Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) represents the most prevalent form of senile dementias. This disease is characterized by the occurrence of extracellular plaques,

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intracellular neurofibrillary tangles, a loss of neurons and synapses, hippocampus and cerebral atrophy, and memory loss. Extracellular plaques consist mainly of beta-amyloid peptides (Aβ), which are generated through proteolytical cleavage of the larger amyloid precursor protein (APP). Mutations in the APP gene as well as in the two presenilin genes (PSEN1/PSEN2) are responsible for the majority of familial AD (FAD) cases. All these mutations lead to an enhanced Abeta deposition in which mostly the generation of the longer Aβ variant containing 42 amino acids ($A\beta_{42}$) is favored. In the current chapter, we will discuss the clinical features of AD, mild cognitive impairment being a transitional stage between the cognitive changes during normal aging and AD, as well as background in AD genetics, pathology, transgenic animal models, current and future therapy options, and risk factors as diabetes and hypertension.

Keywords
Alzheimer's disease • Mild cognitive impairment • Genetics • Pathology • Transgenic animal models • Therapy • Risk factors • Diabetes • Hypertension • Physical activity • Immunotherapy • Amyloid

Brief History

When Alois Alzheimer presented the case of his patient Auguste D. at the Tübingen meeting of the Southwest German Psychiatrists in 1906, he did not attract much attention from his colleagues. The young medical doctor likely would not have believed that, 100 years later, the disease which now holds his name would be the most common cause of dementia and a source of a critical medical and economical problem.

Long before Alois Alzheimer, ancient scholars recognized that a loss of mental function often accompanied aging. The ancient Egyptians were aware that a major memory disorder afflicted the elderly. However, literature gives credit to the ancient Greeks for developing the term dementia derived from the prefix *de* (not) and *mens* (mind). The Roman physician Galen (130–201 a.d.) thought that age-associated mental disorders resulted from primary and secondary cerebral impairments. In the European middle ages, the topic of senile dementia received less attention, perhaps due in part to deadly epidemics and religious beliefs about diseases as punishments for sins.

Until the nineteenth century, there was no major progress in the understanding of the concept of dementia. Gradually, the broad topic of dementia was behaviorally categorized. In the seventeenth century, the anatomical appearance of the brain was inspected as the source of dementia as the dissection of the human brain became tolerated in Europe. However, no remarkable correlations between dementia and brain atrophy was recognized (reviewed in Boller, Forbes; 1998).

Alois Alzheimer was born in a small Bavarian village near Würzburg in 1864 and studied medicine in Berlin, Tübingen and Würzburg. When he was working at the

Municipal Asylum for the Mentally Ill and Epileptics in Frankfurt, he met the 51-year-old Auguste Deter. In 1901, she was admitted to the asylum and was examined by Dr. Alzheimer himself. She showed some remarkable symptoms such as memory impairment, hallucinations, aphasia, and paranoia. Auguste D. died in April of 1906 from septicemia (reviewed in Maurer et al., 1997). By that time, Alzheimer was working in Munich, but he asked for her records and brain to study it in his neuropathology laboratory. At the Tübingen meeting of the Southwest German Psychiatrists, Alzheimer presented Auguste D.'s symptoms and reported the histopathological features that are now associated with Alzheimer's disease: neuron loss, extracellular amyloid plaques, and intracellular neurofibrillary tangles. However, he did not claim that he had discovered a new disease. The term "Alzheimer's disease" appeared several years later in the 8th edition of the Handbook of Psychiatry by Emil Kraepelin to describe a progressive young onset dementia distinct from the senile dementia. Interestingly, Alzheimer's case notes were lost. About 100 years later, his records were discovered by Konrad Maurer, who published them in 1997 and included a photograph of Auguste D. and examples of Alzheimer's handwritten notes. For a long time, Alzheimer's disease was considered only a very rare disease affecting young patients and distinct from senile dementia. It took about seven decades to unify Alzheimer's disease and senile dementia under the name "dementia of the Alzheimer's type." Since then, the interest in Alzheimer's disease has increased. More money and efforts have been invested in this field, and research has been furthered by advances in the biochemical techniques, magnetic resonance imaging (MRI), positron emission tomography (PET), and genetic linkage studies.

Clinical Features

Alzheimer's disease patients show various clinical symptoms, with mild forgetfulness as the result of short-term memory loss being one of the initial problems (Blennow et al., 2006). The most common symptomatic pattern begins with gradually worsening ability to remember new information. Later on, anxiety, aggressiveness and a progressive loss of memory, judgment, reasoning, and orientation become evident. A variety of warning signs, including challenges in planning and solving problems, difficulties in performing familiar tasks at home, work or at leisure, confusion with time and place, or changes in mood or personality among others, can be usually detected. On average, AD patients live from 6 to 10 years after diagnosis, though the disease can last for as many as 20 years. It is estimated that neurodegeneration starts already 20–30 years before clinical symptoms become obvious. During this preclinical stage, there is a silent phase of plaque and tangle accumulation leading to the appearance of clinical manifestations after reaching a certain threshold level. Often this phase is designated as mild cognitive impairment (MCI).

Mild Cognitive Impairment

As the diagnosis in the early stages of AD is often difficult, the definition MCI has been established, representing a transitional stage between the cognitive changes during normal aging and AD. Individuals with MCI have mild, but measurable, changes in thinking abilities that are noticeable to the person affected and to family members and friends, but that do not affect the individual's ability to carry out everyday activities. MCI can present with a variety of symptoms and it is termed "amnestic MCI," when memory loss is the predominant symptom. This includes memory complaints, preferably corroborated by an informant, beyond that expected for their age and level of education. At the same time, general cognitive functions and activities of daily living are not affected. Most of the amnestic MCI patients will progress to AD at a rate of 10–15 % per year, which is in clear contrast with healthy control subjects who convert at a rate of 1–2 % per year; however, complete remission does also occur in the vast majority of cases (reviewed in Blennow et al., 2006).

Diagnosis of Alzheimer's Disease

To date a definite diagnosis of AD is only possible following brain autopsy. It is a clinical diagnosis based on neuropsychological evaluation, laboratory parameters, and neuroimaging. Heteroanamnesis is of high diagnostic relevance as, like in other psychiatric diseases, insight into the disease and cooperativeness of the patient is often constricted. Most commonly the diagnosis is made by the patients' primary care physician and is substantiated by neurological and psychiatric examinations serving as a verification of the estimated diagnosis and accordingly exclusion of other disorders. A variety of screening methods is currently in use to detect neuropsychological deficits. The Mini Mental State Examination (MMSE) is the most commonly used test for cognitive disorders. Within a test period of 30 min, the examiner analyzes spatial and temporal orientation, memory function, attention, calculating and speech comprehension, and command of language by asking simple questions. Laboratory investigation of blood and cerebrospinal fluid is important for the potential reversible cause of dementia (e.g., vitamin B12 deficiency, thyroid gland dysfunction). The analysis of the cerebrospinal fluid is nowadays an established component in the diagnosis of AD. Increased levels of tau protein and hyperphosphorylated tau protein in addition to reduced levels of $A\beta_{42}$ represent valid biomarkers.

Neuroimaging techniques in the routine diagnosis of AD are first and foremost used to exclude other relevant disease causes that could cause cognitive decline. Using computer tomography (CT) or magnetic resonance imaging (MRI), other potentially reversible causes of dementia-like syndromes, including brain tumors, subdural hematoma, stroke, or normal pressure hydrocephalus, can be identified and accordingly excluded. In addition, detection of hippocampal atrophy using MRI can be used to detect AD with 80–90 % accuracy. More recent methods combine

positron emission tomography (PET) with radiolabeled substances like fluorodeoxyglucose (FDG), amyloid-binding dyes like Pittsburgh compound B (PIB), or florbetaben to detect amyloid plaques in the brain during life. This type of analysis might help to establish an early diagnosis of AD in a noninvasive way and might also prove to be a useful method to monitor clinical trials.

Epidemiology and Pathology of Alzheimer's Disease

The pathological features of AD were first published by the German psychiatrist Alois Alzheimer in a case report in 1907 describing a 51-year-old female patient with "a curious disease of the cortex." AD is the most common cause of dementia in the Western world and is estimated that in populations after age 65, the likelihood of developing dementia roughly doubles every 5 years, causing an enormous economical burden. Current estimations act on the assumption of ~5.4 million people aged 65 and older to be affected by AD in the USA (Alzheimer's Association Report 2012). Women are more often affected than men (3.4 million women vs. 1.8 million men in the USA), which is mainly attributed to the fact that, on average, women live longer than men. Aging is the most obvious risk factor for the disease but also other associated with vascular diseases, including hypertension, hypercholesterolemia, atherosclerosis, or coronary heart disease, have been suggested by epidemiological studies.

Neuropathologically AD is characterized by the presence of a huge amount of extracellular plaques (Fig. 1) and neurofibrillary tangles (NFT) (Fig. 2) within cortical areas of the brain. These lesions are not specific to AD as they can be found also in the normal elderly population or other disease conditions. In AD,

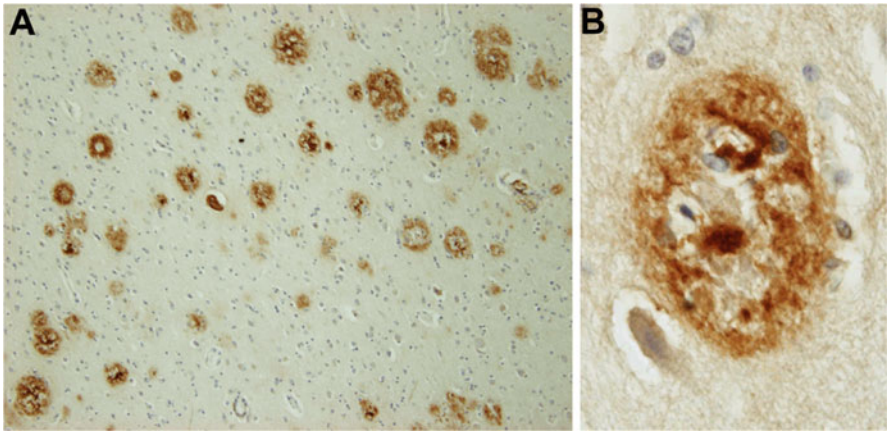

Fig. 1 Immunohistochemical staining against Abeta in plaques. (**a**) Alzheimer case with many plaques in the cortex. (**b**) High-power view of a plaque

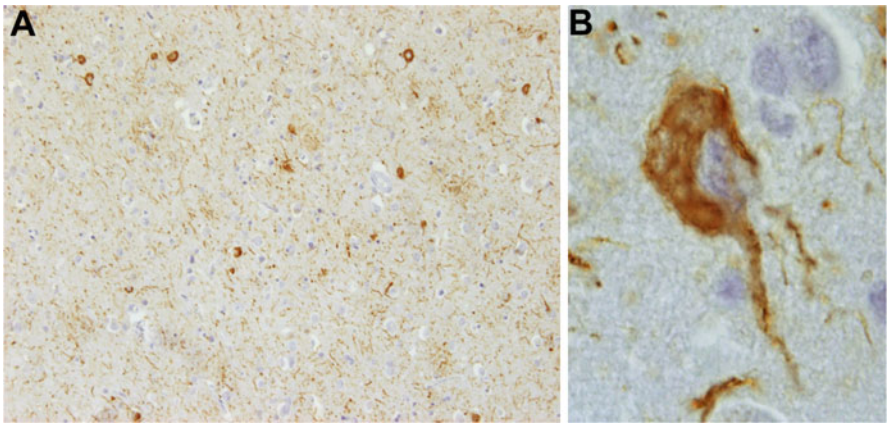

Fig. 2 Immunohistochemical staining against hyperphosphorylated Tau in tangles. (**a**) Alzheimer case with many tangles in the cortex. (**b**) High-power view of a tangle

however, they occur to a much larger extent and come along with accompanying alterations like massive neuronal cell loss and the presence of amyloid deposits within meningeal and cortical blood vessel walls (cerebral amyloid angiopathy, CAA). Whereas plaques are located within the extracellular space, neurofibrillary tangles occur within degenerating neurons. Plaques are mainly composed of a 39–43 amino acid peptide called β-amyloid or Aβ, which is derived by proteolytical cleavage from the larger amyloid precursor protein (APP). Two different forms of plaques exist which are subdivided into neuritic and diffuse plaques based on their composition. Neuritic plaques are microscopic foci of extracellular amyloid deposition and associated axonal and dendritic injury. They are surrounded by dystrophic neurites which are enlarged bulbous structures containing neurofilaments, laminated bodies, mitochondria, or dense lysosomes. In contrast, diffuse plaques are composed of mostly unformed peptides and have only minute wisps of formed filamentous amyloid. Plaque deposition is also common in individuals suffering from Down syndrome (DS) having a triplication of the APP carrying chromosome 21.

Neurofibrillary tangles (NFTs) mainly consist of the microtubule-associated protein Tau. It has been demonstrated that NFTs and neuronal cell loss occur much later in the disease course than extracellular Aβ deposition. However, a correlation between dementia and plaques is vague, whereas a close correlation between dementia and tangles and neuron loss has been established. The current point of view is that Aβ pathology induces NFT formation, whereas tangles are responsible for causing neuronal damage.

Inflammatory processes are a further prominent characteristic of the AD brain, as there is extensive activation of astrocytes and microglia cells, representing the two primary glial cell types involved in a neuroinflammatory response. In addition, activated complement fragments, as well as inflammatory cytokines are associated with plaques and tangles (reviewed in Selkoe, 2001).

Neurofibrillary Tangles and the Tau Protein

NFTs are intracellular deposits or aggregates in the perikarya of neurons and apical dendrites that consist of paired helical filaments (PHFs) (Fig. 2). They are critical lesions in AD but not specific to the disorder because they are also well-known in other neurodegenerative conditions like frontotemporal dementia and other dementias. The principal biochemical component of PHF is tau, a protein belonging to the family of microtubule-associated proteins (reviewed in Sergeant et al., 2008). Tau is abundantly expressed in the central nervous system, with at least six different isoforms being present in the brain, resulting from alternative mRNA splicing of a single gene (352–441 amino acids). One of the normal roles of tau is assembly and stabilization of microtubules, a process controlled by phosphorylation at distinct sites in the tau protein. So far, more than 25 phosphorylation sites have been identified in PHF-tau isolated from human brain material. It is suggested that hyperphosphorylation leads to a microtubule destabilization by tau dissociation from the microtubules and spontaneous formation of insoluble PHF and neurofibrillary tangles. Several protein kinases, including cyclin-dependent kinase 5, glycogen synthase kinase 3β (GSK-3β), or mitogen-activated protein kinase, have been identified to be involved, suggesting that multiple phosphorylation cascades participate.

Tangles are not pathognomonic for AD and have been detected in a variety of other neurodegenerative diseases, including frontotemporal lobar degeneration or supranuclear palsy. Although it is not yet completely clear if tangle formation is a cause or consequence of AD, there is accumulating evidence that tangles are formed as an effect of Aβ accumulation and plaque deposition. The occurrence and localization of NFTs seems to correlate better with the observed cognitive deficits as plaque deposition and is therefore also used to classify the disease state in AD.

Genetics

AD is a heterogeneous disorder with both familial and sporadic forms which differ in the age of onset, as well as in duration and course of the disease. In the very rare familial forms, symptoms occur as a general rule before the age of 60–65 years. They represent only a fraction of the total number of disease cases, whereas the sporadic forms occurring at later ages represent the predominant part. Except for the generally much earlier age of onset, there is no major clinical or neuropathological difference between familial and the most abundant sporadic form. Almost 20 years ago, it was demonstrated that a familial, autosomal dominant variant in a Swedish family is caused by a mutation in the *APP* gene on chromosome 21 (Fig. 3). The occurrence of abundant amyloid pathology in Down syndrome patients can be explained by a gene-dose effect, as DS patients carry an extra copy of chromosome 21.

Further genetic studies led to the identification of more than 25 APP mutations, as well as to the identification of numerous mutations in the presenilin 1 gene (*PSEN1*)

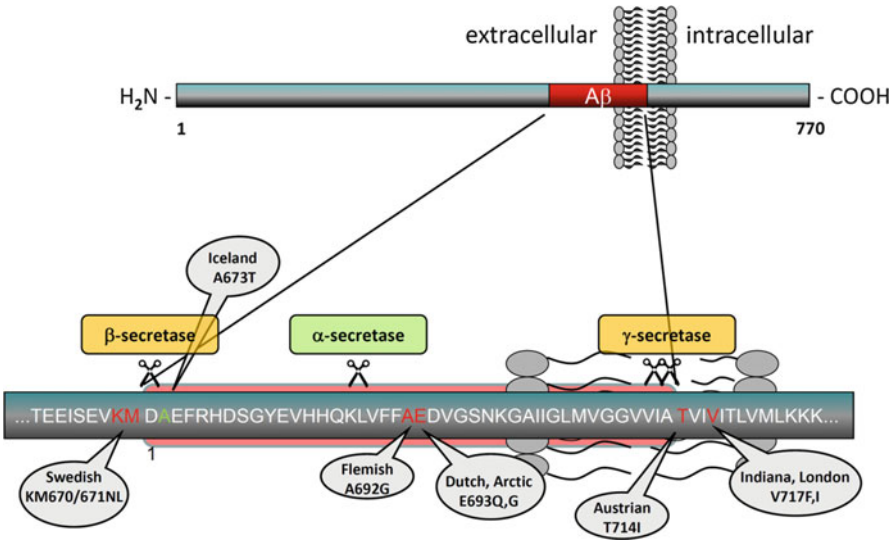

Fig. 3 Drawing of the amyloid precursor protein (*APP*). APP is a type I transmembrane protein. Aβ is released by the activity of β- and γ-secretases, whereas α-secretase inhibits Aβ formation by cleaving within the Aβ sequence. Also shown are the positions of amino acid changes caused by mutations in the APP gene

(>150) on chromosome 14 and the presenilin 2 (*PSEN2*) gene on chromosome 1 (http://www.alzforum.org). These mutations are responsible for the majority of FAD cases and generally cause an early disease onset (in part at the age of 30). Usually these mutations lead to an altered APP processing and an increased amount of Aβ peptides (reviewed in Rademakers, Rovelet-Lecrux; 2009). A common feature of all mutations seems to be an increase in the formation of the toxic $A\beta_{42}$ variant relative to other Aβ species. For example, the Swedish APP mutation (K670N, M671L) is located outside of the transmembrane domain in which β-secretase cleavage generates the N-terminal end of Aβ peptides, leading to increased levels of both $A\beta_{40}$ and $A\beta_{42}$. Interestingly, an APP mutation (A673T) recently identified in an Icelandic population protects against Alzheimer's disease and cognitive decline in the elderly. This substitution is adjacent to the β-cleavage site in APP and results in an approximately 40 % reduction in the formation of amyloidogenic peptides in in vitro assays.

Whereas mutations in *APP* and the *PSEN* genes exclusively affect the familial early onset forms of AD, the situation is much less clear in the majority of the sporadic cases. An association with the epsilon 4 allele of the apolipoprotein E (*ApoE*) gene was identified which is considered to be the so far only assured and consistently replicated genetic risk factor in sporadic AD. ApoE is a major apolipoprotein involved in regulating cholesterol uptake and release. It is produced mainly by astrocytes and acts a major lipid carrier in the cerebrospinal fluid.

APP Processing and Production of Aβ

The toxic agents in AD are considered to be the Aβ peptides. They are derived from the larger amyloid precursor protein by successive proteolytical cleavage events (reviewed in De Strooper et al., 2010). APP is a large type I transmembrane protein with a large extracellular domain and a small cytoplasmic tail (Fig. 3). The processing occurs in two different ways: (1) Non-amyloidogenic processing: In the non-amyloidogenic pathway, the generation of toxic Aβ peptides is precluded by a sequential cleavage of α- and γ-secretase (Fig. 4). Cleavage at the alpha-site occurs within the Aβ region and leads to the generation of a membrane-bound fragment (C83) and the release of soluble sAPPα which has neuroprotective properties. It is carried out by members of the family of disintegrin-metalloproteases, namely, ADAM10 and TACE (TNF-α converting enzyme). (2) Amyloidogenic processing: In the pathologically relevant amyloidogenic processing pathway, APP is initially cleaved by β-secretase, leading to the formation of a 99 amino acid-long membrane-bound fragment (C99). This cleavage is predominantly carried out by the enzyme BACE1 ("beta-site APP cleaving enzyme 1"), a member of the family of aspartyl proteases. Both C83 and C99 can be subsequently cleaved by the γ-secretase complex, leading to the formation of Aβ peptides from C99 and p3, a peptide of so far unknown function, from C83. The exact cleavage site is somewhat variable,

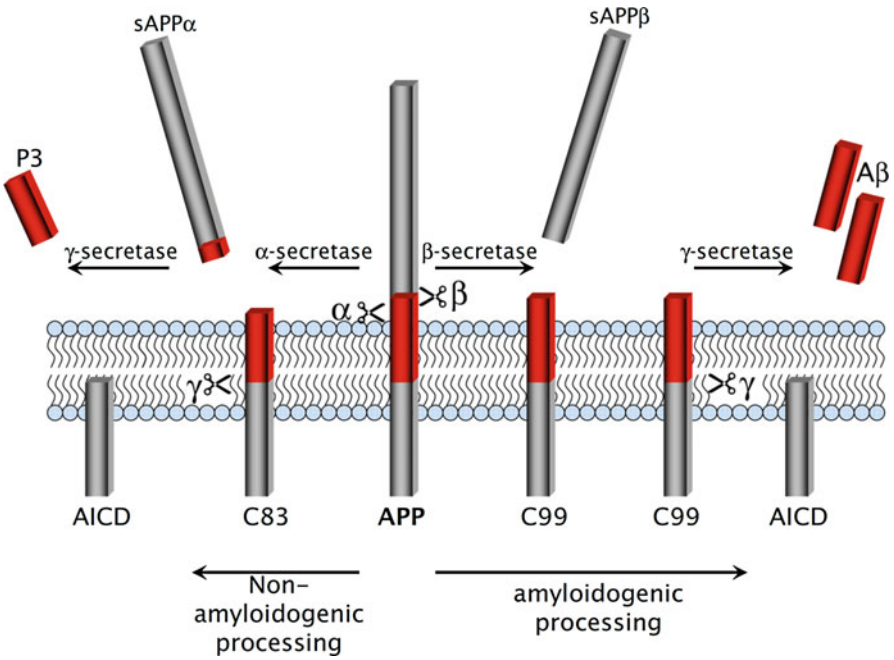

Fig. 4 Closer view on the sequence of cleavages of APP by the different secretases

resulting in Aβ peptides of varying length from mainly 38–43 amino acid residues, with $A\beta_{40}$ being the most abundant species in the healthy brain (>90 %).

This γ-secretase complex consists of different components, namely, presenilin (PSEN), nicastrin, APH-1 ("anterior pharynx defective 1"), and PEN-2 ("presenilin enhancer 2"). This cleavage is unusual as it occurs in a hydrophobic environment within the plasma membrane (Fig. 4). It was shown that $A\beta_{40}$ is the major component of cerebral vascular amyloid deposits, while $A\beta_{42}$ is the main component of neuritic plaques (reviewed in Selkoe, 2001). In general, $A\beta_{42}$ species are more hydrophobic and have a higher tendency to aggregate than $A\beta_{40}$. Furthermore, $A\beta_{42}$ is more neurotoxic and crucial for plaque formation. More recently, amino- and carboxy-terminally truncated Aβ peptides are gaining interest, as they differ in their physicochemical properties. The loss of hydrophilic amino acids renders amino-terminally truncated Aβ peptides to be more hydrophobic, leading to a decreased solubility, faster aggregation kinetics and increased toxicity, as shown by in vitro experiments. Instead of aspartate that is normally found at the first position of Aβ, these Aβ forms possess different N-termini. A variety of N-terminal-truncated Aβ variants have been reported in human AD brains with $A\beta_{pE3–42}$ (starting with a pyroglutamate residue at position 3) or $A\beta_{4–42}$ (starting with phenylalanine at position 4) being the most abundant. Several proteases have been proposed to be involved in the generation of N-truncated Aβ species, including aminopeptidase A, meprin-β, or neprilysin; however, the exact enzymatic activities are in most cases not fully identified (reviewed Bayer, Wirths; 2014).

The Amyloid Cascade Hypothesis

The amyloid cascade hypothesis of AD acts on the assumption of a central role of the beta-amyloid peptide in the pathological cascade which is followed by further neuropathological alterations like neurofibrillary tangle formation. Whereas mutations in APP and PSEN genes leading to increased Aβ production are considered to be the point of origin in familial forms of AD, for the most abundant sporadic form only risk factors including aging, hypertension, obesity, or severe head trauma are known. It is further assumed that in contrast to FAD not increased production but deficient Aβ clearance is the major mechanism leading to enhanced overall Aβ levels. The hypothesis claims that an accumulation of hydrophobic of $A\beta_{40}$ and $A\beta_{42}$ and the formation of insoluble extracellular plaques trigger a cascade of mischievous changes eventually resulting in synapse loss, neuron loss, brain atrophy, and dementia (reviewed in Hardy, Selkoe; 2002). Several observations support this theory: (1) A major strength of this hypothesis is its consistency with the findings obtained from AD genetics, as all autosomal dominant mutations in *APP* and the presenilin genes shift APP processing to the amyloidogenic pathway, thereby producing more $A\beta_{42}$. In addition, mutations in the *Tau* gene lead to the development of tangle but not amyloid pathology, which suggests that amyloid pathology is upstream of tangle pathology. (2) Data from double-transgenic mouse models harboring both amyloid and tau pathology also points into this direction, as tau

pathology is mostly exacerbated while amyloid pathology remains rather unchanged. (3) The apolipoprotein E ε4 allele (*APOE4*), the most important risk factor for late-onset AD (LOAD), is associated with increased Aβ deposition and reduced Aβ clearance. On the other hand, a conclusive link between amyloid and tangle formation in terms of a biochemical pathway has not been established, and there are also arguments challenging the validity of the hypothesis. (1) The localization and amount of amyloid plaque pathology in the brain does not correlate well with the severity of dementia. (2) Non-demented individuals often harbor robust plaque pathology but show no signs of dementia. (3) Many of the currently available AD mouse models harbor abundant amyloid plaque pathology but do not show appropriate memory deficits or neuron loss.

There is growing evidence that intraneuronal Aβ might represent a key contributor in AD pathology (reviewed in Bayer, Wirths, 2010). The first observations of intracellular Aβ in neurons were made nearly 30 years ago, and it was shown in subsequent studies that intraneuronal Aβ accumulates in AD vulnerable brain regions including pyramidal neurons of the hippocampus and entorhinal cortex. Data from transgenic AD mouse models reinforced the importance of intraneuronal Aβ as in most models that harbor a considerable neuron loss a correlation with intraneuronal Aβ accumulations could be established (Fig. 5).

Risk Factors

Apolipoprotein E

Although the specific gene mutations can account for the majority of FAD cases, the genetic risk factors for sporadic AD are less well defined. To date, the strongest genetic risk factor for sporadic AD is the epsilon (ε) 4 allele of the apolipoprotein E (*APOE4*) gene (reviewed in Selkoe, 2001). ApoE normally functions as a major carrier of lipoproteins within the CNS. It is produced mainly by astrocytes, and it delivers essential lipoproteins to neurons via receptor-mediated endocytosis. There are three alleles of the gene (E2, E3, E4) which encode for three separate ApoE isoforms and differ by only a single amino acid. In the Caucasian population, the *APOE3* allele is the most common one, while carriers of a single copy of *APOE4* have a three- to fourfold greater chance of developing AD relative to noncarriers. The risk for AD is increased up to 15-fold for individual homozygous for the E4 allele. In AD patients, the *APOE4* genotype is associated with the formation of more neuritic plaques and neurofibrillary tangles and greater brain atrophy. On the contrary, the *APOE2* allele has a protective effect, evidenced by a decreased allele frequency in AD cases.

While the precise contribution of ApoE4 to the development and progression of AD currently remains unclear, recent research suggests that ApoE4 can influence AD pathology in both Aβ-dependent and Aβ-independent ways. In addition to its role as a lipoprotein carrier, ApoE affects Aβ dynamics within the CNS. It has been shown that ApoE4 binds Aβ less efficiently than ApoE3 or ApoE2. Those Aβ

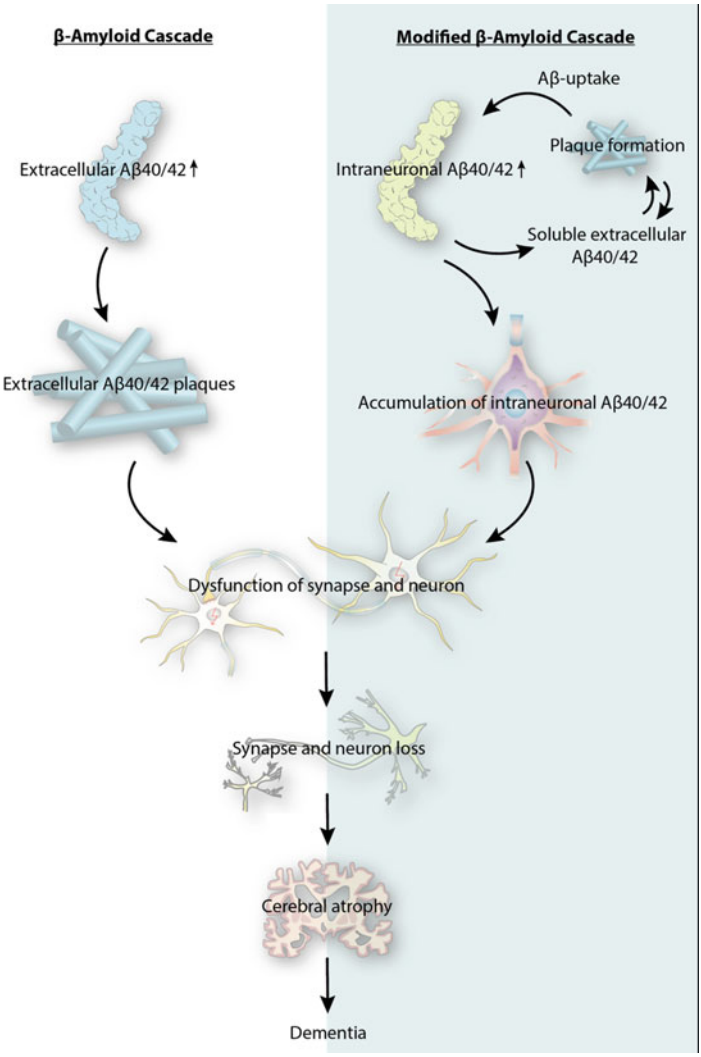

Fig. 5 Scheme of the β-amyloid cascade hypothesis

particles that do form complexes with ApoE4 are cleared more slowly across the blood–brain barrier relative to Aβ bound to the other ApoE isoforms. Studies employing ApoE replacement mice, in which murine ApoE has been replaced by the respective human isoforms corroborated a substantial effect on the clearance of Aβ, with ApoE4 being much less efficient. Certain studies also suggest that ApoE promotes neuronal uptake of soluble Aβ and facilitates its degradation. However, it remains to be clarified whether Aβ endocytosis occurs in an ApoE isoform-specific fashion. ApoE4 can also enhance the toxicity of Aβ oligomers and increase Aβ aggregation to a greater extent than other ApoE types.

Outside of its interaction with Aβ, ApoE4 alone can be harmful to neuronal function. Structural differences in the ApoE4 protein make it more susceptible to proteolysis than other ApoE isoforms. The cleavage of ApoE4 produces neurotoxic fragments that interfere with mitochondrial respiration and induce neurofibrillary tangle-like inclusions within neurons. Intact ApoE4 is detrimental to synaptic plasticity and microtubule stability. In comparison to other forms of ApoE, ApoE4 negatively affects neurite outgrowth and neurogenesis. Neuron-derived ApoE4 also promotes tau phosphorylation and thus the disruption of microtubules and axonal transport.

Diabetes

Alzheimer's disease and diabetes represent growing chronic age-related diseases associated with demographic changes in the population worldwide (reviewed in Mayeux, Stern; 2012). Insulin hormone is secreted in response to the increase in blood glucose after a meal ingestion to stimulate glucose uptake and storage as glycogen in the muscles and liver cells. Type 2 diabetes mellitus (DMII), or non-insulin-dependent diabetes mellitus, is the most common form of diabetes. DMII is defined by high blood glucose due to reduced insulin sensitivity (insulin resistance) mainly in the liver, muscle, and fat cells. To overcome this, the pancreas increases insulin secretion. Eventually, it fails to produce sufficient insulin which leads to hyperglycemia and DMII. Several epidemiological studies have revealed an interesting relationship between AD and DMII. Longitudinal studies which followed groups of patients for several years showed that DMII patients have approximately double risk to develop AD in comparison to nondiabetic controls. This risk might be higher depending on the duration the patient suffered from diabetes. Furthermore, it has been shown that DMII patients suffer from some cognitive impairment as revealed by cognitive tasks. Moreover, the prevalence of DMII among AD patients is higher in comparison to non-demented age-matched controls.

Insulin and insulin receptors (IRs) are ubiquitously distributed throughout the brain. Besides its role in regulation of glucose metabolism and food intake, insulin affects numerous brain functions such as cognition and memory through complex insulin/IR signaling pathways. There is a controversy in explaining the interaction between AD and DMII. A similarity between AD and DMII is the insulin resistance and the low insulin levels in the CNS. As the amyloid hypothesis is a central hypothesis in AD pathology, many studies have suggested that insulin can influence Aβ production or degradation. Insulin degrading enzyme (IDE) has a critical role in degrading Aβ in addition to other substrates such as insulin and amylin. When the levels of insulin are highly increased, insulin competes with Aβ for IDE and results in accumulation of Aβ. However, insulin is known to increase the levels of IDE expression and thus amyloid degradation. Also, insulin elevates APP/Aβ trafficking from the trans-golgi network to the plasma membrane, leading to the secretion of Aβ into the extracellular environment at the expense of the more toxic intracellular levels of Aβ. Taken together, optimized brain insulin levels promote Aβ clearance,

while either low or high CNS levels may promote AD progression. In addition to its influence on amyloid accumulation, insulin is involved in regulating Tau phosphorylation through GSK-3β which is downstream the insulin signaling pathway.

Insulin represents an important neurotrophic and neuroprotective factor associated with protein restoration. Chronic hyperinsulinemia and insulin resistance downregulate the insulin receptors in the blood–brain barrier and reduce insulin transport to the brain. Therefore, the reduced response to insulin in the brain in DMII can render neurons more prone to neurotoxicity. Also, insulin might modulate the cognitive functions in AD by its effect on neurotransmission. Several studies have shown that the restoration of insulin activity can reverse the acetylcholine reduction, a neurotransmitter dramatically reduced in the brain of AD, and restore memory performance. Similar effects of insulin are seen on serotonin.

The vascular association between DMII and AD remains puzzling. Both share the same risk factors such as obesity and lifestyle. On the other hand, DMII complications such as hypertension, hyperlipidemia, and increased blood viscosity might lead to poor perfusion and cognitive impairments. Low blood flow to the brain might impair the delivery of nutrients to the brain, reduce Aβ clearance, and upregulate APP expression. Moreover, DMII-induced hyperglycemia has adverse effects such as oxidative stress and vascular inflammation.

Many studies have shown that administration of insulin may have beneficial effects on memory and cognitive status of AD patients, especially in the early stages of the disease. Moreover, some studies suggest that diabetic patients treated with insulin show reduced risk of developing AD. The role of insulin is supported by findings from animal models where administration of insulin might improve behavioral deficits. Furthermore, depletion of insulin in AD models exacerbated AD pathology. Pilot studies employing intranasal insulin in patients with AD and mild cognitive impairment led to promising results with improved delayed memory and preserved general cognition.

Hypertension

Hypertension is a chronic cardiovascular condition in which diastolic blood pressure is $\geq$90 mmHg and systolic blood pressure is $\geq$140 mmHg. It is best known as a primary risk factor for stroke, aneurysm, and heart attack. However, several longitudinal studies have also implicated hypertension as major risk factor for the development of AD. High blood pressure correlates with a greater likelihood of future cognitive decline and AD diagnosis. The effects of hypertension appear to be most prominent in mid-life, as a late-life increase in blood pressure is not associated with a greater chance of AD diagnosis.

The precise mechanisms by which hypertension raises one's risk of AD are currently debated. It is hypothesized that hypertension may contribute to AD pathology by causing abnormal vascular function that leads to a variety of secondary insults to the brain. These include lacunae formation, cerebral hypoperfusion, small vessel damage, white matter abnormalities, and decreased hippocampal and cortical

volume. Animal models of hypertension suggest this condition increases Aβ, further exacerbating vascular damage due to increased blood pressure.

Given the link between hypertension and AD, studies were conducted to examine if antihypertensive treatment could decrease the risk of developing dementia. The treatments used included diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers. Results from these studies were mixed, with different meta-analyses suggesting antihypertensive drugs either decreased the risk of cognitive decline and dementia or had no protective effect.

Physical Activity

Physical activity can have an enormous impact on health by inducing a variety of vascular and molecular changes and providing protection against age-related diseases. Epidemiological studies suggest that physical activity may have a protective effect against neurological diseases associated with age, such as Parkinson's and Alzheimer's disease. In general, the majority of studies have shown a beneficial relationship between physical activity and cognition. Several prospective studies have shown a reduced risk to develop AD and other forms of dementia in cognitively and physically active individuals. Also, retrospective studies found that people who were physically and cognitively active in their mid-life were less prone to develop AD later in life. Additionally, some studies that involved physical or cognitive intervention in AD or patients with mild cognitive impairment revealed improvements either in cognitive state or other aspects of dementia, such as verbal fluency, mobility, and depression.

Many pathophysiological pathways have been hypothesized to explain the relationship between exercise and mental health. Reactive oxygen species (ROS) are chemically active molecules that are produced by certain cell types and organelles. During periods of stress, the level of ROS rise, causing cellular damage that is described as oxidative stress. However, ROS are important for cell signaling. Exercise induces the production of ROS, which leads to the production of antioxidants and DNA repair. This counteracts the effects of oxidative stress seen in many diseases including AD. Many studies have highlighted two neurotrophic factors: brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), which get elevated with exercise. BDNF is important for synaptic plasticity, learning, memory, and modulation of depression. IGF-1 promotes neurogenesis and proliferation of new progenitor cells. Studies have shown that IGF-1 is inversely correlated with Aβ levels. In addition, exercise increases the levels of several neurotransmitters, such as acetylcholine, which usually drops in AD.

Exercise affects cerebral blood flow to the cortical and subcortical areas due to several factors. Some of these factors are released by skeletal muscles during exercise. Adenosine triphosphate (ATP) is a vasodilator which is elevated during exercise and is involved in normal cerebral perfusion. Nitric oxide, which is secreted by endothelial cells, increases during exercise and improves angiogenesis and

cerebral blood flow. Also, exercise increases the levels of the vascular endothelial growth factor which is an essential determinant involved in capillary formation.

The promising findings from the clinical studies encouraged researches to prove the effects of exercising on AD pathology and to find the responsible biological pathways. Many studies used AD mouse models for different forms of exercising. Unfortunately, animal studies have yet provided a clear answer in that regard. Some of the studies showed improvement in pathology or behavior while others showed no effect or even worsening in the pathology. This might be due to many variations among these studies. Different AD models were used which represent different disease stages and thus respond differently to the exercise intervention. Also, different interventions were applied including physical activity (voluntary and involuntary), cognitive tasks, a social environment, or combination of all three. An important factor is the time when the intervention starts, as well as its duration. Nevertheless, data suggest that exercise may be more beneficial before the progression of AD (protective) or in patients of MCI.

Current Therapeutic Interventions

As long as no effective causal treatment is available, attention is turned on a small number of approved drugs for symptomatic treatment (Blennow et al., 2006). These drugs temporarily improve symptoms; however, the effectiveness of these drugs varies across the population, and none of the treatments available today alters the underlying course of this disease. The cholinergic hypothesis of AD states that memory disturbance and other cognitive symptoms can be caused by degeneration of cholinergic neurons in the basal forebrain, leading to a deficit in the levels of the neurotransmitter acetylcholine. By the application of acetylcholinesterase inhibitors, the availability of acetylcholine is increased, due to blockage of the activity of acetylcholinesterase, the enzyme responsible for degradation of acetylcholine in the synaptic cleft. This in turn leads to an enhanced cholinergic neurotransmission. These inhibitors cannot prevent AD progression, but can temporarily mitigate some of the symptoms. It has been shown that they can be effective for up to 2 years without severe side effects, and they are indicated for mild to moderate AD.

A second approved drug is memantine that belongs to the group of *N*-methyl-D-aspartate (NMDA) receptor antagonists. Under physiological conditions, activation of these receptors by the neurotransmitter glutamate is blocked by magnesium, and activation occurs only temporarily due to high levels of glutamate, leading to calcium influx and signal transduction. In neurodegenerative disorders, like in AD, a sustained low-level activation of NMDA receptors can be caused by increased glutamatergic activity leading to an impaired neuronal function. It is suggested that memantine protects neurons from glutamate-induced cell death, without disturbance of the physiological NMDA receptor activation. This drug is approved for moderate to severe AD.

New Approaches for Alzheimer Therapy

Targeting Secretases

One intensively followed approach is the blockage of the protease-dependent production of Aβ peptides (reviewed in De Strooper et al., 2010). It has been shown that BACE1 knockout mice have an abolished Aβ peptide production in the absence of a relevant clinical phenotype. Based on this observation, BACE1 inhibitors have been developed, which were able to reduce Aβ production in animal models of AD. However, there is a possibility of side effects as it is hard to believe that BACE1 has evolved to generate Aβ exclusively. Indeed, recent studies suggest that β-secretase might be responsible for the cleavage of additional substrates including a Golgi resident sialyltransferase or neuregulin 1, an axonally expressed factor required for glial cell development and myelination. Similar approaches have been carried out for γ-secretase inhibition. One major problem is the occurrence of adverse effects, as γ-secretase cleaves a variety of other substrates besides APP. One important target is the Notch receptor, and it has been shown that its inhibition has serious consequences like the development of skin cancer as it plays a major role in, for instance, regulation of cellular differentiation. Recently novel γ-secretase inhibitors have been developed that do not affect Notch signaling, and they have been shown to possess a good tolerability in early clinical studies. An alternative approach is partial inhibition of γ-secretase activity using inhibitor concentrations that effectively reduce Aβ production without affecting Notch signaling. Besides γ-secretase inhibition, another class of compounds is currently under evaluation. These so-called "γ-secretase" modulators (GSMs) have been demonstrated to reduce the levels of toxic longer Aβ species (like $A\beta_{42}$) while leading to a concomitant increase in the production of shorter Aβ peptides (like e.g., $A\beta_{38}$). These shorter peptides are considered to be less toxic; however, there is currently only limited data available on whether this is true in-vivo.

Besides β- and γ-secretase inhibition, a stimulation of α-secretase activity might also be a promising approach, as enhanced alpha-secretase activity shifts APP processing toward the non-amyloidogenic pathway, thereby precluding Aβ production.

Immunotherapy

Since their initial description in transgenic mouse models of AD, immunotherapeutic strategies to combat neurodegenerative diseases have electrified the scientific community (reviewed in Lannfelt et al., 2014). Active immunization using fibrillar Aβ led to reduced Aβ deposition and subsequently similar results were obtained using passive immunization approaches with antibodies detecting Aβ. Currently alternative mechanisms are discussed. In the microglial activation hypothesis, antibodies enter the CNS and bind to amyloid plaques, thereby inducing an increased clearance by microglial cells via their Fc receptors. Alternatively, antibodies bind to Aβ

peptides in the bloodstream, thereby shifting the distribution of Aβ between the brain and the periphery. This leads to a net efflux of Aβ from the central nervous system to plasma, where it is degraded. In the latter mechanism, which is also known as the "peripheral sink hypothesis," it is not required that antibodies enter the brain. Finally, therapeutic antibodies might directly inhibit Aβ toxicity by neutralizing soluble oligomeric Aβ peptides.

These results in transgenic mice were the origin for a pilot clinical trial using active immunization against pre-aggregated $A\beta_{42}$. Active immunization involves administration of a vaccine containing antigens or other stimuli designed to elicit an immune response leading to antibody generation in the immunized individual. This trial, however, had to be halted because 6 % of the patients developed encephalitis, and it has been suggested that this was due to a T-cell response against portions of the peptide. Autopsy results from this trial revealed that vaccination led to almost complete clearance of Aβ deposits but also caused severe cerebral inflammation as a side effect. In ongoing active vaccination trials, the N-terminal part of Aβ is conjugated to a carrier protein which is believed to bear a much lower risk of T-cell-mediated side effects. In addition, novel passive immunization studies with humanized anti-Aβ monoclonal antibodies are also currently under evaluation clinical trials. Reproducible delivery of a defined amount of therapeutic antibodies to the patient, in addition to a rapid clearance of these antibodies in the case of side effect development, is the most important potential advantage of passive immunization strategies. On the other hand, repeated infusions are necessary to maintain certain antibody levels over time. A major problem is the lack of an active transport system for antibodies of immunoglobulin (Ig) G isotype in to the human central nervous system, with the consequence, that only a minor fraction of ~0.1 % of the antibodies introduced into the periphery can be detected in the brain or CSF of immunized patients.

Other Therapeutic Approaches

Extracellular plaques in the brains of AD patients are accompanied by local inflammatory reactions in the form of astrocytes and microglia cells. Whether these inflammatory processes are contributors or just a consequence during the disease process is currently unclear. It is known from epidemiological studies that patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) have a decreased risk of developing AD (reviewed in Cole, Frautschy; 2010). Interestingly, a number of these drugs including ibuprofen, indomethacin, and sulindac sulfate have been shown to selectively reduce $A\beta_{42}$ in in vitro studies and some, but not all, NSAIDs seem to be also effective in transgenic AD mouse models leading to a reduced Aβ brain burden. The $A\beta_{42}$ lowering effect is apparently unrelated to the inhibition of cyclooxygenase and can be separated from anti-inflammatory properties of this class of substances. So far, clinical trials using anti-inflammatory drugs showed no beneficial effects in AD patients, and it is suggested that they might be protective during mid-life but not effective in interfering with the degenerative process in patients with established pathological changes.

A related epidemiological finding in terms of reduced AD risk was reported in individuals taking cholesterol-lowering drugs such as statins. Studies in cell culture and AD transgenic mice also suggested that these drugs are able to reduce Aβ levels and Aβ deposition. The exact role of cholesterol in Aβ metabolism is not yet clear, and recent treatment trials did not demonstrate significant effects on plasma or cerebrospinal fluid $A\beta_{42}$ levels. A recent Cochrane data review stated that there is insufficient evidence to recommend statins for the treatment of dementia.

Copper (Cu) adsorbs on Aβ and accumulates in extracellular Aβ plaques. This observation, together with in vitro studies showing that copper increases Aβ aggregation, suggested that it might be a risk factor for AD. However, it has been shown that copper treatment of culture cells prevents the generation of Aβ peptides. These in vitro data were corroborated by independent studies in transgenic mouse models of AD, showing a protective effect of copper treatment. To validate these data, AD patients were treated for 12 months with copper in a randomized, placebo-controlled study; however, no beneficial or adverse effects on cognition were reported.

Transgenic Mouse Models of Alzheimer's Disease

In recent years transgenic mice overexpressing the human APP gene have been well established as the model of choice in modern AD research. With the knowledge of mutations that cause AD in humans, it became possible to mimic, at least in part, some key features of AD pathology (reviewed in Duyckaerts et al., 2008 and Götz, Ittner; 2008). As a general rule, these transgenic mice are generated by microinjection of DNA-construct which carries the gene of interest under the control of a tissue-specific promoter. The choice of the promoter has influence on the expression profile and determines if the transgene is expressed ubiquitously or only in certain cell types (e.g., neurons or glial cells). Alternatively the so-called "knock-in" models are used in which endogenous mouse genes are replaced by their human mutant counterpart. This approach has the advantage that expression of the gene of interest is controlled by the endogenous promoter; however, expression levels might be too low to create pathological changes. Since the early 1990s, many different laboratories have developed APP-based transgenic AD mouse models which show a variety of pathological alterations, including extracellular plaque deposition, changes in synaptic transmission, or robust inflammatory responses.

An initial breakthrough was the development of the PDAPP model which expresses human APP with the "Indiana" mutation and shows an age-dependent deposition of extracellular β-amyloid plaques and an associated inflammatory reaction in the form of astro- and microgliosis. The validity of this model was corroborated shortly after by the generation of another transgenic mouse line (Tg2576) overexpressing human APP with the "Swedish" mutation. These mice also displayed extracellular plaques, gliosis, and moderate behavioral deficits. These models were successful due to high levels of APP expression which were six- to tenfold higher than endogenous murine APP levels. Over the years several APP-transgenic mouse models have been developed showing a disease-related phenotype to a greater or

lesser extent. Some of these models show early pathological changes like deficits in synaptic transmission, disturbed long-term potentiation or deficits in working memory. It is of interest that these findings often occur before the onset of extracellular plaque pathology and that they represent alterations which can be explained by plaque preceding changes like intraneuronal accumulation of Aβ peptides. In recent years, it has been demonstrated in different APP-transgenic models that plaque formation is preceded by in part massive Aβ accumulation within neurons. In a double-transgenic model expressing both mutant APP and mutant PSEN1, a transient intraneuronal Aβ accumulation was described which decreases with a concomitant increase in extracellular plaque pathology. This corroborates findings from postmortem studies in young Down syndrome patients (these patients develop AD-typical alterations in the third decade of life) which also show diminished intraneuronal Aβ at time points of augmented extracellular β-amyloid pathology.

More recently, several transgenic mouse models expressing only N-terminally truncated Aβ peptide variants (e.g., Tg4-42 expressing $A\beta_{4-42}$ under the control of a neuron-specific promoter) have been described. These mice do not overexpress the amyloid precursor protein which in some existing models influences the behavioral and neuropathological phenotype in an undesired manner. In addition, Tg4-42 mice lack familial AD mutations making them better suited to study the most abundant sporadic form of AD and present with robust neuron loss and behavioral deficits. These mice show a robust intraneuronal accumulation of Aβ peptides while extracellular plaques are largely absent which strengthens the hypothesis that intraneuronal Aβ, neuron loss, and cognitive decline are intimately linked (reviewed in Bayer, Wirths; 2014).

Outlook

In the current chapter, we discussed the clinical features of AD, mild cognitive impairment, and why the identification of the genes involved in AD was so important and to unravel the cascade of pathological events. Besides the identification of novel genes and pathways, it will be important to further develop improved animal models to test future therapy options. They will be equally instrumental to decipher the role of the risk factors diabetes and hypertension and potential protective factors like physical activity.

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