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Neurodegenerative Diseases

Pathology, Mechanisms, and Potential Therapeutic Targets

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Preface

The World Health Organization has estimated that neurological disorders account for 11% of all deaths worldwide and over one billion years of healthy life lost through disability. Indeed their document, "Neurological disorders: public health challenges" (http://www.who.int/mental_health/neurology/neurological_disorders_report_web.pdf), emphasizes that internationally we face a global public health challenge with policy-makers and health-care providers poorly prepared for a rise in neurological disorders, and the disabilities associated with increasing aged populations. This report stresses the need for (1) disease prevention, (2) reducing disease severity and progression through early detection, and (3) improved interventions. The pathway to improving interventions and reducing the severity of disease for individuals requires basic and clinical insights into neuropathologies. Only with an understanding of disease mechanisms can sites for drug discovery be identified and targeted. Insight into disease processes and mechanisms will impact upon diagnosis, therapeutic management, and patient care.

Our understanding of the molecular and cellular neurobiology of neurodegenerative conditions has grown substantially in the twenty-first century with stunning insights in the last 5 years underpinned by new views of underlying disease pathobiology. Long subclinical phases in many neuropathologies are now receiving attention, especially when taken in the context of the unique discoveries arising from analyses of disease biomarkers and contemporary neuroimaging. Here in particular "omics-related" research in animal and cellular models and in human postmortem tissue has allowed the definition of disease mechanisms and therapeutic targets that have to be considered as totally mind-boggling.

Neurodegenerative Diseases: Pathology, Mechanisms, and Potential Therapeutic Targets provides a comprehensive coverage of these latest advances not only in the major neurodegenerative conditions, but also in other neurological conditions which involve progressive neuronal injury and dysfunction. Our strategy was deliberately threefold and features contributions from leading international researchers from both clinical and basic perspectives, plus coverage of pathobiological mechanisms and emergent technologies whose wide-ranging application have driven progress. Not only does the coverage include the traditional neurodegenerative pathologies,

Alzheimer's, Parkinson's, and Huntington's diseases, but a diverse group of conditions where neurodegeneration and downstream events occur are also placed in their up-to-date context. We include chapters on some diseases not classically considered "neurodegenerative disorders" including schizophrenia and epilepsy, but for which there is evidence that these disorders can be associated with a progressive neurodegenerative phenotype. While the projected financial impact of treating Alzheimer's disease is daunting because of the number of individuals who will be affected, the costs of diseases like schizophrenia are higher on a per patient basis because the disease starts early in life and frequently limits an individual's lifetime productivity.

Although there is variance across neurodegenerative conditions in terms of diagnosis and clinical management, several common themes emerge. In many of these disorders, the disease process including pathology likely starts before there is any functional evidence of disease. Thus, much like cholesterol or LDL levels can be used as screening tools to identify individuals who are at risk for future cardiovascular disease, there is a critical need to develop and validate specific biomarkers of these various disorders. It is also clear that advances in neuroimaging have had a huge impact over the last decade, with whole-brain tractography and diagnostic imaging agents providing significant advances. Finally, as was required for the development of statins (the class of drugs that reduce cholesterol synthesis), it will be critical to understand both basic biological mechanisms and how diseases change this biology.

For every neurological disease, there is unmet need for disease-modifying therapeutic treatments that arrest underlying pathological processes. While progress has been made in individual conditions through genetic, pathological, and biological studies of disease mechanisms, it is clear that these research efforts must continue in the quest for effective therapeutic agents. Mechanistic insights inform the design of disease-modifying therapies. In this volume there are numerous insights into potential therapeutic targets, which offer translational opportunities. As well as coverage of different disease pathologies, common themes emerging in this volume include the neurobiology of misfolded proteins and proteostasis, which involves both autophagic and proteasomal mechanisms. New evidence for the roles of glial cells in neuropathology focuses attention on neuroinflammation in neurodegeneration, a phenomenon which rates frequent mention here across disease conditions.

Finally, the editors extend their sincere thanks to all authors for their patience, commitment, and overall effort to making this highly international volume such a success. The continued support of Michal Koy and other staff at Springer is generously acknowledged.

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Part I
Major Neurodegenerative Conditions

Chapter 1
Alzheimer's Disease: Insights from Genetic Mouse Models and Current Advances in Human IPSC-Derived Neurons

Anne E. Harasta and Lars M. Ittner

Abstract Alzheimer's disease was first described in 1906 and since then tremendous efforts have been made to fully understand the disease pathology and to find a cure for this neurodegenerative disease. The diagnosis of Alzheimer's is still difficult, especially in early stages of the disease. Current treatment of Alzheimer's only ameliorates the symptoms but fails to provide a therapy. Over the last decades, animal models have been proven valuable in elucidating insights of the pathology. In vitro models using patient-derived cells are currently emerging and hold great promise in understanding the disease pathophysiology. Here, we introduce the neurobiology and genetic features of Alzheimer's and describe what we have learned from studies employing mouse models and patient-derived induced pluripotent stem cells.

Keywords Amyloid-β • Tau • Mouse models • Alzheimer's disease • Excitotoxicity • Induced pluripotent stem cells

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Abbreviations

3D	Three-dimensional
ABAD	Aß-binding alcohol dehydrogenase
AD	Alzheimer's disease
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ	Amyloid-β
BDNF	Brain-derived neurotropic factor
BiP	Binding immunoglobulin protein
cAMP	Cyclic adenosine monophosphate
cdk5	Cyclin-dependent kinase
CHOP	CCAAT-enhancer-binding protein homologous protein
CRISPR	Clustered regularly interspaced palindromic repeats
CSF	Cerebrospinal fluid
CT	Computed tomography
CTF83	Carboxy-terminal fragment produced by the α-secretase
CTF99	Carboxy-terminal fragment produced by the β-secretase
DS	Down syndrome
FAD	Familial AD
FTD	Frontotemporal dementia
GSI	β-Secretase inhibitor
GSK3β	Glycogen synthase kinase 3β
GSM	β-Secretase modulator
hiPSCs	Human induced pluripotent stem cells
htau	Human tau
IPSCs	Induced pluripotent stem cells
MAPT	Microtubule-associated protein tau
MB	Methylene blue
miRNA	Micro RNA
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
NFTs	Neurofibrillary tangles
NMDARs	N-methyl-D-aspartate receptors
NO	Nitric oxide
NR1	NMDAR subunit 1
NR2A-NR2D	NMDAR subunit 2A-D
NR3A-NR2B	NMDAR subunit 3A-B
PDGF	Platelet-derived growth factor
PET	Positron emission tomography
PIB	Pittsburgh Compound-B
PP2A	Protein phosphatase 2A
PrPC	Cellular prion protein

PSD95	Postsynaptic density protein 95
PSEN	Presenilin protein
RNA	Ribonucleic acid
ROS	Reactive oxidative species
SAD	Sporadic AD
sAPPα/sAPPβ	Soluble extracellular APP fragment after α- or β-secretase processing, respectively
shRNA	Short hairpin RNA
SNP	Single-nucleotide polymorphism
ZFNs	Zinc-finger nucleases

1.1 General Overview of AD

In 1906, the German physician Alois Alzheimer reported the case of Auguste Deter, a 51-year-old patient with severe memory loss and profound personality changes. In his postmortem analysis on brain slices of his patient, Alois Alzheimer observed not only severe shrinkage of the brain but also the two main hallmarks of this neurodegenerative disease, extracellular senile plaques and neurofibrillary tangles (NFTs) inside cells [1]. This degenerative neurological disorder is today known as Alzheimer's disease (AD) and represents the most common cause of dementia.

Almost eight decades later, researchers discovered that the senile plaques comprise fibrillar forms of amyloid-β (Aβ) [2] and that NFTs are composed of hyperphosphorylated forms of the microtubule-associated protein tau [3, 4].

Since the early discoveries of AD, numbers of PubMed entries for "Alzheimer's disease" rapidly grow every year and underlying mechanisms of disease pathophysiology and progression are increasingly understood, including with the help of animal models. Here, we introduce the neuropathology and genetic features of AD and describe what we have learned from recent studies using mouse models and induced pluripotent stem cells (IPSCs).

1.2 APP Processing and β-Amyloid Plaques

The major protein component of senile plaques is a 40–42 amino-acid polypeptide termed Aβ ($A\beta_{40}$ and $A\beta_{42}$), which derives from sequential cleavage of the amyloid-β precursor protein (APP) [2, 5]. APP undergoes sequential cleavage by α- or β-secretase that initiates two different pathways. When the α-secretase cleaves APP, a soluble extracellular fragment is formed (sAPPα) next to a carboxyl-terminal fragment (CTF83), which is further cut by a complex of proteins called γ-secretase, whose catalytic core is formed by presenilin proteins (PSEN1 and PSEN2). This

pathway is also known as the non-amyloidogenic pathway as the formation of $A\beta_{40}$ and $A\beta_{42}$ is prevented. Alternatively, APP can be cleaved by the β-secretase, resulting in a soluble extracellular fragment (sAPPβ) and a carboxy-terminal fragment (CTF-β or CTF99). The latter is further processed by the γ-secretase, leading to fragments of either 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acids in length. This process is critical, since it dictates the length of the final Aβ, with $A\beta_{40}$ being the most common fragment, and $A\beta_{42}$ the less common but most neurotoxic form (reviewed in [6]). Thus, Aβ formation requires sequential cleavage of APP by the β- and γ-secretase. The hydrophobic nature of Aβ enables clustering and self-aggregation, eventually leading to the deposition as amyloid plaques. In familial AD (FAD), which accounts for only 1% of all AD cases, autosomal dominant mutations have been found in the genes encoding for APP and components of the γ-secretase, PSEN1 and PSEN2. Overall, these mutations lead to increased Aβ production and shift the γ-secretase cleavage to an increase in $A\beta_{42}$ production [7, 8].

Recent evidence suggests that the small weight Aβ oligomers and protofibrils correlate best with the observed neurotoxic effects in AD [9–11]. These aggregates of Aβ were shown to interfere with receptors present in the synaptic cleft, leading to disruptions in signal transduction. On presynaptic neurons, spherical Aβ oligomers were found to impair the function of the neuron-specific Na^+/K^+-ATPaseα3 subunit causing calcium dyshomeostasis, ultimately resulting in neuronal death [12].

The main focus of research however leads towards the N-methyl-D-aspartate receptors (NMDARs). NMDAR signaling is involved in the regulation of neuronal plasticity, promotes cell survival, and, under certain circumstances, leads to cell death (reviewed in [13]). NMDARs assemble as heterotetramers of two obligatory NR1 subunits in combination with two additional NR2 (NR2A–NR2D) and/or NR3 (NR3A–NR3B) subunits. The vast majority of central NMDARs, however, assemble as diheteromers of NR1/NR2A or NR1/NR2B (reviewed in [13]). Historically, NR2A-type NMDRs are mainly linked to downstream signaling pathways promoting cell survival, whereas NR2B containing NMDRs are associated with cell death signaling [14]. Aβ was shown to mediate NMDAR internalization after binding the synaptic α7-nicotinergic receptor and therefore negatively regulating active NMDAR sites on the post-synapse [15, 16]. Furthermore, regulation of active NMDAR expression levels on the postsynaptic cell membrane is controlled by phosphorylation of the NMDAR subunit NR2B via the Scr kinase Fyn. Binding of oligomeric Aβ to the cellular prion protein (PrP^C) was shown to activate Fyn, which then phosphorylates NR2B [17]. This in turn stabilizes the interaction of the NR2B subunit with the synaptic scaffolding protein postsynaptic density protein 95 (PSD95) [18], resulting in an initial increase of active NMDAR sites on the postsynaptic membrane followed by the internalization of NMDARs from the synapse [19]. Binding of PSD95 to the NMDAR NR2B subunit links the NMDAR activity to the production of nitric oxide (NO), a signaling molecule that mediates NMDAR-dependent exocitotoxicity [20, 21]. In addition, internalization of active NMDARs from the postsynaptic membrane results in an overall decreased calcium influx into the dendritic spines, leading to spine shrinkage and eventually to the loss of the synaptic site [22].

1.3 Tau Phosphorylation and Neurofibrillary Tangles

Neurofibrillary tangles (NFTs) constituted by the tau protein are the second hallmark of AD. The *MAPT* (*microtubule-associated protein tau*) gene encoding tau contains 15 exons, with the major tau protein isoform being encoded by 11 exons [23]. By alternative mRNA splicing of exons 2, 3, and 10, six major tau isoforms are produced in the adult human brain. They differ by the presence or absence of one or two short inserts in the amino-terminal half (0N, 1N, or 2N, respectively), and have either three or four microtubule-binding repeat motifs in the carboxy-terminal half (3R or 4R) (Fig. 1.1).

3R and 4R tau isoforms are expressed at a 1:1 ratio in the mature human brain, though during embryonic brain development only 3R tau isoforms are present (reviewed in [24]). While mice also express only 3R tau isoforms during brain development, in contrast to humans, mature mice express only four-repeat tau isoforms (4R0N, 4R1N, or 4R2N) in the brain. Tau is enriched in neurons, but it is also expressed in other cell types such as oligodendrocytes [25]. Under physiological conditions, tau interacts with many different proteins, and has been implicated in cell signaling, neuronal development, and cell survival [24, 26]. Cellular localization of tau is tightly regulated, with the majority of tau protein located in the axon, where it is known to interact with microtubules and regulates axonal transport. However, tau is also present albeit at low levels in dendrites, where it regulates scaffolding of proteins like the kinase Fyn [27]. This kinase, in turn, phosphorylates the NMDARs as mentioned earlier, thereby mediating its interactions with the postsynaptic density protein 95 (PSD95) [28].

Due to the high numbers of serine and threonine residues, tau is a phosphoprotein and thus targeted by various kinases, the best characterized being the glycogen synthase kinase 3β (GSK3β) and the cyclin-dependent kinase (cdk5). The phosphorylation state of tau is furthermore tightly regulated by phosphatases like the protein phosphatase 2A (PP2A) [29]. Under pathological conditions, tau becomes "hyperphosphorylated," which means that tau is phosphorylated to a higher degree

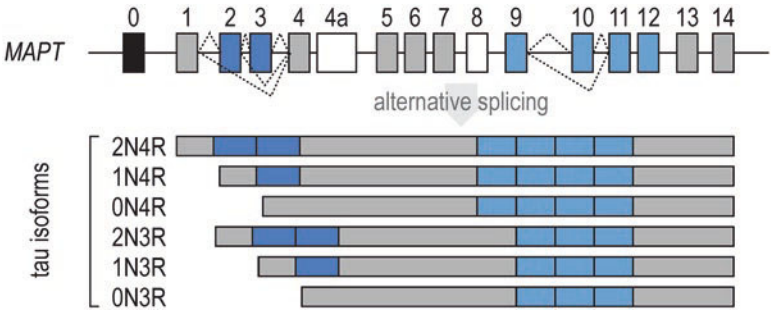

Fig. 1.1 Alternative splicing of tau. The *MAPT* (*microtubule-associated protein tau*) gene encoding tau contains 15 exons (0–14). Alternative mRNA splicing of exons 2, 3, and 10 produces six major tau isoforms in the adult human brain (top). They differ by the presence or absence of one or two short inserts in the amino-terminal half (0N, 1N, or 2N, respectively), and have either three or four microtubule-binding repeat motifs in the carboxy-terminal half (3R or 4R, bottom)

at physiological sites and at de novo—"pathological" sites. This hyperphosphorylation results in tau mislocalization [30] as well as the dissociation of tau from the microtubules and hence compromises microtubule dynamics and axonal transport [31]. Pathological phosphorylation of tau increases the capability of tau to form higher molecular aggregates, which eventually lead to the formation of NFTs. However, there is a growing body of evidence suggesting that the neurotoxic effects of tau are exerted by small soluble aggregates of tau rather than the insoluble tangles [32, 33].

It should be mentioned that no mutations in the gene encoding tau, *MAPT*, have been identified in AD patients. However, NFTs are also abundant in the absence of Aβ plaques in a number of different neurological disorders that are closely related to AD, where mutations have been linked to the *MAPT* gene. Several of these mutations have been expressed in transgenic mice and were investigated to model histopathological characteristics of AD [34, 35].

1.4 Risk Factors of AD

As mentioned earlier, genetic mutations causing early-onset FAD account for only 1% of the total number of AD. The greatest known risk factor, other than genetic mutations, for AD is advancing age. However, in the complex etiology of AD, life style choices, environmental as well as various genetic factors seem to play a crucial role [36]. In line with that, insulin resistance and decreased glucose metabolism might be a risk factor for sporadic AD (SAD) [37, 38]. Of the dozen genes identified until today, only the apolipoprotein E (ApoE) gene has been confirmed as a risk gene, which may be a factor in 20–25% of SAD [39, 40]. The human ApoE protein is a lipoprotein and exists as three major isoforms (ApoE2, ApoE3, and ApoE4). Genetic analysis identified ApoE4 as the major risk factor for AD [41], and neuropathological examination suggests that allele dosage is associated with increased Aβ load, Aβ oligomers, and plaque accumulation in the brain [42–44].

1.5 Mouse Models for AD

The histopathological hallmarks are indistinguishable when FAD is compared with SAD, and the identification of pathogenic mutations in the *APP*, *PSEN1,* and *PSEN2* genes leading to the accumulation of Aβ in patients with early-onset FAD resulted in the formulation of the "Amyloid Cascade Hypothesis." According to this hypothesis, the accumulation of Aβ leads to a pathogenic cascade, eventually resulting in tau pathology, memory deficits, and neuronal loss. Focusing on Aβ and tau pathology, multiple transgenic animal models have been generated, aiming to recapitulate important aspects of the human disease [45]. Since then, transgenic mouse models have become instrumental in understanding AD pathology and are now the major in vivo tool for AD research. Their contribution to the understanding of AD pathology will be reviewed, focusing on fundamental studies and high lighting recent insights into the disease.

The first APP transgenic mouse, that reproduced AD-like pathology, was introduced in 1995 and expressed high levels of the human mutant V717F-form of APP, under control of the platelet-derived growth factor (PDGF) mini-promoter [46]. These PDAPP mice presented with extensive depositions of extracellular amyloid plaques and neuritic dystrophies. Aβ depositions were formed initially in the hippocampus, followed by plaque formations in cortical areas. Behaviorally, PDAPP mice showed age-dependent memory impairments when tested in the Morris Water Maze paradigm, which correlated with Aβ aggregation [47]. A plethora of APP mutant mouse strains have since been generated, with the most popular examples being the J20 (hAPPswe/V717F), APP23 (hAPPswe), and the Tg2576 (hAPPswe) strains [48–50]. Altering γ-secretase activity by expression of the M146L PSEN1 mutation in an APP transgenic background harboring the Swedish (KM670/671NL) mutation resulted in increased $A\beta_{42}$ production and deposition as well as neuronal loss, which was reported even before Aβ plaque formation was observed [51, 52]. Although most of these transgenic mice fail to model all aspects of AD pathology, with most of them lacking neuronal loss or tau pathology, these and other strains recapitulate Aβ plaque formation and memory impairments and have become the most commonly used tools to study AD-related pathological mechanisms in vivo [45].

As mentioned earlier, in AD patients no mutations have been identified in the gene encoding tau, *MAPT*. However, NFTs are also found in a heterogeneous group of neurodegenerative disorders described as frontotemporal dementia (FTD), where mutations have been identified in the *MAPT* gene. Several of these mutations have been expressed in transgenic mice and they have significantly contributed to our current understanding of the pathophysiology of tau, not only FTD but also in AD. The expression of human mutant P301L tau reproduced aggregation and NFT formation in mice for the first time successfully [53, 54] and recent models have built on their success.

In line with this insight, the link between Aβ toxicity and tau pathology was established when the APP transgenic Tg2576 mouse line was crossed with the JNPL3 (P301L) tau transgenic mice [55]. The double transgenic animals exhibited neurofibrillary tangle pathology that was substantially enhanced in the limbic system and olfactory cortex compared to the parental JNPL3 strain, whereas Aβ deposition was not altered in the progenies. Similarly, crossing the APP23 and JNPL3 increased tau phosphorylation and aggravated preexisting NFT pathology [56]. Interestingly, intracerebral injection of brain extracts from aged APP mutant mice (APP23) as well as synthetic $A\beta_{42}$ fibrils into tau transgenic mice (JNPL3 or into pR5 strain) accelerated tau phosphorylation and NFT formation [56, 57], suggesting that it is Aβ itself rather than a cleavage product of APP that promotes tau pathology. Furthermore, intracerebral injections of AD patient and APP23 transgenic brain extracts were employed to answer the question if seeding Aβ can induce amyloidosis. Indeed, these injections caused subsequent Aβ deposits in APP23 transgenic mice [58]. Injections of APP23 and APP/PSEN1 into APP23 and APP/PSEN1 mice resulted in four different types of pathology, suggesting that exogenously induced amyloidosis depends on both the host and the source of the agent, bearing similarities to prion disease [59].

Tau pathology follows a distinct pattern and hence cell to cell transmission is one hypothesis of central tau spreading [60, 61]. Trans-synaptic spreading from the entorhinal cortex to the hippocampus has been suggested, with tau being secreted into the extracellular space from neurons independent of cell death [62, 63]. The cellular mechanism regulating tau release is not well understood, but a recent study showed that neuronal activity triggers tau release into the extracellular space and hence linked trans-synaptic spread of tau pathology with synaptic activity itself [64]. In vivo data also suggested that tau shows similarity to a prion-like spreading mechanism. For example, injection of brain extracts from mutant P301S tau-expressing mice into the brain of transgenic wild-type tau-expressing mice, which normally do not show filamentous tau aggregates, induced assembly of human tau into filaments [65] and spreading of pathology was shown to be determined by connectivity in contrast to proximity [66].

The hypothesis that Aβ and tau do not act in isolation but show rather synergistic roles in AD is further promoted by findings that a reduction of tau levels in APP mutants ameliorates Aβ-induced deficits without changing Aß depositions [67]. Tau is critical in mediating the interaction of Fyn and the NMDAR subunit NR2B, which eventually recruits PSD95 into a multi-protein complex that regulates excitotoxicity as already mentioned above. Elevated levels of synaptic Aβ were shown to cause an over-activation of NMDARs resulting in downstream toxicity. In a situation, where both phosphorylated tau and Aβ are elevated, more Fyn is recruited into the dendritic spines causing an augmenting toxic effect of Aß [27].

Moreover, when Fyn is excluded from the spines, either by deleting the tau domain responsible for Fyn localization, by deleting tau completely [27], or by depleting Fyn itself [68], neuronal excitotoxicity is abolished, strongly suggesting a toxic Fyn-tau-amyloid triad [69]. Additionally, Aβ-mediated calcium elevation via NMDARs has been demonstrated to increase tau phosphorylation via the AMPK and PAR-1/MARK pathway on an epitope that has been associated with a late disease phase [70, 71].

The first triple AD mouse model (3xTg-AD) combined the M146V PSEN1 with the Swedish APP mutation and co-expressed mutant tau (P301L) [72, 73]. This model presented with amyloid plaques as well as NFT formation at the age of 2–4 months, and exhibited behavioral and neuronal symptoms of AD including synaptic dysfunction and LTP deficits [72–74]. Another triple transgenic mouse model was established, co-expressing mutant tau (P301L), PSEN2 (N141I) and the Swedish APP mutation. These $^{\text{triple}}$AD mice develop tau and amyloid deposits in an age-dependent manner, starting with tau accumulation at an age of 4 months [70, 75]. The observed impairment in spatial learning and memory appears to be independent of brain pathology. Crossing the double mutant AD model expressing the human tau P301L mutation in combination with the Swedish and London (V717I) APP mutation with a human PSEN1 (A246E) transgenic mouse resulted in the PLB1-triple strain [76]. These animals show age-related neuropathology including intraneural and oligomeric Aβ accumulation as well as hyperphosphorylated tau at the age of 6 months. Amyloid plaques are low in number at an age of 21 months and these animals show no overt formation of NFT pathology. Furthermore these animals reveal cognitive deficits as well as impaired hippocampal plasticity [76].

In order to overcome inherent caveats that naturally arise with the overexpression of human mutated forms of any protein in rodent models, more recent studies have manipulated the endogenous mouse APP or PSEN1 genes to create humanized mouse models [77, 78]. Employing a knock-in approach, either the Swedish mutation alone or in combination with the Beyreuther/Iberian (I716F) mutations ($App^{NL/NL}$ and $App^{NL\text{-}F/NL\text{-}F}$, respectively) was introduced into the mouse APP locus [78]. These models show endogenous levels of APP while robustly overproducing $A\beta_{42}$. $App^{NL\text{-}F/NL\text{-}F}$ mice produced more $A\beta_{42}$ levels than the $App^{NL/NL}$ strain and also showed a higher $A\beta_{42}/A\beta_{40}$ ratio. These changes were accompanied by a more pronounced and age-dependent Aβ pathology, signs of neuroinflammation, synaptic loss, as well as memory impairments. Interestingly, no memory deficits were observed in the $App^{NL/NL}$ mutants, suggesting that the underlying mechanisms are independent of C-terminal fragment β (CTF-β) levels, a hypothesis which was based on the overexpression of mutant human APP forms in former studies [79]. A third mouse line harboring the Arctic (APP E693G) mutation in addition to the Swedish and Beyreuther/Iberian mutations ($App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$) showed a more pronounced Aβ pathology as well as behavioral alterations [78]. Moreover, the $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ strain presented with additional, subcortical amyloidosis, consistent with the histopathology of patients carrying this mutation. Interestingly, transgenic mice overexpressing human APP, harboring the Arctic mutation, have failed to recapitulate this phenotype so far [80]. Another approach to model AD pathophysiology in mice without the overexpression of mutant transgenes was described recently by crossing the humanized APP and PSEN1 FAD knock-in mice with mice expressing human tau (htau), on a mouse MAPT null background (APP/PSEN1/htau) [77]. These mice show a mild and age-dependent plaque formation as well as tau hyperphosphorylation. Behaviorally, these mutant mice revealed reduced motility at old ages and exaggerated fear responses, which was due to a synergistic interaction between Aβ and phosphorylated tau. Hence, these new mouse models provide valid tools to distinguish facts from artifacts in the phenotypes of commonly used AD models and will be instrumental in validating pathway analysis that may link Aβ amyloidosis to tauopathy and genetic risk factors beyond ApoE.

ApoE4 may contribute to AD through at least two distinctive pathways, one of which is amyloid-dependent [81]. Crossing ApoE null mutants with the PDAPP mice strongly attenuated Aβ levels and plaque loads in the brain [82], whereas viral mediated overexpression of ApoE4 augmented Aβ depositions [83]. Detailed analysis of various Aβ parameters in aging APPV717F transgenic mice expressing either mouse apoE, no apoE or human ApoE2, ApoE3, or ApoE4 demonstrated that ApoE facilitates Aß fibril formation [84]. Furthermore, it has been suggested that the ApoE4 genotype could lead to an earlier impairment of brain insulin signaling, possibly contributing to an earlier onset of AD [85]. Another pathway of ApoE4 mechanisms in AD does not involve amyloid. If neurons are stressed, they overproduce ApoE as part of their repair mechanism. The ApoE4 allele, however, gave rise to toxic products when it was broken down in the organism, which were linked to mitochondrial stress and damage, eventually leading to cell death [80, 86–88]. Increased oxidative stress due to mitochondrial dysfunction has been widely recognized as a contributing factor of AD pathology [89]. Reactive oxidative species

(ROS) are an unavoidable physiological byproduct, which can cause damage to the biological system when present in excess amounts. Neurons overexpressing FAD-causing APP demonstrate mitochondrial fragmentation and structural damage [90, 91]. APP transgenic animals harboring the Swedish and London mutation show early energy dysfunction as demonstrated by a decreased mitochondrial membrane potential, adenosine triphosphate (ATP) level and complex IV activity [92]. Interestingly, these mitochondrial deficits are observed in the presence of elevated Aβ levels, prior to plaque formation [92, 93]. APP/PSEN1 mice, which present with plaque formation at 3 months, present even stronger reductions in mitochondrial membrane potentials and ATP levels compared to age-matched APP mutants. Consequently, Aβ-induced mitochondrial dysfunction starts very early in the progress of pathology and accelerates with increased age and Aβ load [94]. Mitochondrial fragmentation and structural damage was also shown in the brains of the Tg2576 and APP/PS1 transgenic mouse strains [95]. In line with this evidence, crossing the APP mutant J20 strain with a mouse line lacking the mitochondrial enzyme Aβ-binding alcohol dehydrogenase (ABAD) has been shown to increase the generation of ROS as well as causing spatial learning memory deficits [96].

Interestingly, stereotaxic injection of human tau oligomers into wild-type mice caused mitochondrial dysfunction by interfering with the electron transport chain complex I and activated the apoptotic mitochondrial pathway [97]. Although Aβ and tau pathology are the main hallmarks of AD pathology, it still remains to be fully elucidated how they relate to each other. Hence, a close relationship between mitochondrial failure, Aβ, and tau has also been suggested recently. Further analysis of a tau transgenic mouse line (pR5), expressing the P301L mutation, revealed mitochondrial dysfunction including deregulation of mitochondrial respiratory chain complex components as well as antioxidant enzymes [98]. Furthermore, mitochondria of tau pR5 mice show increased vulnerability towards Aβ in vitro [94, 98]. Using quantitative proteomics and functional assays, analysis of the ^{triple}AD model revealed that Aβ and tau act synergistically in amplifying mitochondrial respiratory deficits. Remarkably, deregulation of complex I was related to tau, whereas deregulation of complex IV was Aβ-dependent [75]. These data complement findings in the 3xTg-AD mouse model that showed decreased activation of regulatory enzymes of the mitochondrial complex as well as increased oxidative stress and lipid peroxidation [99]. Disturbed calcium homeostasis was also reported in AD with regard to mitochondrial dysfunction [100]. Consistent with this evidence, aberrations in calcium transients were found in APP as well as tau mutants [101]. Consequently, besides the treatment and/or removal of both Aβ and tau pathology, strategies to protect cells at the mitochondrial level by stabilizing or restoring mitochondrial function or by interfering with the energy metabolism appear to be promising in treating or preventing AD. In line with that, Methylene Blue (MB) has recently attracted some attention as antioxidant and mitochondrial protective effects have been described previously [102]. MB was shown to influence tau aggregation [103, 104] and ameliorate Aβ aggregation and deposition, possibly due to remodeling toxic soluble Aβ oligomers [105]. A recent report showed that MB is able to enhance cell viability by reducing ROS levels [106]. Other antioxidants such as Ginkgo