology is poorly understood (Keshavan et al. 2017). Higher CSF levels of neurofilament light chain protein are detected in early clinical stages that show beginning of the brain atrophy in multiple regions and mild cognitive impairment (Zetterberg et al. 2016). Tau_{total} and neurogranin levels reflect advanced $A\beta^{+}$ clinical stage (Mattsson et al. 2016).

Altered $A\beta_{1-42}$ and neurofilament light chain protein levels are also associated with AD when measured in plasma samples. Long-term studies show that neurofilament light chain levels begin to increase up to 10 years before the onset of dementia symptoms; tau levels in plasma are however ambiguous. One of the reasons for the poor detection of tau proteins in the plasma is the likely degradation of the protein in the bloodstream (de Wolf et al. 2020). Changes in protein levels and biochemistry in blood complicate standardization of blood biomarkers, resulting in a paucity of blood biomarkers for AD.

In terms of invasiveness, urine biomarkers are the most promising ones due to safety and ease of sample collection. Unlike the CSF that is derived from the brain tissue, urine mostly contains secondary metabolites dominated by the biochemistry of visceral organs. Thus, to qualify as an AD biomarker, AD pathology must be corelated with the metabolic function at a systemic level. Sucrose levels and catabolic products of spermidine (N-acetylisoputreanine-γ-lactam) are higher in patients with AD, suggesting changes in glucose and polyamine metabolism (Kurbatova et al. 2020). Recent high-throughput genomic, proteomic, and metabolomic studies report dysregulation of multiple proteins and RNA. However, direct evidence of these changes and their role in AD is lacking.

Neuroimaging of suspect AD patients is a noninvasive, highly informative screening technique. It allows to study the structural features of the brain like gray and white matter volume, ventricular volume, etc. by MRI. PET and SPECT imaging enables probing biochemical functions by using specific radiotracers. MRI scans are commonly used to detect changes in the brain structures of AD patients. AD brains typically show gray matter atrophy in regions of median temporal lobe, insula, and

temporoparietal cortices along with the increase of ventricular volume and reduction in total brain tissue volume (Busatto et al. 2008). The atrophy begins in the median temporal lobe and extends to the temporal neocortex and then to the parietal and frontal lobes. Atrophy in the median temporal lobe coincides with mild cognitive impairment as a clinical symptom, which often develops into typical AD in the following years. Thus, an MRI scan is a robust imaging biomarker for presymptomatic and early-stage AD patients (Whitwell et al. 2007).

Functional brain imaging with PET scans for glucose metabolism (^{18}F-fluorodeoxyglucose) and SPECT scans for blood flow imaging in AD patients shows hypometabolic profile and reduced blood flow in the temporoparietal cortex. Impaired temporoparietal function in clinical cases with mild cognitive impairment develops into typical AD in time (Schroeter et al. 2009). Although functional brain imaging for one or more brain functions is predictive of the high risk to develop AD, it should be complemented with approaches like MRI/CT scans that assess the whole brain structure.

Amyloid deposits in the brain tissue of AD patients can be imaged by Aβ-specific ligands like ^{11}C-PiB, ^{11}C6-OH-BTA-1 (derived from amyloid binding dye thioflavin-T), and tau-specific ligands like ^{18}F-MK-6240, using amyloid PET (Mathis et al. 2003; Marquez and Yassa 2019). The presence of Aβ amyloids however does not correlate with a decline in cognition or AD pathology. Various amyloid imaging experiments show that amyloid deposits appear and increase with age; they may or may not be associated with dementia or AD (Marquez and Yassa 2019).

12.4 Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. Like AD, the definitive diagnosis of PD is also made by brain autopsy of the suspected patients. The clinical symptoms of PD include both motor and nonmotor disorders. The motor disorders affect the normal range and strength of muscle movements like bradykinesia, rigidity, tremors, and unstable posture. These symptoms are caused by lack of dopamine due to loss of dopaminergic neurons in substantia nigra pars compacta. Many of the motor symptoms can be ameliorated by levodopa supplements. The nonmotor disorders include impaired brain function like sleep problems, depression, dementia, and fatigue. These symptoms are caused by the loss of a heterogeneous group of neurotransmitters and do not respond to levodopa; this form of PD is called atypical Parkinsonian syndrome (Kalia and Lang 2015). The presence and magnitude of all these symptoms are highly variable in suspected patients, leading to ambiguity in clinical diagnosis based solely on the symptoms. Moreover, symptoms of PD do not manifest until the loss of 70–80% substantia nigra neurons (Schapira 1999). Tissue analysis, postmortem, in PD patients show the presence of Lewy bodies and Lewy neurites accompanied by loss of substantia nigra neurons (that produce dopamine). The main component of

Lewy bodies and neurites is α-synuclein protein in the amyloid form. Familial forms of PD are caused by mutations in a number of genes: α-synuclein, PARKIN, LRRK2, DJ-1, etc. Screening for mutations in these genes, using PCR-based techniques, in individuals with familial history of PD provides risk assessment and prognostic evaluation.

α-Synuclein (α-Syn) aggregates are central to various forms of dementia—Parkinson's disease, multiple system atrophy, dementia with Lewy bodies, Lewy body variant of AD, and neurodegeneration with brain iron accumulation—which are characterized by the presence of α-Syn aggregates; these conditions are collectively called α-synucleopathies. Detection of α-Syn in CSF, blood, and other body fluids, as a biomarker, is thus the most obvious choice for synuclein-associated pathologies. However, a biomarker should be able to distinguish PD from other synucleopathies, as the etiology, prognosis, and therapy for each condition are typical. In general, total α-Syn levels in PD patients' CSF are lower than in control samples. However, the range of total α-Syn concentration varies considerably; this limits its use as a diagnostic biomarker. Two main sources of variation include contamination from blood (erythrocytes have a high level of α-Syn) and heterogeneity of stage, age, and genetic background of patients enrolled in trials (Mollenhauer et al. 2016; Barbour et al. 2008). Contamination from erythrocyte α-Syn is also a problem when measuring blood α-Syn levels for diagnostic purposes. Some forms of neurodegeneration like progressive supranuclear palsy, caused by extensive neuron damage in the brain stem, show increased levels of CSF α-Syn. In cases with an overlap of synucleopathies and progressive supranuclear palsy (a form of tauopathy), the total CSF α-Syn levels can be normal due to a decrease caused by synucleopathies and an increase caused by progressive supranuclear palsy. Overall, the heterogeneity between synucleopathies, other forms of neurodegeneration and contamination from erythrocytes, renders total CSF α-Syn levels a nonselective biomarker for PD (Parnetti et al. 2019).

Lewy body-associated α-Syn pathology proceeds through the aggregation of monomeric α-Syn to oligomers and fibrils. The oligomeric form of α-Syn is elevated in CSF and blood of PD patients; however, the diagnostic accuracy of the assays used and heterogeneity observed is not fit for clinical use as yet (Eusebi et al. 2017). Modified forms of α-Syn monomers/oligomers—phosphorylated, ubiquitinated, nitrated, oxidized, and truncated—are under investigation for use as a specific and selective biomarker for PD (Schmid et al. 2013). Aggregation of α-Syn depends on the α-Syn concentration; thus, enzymes in cellular pathways that regulate the degradation of α-Syn—autophagy-mediated lysosomal degradation—are also potential biomarkers for PD. A combined reduction in β-glucocerebrosidase and β-hexosaminidase activity in CSF shows a good correlation with PD but lacks accuracy for clinical use (Balducci et al. 2007).

Structural MRI allows detection of dopaminergic lesions and brain atrophy in PD patients that corroborate the clinical symptoms for PD. Functional MRI based on PD-specific patterns of low-frequency fluctuations in the striatum, supplementary motor neurons, frontal gyrus, and occipital cortex can distinguish PD patients from healthy subjects with high accuracy (Wu et al. 2015). A combination approach of

brain MRI, ^{123}I-mIBG SPECT, and ^{18}F-FDG PET enables differential diagnosis of PD and atypical PD. PET scan alone can identify several PD subtypes (Pagano et al. 2016). Although highly specific and informative, none of the neuroimaging modalities are used in the clinic as a routine diagnostic method as yet.

12.5 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease caused by progressive degeneration of spinal cord neurons that results in atrophy of the lower and upper motor neuron function. About 10% of ALS cases are inherited and are called familial ALS, while about 90% are sporadic in etiology. Clinical symptoms of ALS include progressive muscle weakness, spasticity, and twitching that progresses to dysphagia, dysarthria, dyspnea, and limb paralysis due to the death of motor neurons (amyotrophy). The average life expectancy after the onset of symptoms is 2–5 years, and death occurs mainly due to respiratory failure. ALS is a heterogeneous disorder with symptoms overlapping with other neurodegenerative diseases like frontotemporal dementia (FTD) and frontotemporal lobar degeneration (FTLD). Neurodegeneration and neuroinflammation are the molecular hallmarks of ALS. The most common genes (familial form) and proteins (sporadic form) associated with ALS are TDP-43 (Tar DNA binding protein of 43 kDa), FUS (fused in sarcoma), C9ORF72 (chromosome 9 open reading frame 72), and SOD1 (superoxide dismutase 1). In ALS and FTLD, loss of neuronal function is accompanied by the presence of cytoplasmic aggregates of FUS, SOD1, dipeptide repeats, and TDP-43 protein (Ustyantseva et al. 2020).

The earliest symptoms of ALS include weight loss, respiratory insufficiency, and muscle stiffness. Thus, the earliest biomarkers of ALS include monitoring weight loss that is accompanied by a decline in respiratory function. Sniff nasal inspiratory pressure (SNIP) is a volitional noninvasive test that measures inspiratory muscle strength. SNIP performance declines with motor neuron degeneration (Heritier et al. 1994). SNIP is recommended as a predictive biomarker in ALS patients that may need noninvasive ventilation within 3 months (Tilanus et al. 2017). Respiration is controlled by phrenic nerve stimulation; tests for phrenic nerve stimulation, though invasive, also acts as a biomarker for respiratory function. Phrenic nerve conduction studies (PNCSs) measure the diaphragm action potential that allows measuring the nerve function. In ALS patients, PNCSs show increased latency in the nerve stimulation that correlates with weak muscles and poor respiratory function (Evangelista et al. 1995). Twitch trans-diaphragmatic pressure (Tw Pdi) measures inspiratory muscle strength in a nonvolitional manner. Tw Pdi shows a linear decline with the progression of ALS and thus has predictive potential for mortality stratification (Polkey et al. 2017). Both PNCSs and Tw Pdi are performed by electrophysiologists in a clinical setup. Therefore, phrenic nerve tests are slightly invasive and need trained personnel and dedicated infrastructure.

Specific and reliable CSF-blood biomarkers for ALS are not known. Some biomarkers like levels of neurofilament heavy/light chain proteins and tau proteins are elevated in CSF from ALS patients, but these are indicative of extensive axon damage and neuron degeneration, and not ALS specifically. Moreover, their levels plateau out after significant neuron damage. TDP-43 levels in CSF and blood are reported to change, but more evidence is required to classify it as an ALS biomarker (Verber et al. 2019). SOD1, one of the key proteins associated with familial and sporadic ALS, maintains the redox homeostasis in neurons. Loss of function of SOD1 in ALS increases the concentration of biomarkers for oxidative stress in various body fluids, e.g., 8-hydroxy-2′-deoxyguanosine and 3-nitrotyrosine in CSF, 4-hydroxy-2,3-nonenal in serum and CSF, and 8-oxodeoxyguanosine and ^{15}F(2 t)-isoprostane in urine (Verber et al. 2019). However, oxidative stress biomarkers need further investigation and protocol optimization for clinical use.

One of the proposed mechanisms for the spread of ALS is through the extracellular vesicles (microvesicles and exosomes) that carry misfolded proteins to neighboring neurons and induce a prion-like propagation of the disease. ALS-associated proteins, TDP-43, FUS, and SOD1, are found in extracellular vesicles isolated from blood as well as CSF; however, there is no consensus, as yet, about their optimization as a biomarker either for diagnosis or disease progression (Sproviero et al. 2018; Feneberg et al. 2014; Gagliardi et al. 2021).

Key ALS-associated proteins, FUS, TDP-43, and C9ORF72, are RNA binding proteins. FUS and TDP-43 are involved in miRNA biogenesis; thus, loss of function in these proteins is expected to reflect in RNA metabolism. Consequently, RNA levels in the extracellular vesicles in CSF as well as in the blood of ALS patients also change (Kawahara and Mieda-Sato 2012; Morlando et al. 2012). Panels of upregulated and downregulated miRNAs are promising biomarkers, but more research is needed to find optimal candidates.

MRI imaging in ALS patients shows brain atrophy that is not limited to the motor cortex. Reduction in brain volume in ALS is not a reliable prognostic or diagnostic biomarker for ALS; it is mainly used in the clinic to exclude conditions that mimic ALS symptoms (Filippi et al. 2010). Diffusion tensor imaging (DTI), a type of MRI for visualization of white matter, shows loss of white matter in the early stages of ALS. DTI is recommended as a diagnostic biomarker for relatives of familial ALS patients to detect early damage in upper motor neurons (Filippi et al. 2010). PET imaging using ligands like ^{11}C-flumazenil, ^{11}C-WAY 100635, ^{11}C(R)-PK11195, and ^{11}C(l)-deprenyl correlate changes in neural biochemistry with symptoms broadly observed in motor-neuron diseases, but their specificity to ALS is not proven. Thus, more specific ligands for ALS need to be developed for use as ALS biomarkers (Filippi et al. 2010).

12.6 Huntington's Disease

Huntington's disease (HD) is a progressive neurodegenerative disease that is mainly inherited in an autosomal dominant manner. The typical age of onset in adults is 30–40 years. The clinical symptoms of HD include poor motor control, characterized by involuntary movements (chorea), dystonia, decline in cognition, and psychiatric imbalance. At the cellular level, the symptoms originate from the progressive cell death in the striatum, i.e., atrophy of caudate nucleus, putamen, and globus pallidus. The cause for cell death is not precisely understood but is suggested to stem from dysregulated cellular proteostasis that includes synaptic dysfunction, mitochondrial toxicity, and hampered axonal transport. At the molecular level, HD is caused by an expansion in the trinucleotide repeat—CAG—of the huntingtin protein (Htt). An increase in the number of CAG repeats from 36–39 (nonpathological) to >40 leads to pathogenesis. The mutant Htt, with expanded repeats, aggregates into amyloids resulting in cytoplasmic and nuclear Htt-positive inclusion bodies, which are the pathological hallmark of HD (McColgan and Tabrizi 2018; Arrasate and Finkbeiner 2012).

Autosomal dominant nature of HD and monogenic etiology makes genetic screening for CAG expansion in the HTT gene the most robust prognostic biomarker in individuals with a family history of HD. Polymerase chain reaction-based techniques are standard methods to identify and characterize the CAG repeat length and to predict the severity and age of onset in suspected individuals. Mutant Htt protein is detectable in CSF and can be measured in femtomolar concentrations (Wild et al. 2015). The increase in mutant Htt coincides with an increase in CSF tau and neurofilament proteins, suggesting that the source of mutant Htt is damaged neurons. Therefore, mutant Htt can be a diagnostic marker for HD but is not as yet able to delineate the HD progression and prognosis. Mutant Htt levels for HD specific pathology and neurofilament level for global neuron damage are in clinical trials for validation and endpoint use in the clinic (Wild et al. 2015).

Volumetric MRI scans of the striatum are used as biomarkers in the clinic to map the progress of HD. Screening in susceptible individuals shows atrophy in various striatal structures 15–20 years before the onset of clinical symptoms. Changes in gray and white matter in specific areas or at the whole-brain level provide individual-specific changes in the brain structure as HD progresses. Diffusion tensor imaging reports the neuron damage prior to cell death that leads to volume reduction. Thus, MRI is routinely used to diagnose and follow HD progression, but it has little prognostic value (Rodrigues et al. 2018).

PET scans with specific radiotracer ligands are used to measure changes in the dopaminergic neurons (control muscle movements) by ^{11}C-β-CIT and ^{11}C-DTBZ ligands for the presynaptic dopaminergic system and ^{11}C-SCH22390, ^{11}C-raclopride, and ^{11}C-FLB457 ligands for the postsynaptic dopaminergic system. The pre- and postsynaptic dopaminergic systems include the D1 type and D2 type dopamine receptors in the substantia nigra pars compacta and globus pallidus. HD progression correlates with a decrease in D1 and D2 receptor activity (Wilson et al.

2017). Glucose metabolism and blood flow in the brain regions of HD patients are measured using ^{18}F-FDG and ^{15}O-H_2O labeled tracers, respectively. Reduced glucose metabolism in the striatum and cortex is associated with a decline in motor and cognitive functions (Kuwert et al. 1990). Neuroinflammation in HD brains via microglia activation in early HD stages can be measured using ^{11}C-PK11195 as a ligand, which binds to the activated microglial surface protein called translocator protein. Pro-survival cell-signaling via cyclic monophosphate signaling can be measured by ligands like ^{18}F-JNJ42258152 and ^{18}F-MNI-659 against phosphodiesterase 10A enzyme. However, these biomarkers for neuroinflammation and pro-survival signaling need more optimization to be used in clinics (Wilson et al. 2017).

12.7 Future of Biomarkers

Numerous studies show increased levels of tau, neurofibrillary proteins, and inflammatory molecules in the CSF of patients with AD, PD, ALS, and HD. This common CSF profile is indicative of the neuron damage that occurs with the progression of neurodegenerative diseases. A critical mass of neurons has already died before these biomarkers can be detected in the CSF and blood above the baseline, suggesting that they do not reflect early molecular and cellular changes at the onset of the disease. Moreover, with the present biomarkers, the sub-types of the four main neurodegenerative diseases cannot be reliably distinguished. MRI and PET-based neuroimaging provide information on both specific and global changes in the brain for each type of neurodegenerative disease, but they are poor in disease prognosis and have little predictive value.

With high-throughput techniques like the whole-genome sequencing, the whole exome sequencing, RNA sequencing, liquid and gas chromatography coupled to mass spectrometry, etc. becoming more affordable, the emphasis for biomarker discovery is shifting from a single biomarker to a panel of biomarkers that reflect changes not only in the brain but also in various other organs—from a reductionist to a systems biology approach. Biomarker discovery using genomics, proteomics, metabolomics, and lipidomics to find markers for genetic polymorphism, oxidative stress, neuroinflammation, DNA methylation, and neurofilament proteins will allow one to screen, predict the risk, and stratify the population; diagnose the disease onset in the early stages; and follow the progression of the disease and response to therapy at a systemic level. Regulatory miRNAs are promising biomolecules to probe cellular homeostasis. Numerous reports suggest upregulation and downregulation of specific groups of miRNAs in given neurodegenerative disease. However, the lab protocols to identify and measure these biomarkers are not standardized, as yet, so the results are not always reproducible. Moreover, the patient cohort in various studies represents different age groups, stages of the disease, and ethnic background that together makes direct comparison of results challenging.

12.8 Conclusion

Neurodegenerative diseases are a major concern in an aging world, primarily due to the absence of treatment options. Diagnosis, prognosis, and pharmacological intervention in neurodegenerative diseases are hampered due to the lack of optimal biomarkers. The biggest challenges in finding specific and sensitive biomarkers for neurodegenerative diseases are the heterogeneity of the clinical symptoms and the substantial overlap of symptoms between various conditions. Since adult-onset neurodegenerative diseases develop over decades with irreversible cellular damage, predictive and diagnostic biomarker are needed to reduce their burden in the coming years. For neurodegenerative diseases, especially, longitudinal studies with big sample size, standard protocols, age, and stage mapping are critical to characterize molecular and cellular hallmarks for disease risk, onset, progression, and response to therapy, as each of these stages may last for years and can be modified by lifestyle choices.

Conflict of Interest The authors declare no conflict of interest.

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