

Review

# $\beta$ -Amyloid and the Pathomechanisms of Alzheimer's Disease: A Comprehensive View

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Received: 15 September 2017; Accepted: 6 October 2017; Published: 10 October 2017

**Abstract:** Protein dyshomeostasis is the common mechanism of neurodegenerative diseases such as Alzheimer's disease (AD). Aging is the key risk factor, as the capacity of the proteostasis network declines during aging. Different cellular stress conditions result in the up-regulation of the neurotrophic, neuroprotective amyloid precursor protein (APP). Enzymatic processing of APP may result in formation of toxic A $\beta$  aggregates ( $\beta$ -amyloids). Protein folding is the basis of life and death. Intracellular A $\beta$  affects the function of subcellular organelles by disturbing the endoplasmic reticulum-mitochondria cross-talk and causing severe Ca<sup>2+</sup>-dysregulation and lipid dyshomeostasis. The extensive and complex network of proteostasis declines during aging and is not able to maintain the balance between production and disposal of proteins. The effectivity of cellular pathways that safeguard cells against proteotoxic stress (molecular chaperones, aggresomes, the ubiquitin-proteasome system, autophagy) declines with age. Chronic cerebral hypoperfusion causes dysfunction of the blood-brain barrier (BBB), and thus the A $\beta$ -clearance from brain-to-blood decreases. Microglia-mediated clearance of A $\beta$  also declines, A $\beta$  accumulates in the brain and causes neuroinflammation. Recognition of the above mentioned complex pathogenesis pathway resulted in novel drug targets in AD research.

**Keywords:** Alzheimer's disease; protein and lipid dyshomeostasis; APP; intracellular A $\beta$ ; ER-mitochondrial axis; Ca<sup>2+</sup> dysregulation; molecular chaperones; autophagy; neuroinflammation; A $\beta$ -clearance

## 1. An Overview of the Possible Pathomechanisms of Alzheimer's Disease (AD)

Nascent protein chains, emerging from the ribosomes, need to fold properly into unique 3D structures, eventually translocate and then assemble into stable, functionally flexible complexes [1]. In the crowded cellular environment, newly synthesized polypeptide chains are at risk of misfolding, forming stable, toxic aggregates. These species may accumulate in aged animal cells, especially in neurons and can cause cellular damage inducing cell death [2]. Aggregation of specific proteins into protein inclusions and plaques is characteristic for many neurodegenerative diseases (NDDs), including AD, Parkinson's (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Table 1 summarizes the short list of some NDDs and their corresponding misfolded proteins [3].

Molecular pathological classification of neurodegenerative diseases is based on the presence of these pathologically altered, misfolded proteins in the brain as deposits [4]. These proteins and their biochemical modifications can potentially be targeted for therapy or used as biomarkers (e.g., CSF-biomarkers in AD [5]) sometimes combined with neuroimaging modalities [6,7]. The combination of proteinopathies is also frequent.

**Table 1.** Examples for protein conformational, neurodegenerative diseases as well as their corresponding misfolded proteins.

List of Diseases	Misfolded Proteins
Alzheimer's disease	$\beta$ -amyloid ( $A\beta$ ) hyperphosphorylated Tau (pTau) $\alpha$ -synuclein TDP-43
Parkinson's disease	$\alpha$ -synuclein
Huntington disease	huntingtin
Lewy-body dementia	$\alpha$ -synuclein
Amyotrophic lateral sclerosis	TDP-43
Prion diseases	superoxide dismutase prion protein ( $PrP^{Sc}$ )

What is the mechanism of formation of toxic protein aggregates in a living cell? Proteins are structurally dynamic and thus constant surveillance of the proteome by integrated networks of chaperones and protein degradation machineries (including several forms of autophagy) are required to maintain protein homeostasis (proteostasis) [1]. NDDs are considered mostly as pathologies of disturbed protein homeostasis. Proteostasis network declines during aging, triggering neurodegeneration and other chronic diseases associated with toxic protein aggregation [8]. In both aging and AD there is a general decrease in the capacity of the body to eliminate toxic compounds. In AD, toxic  $\beta$ -amyloid ( $A\beta$ ) and hyperphosphorylated Tau (pTau) aggregates may interact with subcellular organelles of the neurons, trigger neuronal dysfunction and apoptosis that lead to memory decline and dementia.

Several studies demonstrate the central role of disturbed protein homeostasis in the pathogenesis of AD. Transcriptional signature of AD is associated with a metastable subproteome at risk for aggregation [9]. A proteostasis signature in healthy brain recapitulates tissue vulnerability to AD [10]: neurons contain suboptimal levels of protein homeostasis components (low levels of aggregation protectors and presence of aggregation promoters).

Aging *per se* is the most important factor of AD and several other neurodegenerative diseases, “the neurobiology of aging and AD is walking down the same road” [11]. AD could be seen as a “maladaptive interaction between human brain evolution and senescence” [12]. Other authors hypothesized that formation of aggregated proteins might be a protective strategy of the aging neurons [13]. Very recent results demonstrate that transposon-mediated genomic instability plays a key role in the aging process [14].

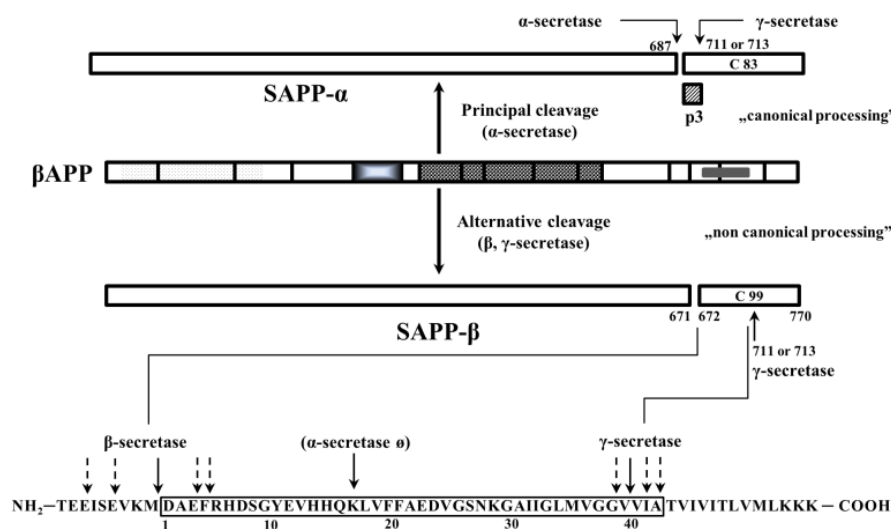
There are numerous hypotheses for understanding the pathogenesis of AD, owing to the multifactorial character of the disease. Some of them (disturbance of the cholinergic system; hypoperfusion, hypoxia in the brain;  $Ca^{2+}$ -signalization problems; neuroinflammation; mitochondrial dyshomeostasis; chronic ER-stress and protein misfolding; decreased  $A\beta$ -clearance, etc.) are not controversial and could be unified into a general broad hypothesis. The common nominator of these hypotheses is the important role of  $A\beta$  in the pathogenesis of AD.

The conventional view of AD is that much of the AD-pathology is driven by an increased load of  $A\beta$  in the brain of patients (“amyloid hypothesis” [15]). During the last 15 years many therapeutic strategies were based on lowering  $A\beta$  in the brain. Up to now, most of the strategies have failed in clinical trials and the relevance of the amyloid hypothesis has often been questioned [16]. Very recent results show that pathophysiological changes begin many years before clinical manifestation of AD and the disease is a multifaceted process [17]. A rare mutation in the Iceland population gave a strong evidence for the important role of  $A\beta$  in the pathomechanism of AD [18]. The core of the amyloid hypothesis stays on and novel clinical trial strategies may hold promise [19].

In the present review article, we summarize the physiological functions of amyloid precursor protein (APP) and the role of amyloid fragments in adult brain. Then we give a short summary on the genetic background of AD, the interaction of A $\beta$  peptides with subcellular organelles, the pathways of A $\beta$  clearance from the brain, the role of neuroinflammation, brain circulation and the blood-brain-barrier (BBB) in the AD pathogenesis. Finally, we discuss very shortly the major trends in drug discovery and the possibilities for prevention and treatment of AD.

## 2. Physiological Functions of the Amyloid Precursor Protein (APP) and Its Metabolites (Amyloid Fragments)

APP is a transmembrane protein with a large extracellular N-terminal domain, a transmembrane domain and a short C-terminal cytoplasmic domain consisting of 59 amino acid residues [20]. There are eight isoforms of APP; the shortest 695 amino acid isoform is highly expressed in the CNS. Studies on APP overexpression demonstrate that it positively modulates cell survival and growth [21]. APP promotes neurite arborization in a *Drosophila* model of brain injury [22]. APP also plays an important role in the formation and maintenance of synapses, neuronal survival and neuritic outgrowth [23–25]. The importance and the functional neurophysiology of the APP-processing pathways and products are widely reviewed by Randall et al. [26]. It is known that APP has two main processing pathways: (1) the canonical, non-amyloidogenic (90%) involving  $\alpha$ -secretase and releasing a truncated form of APP (soluble APP $\alpha$ , SAPP $\alpha$ ) and the C-terminal fragment C-83; and (2) the non-canonical, amyloidogenic pathway (10%), which generates A $\beta$  peptides through the sequential cleavage by  $\beta$ -secretase (BACE) and  $\gamma$ -secretase (Figure 1).



**Figure 1.** Simplified presentation of the amyloid precursor protein (APP) and its processing products. Principal cleavage of APP with  $\alpha$ -secretase results in the formation of water soluble products (SAPP- $\alpha$ , C83 and P3). Alternative (amyloidogenic) cleavage with  $\beta$ - and  $\gamma$ -secretase gives a heterogeneous mixture of  $\beta$ -amyloid (A $\beta$ ) peptides of 37 to 43 amino acids.

It was suggested that the non-amyloidogenic cleavage of APP is localized mostly in the plasma membrane [27] and the amyloidogenic cleavage runs intracellularly. After the biosynthesis of APP, the polypeptide chain is transported to the Golgi apparatus, where it is O- and N-glycosylated, phosphorylated and sulfonated at tyrosine residues [28]. Only 10% of APP goes to the plasma membrane and the majority of APP remains in the Golgi and trans-Golgi network (TGN) according to in vitro studies. APP inserted into the plasma membrane can be internalized via endocytosis (due to the presence of “YENPTY” motif) and reaches the endosome [29]. Several subcellular compartments (trans-Golgi network, lysosomes, and ER-MAM) may participate in APP processing in the cells:

1. Late Golgi compartments could be mainly involved in the generation of intracellular A $\beta$  (iA $\beta$ ) [30].
2. Recent studies have demonstrated that APP is rapidly transported from the Golgi apparatus to the lysosome, where it is processed into A $\beta$  [31]. The adaptor protein 3 (AP-3) mediates rapid delivery of APP to lysosomes.
3. Several studies demonstrated, however, that  $\alpha$ - and  $\beta$ -secretases are present in ER allowing APP processing [32,33]. It was shown that presenilin 1 and 2 (PSN1, PSN2) and  $\gamma$ -secretase activity are located in the special subcompartment of the ER, called mitochondrial associated membrane (MAM). It was hypothesized that MAM is a subcellular site of the amyloidogenic processing of APP and A $\beta$  generation [34,35]. Most recent results demonstrate that vascular dysfunctions, exposures to hypoxia/ischemia increase the amyloidogenic processing of APP and provoke AD pathology by activating  $\beta$ - and  $\gamma$ -secretases [19].

Up to now, most APP-related research has focused on the amyloidogenic properties and toxicity of A $\beta$  peptides, with less emphasis on the normal physiological roles of APP and its other cleavage products. APP family (APP and APP-like proteins) remained a biochemical enigma in brain development and function [36]. Novel studies on the physiological functions of the protein have opened insight into the role of APP beyond AD, emphasizing the neuroprotective role of APP and its metabolites [37]. The short summary of the most important new results are:

1. APP plays a key role in neuronal homeostasis [38] including intracellular transport and signaling. APP acts as a signaling connection that transduces information about extracellular conditions (e.g., neuronal damage) and induces intracellular signaling events. Minute disruptions in APP signaling functions may be major contributors to neuronal dysfunction leading to AD.
2. APP has a trophic function and may be involved in neuronal stem cell development, neuronal survival, neurite outgrowth and neurorepair [21,22,37].
3. Most recent studies proved the results on the key role of APP as a neuroprotective factor [39]. Acute (stroke, cardiac arrest) or chronic (cerebrovascular disease) hypoxic-ischemic conditions cause up-regulation of APP. The protein itself and its soluble extracellular fragment SAPP $\alpha$  can promote neuronal survival. The underlying mechanism is very probably APP-mediated regulation of calcium homeostasis.
4. Among the three major splice isoforms of APP (APP<sub>695</sub>, APP<sub>751</sub>, APP<sub>770</sub>), the predominant neuronal form is APP<sub>695</sub>. Beyond these, closely related APP-like proteins (APLP1, APLP2) exist as members of the APP family. All the APP family members are truly multifunctional proteins and can form large signaling complexes with various transmembrane proteins and intracellular binding partners [40].
5. Canonical and non-canonical processing of APP results in a series of APP fragments, the most important fragments are SAPP $\alpha$ , SAPP $\beta$ , A $\beta$  (40 and 42 AA length) and AICD. Their in vivo functions in the CNS are widely reviewed by Müller et al. [40], here we give a very short summary of their most important role:

#### SAPP $\alpha$ :

rescues memory and spine density in aged rats  
 reduces plaque deposition and tau phosphorylation  
 protects against traumatic brain injury (TBI), neuronal death in transient ischemia and hypoxia  
 stimulates adult neurogenesis

#### AICD:

modulator of gene expression apoptosis and cytoskeletal dynamics [41]  
 indirect regulation of DNA damage response

A $\beta$  40/42:

regulation of lipid homeostasis [42].

regulates neuronal homeostasis in picomolar amounts (stimulating PTP, LTP and memory) when their production and degradation are imbalanced, the peptide forms toxic A $\beta$ -assemblies in higher concentration [43,44].

In summary, it can be stated that APP and its metabolites (SAPP $\alpha$ , AICD, native A $\beta$ ) have important physiological functions in cell mainly in normal neuronal development, neurogenesis, neuronal homeostasis and different signalization pathways.

### 3. Formation and Propagation of Hypertoxic A $\beta$ Structures and the Prionoid Hypothesis

Emerging evidence supports the hypothesis that A $\beta$  monomers in picomolar level possess neuroprotective activity [45]. However, understanding the role of A $\beta$  in the neurodegenerative process of the disease has been the central challenge. The continually (sometimes radically) changing amyloid cascade theory hypothesized a seeding-nucleation model for A $\beta$  aggregation [46] and speculated the following toxic assemblies as causative factors for AD [47]:

1. Fibrillar A $\beta$
2. Soluble, oligomeric A $\beta$ : A $\beta$  dimers; A $\beta$  trimer, tetramer, hexamer; A $\beta$  dodecamer (56kD, A $\beta$ \*56); large A $\beta$  oligomers (“high-n” oligomers)
3. Protofibrils
4. Dispersible A $\beta$  oligomers, protofibrils and fibrils [48,49].

A glossary of A $\beta$ -aggregation products and precise descriptions for preparing different A $\beta$  species are given by Jan et al. [49]. What is the toxic or most toxic A $\beta$ -assembly and what is the role of A $\beta$  in AD? These questions have remained the subject of hot debates [50–52]. Analysis of the soluble and insoluble amyloid material of human AD brain demonstrated the extraordinary heterogeneity of A $\beta$  peptides (26 proteoforms of different peptide chain length including various N- and C-terminal transactions) [53]. The vast majority of A $\beta$  peptides deposited in AD brains is truncated and post translationally modified at the C-terminus.

Not only might the size of A $\beta$ -aggregates be important for toxicity, but also the peptide conformation. A formidable experimental challenge is the analysis of the biophysical properties and conformation of a single A $\beta$ -species (e.g., A $\beta$  1-42), mainly because of their nanoscale dimensions and heterogeneous nature [54]. Bulk techniques (CD or IR spectroscopy) are not able to characterize the heterogeneity and inner conformational properties of amyloid aggregates at the single species level.

The fundamental mechanism of protein folding allows the formation of misfolded protein structures [55,56] leading to many fatal diseases. Protein aggregation is regarded as a side reaction of folding. The various mechanisms of protein homeostasis (folding, unfolding, misfolding, aggregation) are summarized in a paper of Goloubinoff et al. [57]). Cells have different mechanisms to deal with protein misfolding and aggregation, where the molecular chaperone machinery constitutes the first line of defence against misfolded proteins. A misfolded protein can be refolded, degraded or aggregated—the last process may lead to toxic assemblies. Aggregation-prone proteins are constitutively expressed in the cell, creating a chronic stress situation. Enhancers and suppressors of aggregation appear during aging and disease [58] for regulating amyloid formation.

Table 1 listed misfolded proteins (similar to A $\beta$ ) as causative agents of protein misfolding diseases. The toxic conformational transition would be a therapeutic target for polyQ diseases and probably for conformational diseases in general [59]. Misfolding of  $\alpha$ -synuclein is a well-known causative process of PD and LBD [60–62]. The spongiform change of the brain in dementia with Lewy bodies and AD connects these disorders to prion diseases [63]. It is assumed that toxic amyloid forming monomers show intrinsically disordered (ID) nature [64–67].



A $\beta$  may be directly toxic to neuronal cells and synapses. The ID structures of A $\beta$  represent transient intermediates in the aggregation cascade of patient brains. Infectious amyloid material can be generated and this fact binds AD to prion disease [68]. The pathology of AD, the Braak-stages also support the idea that AD spreads from neuron to neuron. It is almost forgotten that at the beginning of the eighties-nineties, AD was suspected of being a prion disease [69]. Recently, it was demonstrated that extracts containing soluble A $\beta$  aggregates induce amyloidosis in mice that otherwise never develop amyloid plaques. Pyroglutamylated A $\beta$  3-42 shows prion-like behavior in mice [70]. Pathological similarities between AD and prion diseases suggest that the formation and spread of the proteinaceous lesions might involve a common molecular mechanism—corruptive protein templating [71–73]. Experimentally, cerebral  $\beta$ -amyloidosis can be exogenously induced by exposure to dilute brain extracts containing aggregated A $\beta$  seeds (seeding-nucleation model). Stohr et al. (in cooperation with Prusiner) [74] have shown that pure A $\beta$  injections into the brain may induce plaques throughout the whole brain within 5 to 6 months. If the peptides were injected into one hemisphere, plaque formation started in both halves of the brain. The amyloid-inducing agent is probably A $\beta$  itself [75], in a special toxic conformation (synonyms: “hypertoxic A $\beta$ ”, “primordial cytotoxic A $\beta$ ”, “prionoid A $\beta$ ”) generated most effectively in the living brain [76]. Amyloid aggregates of one protein are able to directly nucleate amyloid formation of another, different protein: this is called amyloid cross-interaction [77]. A $\beta$  and  $\alpha$ -synuclein proteins (associated with AD and synucleopathies) share similar biophysical and biochemical properties with PrP<sup>Sc</sup> that influences how they misfold, aggregate and propagate in disease [78].

The term “prionoid” was introduced by Aguzzi [79] for self-propagating, transmissible protein aggregates [79,80]. Infectious prions and prionoids can be prepared in the laboratory by protein misfolding cyclic amplification (PMCA; [81–85]). The precise molecular mechanism of the conversion of a non-transmissible protein molecule to the pathogenic form (e.g., PrP<sup>C</sup>→PrP<sup>Sc</sup>) is not completely understood. The available data support the seeding-nucleation model in which the infectious conformer (e.g., PrP<sup>Sc</sup>) is an oligomer that acts as a seed to bind native protein (PrP<sup>C</sup>) and catalyze its conversion into the misfolded form by incorporating into the growing polymer [81,86]. Nath et al. [87] demonstrated the transmission of oligomeric A $\beta$  1-42 after microinjection into primary hippocampal rat neurons via direct neuron-to-neuron transfer. This A $\beta$ -transfer depends on direct cellular connections. As the transferred oligomers accumulate, acceptor cells gradually show beading of tubulin (a sign of neurite damage) and gradual endosomal leakage (a sign of cytotoxicity). This observation supports that intracellular A $\beta$  (iA $\beta$ ) oligomers play a role in neurodegeneration and explain the progression of AD via anatomical connections.

Very recently Condello and Stohr [88] reviewed novel studies on the prionoid character of A $\beta$ . The progressive nature of AD occurs (at least in part) by the self-replication and spreading of A $\beta$  (and tau) aggregates through a prion mechanism. Evidence exists that structural variants of A $\beta$ -prions can propagate their distinct conformation through template—directed folding of native A $\beta$  peptides. As a consequence, the first self-propagating A $\beta$  assembly emerged in the brain, dictates the conformation, anatomical spread and pace of subsequently formed amyloid deposits. It is hypothesized that the existence of diverse clinicopathological phenotypes (observed among AD patients) might be due to different A $\beta$  strains propagated by prion mechanism. According to Qiang et al. the heterogeneity of AD may be the result of diverse A $\beta$  strains. Structural variation in A $\beta$ -fibrils in AD clinical subtypes indicates that there are structural variations (qualitative differences) between A $\beta$  1-40 and A $\beta$  1-42 aggregates in the brain tissue of patients with different AD subtypes of r-AD (rapidly progressive) and t-AD (typical prolonged duration) form [89].

It has remained unclear whether further molecules (co-factors), beside the misfolded prion/prionoid protein, are necessary elements of the infectious agents. Such a co-factor might act as an essential catalyst for prion/prionoid replication, help to stabilize the hypertoxic conformation and increase the biological stability of prions/prionoids. Theoretically, all PMDs have the intrinsic potential to be transmissible [86,90]. Several studies demonstrated Tau-protein transmission [91–96].

Tau and A $\beta$  are probably ready for admission to the prion club [97]. Despite many similarities, there is a difference between prion and prionoid diseases: unlike the classical prions, the prionoids (A $\beta$ , Tau,  $\alpha$ -synuclein, etc.) do not appear to spread infections from one individual to another. There is still no evidence that AD, PD or ALS are infectious under everyday circumstances.

In summary, it can be stated that in the brain A $\beta$  is a very heterogeneous mixture of peptide with a broad range of aggregation grade and conformational variability. A $\beta$  has very probably prionoid character.

#### 4. Short View of the Genetic Background of AD

Although advanced age is the best known risk factor of AD, some individuals may develop AD at a younger age. Hence, based on the time of onset, AD is classified into two types [98]. Early-onset AD (EOAD) typically develops before the age of 65 years, the other form, late-onset AD (LOAD) develops in patients older than 65 years. The production and clearance of A $\beta$  is regulated by a large group of genes. The genetic background of AD is widely reviewed [99–102]; for references see also <http://www.molgen.ua.ac.be/admutations>.

EOAD is caused by rare and mostly autosomal dominantly inherited mutations in APP, PSEN1 and PSEN2. These three genes (APP in chromosome 21q, PSEN1 in 14q and PSEN2 in 1q) are considered the main risk factors for EOAD. The mutations within these three genes affect a common pathogenic pathway in APP synthesis and processing (proteolysis), which lead to excessive production of A $\beta$ . To date, more than 270 highly penetrant mutations have been described in these genes that cause familial AD and many more are discovered each year [103]. To date, 49 APP mutations in 119 families are known to cause AD, most of them are dominantly inherited, but two recessive mutations (A673V and E693V) were also demonstrated to cause AD. Most of the pathogenic APP mutations are either adjacent to or within the cleavage sites for  $\beta$ - and  $\gamma$ -secretase, showing that A $\beta$  formation may be involved in AD pathogenesis [104]. A protective mutation of APP against AD (A673T) was also found in the Iceland population [18].

To date, 215 pathogenic mutations of PSEN1 and 13 mutations of PSEN2 have been identified. These proteins are the catalytically active components of  $\gamma$ -secretase. Mutations in PSEN1 account for up to 50% of EOAD with complete penetrance and early age of onset. Mutations of both PSEN1 and PSEN2 alter  $\gamma$ -secretase activity resulting in an increase in the A $\beta$  42/40 ratio (studies have shown that A $\beta$  1-42 is more amyloidogenic and prone to aggregate than A $\beta$  1-40 in the brain [105]).

Strikingly, very recent studies show that only 5% of EOAD patients are carrying a pathogenic mutation in one of the AD genes (APP/PSEN1/PSEN2) or an apolipoproteinE (ApoE) risk allele  $\epsilon$ 4 [106]. LOAD is genetically far more complex than EOAD with the possible involvement of multiple genes and environmental factors. It is likely that many genes are involved in LOAD pathogenesis, each of small effect and unknown transmission and expression. Most LOAD cases are sporadic with no family history of the disease. Before the era of large scale genome-wide association studies (GWAS), the APOE  $\epsilon$ 4 allele was the only well-established risk factor for LOAD. During the last 6-8 years of the GWAS era, researchers have identified a number of genes that may increase a person's risk for LOAD to varying degrees. GWASs identified many common gene variants with low penetrance. It was very interesting that most of the genes identified by GWASs could be linked with the A $\beta$  cascade or Tau pathology. Most of the important 27 genes involved in LOAD pathogenesis can be clustered within three pathways [102], and some genes participate in two pathways:

##### 1. Lipid metabolism:

APOE	SORL1
ABCA7	DSG2
CLU	

2. Inflammatory response. Neuroinflammation is a pathological hallmark of AD and several genes were found in GWASs:

CD33	MEF2c	HLA-DRB5/HLA-DRB1
CR1	MS4A	ABCA7
TREM2	JNPP5D	CLU
EPHA1		

3. Endocytosis:

PICALM	CDZAP
BIN1	SORL1

Cholesterol metabolism is involved in the pathogenesis of AD. High cholesterol levels in midlife increase the risk of AD [107] and ApoE protein is the major cholesterol carrier. Endocytosis is a critical process in synaptic transmission and response to neural damage. Several genes were found in GWASs, most of them are involved in APP trafficking that plays a key role in AD pathogenesis.

Recent meta-analysis of GWASs has identified additional genes implicated in LOAD: CASS4, PTK2B, NME8, ZCWPW1, CELF1, FERMT2, SLC24A4/RIN3 and DSG2. Functions of these genes are yet poorly characterized.

Advances in sequencing technologies (whole-exome and whole-genome sequencing) resulted in identification of novel genes associated with both EOAD and LOAD. The most important novel genes are: PLD3, UNC5C, AKAP9 and ADAM 10.

Very recent studies have characterized the genetic networks associated with AD from large sampling of postmortem brain tissues [108]. Analysis of routes of possible disease initiation processes uncovered a number of deregulated biological processes and pathways (e.g., neuron differentiation, aging, oxidative stress, chemokine signaling, LTP, etc.)

In summary, AD is a complex neurodegenerative disease with a strong genetic component. With the advance of molecular genetics, linkage studies revealed APP, PSEN1, PSEN2 and APOE as AD genes. GWASs have identified over 20 loci associated with AD risk. These results may help in the development of effective prevention and treatment strategies for AD.

## 5. Effect of Lipids in APP Processing and the Role of APP Metabolites in Lipid Homeostasis

There is a close connection between AD related proteins and lipid metabolism. Several lipid classes and fatty acids have been found to be altered in AD brains. It was demonstrated that A $\beta$  and AICD regulate several lipid metabolism pathways. Inversely, APP processing and thus formation of A $\beta$  and AICD is strongly influenced by the surrounding lipid environment, indicating a bidirectional link between APP proteolysis and lipid metabolism.

The link between AD pathology and lipid homeostasis was strengthened by the discovery that the APOE gene  $\epsilon$ 4 allele is the strongest genetic risk factor for LOAD. ApoE is the major cholesterol carrier, which influences A $\beta$  clearance, aggregation and deposition in an isoform-dependent manner. Studies suggest that ApoE4 protein is less efficient than ApoE2 and ApoE3 in A $\beta$  clearance [109]. APOE  $\epsilon$ 2 allele suppresses the initiation of AD development by lowering the cholesterol levels in synaptic membranes [110].

The surrounding lipid bilayer has strong impact on APP-processing. Increased membrane fluidity may stimulate the non-amyloidogenic APP-processing by reducing APP internalization [111]. The generation of A $\beta$  mainly takes place in lipid rafts, membrane microdomains enriched with cholesterol, gangliosides and other sphingolipids, where APP and the  $\beta$ - and  $\gamma$ -secretases are together. (The non-amyloidogenic APP-cleavage occurs predominantly in non-raft-regions [112].) Modulation of the membrane lipid composition might influence A $\beta$ -generation. Most recently, the bidirectional link and regulatory feedback cycles between APP and its metabolites as well as lipids have been widely reviewed [113,114]. The most important discoveries are:



1. *Cholesterol*: A mutual regulatory feedback cycle exists, in which cholesterol influences APP processing to A $\beta$ , while de novo, cholesterol synthesis is inhibited by high level of A $\beta$ . Modification of cholesterol levels has effects on multiple proteins, not only APP and A $\beta$  [115].
2. *Docosahexaenoic acid (DHA)* is a polyunsaturated fatty acid. There are several epidemiological and experimental indications for a beneficial effects of DHA in preventing AD, at least at very early disease stages.
3. *Plasmalogens (PLs)* represent 22% of the total phospholipid mass in human brain tissue. In AD pathogenesis a vicious circle between PLs and A $\beta$ -generation might be postulated. Accumulated A $\beta$  reduces cerebral PL content and that stimulates  $\gamma$ -secretase activity, which leads to further A $\beta$ -production [114].
4. *Sphingolipid (SL)* impact on AD. Sphingolipids modulate APP-processing and A $\beta$ -aggregation and, inversely, AICD downregulates total sphingolipid biosynthesis [116]. Sphingolipids are major components of lipid raft and the different SL classes have impact in AD-pathogenesis:

*Ceramide* is accumulated in AD-patients. A feed-forward cycle between ceramide and A $\beta$  exists in AD brain: increased ceramide level leads to enhanced A $\beta$  production and that elevates ceramide synthesis, which stimulates A $\beta$ -production. *Sphingosine* content elevates during the progress of AD [117]. In contrast, cerebral *sphingosine-1-phosphate (S1P)* content declines in AD patients, and negatively correlates with the level of A $\beta$ .

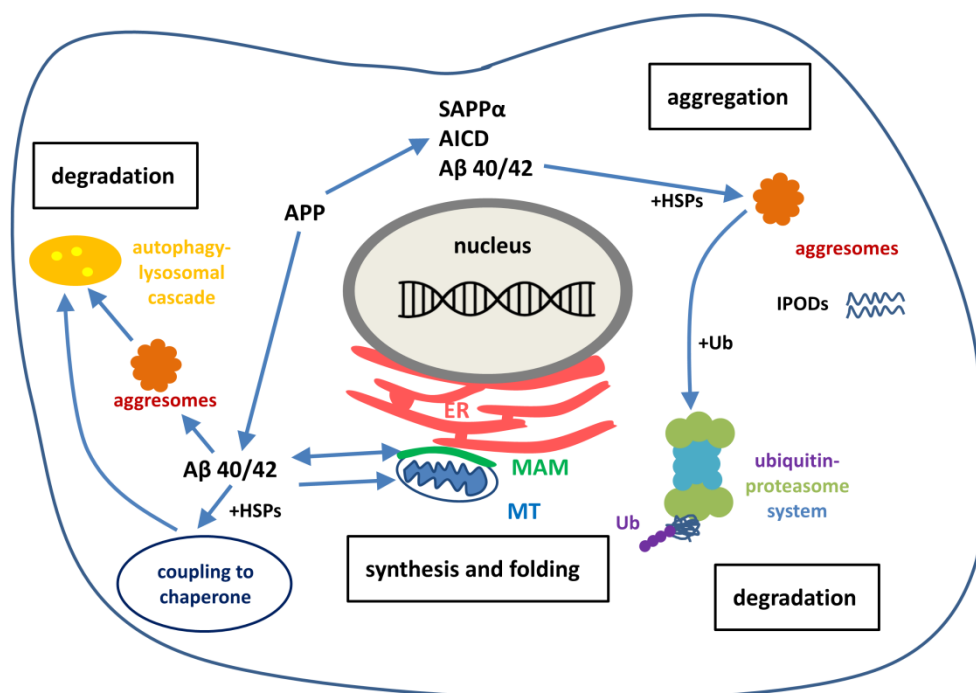
*Gangliosides*, sialic acid containing glycosphingolipids represent a heterogeneous group of different subtypes (GM1, GM1a, GD1b, GT1b GM3, etc.). There is strong link between ganglioside homeostasis and AD, however, further studies are needed to identify the most promising molecular target for AD therapeutic approaches.

In summary, several sphingolipid classes have been shown to affect the proteolytic processing of APP and A $\beta$ -clearance: ceramides and total gangliosides. GM1 and GD3 are associated with an increased A $\beta$  level, while sphingomyelin and GM3 have the opposite effect.

## 6. Dysregulation of Protein Homeostasis in AD

During the last ten years extensive research has been performed for studying the pathomechanism of NDDs. These studies suggest that the first step of initiation of AD and other NDDs is not the accumulation of misfolded protein aggregates in neurons, but rather the severe dysregulation of protein homeostasis. NDDs share common cellular and molecular pathological mechanisms, such as excitotoxicity, calcium dysregulation, oxidative stress, mitochondrial dysfunction, ER stress and the failure of the protective processes for removal of misfolded proteins. The term protein homeostasis (proteostasis) refers to the maintenance of all proteins in the proteome in a conformation, concentration and location that is required for their correct functions [118]. An extensive and complex network of signaling pathways are involved in safeguarding cells and organism against proteotoxic stress [119]. The task of the system is to maintain the balance between the production and disposal of proteins. The proteostasis network contains pathways that regulate the biogenesis, folding, trafficking and degradation of proteins (Figure 2).

The removal of toxic forms of misfolded proteins by aggresomes, autophagy or the ubiquitin-proteasome system (UPS) is especially important in post-mitotic cells, such as neurons, since these cannot be readily replaced. In neurons, the maintenance of proteostasis plays a key role to healthy aging, dysregulation of the network can lead to NDD. APP, SAPP $\alpha$  and A $\beta$  peptides are involved at the onset of AD.



**Figure 2.** Schematic representation of the intracellular proteostasis network. Cytosolic misfolded proteins are bound by molecular chaperones, such as the HSPs. Molecular chaperones may act as aggregate unfolding and refolding enzymes. Autophagy pathways (chaperone-mediated, micro- and macroautophagy) degrade misfolded proteins. Ubiquitinated proteins are degraded by the ubiquitin-proteasome system. Sequestration of degraded misfolded proteins may form compact aggresomes and IPODs. Proteasome resistant aggresomes become enveloped by autophagosome membranes and transported into the lysosomes for autophagy.

### 6.1. The Double Edged Sword: Neurotrophic APP as A Source of Toxic A $\beta$

Aggregation-prone proteins play important roles in the onset of NDDs. APP itself and its soluble extracellular fragment SAPP $\alpha$  are not aggregation-prone and act as protective factors in acute neuronal insults [37–39]. APP, as a neuroprotective protein, may play a key role in neuronal homeostasis, very probably by regulation of Ca<sup>2+</sup>-homeostasis (see Section 2). APP biosynthesis is up-regulated for neuronal survival in acute and chronic stress situations. In an unexpected manner, this mechanism may increase the risk of formation of the aggregation-prone A $\beta$  by processing of APP by  $\beta$ - and  $\gamma$ -secretase.

Analysis of the genetic background of EOAD demonstrated that several mutations of one gene (APP, PSEN-1 or PSEN-2) are satisfactory for manifestation of EOAD owing to continuous formation of toxic A $\beta$  assemblies. The onset of the disease is relatively early (age of 45–65 years, see Section 4), toxic A $\beta$  initiates the pathogenesis.

LOAD is a polygenic disease, and thus also environmental factors are needed for its manifestation beyond the genetic background. Aging is the key risk factor due to the progressive decline of protein homeostasis. In early life, the overexpression of aggregation-prone proteins in the cell can be balanced by the proteostasis network. The capacity of this network declines during aging [8]. (An example: age-related decline of heat-shock factor 1 (HSF1) decreases the activity of chaperones.) Different cellular stress conditions result in APP overexpression in aging brain. Although, the protein itself is neuroprotective, chronic up-regulation of APP may result in the formation of A $\beta$  and neurotoxic A $\beta$  aggregates. A simultaneous reduction or dysfunction of proteostasis capacity during aging results in an accumulation of toxic A $\beta$  aggregates and thus the onset of LOAD. To summarize our present knowledge, APP might play an important role in the pathomechanism of both EOAD and LOAD: APP represents the source of aggregation prone A $\beta$  peptides.

## 6.2. Formation of Intracellular A $\beta$ in Neurons

For many years, extracellular A $\beta$  (eA $\beta$ , particularly eA $\beta$  1-42) has been thought to be one of the primary causes of AD [15,120], mainly because of the extracellular location of senile plaques. This hypothesis was supported by the facts that fibrillar and oligomeric A $\beta$  could be toxic to neurons, activate microglial inflammation, induce Tau-hyperphosphorylation and directly damage membranes. Recent publications demonstrate unequivocally that neuronal (also neurotransmitter) receptors are targets of eA $\beta$ . Strong evidence supports that water-soluble, oligomeric eA $\beta$ -triggered aberrant signaling may cause cognitive dysfunction and consequent neurodegeneration in the brain [121–124].

Experimental studies demonstrated that two pools of A $\beta$  exist in the brain: extracellular and intracellular, and a dynamic relationship exists between them. More and more evidence suggests that eA $\beta$  may have a reduced impact on AD pathology, and eA $\beta$  deposition alone cannot be responsible for the whole AD pathology. The “intracellular A $\beta$  hypothesis” is becoming more and more accepted [125]. This hypothesis assumes that iA $\beta$  accumulation is the early sign of AD and a causative event in disease development. iA $\beta$  is a cytotoxic substance and the eA $\beta$  deposition (in the later stage of AD) is rather the result of cell death and destruction. Mechanism of amyloid plaque formation also suggests an intracellular basis of A $\beta$  pathogenicity [126].

A series of review articles has summarized the mechanism of iA $\beta$  accumulation and the results of iA $\beta$  experiments performed during the last ten years [125,127–132]. iA $\beta$  has been widely detected in neuronal cells and primary human neurons, and has a very broad range of interactions with cell organelles and proteins [133–137]. Very recently, intraneuronal accumulation of A $\beta$  was observed by light and fluorescent microscopy as well as immune-electron microscopy [138]. Studies in several mouse models of AD with intraneuronal expression and accumulation of A $\beta$  have proven that iA $\beta$  causes the onset of early AD related cognitive deficits in transgenic mice [139–142].

The intraneuronal pool of A $\beta$  has a double origin: slow production of iA $\beta$  from APP inside the neurons [127–131,143–145] and uptake from the extracellular space. iA $\beta$  may induce subcellular compartment structural changes, e.g., stable expression of human iA $\beta$  increases the number of Golgi apparatus elements, lysosomes and lipofuscin bodies in the hippocampus in a double APPxPS1 mutant transgenic rat model [146]. iA $\beta$  is selectively resistant to degradation and accumulates in lysosomes. This lysosomal accumulation is mechanistically similar to prion replication in a cyclic process [147,148]. Accumulation of iA $\beta$  takes place in the solid phase and forms a heterogeneous population and results in nonfibrillary iA $\beta$ -species [149]. Aberrant iA $\beta$  accumulation begins in endosomes. The endosomal-lysosomal (EL) system is one of the most important sites of A $\beta$  metabolism. Insoluble iA $\beta$ , resistant against proteases, accumulates in the EL-system and then the iA $\beta$  aggregates trigger neuronal death [150,151]. A $\beta$  accumulation in the EL system (sometimes called late endosome/multivesicular bodies, MVBs) increases lysosomal membrane permeability and causes the release of the lysosomal content (cathepsins) into the cytoplasm starting apoptotic cell death. One of the earliest events in iA $\beta$ -mediated in vitro neurotoxicity is just the release of lysosomal proteases [152].

In summary, a series of experiments demonstrated the formation and accumulation of iA $\beta$ , including the observation of iA $\beta$  by fluorescent and immune-electron microscopy. The presence of iA $\beta$  in the neurons results in interaction with cellular proteins and subcellular organelles.

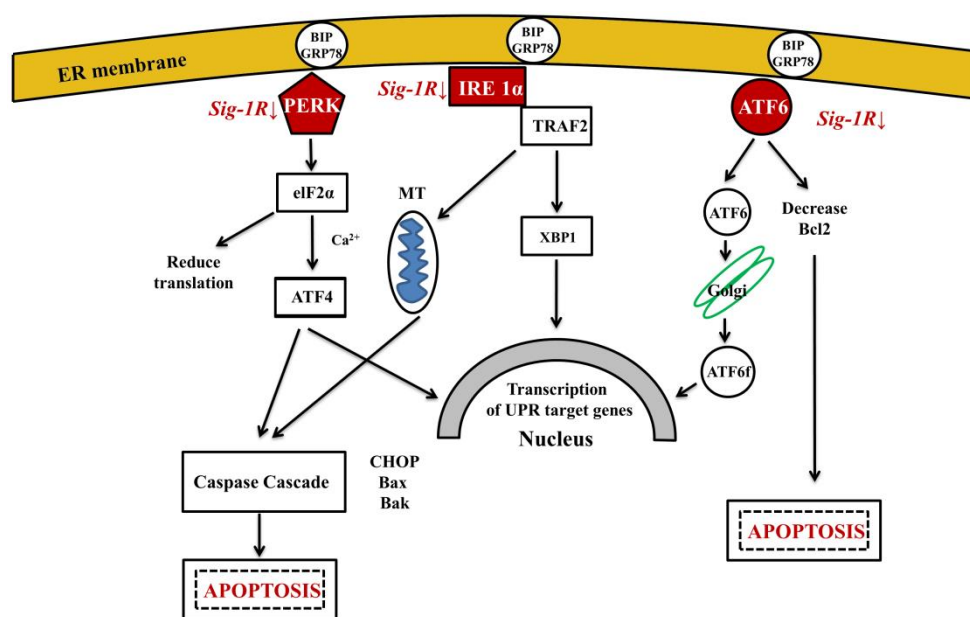
## 6.3. Intracellular A $\beta$ Affects the Function of Subcellular Organelles: Ca<sup>2+</sup> Dysregulation, the ER-Mitochondria Axis and Cross-Talk

Proof of mitochondrial localization of iA $\beta$  is an important result in AD research. Mitochondria play a key role in energy metabolism, their role in aging and AD pathogenesis has been emphasized for years. Mitochondrial A $\beta$  may have a decisive role in the pathological cascade leading to neuronal dysfunction and AD pathology [153–155]. It was found that iA $\beta$  affects mitochondrial failure and dysfunction. Brain hypometabolism and progressive decrease of brain metabolism were early signs of AD and other NDDs [34,156–159]. AD-related hypometabolism is linked with regional vulnerability of neurons in AD-brain [160]. The presence of mitochondrial A $\beta$  correlates well with mitochondrial

dysfunction in the synapses [161]. Changes in the level of individual mitochondrial proteins could serve as early metabolic markers for AD [162]. A $\beta$  was found to impair the import of mitochondrial pre-proteins within the organelle, because of its aggregation with these proteins [163,164].

The endoplasmic reticulum (ER), a dynamic multifunctional organelle, plays a decisive role in Ca<sup>2+</sup> homeostasis, protein folding and transportation and neuronal death. ER is responsible for protein assembly as well as for post-translational modifications of the peptide chains (e.g., glycosylation, disulfide bond formation, etc.). ER also monitors the quality of nascent proteins.

Several factors, including ER Ca<sup>2+</sup>-depletion, metabolic disturbances, oxidative stress and trafficking of proteins through the secretory pathway may initiate misfolded protein accumulation in ER lumen and lead to ER-stress. The possible involvement of ER-stress in the pathogenesis of AD has been studied [165]. It has been shown that ER-stress enhances  $\gamma$ -secretase activity by up-regulating PSEN1 levels and thus it might be involved in A $\beta$ -formation and in the initiation of AD [166]. When cells are exposed to stressful conditions that perturb ER function, unfolded proteins accumulate in ER lumen. For maintaining and re-establishing protein homeostasis, ER activates the unfolded protein response (UPR) [167–169]. Three signaling pathways operate in parallel to sense ER-stress and activate UPR: IRE1 $\alpha$ , PERK and ATF6 [34] (Figure 3). UPR is a bi-functional cellular response to misfolding, having both pro-survival and pro-apoptotic effects. Ca<sup>2+</sup> regulates cell death both at the early and late stages of apoptosis, and severe Ca<sup>2+</sup> dysregulation can promote apoptotic cell death [170]. Recently, the UPR theory has been demonstrated in a *C. elegans* model of AD: basal activity of the UPR was beneficial under normal conditions, however, inducible A $\beta$  peptide expression caused repression of the signaling [171].



**Figure 3.** Three signal pathways of ER-stress activate UPR. ER chaperone GRP78 under normal conditions binds all the three ER-stress sensors (PERK: protein kinase RNA like ER-kinase; IRE1 $\alpha$ : inositol requiring enzyme 1 $\alpha$ ; ATF6: activating transcription factor 6). Under ER-stress GRP78 dissociates from sensors. PERK and IRE1 $\alpha$  will be phosphorylated and oligomerized, ATF6 translocates to the Golgi (Abbreviations: eIF2 $\alpha$ : eukaryotic translation initiation factor 2 $\alpha$ ; XBP1: X-box binding protein 1 (spliced form); TRAF2: TNF-associated factor-2; ATF4: transcriptional activator factor-4; MT: mitochondrium). ATF6, ATF4 and XBP1 activate UPR target genes to enhance the capacity of the ER to cope with unfolded proteins. Activation of Sig-1R inhibits the three branches of UPR.

Very recent experimental results have validated the old Ca<sup>2+</sup> hypothesis of AD: intracellular Ca<sup>2+</sup> (iCa<sup>2+</sup>) plays a key role in the pathomechanism of AD. Dysregulation of iCa<sup>2+</sup> homeostasis has been

suggested as a common proximal cause of neural dysfunction [172,173]. Also targeting  $\text{Ca}^{2+}$ -signaling pathways has been proposed as a novel therapeutic possibility to preserve cognitive functions in AD [174,175].  $\text{iCa}^{2+}$  levels are tightly regulated within a narrow physiological range.  $\text{Ca}^{2+}$  enters the cells via different  $\text{Ca}^{2+}$ -channels (receptor-operated, voltage dependent, store operated channels and sodium/calcium exchanger). Calcium may also be released into the cytoplasm from the ER through inositol-1,4,5-triphosphate ( $\text{IP}_3\text{R}$ ) and ryanodine receptors (RyRs). Different systems counterbalance the  $\text{iCa}^{2+}$  increase in the cell: plasma membrane  $\text{Ca}^{2+}$ -pump and the sarcoendoplasmic reticulum ATP-ase (SERCA) restore physiological  $\text{Ca}^{2+}$ -levels. Mitochondria play a central role also in  $\text{Ca}^{2+}$  buffering and apoptotic signaling. The excess of  $\text{iCa}^{2+}$  can be taken up by mitochondria through the mitochondrial  $\text{Ca}^{2+}$ -uniporter (MCU) [176,177].

ER-to-mitochondria contacts represent important structures linked to multiple cell processes, such as  $\text{Ca}^{2+}$ -signaling, UPR, proliferation and cell death, inflammation, lipid metabolism and autophagy [178]. The ER-mitochondria axis is progressively emerging as a complex signaling platform for cell survival [179,180]. This axis controls cellular  $\text{Ca}^{2+}$ -homeostasis and thus the cellular metabolism, by regulating mitochondrial activity and the action of different hormones. A sub-region of the ER, referred to as MAM (Section 2), is physically and biochemically in contact with the outer mitochondrial membrane. MAM is an inter-organelle interface, its proteins connect the ER to mitochondria. MAMs are intracellular lipid rafts that regulate  $\text{Ca}^{2+}$ -homeostasis and glucose-, phospholipid- and cholesterol metabolism, maintenance of membrane-lipid composition [181,182]. All of these processes are altered in AD.

MAM structure and function as well as ER-mitochondrial interplay have been extensively studied. A series of MAM-associated proteins have been characterized [183,184]. The most important proteins are:

- phosphatidylserine synthase-1 (PSS1)
- fatty acid EoA ligase 4 (FACL4)
- inositol -1,4,5-triphosphate receptors ( $\text{IP}_3\text{Rs}$ )
- voltage-dependent anion channel (VDAC1)
- sigma-1 receptor (Sig-1R)

ER-mitochondria  $\text{Ca}^{2+}$ -transfer is the central process of the communication between the two organelles. A constitutive  $\text{IP}_3$ -receptor mediated ER-MT  $\text{Ca}^{2+}$ -transfer stimulates mitochondrial respiration, increases ATP production and guarantees normal cell bioenergetics [185]. The PSS1 protein is in the ER-side of MAM, it is essential for the transfer of  $\text{Ca}^{2+}$  from the ER at MAM [184]. VDAC1 is localized in the mitochondrial site of MAM and forms bridging complexes for  $\text{Ca}^{2+}$  transport (e.g.,  $\text{IP}_3\text{R}$  1-VDAC1) [186]. The details of the processes have been recently reviewed [187].

AD patients display different early intracellular alteration, such as  $\text{Ca}^{2+}$  and lipid dyshomeostasis, increased ROS-levels, impairments in autophagy, axonal transport and mitochondrial dynamics. MAM is the central hub of these activities [178] and might be the place of APP-processing and  $\text{iA}\beta$  production (see Section 2). In vitro studies demonstrated that APP 695 accumulates in the ER upon ER-stress [188]. Katayama et al. [189] found that the loss or dysregulation of UPR can initiate neuronal degeneration through  $\text{A}\beta$  generation as a consequence of APP processing. It has been proposed that MAM alterations (e.g., upregulation) are the background of LOAD [190]. Pathogenic level of  $\text{A}\beta$  strengthens ER-to-mitochondria  $\text{Ca}^{2+}$ -transfer [191] and an increased ER-mitochondria coupling has been observed in different AD models [191].

In summary,  $\text{iA}\beta$  might be localized in the mitochondria, affecting mitochondrial failure and dysfunction.  $\text{iA}\beta$  disturbs the ER-mitochondrial interplay, the ER-to-mitochondria  $\text{Ca}^{2+}$ -transfer and thus cellular  $\text{Ca}^{2+}$ -homeostasis.



#### 6.4. Molecular Chaperones in AD

Molecular chaperones belong to the most important factors in proteostasis. They form the first line of defence of cells against protein misfolding and subsequent aggregation [192]. Analysis of the human intracellular “chaperome” identified 332 chaperone and co-chaperone genes that were placed into nine families [193]. Five of these chaperone families correspond to heat-shock proteins (HSPs: HSP90, HSP70, HSP60, HSP40 and small HSPs.). Chronic proteotoxic stress results in the induction of the heat shock response (HSR) by the activation of the transcription factor HSF1. This activation leads to the rapid expression of the HSP molecular chaperones [194]. HSPs play various roles in most cellular processes e.g., they regulate stress responses, traffic proteins, buffer apoptotic signalling, shuttle misfolded proteins for degradation by the proteasome or by autophagy. The roles of individual HSPs in proteostasis are widely reviewed [195–197].

Human brain chaperone levels decrease during aging, and cellular senescence of neurons further decreases chaperone activity with age and in NDDs. Therapeutic application or up-regulation of HSPs represent novel possibilities for treatment of NDDs. Overexpression of individual HSPs can be an over-simplification owing to the interdependence of chaperones and compensatory mechanisms within the chaperome. As an example, the chaperone-mediated degradation of aggregation-prone proteins by autophagy required the concerted actions of HSP70 and HSP40 as well as two co-chaperones Hip and Hop [198]. Overexpression of HSP70 in mice could prevent the aggregation of A $\beta$  and the associated toxicity [199]. It was found that A $\beta$  was co-localized with HSPB8 in AD brain [200]. Sequestering proteins into chaperone-enriched aggregates prevented an age-related decline in proteostasis and prolonged lifespan in *C. elegans* [201]. HSPs very probably inhibit aggregate fragmentation to cytotoxic oligomers [202] and facilitate degradation of the aggregates by chaperone-mediated autophagy. A complex chaperon network in ER lumen orchestrates the secretory protein folding [203]. This network ensures proper folding of native proteins in a crowded cellular environment accomplishing highly efficient production of the secretome [204]. Glucose regulated protein 78 (BiP or GRP78) and calreticulin (CRP55) as chaperones of the HSP family stabilize the cell. Sig-1R, a molecular chaperone is also a cytoprotective protein by participating in Ca<sup>2+</sup>-regulation at MAM. Sig-1R helps Ca<sup>2+</sup>-homeostasis by stabilizing IP3 receptor type 3 (IP3R3) [184]. Sig-1R agonists have been used in the therapy of neuropsychiatric diseases (reviewed in [205]).

To summarize these studies, HSP-s play a crucial role in proteostasis. The levels of HSPs decrease during aging and thus formation of misfolded proteins is increased. Simultaneously, HSP-dependent degradation and clearance of amyloid is decreased resulting in a growing imbalance in the mechanism of proteostasis.

#### 6.5. Aggresome Activity for Maintaining Proteostasis

Protein aggregation and the formation of inclusions are closely associated with neuronal degeneration [206]. Aggregation alone provides strong evidence that proteostasis has been disrupted in these diseases. We are only just beginning to understand the complex cellular processes that actively promote the formation of inclusions in cells. It is still not clear that formation of a protein inclusion is a cause or a consequence of NDDs, but it may be detrimental to suppress this process with anti-aggregating small molecules [119].

It is now clear that cells are able to actively sequester misfolded and aggregating proteins into subcellular compartments. (These compartments are not organelles but spatially distinct regions in the cell.) This compartmentalization minimizes the risk that misfolded proteins pose to cells (e.g., nucleation of further proteins) and opens the door for degradation or resolubilization. Several mechanisms for formation of inclusions have been described in the cell:

1. Formation of aggresomes or aggresome-like structures [207]. In aggresomes the sequestered proteins are associated with chaperones and proteasome subunits [208].

2. Insoluble protein deposits (IPODs): the proteins within are not ubiquitinated and mostly form amyloid fibrils [209].
3. Juxtannuclear quality control (JUNQ) [209] inclusions contain ubiquitinated proteins, proteasome subunits and chaperones (e.g., HSP70). JUNQ was proposed to be a cellular quality control center in which soluble misfolded proteins or aggregates from the cytosol accumulate for proteosomal degradation and refolding [209].

#### 6.6. Balancing Proteostasis by Protein Degradation

The ubiquitin-proteasome system (UPS) is the primary selective system in eukaryotic cells, a precise mechanism that maintains the proper concentration of many regulatory proteins of the cell. In addition, UPS is a key component of the proteostasis network to terminate damaged proteins [210]. The UPS degrades more than 80% of normal and abnormal, misfolded intracellular proteins [211].

Ubiquitination is one of the most abundant posttranslational modification in cellular signaling that regulates a broad range of cellular pathways. Ubiquitin (Ub) labels substrate proteins via a highly ordered multi-step enzymatic cascade. In the CNS, Ub contributes to neuronal growth and development, excitability, neurotransmission, long term potentiation as well as synapse formation and elimination [212]. Maintenance of the Ub-proteasome system is central to neuronal health, as neurons are very sensitive to prolonged Ub deficiency which leads to cell death [213]. The ubiquitination machinery is a highly promising target for human therapy [213–215] as much evidence supports the role of compromised Ub-homeostasis in the pathophysiology of NDDs. Probably the most important evidence for the role of Ub in NDDs came from the widely observed enrichment of Ub in cytoplasmic inclusion bodies of many NDDs. Studies on genetically modified, Ub deficient mice demonstrate that the maintenance of Ub homeostasis is necessary for neuronal protection [216].

Components of the Ub-proteasome system are very important potential targets in AD management. The recent therapeutic developments are summarized in [214,215].

The autophagy-lysosome system. The broad term autophagy is a process by which cytoplasmic constituents (e.g., organelles, macromolecules) are degraded by the lysosome [215,217]. Molecular definitions and different subtypes of autophagy were reviewed in 2017 [218]. Autophagy involves the formation of double membrane cytosolic vesicles (known as phagosomes), which transport the engulfed long-lived proteasome resistant proteins and particular organelles (e.g., mitochondria) to the lysosomes for degradation [219]. Failure of each form of autophagy (microautophagy and endosomal microautophagy, chaperone mediated autophagy, macroautophagy) may play an important role in the initiation of NDDs. Mutations of genes regulating autophagy cause NDDs, such as AD, across the age spectrum with exceptional frequency [220]. Recent GWASs suggested that compromised autophagic-lysosomal mechanisms underlie (and thus contribute to) NDDs. Lysosomal pH is acidic and defective lysosomal acidification might cause autophagy failure and thus AD [221]. Numerous studies show lysosomal dysfunction in AD, including perturbed trafficking of lysosomal enzymes and, as a consequence, accumulation of APP metabolites. This evidence leads to the hypothesis that endo-lysosomal and autophagic dysfunction might be a driving factor for AD [222]. Autophagy appears to be mechanistically linked to disease progression in AD [223]. Autophagy vacuoles are abundant in AD neurons and contain substantial amounts of A $\beta$  [224]. Increased autophagy compartments occur at early stages of the disease, when amyloid deposits are not visible. However, autophagy declines with the disease progression. Both the maturation and transport of autophagosomes are impaired and thus, A $\beta$ -containing autophagosomes appear in AD [224,225]. Impaired lysosomal proteolysis may also contribute to A $\beta$  accumulation. Recent studies focuses on the relationship between aging and autophagy gave interesting results [226,227]: senescence of cells was associated with decreased autophagy, decreased expression of autophagy genes. It occurred as the result of the increased methylation of autophagy genes by the DNA methyl transferase DNMT3. Researchers identified the gene encoding ATG5, an autophagy protein involved in the elongation of autophagosomes, as one

of the highly methylated autophagy genes. Evidence demonstrates the role of both cytoplasmic and nuclear mechanisms in the regulation of autophagy [228].

In summary, different protein degradation systems (UPS, several autophagy pathways) serve to maintain proteostasis in cells. There is a great hope that UPS and the autophagy-lysosomal cascade may provide therapeutic targets for the treatments of NDDs, among them in AD [229]. Up-regulation of degradative pathways might be a therapeutic method, however, many unknown factors should still be studied before therapeutic application [119,220].

## 7. Vascular Risk Factors: The Role of the Neurovascular Unit and the BBB in AD

A $\beta$  clearance from the brain is mediated by various mechanisms, such as phagocytosis by glial cells, enzymatic degradation, transport to the cerebrospinal fluid (CSF) with subsequent re-absorption into the venous circulation and direct transport across the BBB [230].

It is well-known that chronic cerebral hypoperfusion and BBB dysfunction could precede the manifestation of AD by years or even decades [231]. Hypoxia activates the amyloidogenic processing of APP leading to A $\beta$  accumulation in the brain [19]. Vascular dysfunction can provoke AD pathology by activating  $\beta$ - and  $\gamma$ -secretases.

The neurovascular unit (NVU) includes clusters of glial cells, neurons and pericytes and undergoes functional changes during aging [232]. These changes may contribute to neuronal injury and cognitive deficit. The BBB is formed by the capillary endothelium, the basement membrane, and the surrounding pericytes and astrocyte end-feet. The BBB operates within the NVU. The role of vascular dysfunction in the pathogenesis of AD has recently been reviewed [233].

Much evidence demonstrates that age and cardiovascular conditions initiate cerebral hypoperfusion (CCH) that increases the risk of AD. Chronic CCH causes BBB dysfunction [233]. The BBB may have fundamental, even though causal role in AD [234]. Three mechanisms are considered to participate in the initiation of the disease: decreased bulk flow, decreased BBB clearance of A $\beta$  and decreased insulin transport:

1. Decreased bulk flow occurs because of reduced production of CSF. With aging (and even more so in AD) the reabsorption of CSF back into the circulation is reduced [235]. CSF levels of neurotoxic substances, such as A $\beta$  increase, this process is further aggravated by a slowing of the glymphatic circulation [234,236] and BBB disruption. Neuroinflammation might be the background of these changes.
2. In AD, the BBB undergoes functional and structural changes that disrupt its gate function, impair energy supply to the brain, reduce the clearance of A $\beta$  and produce neurotoxic molecules. Decreased BBB clearance of A $\beta$  leads to an increased amyloid burden in the brain and the initiation of AD [237]. The brain-to-blood efflux of A $\beta$  is mediated by the lipoprotein receptor related protein (LRP-1), and the ABC subfamily B member 1, also termed P-glycoprotein (P-gp). Data from mouse models suggest that LRP-1 and P-gp expressions decline with age [238] suggesting a possible path for increased A $\beta$  deposition and decreased clearance. Patients with AD show decreased levels of LRP-1 and P-gp [239] in BBB cells, leading to increased oxidation of LRP-1 and decreased level of P-gp activity. Knockdown of LRP-1 in mice recapitulates the key features of AD predicted by the neurovascular hypothesis (decreased A $\beta$ -clearance, increased brain levels of A $\beta$  and impaired cognition). Once initiated, decreased clearance of A $\beta$  can further impair LRP-1 function by oxidation. Decreasing A $\beta$  levels in animal models of AD restores A $\beta$  clearance and cognition [240]. Inflammation also impairs A $\beta$ -clearance from the brain and increases pericyte uptake of A $\beta$ . This mechanism may account for the loss of pericytes seen in AD [241]. Taken together, BBB impairment can promote or even initiate AD: BBB dysfunction causes oxidative stress. Inflammation and mitochondrial dysfunction can mediate a vicious circle causing neuronal damage and increase of A $\beta$  level in the brain [233]. BBB-mediated A $\beta$  accumulation is predominantly based on reduced efflux across the barrier.

3. The brain is dependent on BBB transport of insulin; this hormone has neurotrophic and neuroprotective functions in the CNS. In AD, the transport of insulin across the BBB is decreased. Such a transport defect reinforces that insulin resistance occurs in the brain of AD patients [242]. In clinical studies, delivery of insulin to CNS could improve some aspects of cognition of AD patients within 15 min [243].

In summary, aging and cardiovascular conditions initiate cerebral hypoperfusion and BBB dysfunction that may mediate decreased A $\beta$  clearance and the onset of AD.

## 8. AD as An Inflammatory Disease: The Role of Microglia

Neuroinflammation is one of the main triggers of neurodegeneration. The concept of linking AD to neuroinflammation emerged in the early 90s when it became evident that an altered immune response could be observed in AD. Neuroinflammation is primarily driven by microglia, perivascular myeloid cells and astrocytes, acting as triggers for AD pathogenesis either independently or in combination with the effects of A $\beta$  [244]. Microglia and astrocytes are two major cell components of the CNS innate immune system. Microglia play a macrophage-like role in the immune defense. Microglia exerted neuroprotective activity by degrading A $\beta$  in an A $\beta$  overexpressing AD mouse model (APPxPS1) [245]. Microglia-mediated clearance of A $\beta$  occurs through the TLR4 receptor. As A $\beta$  is a TLR4 ligand, chronic exposure of the receptors to A $\beta$  can result in TLR-signaling dysfunction and inflammation [246]. A $\beta$  aggregates may also interact with other microglial receptors like CD14, CD36, CD47 and RAGE. Inflammasome activation occurs through the binding of A $\beta$  to CD36 [247]. Inflammasomes link vascular disease with neuroinflammation and brain disorders [248]. Microglia regularly monitor their environment and regulate tissue homeostasis through the scavenging functions. Resident microglia have functions related to immune surveillance [249], adult neurogenesis and refinement of synaptic network by synapse pruning [250]. Contrary, novel experiments have demonstrated that microglia mediate synaptic loss in an early-AD mouse model [251]. A $\beta$  1-40 dimer was injected into the brains of wild type mice and a severe synaptic loss was found in 72 hours and postsynapses contained C1q and C3 complement proteins. Blocking the complement pathway (e.g., with anti-C1q antibodies) prevented synaptic loss.

In summary, microglia are a double edged sword playing double role in the onset of AD. Several experimental and epidemiological findings have demonstrate that microglia play a crucial role in A $\beta$  removal by reduction of A $\beta$  plaques, and thus attenuating the disease. On the contrary, other reports propose the harmful role of the activated microglia in AD: A $\beta$  stimulation increased the production of pro-inflammatory cytokines such as TNF $\alpha$  and IL1 $\beta$  [252]. Microglia cells are needed for the protection of neurons (the brain is unprotected against injuries without microglia). Selective inhibition of microglia to switch off the formation of pro-inflammatory cytokines would be a realistic way for attenuating neuroinflammation in AD brain.

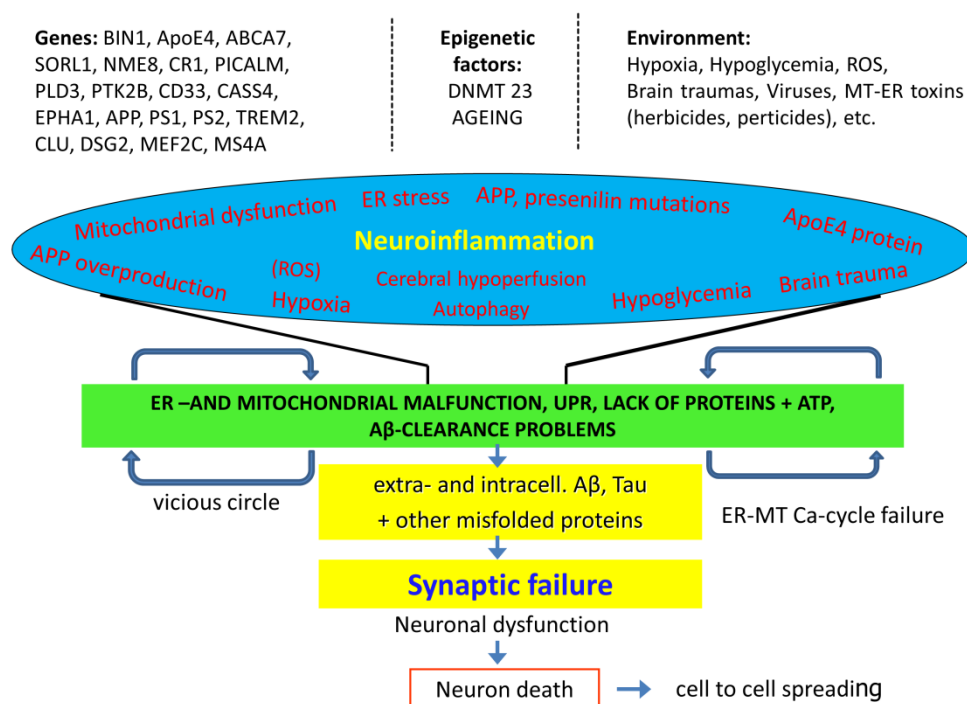
## 9. Conclusions: Major Trends in AD Prevention and Drug Discovery

The precise molecular mechanisms of AD are still not fully understood. The role of very important factors, such as APP and microglia is controversial in the initiation of AD. There is no breakthrough in the development of AD drugs [253]. However, it is clear that AD is a multifactorial disease with complex genetic background, and protein dyshomeostasis and aging are the main risk factors. Figure 4 shows the complexity of AD.

The main problem of the initiation and progression of AD is the dysregulation and imbalance of the proteostasis network in aging. It occurs years or decades before AD is manifested. We know already the sequence of changes that occurs during the asymptomatic phase of AD.

There is a great progress towards the understanding of the structural and physical properties of the amyloid state, the kinetics and mechanism of its formation, and the nature and origin of its links with the disease. We should understand the nature of protein homeostasis in healthy cells as

well as the effect of suppression or inhibition of protein aggregation and amyloid formation to the pathogenesis of AD.



**Figure 4.** Genes of high and low penetrance, as well as epigenetic and environmental factors are participating in AD pathogenesis. Brain traumas, vascular problems (cerebral hypoperfusion, hypoxia, hypoglycemia), declining amyloid degradation and clearance with aging are involved in ER- and mitochondrial dysfunction and protein dyshomeostasis. Amyloid accumulation leads to neuroinflammation, synaptic failure, neuronal dysfunction and death.

Over the last decade, more than 50 drug candidates have successfully passed phase II clinical trials, but none has successfully used in phase III [254]. Most of the drug candidates targeted different forms of A $\beta$  with the aim of removing plaques or stopping them from developing. The failure of these trials changed the aim of AD drug research, the novel aims are prevention of the disease and “to stop Alzheimer’s before it starts” [253]. Prevention strategies [113,255] and new AD-drug targets have been outlined with this aim [119,254,256–258]:

1. Management of modifiable risk factors for dementia [255]. Beyond regular physical activity and decreasing cardiovascular risk, the nutritional factors are in the center of the research. Nutritional approaches have shown limited beneficial effects in clinical studies. The identification of apoE4 as the most prevalent genetic risk factor for AD emphasized the crucial role of lipids in the pathomechanism of AD (see Section 5). Based on the results of apoE and lipid experiments, novel methods are offered: different supplements can be combined in multinutritional approaches [113].
2. Development of compounds acting on the main stages of the pathogenesis of disease (the so called “disease modifying agents”). These drugs could potentially slow down the development of functional (and structural) abnormalities in the CNS, providing improvements of cognitive functions. Potential proteostasis-based therapeutics improving the imbalance between protein production and degradation belong to this group.
3. Focused design of multitargeted drugs acting on multiple molecular targets involved in the pathogenesis of A $\beta$ . Some enzyme inhibitors, (e.g., Sig-1R agonists), anti-inflammatory agents and antioxidants are in these groups.



4. Repositioning of old drugs for new, anti-AD application offers a very attractive approach facilitating clinical trials. The oncolytic drugs bexarotene, tamibarotene, isotretinoin and long-acting insulin analogs belong to this group.

A number of radically new approaches for targeting key stages in AD pathogeneses were proposed and gave hope for successful development of novel therapeutics for AD treatments.

**Acknowledgments:** This work was supported by the grants KTIA\_13\_NAP-A-III/7 and GINOP-2.3.2-15-2016-00060, GINOP-2.3.2-15-2016-00034.

**Author Contributions:** Each author contributed to the writing of the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

A $\beta$	$\beta$ -amyloid peptide
AD	Alzheimer's disease
AICD	APP intracellular domain
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
ATF 6	activating transcription factor 6
ATP	Adenosine triphosphate
BACE	Beta-site APP cleaving enzyme
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2 like protein 4
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
BiP (GRP78)	Binding immunoglobulin protein
CCH	Chronic cerebral hypoperfusion
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNMT	DNA methyl transferum
EOAD	Early onset AD
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
GWAS	Genome wide association studies
HD	Huntington disease
HSP	heat shock protein
IRE1 $\alpha$	inositol requiring enzyme 1 $\alpha$
iA $\beta$	Intracellular A $\beta$
IP <sub>3</sub> R	Inositol-triphosphate receptor
IL1 $\beta$	Interleukin 1 $\beta$
ID	Intrinsically disordered
IPOD	Insoluble protein deposits
JNK	c-Jun amino terminal kinase
LBD	Lewy body disease
LOAD	Late onset AD
LRP1	Lipoprotein receptor related protein-1
LTP	Long term potentiation
MAM	Mitochondrion associated membrane
MVB	Multivesicular body
NDD	Neurodegenerative disease
NVU	Neurovascular unit
PD	Parkinson disease
P-gp	P-glycoprotein

PERK	Protein kinase RNA like ER-kinase
PM	Plasma membrane
PSN1, PSN2	Presenilin 1 and 2
PrP	prion protein
ROS	Reactive oxygene species
SAPP $\alpha$	Soluble APP- $\alpha$
Sig-1R	Sigma1 receptor
SERCA	Sarcoendoplasmic reticulum ATP-ase
SOS	Superoxide dismutase
TLR4	Toll-like receptor 4
TBI	Traumatic brain injury
TGN	Trans Golgi network
TNF $\alpha$	Tumor necrosis factor a
Ub	Ubiquitin
UPR	Unfolded protein response
UPS	Ubiquitin-proteasome system

## References

1. Goloubinoff, P. Mechanisms of protein homeostatis in health, aging and disease. *Swiss Med. Wkly.* **2016**, *146*, w14306. [[PubMed](#)]
2. Khanam, H.; Ali, A.; Asif, M.; Shamsuzzaman. Neurodegenerative diseases linked to misfolded proteins and their therapeutic approaches: A review. *Eur. J. Med. Chem.* **2016**, *124*, 1121–1141. [[CrossRef](#)] [[PubMed](#)]
3. Penke, B.; Bogar, F.; Fulop, L. Protein folding and misfolding, endoplasmic reticulum stress in neurodegenerative diseases: In trace of novel drug targets. *Curr. Protein Pept. Sci.* **2016**, *17*, 169–182. [[CrossRef](#)] [[PubMed](#)]
4. Kovacs, G.G. Molecular pathological classification of neurodegenerative diseases: Turning towards precision medicine. *Int. J. Mol. Sci.* **2016**, *17*, 189. [[CrossRef](#)] [[PubMed](#)]
5. Arneric, S.P.; Batrla-Utermann, R.; Beckett, L.; Bittner, T.; Blennow, K.; Carter, L.; Dean, R.; Engelborghs, S.; Genius, J.; Gordon, M.F.; et al. Cerebrospinal fluid biomarkers for Alzheimer’s disease: A view of the regulatory science qualification landscape from the coalition against major diseases csf biomarker team. *J. Alzheimers Dis.* **2017**, *55*, 19–35. [[CrossRef](#)] [[PubMed](#)]
6. Rathore, S.; Habes, M.; Iftikhar, M.A.; Shacklett, A.; Davatzikos, C. A review on neuroimaging-based classification studies and associated feature extraction methods for Alzheimer’s disease and its prodromal stages. *NeuroImage* **2017**, *155*, 530–548. [[CrossRef](#)] [[PubMed](#)]
7. Chen, G.; Shu, H.; Ward, B.D.; Antuono, P.G.; Zhang, Z.; Li, S.J. Staging Alzheimer’s disease risk by sequencing brain function and structure, cerebrospinal fluid, and cognition biomarkers. *J. Alzheimers Dis.* **2016**, *54*, 983–993. [[CrossRef](#)] [[PubMed](#)]
8. Wang, Y.C.; Lauwers, E.; Verstreken, P. Presynaptic protein homeostasis and neuronal function. *Curr. Opin. Genet. Dev.* **2017**, *44*, 38–46. [[CrossRef](#)] [[PubMed](#)]
9. Ciryam, P.; Kundra, R.; Freer, R.; Morimoto, R.I.; Dobson, C.M.; Vendruscolo, M. A transcriptional signature of Alzheimer’s disease is associated with a metastable subproteome at risk for aggregation. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 4753–4758. [[CrossRef](#)] [[PubMed](#)]
10. Freer, R.; Sormanni, P.; Vecchi, G.; Ciryam, P.; Dobson, C.M.; Vendruscolo, M. A protein homeostasis signature in healthy brains recapitulates tissue vulnerability to Alzheimer’s disease. *Sci. Adv.* **2016**, *2*, e1600947. [[CrossRef](#)] [[PubMed](#)]
11. Boehm, J.; Fernandes, K.; Leclerc, N.; Robitaille, R. The neurobiology of aging and Alzheimer’s disease: Walking down the same road? *Eur. J. Neurosci.* **2013**, *37*, 1885–1886. [[CrossRef](#)] [[PubMed](#)]
12. Neill, D. Should Alzheimer’s disease be equated with human brain ageing?: A maladaptive interaction between brain evolution and senescence. *Ageing Res. Rev.* **2012**, *11*, 104–122. [[CrossRef](#)] [[PubMed](#)]
13. Doehner, J.; Genoud, C.; Imhof, C.; Krstic, D.; Knuesel, I. Extrusion of misfolded and aggregated proteins—A protective strategy of aging neurons? *Eur. J. Neurosci.* **2012**, *35*, 1938–1950. [[CrossRef](#)] [[PubMed](#)]

14. Sturm, A.; Perczel, A.; Ivics, Z.; Vellai, T. The piwi-pirna pathway: Road to immortality. *Aging Cell* **2017**, *16*, 906–911. [[CrossRef](#)] [[PubMed](#)]
15. Hardy, J.; Allsop, D. Amyloid deposition as the central event in the etiology of alzheimers-disease. *Trends Pharmacol. Sci.* **1991**, *12*, 383–388. [[CrossRef](#)]
16. Karran, E.; Mercken, M.; De Strooper, B. The amyloid cascade hypothesis for Alzheimer’s disease: An appraisal for the development of therapeutics. *Nat. Rev. Drug Discov.* **2011**, *10*, 698–712. [[CrossRef](#)] [[PubMed](#)]
17. Aisen, P.S.; Cummings, J.; Jack, C.R.; Morris, J.C.; Sperling, R.; Frolich, L.; Jones, R.W.; Dowsett, S.A.; Matthews, B.R.; Raskin, J.; et al. On the path to 2025: Understanding the Alzheimer’s disease continuum. *Alzheimers Res. Ther.* **2017**, *9*, 60. [[CrossRef](#)] [[PubMed](#)]
18. Jonsson, T.; Atwal, J.K.; Steinberg, S.; Snaedal, J.; Jonsson, P.V.; Bjornsson, S.; Stefansson, H.; Sulem, P.; Gudbjartsson, D.; Maloney, J.; et al. A mutation in app protects against Alzheimer’s disease and age-related cognitive decline. *Nature* **2012**, *488*, 96–99. [[CrossRef](#)] [[PubMed](#)]
19. Salminen, A.; Kauppinen, M.; Kaamiranta, K. Hypoxia/ischemia activate processing of amyloid precursor protein: Impact of vascular dysfunction in the pathogenesis of Alzheimer’s disease. *J. Neurochem.* **2017**, *140*, 536–549. [[CrossRef](#)] [[PubMed](#)]
20. Reinhard, C.; Hebert, S.S.; De Strooper, B. The amyloid-beta precursor protein: Integrating structure with biological function. *EMBO J.* **2005**, *24*, 3996–4006. [[CrossRef](#)] [[PubMed](#)]
21. Thinakaran, G.; Koo, E.H. Amyloid precursor protein trafficking, processing, and function. *J. Biol. Chem.* **2008**, *283*, 29615–29619. [[CrossRef](#)] [[PubMed](#)]
22. Leyssen, M.; Ayaz, D.; Hebert, S.S.; Reeve, S.; De Strooper, B.; Hassan, B.A. Amyloid precursor protein promotes post-developmental neurite arborization in the drosophila brain. *EMBO J.* **2005**, *24*, 2944–2955. [[CrossRef](#)] [[PubMed](#)]
23. Roch, J.M.; Masliah, E.; Rochlevecq, A.C.; Sundsmo, M.P.; Otero, D.A.C.; Veinbergs, I.; Saitoh, T. Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid beta/a4 protein-precursor. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7450–7454. [[CrossRef](#)] [[PubMed](#)]
24. Turner, P.R.; O’Connor, K.; Tate, W.P.; Abraham, W.C. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog. Neurobiol.* **2003**, *70*, 1–32. [[CrossRef](#)]
25. Lee, K.J.; Moussa, C.E.H.; Lee, Y.; Sung, Y.; Howell, B.W.; Turner, R.S.; Pak, D.T.S.; Hoe, H.S. Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines. *Neuroscience* **2010**, *169*, 344–356. [[CrossRef](#)] [[PubMed](#)]
26. Randall, A.D.; Witton, J.; Booth, C.; Hynes-Allen, A.; Brown, J.T. The functional neurophysiology of the amyloid precursor protein (app) processing pathway. *Neuropharmacology* **2010**, *59*, 243–267. [[CrossRef](#)] [[PubMed](#)]
27. Lammich, S.; Kojro, E.; Postina, R.; Gilbert, S.; Pfeiffer, R.; Jasionowski, M.; Haass, C.; Fahrenholz, F. Constitutive and regulated alpha-secretase cleavage of Alzheimer’s amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3922–3927. [[CrossRef](#)] [[PubMed](#)]
28. Lai, A.; Sisodia, S.S.; Trowbridge, I.S. Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. *J. Biol. Chem.* **1995**, *270*, 3565–3573. [[CrossRef](#)] [[PubMed](#)]
29. Perez, R.G.; Soriano, S.; Hayes, J.D.; Ostaszewski, B.; Xia, W.M.; Selkoe, D.J.; Chen, X.H.; Stokin, G.B.; Koo, E.H. Mutagenesis identifies new signals for beta-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including a beta 42. *J. Biol. Chem.* **1999**, *274*, 18851–18856. [[CrossRef](#)] [[PubMed](#)]
30. Grimm, H.S.; Behr, D.; Lichtenthaler, S.F.; Shearman, M.S.; Beyreuther, K.; Hartmann, T. Gamma-secretase cleavage site specificity differs for intracellular and secretory amyloid beta. *J. Biol. Chem.* **2003**, *278*, 13077–13085. [[CrossRef](#)] [[PubMed](#)]
31. Tam, J.H.K.; Seah, C.; Pasternak, S.H. The amyloid precursor protein is rapidly transported from the golgi apparatus to the lysosome and where it is processed into beta-amyloid. *Mol. Brain* **2014**, *7*, 54. [[CrossRef](#)] [[PubMed](#)]
32. Chyung, A.S.C.; Greenberg, B.D.; Cook, D.G.; Doms, R.W.; Lee, V.M.Y. Novel beta-secretase cleavage of beta-amyloid precursor protein in the endoplasmic reticulum intermediate compartment of nt2n cells. *J. Cell. Biol.* **1997**, *138*, 671–680. [[CrossRef](#)] [[PubMed](#)]

33. Shin, R.W.; Saido, T.C.; Maeda, M.; Kitamoto, T. Novel alpha-secretase cleavage of Alzheimer's amyloid beta precursor protein in the endoplasmic reticulum of cos7 cells. *Neurosci. Lett.* **2005**, *376*, 14–19. [[CrossRef](#)] [[PubMed](#)]
34. Placido, A.I.; Pereira, C.M.F.; Duarte, A.I.; Candeias, E.; Correia, S.C.; Santos, R.X.; Carvalho, C.; Cardoso, S.; Oliveira, C.R.; Moreira, P.I. The role of endoplasmic reticulum in amyloid precursor protein processing and trafficking: Implications for Alzheimer's disease. *BBA-Mol. Basis Dis.* **2014**, *1842*, 1444–1453. [[CrossRef](#)] [[PubMed](#)]
35. Vetrivel, K.S.; Thinakaran, G. Membrane rafts in Alzheimer's disease beta-amyloid production. *BBA-Mol. Cell. Biol. Lipid* **2010**, *1801*, 860–867. [[CrossRef](#)] [[PubMed](#)]
36. Nalivaeva, N.N.; Turner, A.J. The amyloid precursor protein: A biochemical enigma in brain development, function and disease. *FEBS Lett.* **2013**, *587*, 2046–2054. [[CrossRef](#)] [[PubMed](#)]
37. Dawkins, E.; Small, D.H. Insights into the physiological function of the beta-amyloid precursor protein: Beyond Alzheimer's disease. *J. Neurochem.* **2014**, *129*, 756–769. [[CrossRef](#)] [[PubMed](#)]
38. van der Kant, R.; Goldstein, L.S. Cellular functions of the amyloid precursor protein from development to dementia. *Dev. Cell* **2015**, *32*, 502–515. [[CrossRef](#)] [[PubMed](#)]
39. Hefter, D.; Draguhn, A. App as a protective factor in acute neuronal insults. *Front. Mol. Neurosci.* **2017**, *10*. [[CrossRef](#)] [[PubMed](#)]
40. Müller, U.C.; Deller, T.; Korte, M. Not just amyloid: Physiological functions of the amyloid precursor protein family. *Nat. Rev. Neurosci.* **2017**, *18*, 281–298. [[CrossRef](#)] [[PubMed](#)]
41. Bukhari, H.; Glotzbach, A.; Kolbe, K.; Leonhardt, G.; Loosse, C.; Muller, T. Small things matter: Implications of app intracellular domain acid nuclear signaling in the progression and pathogenesis of Alzheimer's disease. *Prog. Neurobiol.* **2017**, *156*, 189–213. [[CrossRef](#)] [[PubMed](#)]
42. Grimm, M.O.; Grimm, H.S.; Hartmann, T. Amyloid beta as a regulator of lipid homeostasis. *Trends Mol. Med.* **2007**, *13*, 337–344. [[CrossRef](#)] [[PubMed](#)]
43. Pearson, H.A.; Peers, C. Physiological roles for amyloid beta peptides. *J. Physiol.-Lond.* **2006**, *575*, 5–10. [[CrossRef](#)] [[PubMed](#)]
44. Hiltunen, M.; van Groen, T.; Jolkkonen, J. Functional roles of amyloid-beta protein precursor and amyloid-beta peptides: Evidence from experimental studies. *J. Alzheimers Dis.* **2009**, *18*, 401–412. [[CrossRef](#)] [[PubMed](#)]
45. Giuffrida, M.L.; Caraci, F.; De Bona, P.; Pappalardo, G.; Nicoletti, F.; Rizzarelli, E.; Copani, A. The monomer state of beta-amyloid: Where the Alzheimer's disease protein meets physiology. *Rev. Neurosci.* **2010**, *21*, 83–93. [[CrossRef](#)] [[PubMed](#)]
46. Wogulis, M.; Wright, S.; Cunningham, D.; Chilcote, T.; Powell, K.; Rydel, R.E. Nucleation-dependent polymerization is an essential component of amyloid-mediated neuronal cell death. *J. Neurosci.* **2005**, *25*, 1071–1080. [[CrossRef](#)] [[PubMed](#)]
47. Sakono, M.; Zako, T. Amyloid oligomers: Formation and toxicity of a beta oligomers. *FEBS J.* **2010**, *277*, 1348–1358. [[CrossRef](#)] [[PubMed](#)]
48. Upadhaya, A.R.; Capetillo-Zarate, E.; Kosterin, I.; Abramowski, D.; Kumar, S.; Yamaguchi, H.; Walter, J.; Fandrich, M.; Staufenbiel, M.; Thal, D.R. Dispersible amyloid beta-protein oligomers, protofibrils, and fibrils represent diffusible but not soluble aggregates: Their role in neurodegeneration in amyloid precursor protein (app) transgenic mice. *Neurobiol. Aging* **2012**, *33*, 2641–2660. [[CrossRef](#)] [[PubMed](#)]
49. Jan, A.; Hartley, D.M.; Lashuel, H.A. Preparation and characterization of toxic a beta aggregates for structural and functional studies in Alzheimer's disease research. *Nat. Protoc.* **2010**, *5*, 1186–1209. [[CrossRef](#)] [[PubMed](#)]
50. Hardy, J. The amyloid hypothesis for Alzheimer's disease: A critical reappraisal. *J. Neurochem.* **2009**, *110*, 1129–1134. [[CrossRef](#)] [[PubMed](#)]
51. Benilova, I.; Karran, E.; De Strooper, B. The toxic a beta oligomer and Alzheimer's disease: An emperor in need of clothes. *Nat. Neurosci.* **2012**, *15*, 349–357. [[CrossRef](#)] [[PubMed](#)]
52. Brouillette, J.; Caillierez, R.; Zommer, N.; Alves-Pires, C.; Benilova, I.; Blum, D.; De Strooper, B.; Buee, L. Neurotoxicity and memory deficits induced by soluble low-molecular-weight amyloid-beta(1-42) oligomers are revealed in vivo by using a novel animal model. *J. Neurosci.* **2012**, *32*, 7852–7861. [[CrossRef](#)] [[PubMed](#)]
53. Wildburger, N.C.; Esparza, T.J.; LeDuc, R.D.; Fellers, R.T.; Thomas, P.M.; Cairns, N.J.; Kelleher, N.L.; Bateman, R.J.; Brody, D.L. Diversity of amyloid-beta proteoforms in the Alzheimer's disease brain. *Sci. Rep.* **2017**, *7*. [[CrossRef](#)] [[PubMed](#)]

54. Ruggeri, F.S.; Habchi, J.; Cerreta, A.; Dietler, G. Afm-based single molecule techniques: Unraveling the amyloid pathogenic species. *Curr. Pharm. Des.* **2016**, *22*, 3950–3970. [[CrossRef](#)] [[PubMed](#)]
55. Dobson, C.M. The structural basis of protein folding and its links with human disease. *Philos. T. Roy. Soc. B* **2001**, *356*, 133–145. [[CrossRef](#)] [[PubMed](#)]
56. Dobson, C.M. Protein folding and misfolding. *Nature* **2003**, *426*, 884–890. [[CrossRef](#)] [[PubMed](#)]
57. Goloubinoff, P. Recent and future grand challenges in protein folding, misfolding and degradation. *Front. Mol. Biosci.* **2014**, *1*. [[CrossRef](#)] [[PubMed](#)]
58. Stroo, E.; Koopman, M.; Nollen, E.A.A.; Mata-Cabana, A. Cellular regulation of amyloid formation in aging and disease. *Front. Neurosci.* **2017**, *11*. [[CrossRef](#)] [[PubMed](#)]
59. Nagai, Y.; Inui, T.; Popiel, H.A.; Fujikake, N.; Hasegawa, K.; Urade, Y.; Goto, Y.; Naiki, H.; Toda, T. A toxic monomeric conformer of the polyglutamine protein. *Nat. Struct. Mol. Biol.* **2007**, *14*, 332–340. [[CrossRef](#)] [[PubMed](#)]
60. Kovari, E.; Horvath, J.; Bouras, C. Neuropathology of lewy body disorders. *Brain Res. Bull.* **2009**, *80*, 203–210. [[CrossRef](#)] [[PubMed](#)]
61. Breydo, L.; Wu, J.W.; Uversky, V.N. Alpha-synuclein misfolding and parkinson's disease. *BBA-Mol. Basis Dis.* **2012**, *1822*, 261–285. [[CrossRef](#)] [[PubMed](#)]
62. Lundblad, M.; Decressac, M.; Mattsson, B.; Bjorklund, A. Impaired neurotransmission caused by overexpression of alpha-synuclein in nigral dopamine neurons. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 3213–3219. [[CrossRef](#)] [[PubMed](#)]
63. Sherzai, A.; Edland, S.D.; Masliah, E.; Hansen, L.; Pizzo, D.P.; Corey-Bloom, J. Spongiform change in dementia with lewy bodies and Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **2013**, *27*, 157–161. [[CrossRef](#)] [[PubMed](#)]
64. Uversky, V.N. Mysterious oligomerization of the amyloidogenic proteins. *FEBS J.* **2010**, *277*, 2940–2953. [[CrossRef](#)] [[PubMed](#)]
65. Uversky, V.N. Targeting intrinsically disordered proteins in neurodegenerative and protein dysfunction diseases: Another illustration of the d-2 concept. *Expert Rev. Proteom.* **2010**, *7*, 543–564. [[CrossRef](#)] [[PubMed](#)]
66. Turoverov, K.K.; Kuznetsova, I.M.; Uversky, V.N. The protein kingdom extended: Ordered and intrinsically disordered proteins, their folding, supramolecular complex formation, and aggregation. *Prog. Biophys. Mol. Biol.* **2010**, *102*, 73–84. [[CrossRef](#)] [[PubMed](#)]
67. Uversky, V.N. Intrinsically disordered proteins and novel strategies for drug discovery. *Expert Opin. Drug Dis.* **2012**, *7*, 475–488. [[CrossRef](#)] [[PubMed](#)]
68. Griffith, J.S. Self-replication and scrapie. *Nature* **1967**, *215*, 1043–1044. [[CrossRef](#)] [[PubMed](#)]
69. Prusiner, S.B. Some speculations about prions, amyloid, and Alzheimer's-disease. *N. Engl. J. Med.* **1984**, *310*, 661–663. [[CrossRef](#)] [[PubMed](#)]
70. Nussbaum, J.M.; Schilling, S.; Cynis, H.; Silva, A.; Swanson, E.; Wangsanut, T.; Tayler, K.; Wiltgen, B.; Hatami, A.; Ronicke, R.; et al. Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid-beta. *Nature* **2012**, *485*, 651–655. [[CrossRef](#)] [[PubMed](#)]
71. Eisele, Y.S.; Bolmont, T.; Heikenwalder, M.; Langer, F.; Jacobson, L.H.; Yan, Z.X.; Roth, K.; Aguzzi, A.; Staufenbiel, M.; Walker, L.C.; et al. Induction of cerebral beta-amyloidosis: Intracerebral versus systemic a beta inoculation. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12926–12931. [[CrossRef](#)] [[PubMed](#)]
72. Eisele, Y.S.; Obermuller, U.; Heilbronner, G.; Baumann, F.; Kaeser, S.A.; Wolburg, H.; Walker, L.C.; Staufenbiel, M.; Heikenwalder, M.; Jucker, M. Peripherally applied a beta-containing inoculates induce cerebral beta-amyloidosis. *Science* **2010**, *330*, 980–982. [[CrossRef](#)] [[PubMed](#)]
73. Jucker, M.; Walker, L.C. Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann. Neurol.* **2011**, *70*, 532–540. [[CrossRef](#)] [[PubMed](#)]
74. Stohr, J.; Watts, J.C.; Mensinger, Z.L.; Oehler, A.; Grillo, S.K.; DeArmond, S.J.; Prusiner, S.B.; Giles, K. Purified and synthetic Alzheimer's amyloid beta (a beta) prions. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11025–11030. [[CrossRef](#)] [[PubMed](#)]
75. Auer, S.; Dobson, C.M.; Vendruscolo, M.; Maritan, A. Self-templated nucleation in peptide and protein aggregation. *Phys. Rev. Lett.* **2008**, *101*. [[CrossRef](#)] [[PubMed](#)]
76. Polyimenidou, M.; Cleveland, D.W. Prion-like spread of protein aggregates in neurodegeneration. *J. Exp. Med.* **2012**, *209*, 889–893. [[CrossRef](#)] [[PubMed](#)]



77. Luo, J.H.; Warmlander, S.K.T.S.; Graslund, A.; Abrahams, J.P. Cross-interactions between the Alzheimer disease amyloid-beta peptide and other amyloid proteins: A further aspect of the amyloid cascade hypothesis. *J. Biol. Chem.* **2016**, *291*, 16485–16493. [[CrossRef](#)] [[PubMed](#)]
78. Ugalde, C.L.; Finkelstein, D.I.; Lawson, V.A.; Hill, A.F. Pathogenic mechanisms of prion protein, amyloid-beta and alpha-synuclein misfolding: The prion concept and neurotoxicity of protein oligomers. *J. Neurochem.* **2016**, *139*, 162–180. [[CrossRef](#)] [[PubMed](#)]
79. Aguzzi, A.; Rajendran, L. The transcellular spread of cytosolic amyloids, prions, and prionoids. *Neuron* **2009**, *64*, 783–790. [[CrossRef](#)] [[PubMed](#)]
80. Aguzzi, A.; Falsig, J. Prion propagation, toxicity and degradation. *Nat. Neurosci.* **2012**, *15*, 936–939. [[CrossRef](#)] [[PubMed](#)]
81. Soto, C.; Saborio, G.P.; Anderes, L. Cyclic amplification of protein misfolding: Application to prion-related disorders and beyond. *Trends Neurosci.* **2002**, *25*, 390–394. [[CrossRef](#)]
82. Saa, P.; Castilla, J.; Soto, C. Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. *J. Biol. Chem.* **2006**, *281*, 35245–35252. [[CrossRef](#)] [[PubMed](#)]
83. Murayama, Y.; Yoshioka, M.; Yokoyama, T.; Iwamaru, Y.; Imamura, M.; Masujin, K.; Yoshida, S.; Mohri, S. Efficient in vitro amplification of a mouse-adapted scrapie prion protein. *Neurosci. Lett.* **2007**, *413*, 270–273. [[CrossRef](#)] [[PubMed](#)]
84. Gonzalez-Montalban, N.; Makarava, N.; Ostapchenko, V.G.; Savtchenk, R.; Alexeeva, I.; Rohwer, R.G.; Baskakov, I.V. Highly efficient protein misfolding cyclic amplification. *PLoS Pathog.* **2011**, *7*, e1001277. [[CrossRef](#)] [[PubMed](#)]
85. Moreno-Gonzalez, I.; Soto, C. Misfolded protein aggregates: Mechanisms, structures and potential for disease transmission. *Semin. Cell Dev. Biol.* **2011**, *22*, 482–487. [[CrossRef](#)] [[PubMed](#)]
86. Soto, C.; Estrada, L.; Castilla, J. Amyloids, prions and the inherent infectious nature of misfolded protein aggregates. *Trends Biochem. Sci.* **2006**, *31*, 150–155. [[CrossRef](#)] [[PubMed](#)]
87. Nath, S.; Agholme, L.; Kurudenkandy, F.R.; Granseth, B.; Marcusson, J.; Hallbeck, M. Spreading of neurodegenerative pathology via neuron-to-neuron transmission of beta-amyloid. *J. Neurosci.* **2012**, *32*, 8767–8777. [[CrossRef](#)] [[PubMed](#)]
88. Condello, C.; Stohr, J. Abeta propagation and strains: Implications for the phenotypic diversity in Alzheimer's disease. *Neurobiol. Dis.* **2017**. [[CrossRef](#)] [[PubMed](#)]
89. Qiang, W.; Yau, W.M.; Lu, J.X.; Collinge, J.; Tycko, R. Structural variation in amyloid-beta fibrils from Alzheimer's disease clinical subtypes. *Nature* **2017**, *541*, 217–221. [[CrossRef](#)] [[PubMed](#)]
90. Brundin, P.; Melki, R.; Kopito, R. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat. Rev. Mol. Cell. Biol.* **2010**, *11*, 301–307. [[CrossRef](#)] [[PubMed](#)]
91. Clavaguera, F.; Bolmont, T.; Crowther, R.A.; Abramowski, D.; Frank, S.; Probst, A.; Fraser, G.; Stalder, A.K.; Beibel, M.; Staufenbiel, M.; et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* **2009**, *11*, 909–913. [[CrossRef](#)] [[PubMed](#)]
92. Frost, B.; Jacks, R.L.; Diamond, M.I. Propagation of tau misfolding from the outside to the inside of a cell. *J. Biol. Chem.* **2009**, *284*, 12845–12852. [[CrossRef](#)] [[PubMed](#)]
93. Novak, P.; Prcina, M.; Kontsekova, E. Tauons and prions: Infamous cousins? *J. Alzheimers Dis.* **2011**, *26*, 413–430. [[PubMed](#)]
94. Liu, L.; Drouet, V.; Wu, J.W.; Witter, M.P.; Small, S.A.; Clelland, C.; Duff, K. Trans-synaptic spread of tau pathology in vivo. *PLoS ONE* **2012**, *7*, e31302. [[CrossRef](#)] [[PubMed](#)]
95. De Calignon, A.; Polydoro, M.; Suarez-Calvet, M.; William, C.; Adamowicz, D.H.; Kopeikina, K.J.; Pitstick, R.; Sahara, N.; Ashe, K.H.; Carlson, G.A.; et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* **2012**, *73*, 685–697. [[CrossRef](#)] [[PubMed](#)]
96. Jaworski, T.; Kügler, S.; Van Leuven, F. Modeling of tau-mediated synaptic and neuronal degeneration in Alzheimer's disease. *Int. J. Alzheimers Dis.* **2010**, *2010*, 573138. [[CrossRef](#)] [[PubMed](#)]
97. Hall, G.F.; Patuto, B.A. Is tau ready for admission to the prion club? *Prion* **2012**, *6*, 223–233. [[CrossRef](#)] [[PubMed](#)]
98. Blennow, K.; de Leon, M.J.; Zetterberg, H. Alzheimer's disease. *Lancet* **2006**, *368*, 387–403. [[CrossRef](#)]
99. Tanzi, R.E. The genetics of Alzheimer disease. *CSH Perspect. Med.* **2012**, *2*, a006296. [[CrossRef](#)] [[PubMed](#)]
100. Piaceri, I.; Nacmias, B.; Sorbi, S. Genetics of familial and sporadic Alzheimer's disease. *Front. Biosci. (Elite Ed.)* **2013**, *5*, 167–177. [[CrossRef](#)] [[PubMed](#)]

101. Bettens, K.; Sleegers, K.; Van Broeckhoven, C. Genetic insights in Alzheimer's disease. *Lancet Neurol.* **2013**, *12*, 92–104. [[CrossRef](#)]
102. Giri, M.; Zhang, M.; Lu, Y. Genes associated with Alzheimer's disease: An overview and current status. *Clin. Interv. Aging* **2016**, *11*, 665–681. [[CrossRef](#)] [[PubMed](#)]
103. Karch, C.M.; Cruchaga, C.; Goate, A.M. Alzheimer's disease genetics: From the bench to the clinic. *Neuron* **2014**, *83*, 11–26. [[CrossRef](#)] [[PubMed](#)]
104. De Jonghe, C.; Esselens, C.; Kumar-Singh, S.; Craessaerts, K.; Serneels, S.; Checler, F.; Annaert, W.; Van Broeckhoven, C.; De Strooper, B. Pathogenic app mutations near the gamma-secretase cleavage site differentially affect abeta secretion and app c-terminal fragment stability. *Hum. Mol. Genet.* **2001**, *10*, 1665–1671. [[CrossRef](#)] [[PubMed](#)]
105. Esler, W.P.; Wolfe, M.S. Biomedicine—A portrait of Alzheimer secretases—New features and familiar faces. *Science* **2001**, *293*, 1449–1454. [[CrossRef](#)] [[PubMed](#)]
106. Cacace, R.; Sleegers, K.; Van Broeckhoven, C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimers Dement.* **2016**, *12*, 733–748. [[CrossRef](#)] [[PubMed](#)]
107. Solomon, A.; Kivipelto, M.; Wolozin, B.; Zhou, J.F.; Whitmer, R.A. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. *Dement. Geriatr. Cogn. Disord.* **2009**, *28*, 75–80. [[CrossRef](#)] [[PubMed](#)]
108. Zhang, B.; Tran, L.; Emilsson, V.; Zhu, J. Characterization of genetic networks associated with Alzheimer's disease. *Methods Mol. Biol.* **2016**, *1303*, 459–477. [[PubMed](#)]
109. Holtzman, D.M.; Herz, J.; Bu, G.J. Apolipoprotein e and apolipoprotein e receptors: Normal biology and roles in Alzheimer disease. *CSH Perspect. Med.* **2012**, *2*, a006312. [[CrossRef](#)] [[PubMed](#)]
110. Oikawa, N.; Hatsuta, H.; Murayama, S.; Suzuki, A.; Yanagisawa, K. Influence of apoe genotype and the presence of Alzheimer's pathology on synaptic membrane lipids of human brains. *J. Neurosci. Res.* **2014**, *92*, 641–650. [[CrossRef](#)] [[PubMed](#)]
111. Kojro, E.; Gimpl, G.; Lammich, S.; Marz, W.; Fahrenholz, F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase adam 10. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5815–5820. [[CrossRef](#)] [[PubMed](#)]
112. Harris, B.; Pereira, I.; Parkin, E. Targeting adam10 to lipid rafts in neuroblastoma sh-sy5y cells impairs amyloidogenic processing of the amyloid precursor protein. *Brain Res.* **2009**, *1296*, 203–215. [[CrossRef](#)] [[PubMed](#)]
113. Grimm, M.O.; Michaelson, D.; Hartmann, T. Omega-3 fatty acids, lipids and apoe lipidation in Alzheimer's disease: A rationale for multi-nutrient dementia prevention. *J. Lipid Res.* **2017**. [[CrossRef](#)] [[PubMed](#)]
114. Grimm, M.O.W.; Mett, J.; Grimm, H.S.; Hartmann, T. App function and lipids: A bidirectional link. *Front. Mol. Neurosci.* **2017**, *10*, 63. [[CrossRef](#)] [[PubMed](#)]
115. Wood, W.G.; Li, L.; Muller, W.E.; Eckert, G.P. Cholesterol as a causative factor in Alzheimer's disease: A debatable hypothesis. *J. Neurochem.* **2014**, *129*, 559–572. [[CrossRef](#)] [[PubMed](#)]
116. Grimm, M.O.; Grosgen, S.; Rothhaar, T.L.; Burg, V.K.; Hundsdorfer, B.; Hauptenthal, V.J.; Friess, P.; Muller, U.; Fassbender, K.; Riemenschneider, M.; et al. Intracellular app domain regulates serine-palmitoyl-coa transferase expression and is affected in Alzheimer's disease. *Int. J. Alzheimers Dis.* **2011**, *2011*, 695413. [[CrossRef](#)] [[PubMed](#)]
117. He, X.X.; Huang, Y.; Li, B.; Gong, C.X.; Schuchman, E.H. Deregulation of sphingolipid metabolism in Alzheimer's disease. *Neurobiol. Aging* **2010**, *31*, 398–408. [[CrossRef](#)] [[PubMed](#)]
118. Balch, W.E.; Morimoto, R.I.; Dillin, A.; Kelly, J.W. Adapting proteostasis for disease intervention. *Science* **2008**, *319*, 916–919. [[CrossRef](#)] [[PubMed](#)]
119. Yerbury, J.J.; Ooi, L.; Dillin, A.; Saunders, D.N.; Hatters, D.M.; Beart, P.M.; Cashman, N.R.; Wilson, M.R.; Ecroyd, H. Walking the tightrope: Proteostasis and neurodegenerative disease. *J. Neurochem.* **2016**, *137*, 489–505. [[CrossRef](#)] [[PubMed](#)]
120. Hardy, J.; Selkoe, D.J. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **2002**, *297*, 353–356. [[CrossRef](#)] [[PubMed](#)]
121. Krafft, G.A.; Klein, W.L. Addls and the signaling web that leads to Alzheimer's disease. *Neuropharmacology* **2010**, *59*, 230–242. [[CrossRef](#)] [[PubMed](#)]
122. Schliebs, R.; Arendt, T. The cholinergic system in aging and neuronal degeneration. *Behav. Brain Res.* **2011**, *221*, 555–563. [[CrossRef](#)] [[PubMed](#)]

123. Patel, A.N.; Jhamandas, J.H. Neuronal receptors as targets for the action of amyloid-beta protein (a beta) in the brain. *Expert Rev. Mol. Med.* **2012**, *14*. [[CrossRef](#)] [[PubMed](#)]
124. Xu, Y.Q.; Yan, J.Q.; Zhou, P.; Li, J.J.; Gao, H.M.; Xia, Y.; Wang, Q. Neurotransmitter receptors and cognitive dysfunction in Alzheimer's disease and parkinson's disease. *Prog. Neurobiol.* **2012**, *97*, 1–13. [[CrossRef](#)] [[PubMed](#)]
125. Hartmann, T. Intracellular biology of Alzheimer's disease amyloid beta peptide. *Eur. Arch. Psychiatry Clin. Neurosci.* **1999**, *249*, 291–298. [[CrossRef](#)] [[PubMed](#)]
126. Friedrich, R.P.; Tepper, K.; Roznicke, R.; Soom, M.; Westermann, M.; Reymann, K.; Kaether, C.; Fandrich, M. Mechanism of amyloid plaque formation suggests an intracellular basis of a beta pathogenicity. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1942–1947. [[CrossRef](#)] [[PubMed](#)]
127. Gimenez-Llort, L.; Blazquez, G.; Canete, T.; Johansson, B.; Oddo, S.; Tobena, A.; LaFerla, F.M.; Fernandez-Teruel, A. Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: A role for intraneuronal amyloid. *Neurosci. Biobehav. Rev.* **2007**, *31*, 125–147. [[CrossRef](#)] [[PubMed](#)]
128. Li, M.; Chen, L.; Lee, D.H.S.; Yu, L.C.; Zhang, Y. The role of intracellular amyloid beta in Alzheimer's disease. *Prog. Neurobiol.* **2007**, *83*, 131–139. [[CrossRef](#)] [[PubMed](#)]
129. Gouras, G.K.; Tampellini, D.; Takahashi, R.H.; Capetillo-Zarate, E. Intraneuronal beta-amyloid accumulation and synapse pathology in Alzheimer's disease. *Acta Neuropathol.* **2010**, *119*, 523–541. [[CrossRef](#)] [[PubMed](#)]
130. Bayer, T.A.; Wirths, O. Intracellular accumulation of amyloid-beta—A predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. *Front. Aging Neurosci.* **2010**, *2*, 8. [[CrossRef](#)] [[PubMed](#)]
131. Zhang, Y. Intracellular amyloid  $\beta$ : A modification to the amyloid hypothesis in Alzheimer's disease. In *Lipids and Cellular Membranes in Amyloid Diseases*; Jelinek, R., Ed.; Wiley: New York, NY, USA, 2011; pp. 143–159.
132. Zheng, L.; Cedazo-Minguez, A.; Hallbeck, M.; Jerhammar, F.; Marcusson, J.; Terman, A. Intracellular distribution of amyloid beta peptide and its relationship to the lysosomal system. *Transl. Neurodegener.* **2012**, *1*, 19. [[CrossRef](#)] [[PubMed](#)]
133. Foldi, I.; Datki, Z.L.; Szabo, Z.; Bozso, Z.; Penke, B.; Janaky, T. Proteomic study of the toxic effect of oligomeric abeta1-42 in situ prepared from iso-abeta1-42. *J. Neurochem.* **2011**, *117*, 691–702. [[PubMed](#)]
134. Juhasz, G.; Foldi, I.; Penke, B. Systems biology of Alzheimer's disease: How diverse molecular changes result in memory impairment in ad. *Neurochem. Int.* **2011**, *58*, 739–750. [[CrossRef](#)] [[PubMed](#)]
135. Verdier, Y.; Penke, B. Polypeptide and protein aggregates with neurotoxic properties in neurodegenerative disorders: Emphasis on beta-amyloid-induced dysfunction of receptor-mediated signal transduction pathways. *Curr. Signal Transduct. Ther.* **2006**, *1*, 97–112. [[CrossRef](#)]
136. Verdier, Y.; Foldi, I.; Sergeant, N.; Fulop, L.; Penke, Z.; Janaky, T.; Szucs, M.; Penke, B. Characterization of the interaction between a beta 1-42 and glyceraldehyde phosphodehydrogenase. *J. Pept. Sci.* **2008**, *14*, 755–762. [[CrossRef](#)] [[PubMed](#)]
137. Penke, B.; Toth, A.M.; Foldi, I.; Szucs, M.; Janaky, T. Intraneuronal ss-amyloid and its interactions with proteins and subcellular organelles. *Electrophoresis* **2012**, *33*, 3608–3616. [[CrossRef](#)] [[PubMed](#)]
138. Takahashi, R.H.; Nagao, T.; Gouras, G.K. Plaque formation and the intraneuronal accumulation of beta-amyloid in Alzheimer's disease. *Pathol. Int.* **2017**, *67*, 185–193. [[CrossRef](#)] [[PubMed](#)]
139. Casas, C.; Sergeant, N.; Itier, J.M.; Blanchard, V.; Wirths, O.; van der Kolk, N.; Vingtdoux, V.; van de Steeg, E.; Ret, G.; Canton, T.; et al. Massive ca1/2 neuronal loss with intraneuronal and n-interminal truncated a beta(42) accumulation in a novel Alzheimer transgenic model. *Am. J. Pathol.* **2004**, *165*, 1289–1300. [[CrossRef](#)]
140. Billings, L.M.; Oddo, S.; Green, K.N.; McGaugh, J.L.; LaFerla, F.M. Intraneuronal abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron* **2005**, *45*, 675–688. [[CrossRef](#)] [[PubMed](#)]
141. Tomiyama, T.; Matsuyama, S.; Iso, H.; Umeda, T.; Takuma, H.; Ohnishi, K.; Ishibashi, K.; Teraoka, R.; Sakama, N.; Yamashita, T.; et al. A mouse model of amyloid beta oligomers: Their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss in vivo. *J. Neurosci.* **2010**, *30*, 4845–4856. [[CrossRef](#)] [[PubMed](#)]

142. Abramowski, D.; Rabe, S.; Upadhaya, A.R.; Reichwald, J.; Danner, S.; Staab, D.; Capetillo-Zarate, E.; Yamaguchi, H.; Saido, T.C.; Wiederhold, K.H.; et al. Transgenic expression of intraneuronal a beta(42) but not a beta(40) leads to cellular a beta lesions, degeneration, and functional impairment without typical Alzheimer's disease pathology. *J. Neurosci.* **2012**, *32*, 1273–1283. [[CrossRef](#)] [[PubMed](#)]
143. Claeysen, S.; Cochet, M.; Donneger, R.; Dumuis, A.; Bockaert, J.; Giannoni, P. Alzheimer culprits: Cellular crossroads and interplay. *Cell Signal.* **2012**, *24*, 1831–1840. [[CrossRef](#)] [[PubMed](#)]
144. Burgos, P.V.; Mardones, G.A.; Rojas, A.L.; daSilva, L.L.P.; Prabhu, Y.; Hurley, J.H.; Bonifacino, J.S. Sorting of the Alzheimer's disease amyloid precursor protein mediated by the ap-4 complex. *Dev. Cell* **2010**, *18*, 425–436. [[CrossRef](#)] [[PubMed](#)]
145. Nixon, R.A. Autophagy in neurodegenerative disease: Friend, foe or turncoat? *Trends Neurosci.* **2006**, *29*, 528–535. [[CrossRef](#)] [[PubMed](#)]
146. Lopez, E.M.; Bell, K.F.S.; Ribeiro-da-Silva, A.; Cuello, A.C. Early changes in neurons of the hippocampus and neocortex in transgenic rats expressing intracellular human a-beta. *J. Alzheimers Dis.* **2004**, *6*, 421–431. [[CrossRef](#)] [[PubMed](#)]
147. Gajdusek, D.C. Transmissible and non-transmissible amyloidoses: Autocatalytic post-translational conversion of host precursor proteins to beta-pleated sheet configurations. *J. Neuroimmunol.* **1988**, *20*, 95–110. [[CrossRef](#)]
148. Prusiner, S.B.; Dearmond, S.J. Prions causing nervous-system degeneration. *Lab. Investig.* **1987**, *56*, 349–363. [[PubMed](#)]
149. Glabe, C. Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *J. Mol. Neurosci.* **2001**, *17*, 137–145. [[CrossRef](#)]
150. Nagele, R.G.; D'Andrea, M.R.; Anderson, W.J.; Wang, H.Y. Intracellular accumulation of beta-amyloid(1-42) in neurons is facilitated by the alpha 7 nicotinic acetylcholine receptor in Alzheimer's disease. *Neuroscience* **2002**, *110*, 199–211. [[CrossRef](#)]
151. Burdick, D.; Kosmoski, J.; Knauer, M.F.; Glabe, C.G. Preferential adsorption, internalization and resistance to degradation of the major isoform of the Alzheimer's amyloid peptide, a beta 1–42, in differentiated pc12 cells. *Brain Res.* **1997**, *746*, 275–284. [[CrossRef](#)]
152. Ditaranto, K.; Tekirian, T.L.; Yang, A.J. Lysosomal membrane damage in soluble a beta-mediated cell death in Alzheimer's disease. *Neurobiol. Dis.* **2001**, *8*, 19–31. [[CrossRef](#)] [[PubMed](#)]
153. Lustbader, J.W.; Cirilli, M.; Lin, C.; Xu, H.W.; Takuma, K.; Wang, N.; Caspersen, C.; Chen, X.; Pollak, S.; Chaney, M.; et al. Abad directly links a beta to mitochondrial toxicity in Alzheimer's disease. *Science* **2004**, *304*, 448–452. [[CrossRef](#)] [[PubMed](#)]
154. Chen, X.; Yan, S.D. Mitochondrial abeta: A potential cause of metabolic dysfunction in Alzheimer's disease. *IUBMB Life* **2006**, *58*, 686–694. [[CrossRef](#)] [[PubMed](#)]
155. Lin, M.T.; Beal, M.F. Alzheimer's app mangles mitochondria. *Nat. Med.* **2006**, *12*, 1241–1243. [[CrossRef](#)] [[PubMed](#)]
156. O'Brien, R.J.; Wong, P.C. Amyloid precursor protein processing and Alzheimer's disease. *Annu. Rev. Neurosci.* **2011**, *34*, 185–204. [[CrossRef](#)] [[PubMed](#)]
157. Blair, J.A.; Siedlak, S.L.; Wolfram, J.A.; Nunomura, A.; Castellani, R.J.; Ferreira, S.T.; Klein, W.L.; Wang, Y.; Casadesus, G.; Smith, M.A.; et al. Accumulation of intraneuronal amyloid-beta is common in normal brain. *Curr. Alzheimer Res.* **2014**, *11*, 317–324. [[CrossRef](#)] [[PubMed](#)]
158. Alberdi, E.; Wyssenbach, A.; Alberdi, M.; Sanchez-Gomez, M.V.; Cavaliere, F.; Rodriguez, J.J.; Verkhratsky, A.; Matute, C. Ca<sup>2+</sup>-dependent endoplasmic reticulum stress correlates with astrogliosis in oligomeric amyloid-treated astrocytes and in a model of Alzheimer's disease. *Aging Cell* **2013**, *12*, 292–302. [[CrossRef](#)] [[PubMed](#)]
159. Endres, K.; Reinhardt, S. Er-stress in Alzheimer's disease: Turning the scale? *Am. J. Neurodegener Dis.* **2013**, *2*, 247–265. [[PubMed](#)]
160. Oh, H.; Madison, C.; Baker, S.; Rabinovici, G.; Jagust, W. Dynamic relationships between age, amyloid-beta deposition, and glucose metabolism link to the regional vulnerability to Alzheimer's disease. *Brain* **2016**, *139*, 2275–2289. [[CrossRef](#)] [[PubMed](#)]
161. Roussel, B.D.; Kruppa, A.J.; Miranda, E.; Crowther, D.C.; Lomas, D.A.; Marciniak, S.J. Endoplasmic reticulum dysfunction in neurological disease. *Lancet Neurol.* **2013**, *12*, 105–118. [[CrossRef](#)]



162. Volgyi, K.; Juhasz, G.; Kovacs, Z.; Penke, B. Dysfunction of endoplasmic reticulum (er) and mitochondria (mt) in Alzheimer's disease: The role of the er-mt cross-talk. *Curr. Alzheimer Res.* **2015**, *12*, 655–672. [[CrossRef](#)] [[PubMed](#)]
163. Mossmann, D.; Vogtle, F.N.; Taskin, A.A.; Teixeira, P.F.; Ring, J.; Burkhart, J.M.; Burger, N.; Pinho, C.M.; Tadic, J.; Loreth, D.; et al. Amyloid-beta peptide induces mitochondrial dysfunction by inhibition of preprotein maturation. *Cell Metab.* **2014**, *20*, 662–669. [[CrossRef](#)] [[PubMed](#)]
164. Cenini, G.; Rub, C.; Bruderek, M.; Voos, W. Amyloid beta-peptides interfere with mitochondrial preprotein import competence by a coaggregation process. *Mol. Biol. Cell.* **2016**, *27*, 3257–3272. [[CrossRef](#)] [[PubMed](#)]
165. Hosoi, T.; Matsuzaki, S.; Miyahara, T.; Shimizu, K.; Hasegawa, Y.; Ozawa, K. Possible involvement of 15-deoxy-delta(12,14)-prostaglandin j(2) in the development of leptin resistance. *J. Neurochem.* **2015**, *133*, 343–351. [[CrossRef](#)] [[PubMed](#)]
166. Ohta, K.; Mizuno, A.; Li, S.M.; Itoh, M.; Ueda, M.; Ohta, E.; Hida, Y.; Wang, M.X.; Furoi, M.; Tsuzuki, Y.; et al. Endoplasmic reticulum stress enhances gamma-secretase activity. *Biochem. Biophys. Res. Commun.* **2011**, *416*, 362–366. [[CrossRef](#)] [[PubMed](#)]
167. Hetz, C. The unfolded protein response: Controlling cell fate decisions under er stress and beyond. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 89–102. [[CrossRef](#)] [[PubMed](#)]
168. Pereira, C.M. Crosstalk between endoplasmic reticulum stress and protein misfolding in neurodegenerative diseases. *ISRN Cell Biol.* **2013**, *2013*. [[CrossRef](#)] [[PubMed](#)]
169. Scheper, W.; Hoozemans, J.J.M. The unfolded protein response in neurodegenerative diseases: A neuropathological perspective. *Acta Neuropathol.* **2015**, *130*, 315–331. [[CrossRef](#)] [[PubMed](#)]
170. Bahar, E.; Kim, H.; Yoon, H. Er stress-mediated signaling: Action potential and ca(2+) as key players. *Int. J. Mol. Sci.* **2016**, *17*, 1558. [[CrossRef](#)] [[PubMed](#)]
171. Safra, M.; Ben-Hamo, S.; Kenyon, C.; Henis-Korenblit, S. The ire-1 er stress-response pathway is required for normal secretory-protein metabolism in c. *Elegans*. *J. Cell Sci.* **2013**, *126*, 4136–4146. [[CrossRef](#)] [[PubMed](#)]
172. Magi, S.; Castaldo, P.; Macri, M.L.; Maiolino, M.; Matteucci, A.; Bastioli, G.; Gratteri, S.; Amoroso, S.; Lariccia, V. Intracellular calcium dysregulation: Implications for Alzheimer's disease. *Biomed. Res. Int.* **2016**, *2016*. [[CrossRef](#)] [[PubMed](#)]
173. Berridge, M.J. Calcium regulation of neural rhythms, memory and Alzheimer's disease. *J. Physiol.-Lond.* **2014**, *592*, 281–293. [[CrossRef](#)] [[PubMed](#)]
174. Popugaeva, E.; Pchitskaya, E.; Bezprozvanny, I. Dysregulation of neuronal calcium homeostasis in Alzheimer's disease—A therapeutic opportunity? *Biochem. Biophys. Res. Commun.* **2017**, *483*, 998–1004. [[CrossRef](#)] [[PubMed](#)]
175. Briggs, C.A.; Chakroborty, S.; Stutzmann, G.E. Emerging pathways driving early synaptic pathology in Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **2017**, *483*, 988–997. [[CrossRef](#)] [[PubMed](#)]
176. Liao, Y.; Dong, Y.; Cheng, J. The function of the mitochondrial calcium uniporter in neurodegenerative disorders. *Int. J. Mol. Sci.* **2017**, *18*, 248. [[CrossRef](#)] [[PubMed](#)]
177. De Stefani, D.; Rizzuto, R.; Pozzan, T. Enjoy the trip: Calcium in mitochondria back and forth. *Annu. Rev. Biochem.* **2016**, *85*, 161–192. [[CrossRef](#)] [[PubMed](#)]
178. Filadi, R.; Theurey, P.; Pizzo, P. The endoplasmic reticulum-mitochondria coupling in health and disease: Molecules, functions and significance. *Cell Calcium* **2017**, *62*, 1–15. [[CrossRef](#)] [[PubMed](#)]
179. Paillusson, S.; Stoica, R.; Gomez-Suaga, P.; Lau, D.H.W.; Mueller, S.; Miller, T.; Miller, C.C.J. There's something wrong with my mam; the er-mitochondria axis and neurodegenerative diseases. *Trends Neurosci.* **2016**, *39*, 146–157. [[CrossRef](#)] [[PubMed](#)]
180. Joshi, A.U.; Kornfeld, O.S.; Mochly-Rosen, D. The entangled er-mitochondrial axis as a potential therapeutic strategy in neurodegeneration: A tangled duo unchained. *Cell Calcium* **2016**, *60*, 218–234. [[CrossRef](#)] [[PubMed](#)]
181. Schon, E.A.; Area-Gomez, E. Mitochondria-associated er membranes in Alzheimer disease. *Mol. Cell. Neurosci.* **2013**, *55*, 26–36. [[CrossRef](#)] [[PubMed](#)]
182. Schon, E.A.; Area-Gomez, E. Is Alzheimer's disease a disorder of mitochondria-associated membranes? *J. Alzheimers Dis.* **2010**, *20*, S281–S292. [[CrossRef](#)] [[PubMed](#)]
183. Csordas, G.; Renken, C.; Varnai, P.; Walter, L.; Weaver, D.; Buttle, K.F.; Balla, T.; Mannella, C.A.; Hajnoczky, G. Structural and functional features and significance of the physical linkage between er and mitochondria. *J. Cell Biol.* **2006**, *174*, 915–921. [[CrossRef](#)] [[PubMed](#)]



184. Hayashi, T.; Rizzuto, R.; Hajnoczky, G.; Su, T.P. Mam: More than just a housekeeper. *Trends Cell Biol.* **2009**, *19*, 81–88. [[CrossRef](#)] [[PubMed](#)]
185. Cardenas, C.; Miller, R.A.; Smith, I.; Bui, T.; Molgo, J.; Muller, M.; Vais, H.; Cheung, K.H.; Yang, J.; Parker, I.; et al. Essential regulation of cell bioenergetics by constitutive insp(3) receptor ca<sup>2+</sup> transfer to mitochondria. *Cell* **2010**, *142*, 270–283. [[CrossRef](#)] [[PubMed](#)]
186. Szabadkai, G.; Bianchi, K.; Varnai, P.; De Stefani, D.; Wieckowski, M.R.; Cavagna, D.; Nagy, A.I.; Balla, T.; Rizzuto, R. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial ca<sup>2+</sup> channels. *J. Cell Biol.* **2006**, *175*, 901–911. [[CrossRef](#)] [[PubMed](#)]
187. Filadi, R.; Pozzan, T. Generation and functions of second messengers microdomains. *Cell Calcium* **2015**, *58*, 405–414. [[CrossRef](#)] [[PubMed](#)]
188. Domingues, S.C.; Henriques, A.G.; Wu, W.; Da Cruz e Silva, E.F.; Da Cruz e Silva, O.A. Altered subcellular distribution of the Alzheimer’s amyloid precursor protein under stress conditions. *Ann. N. Y. Acad. Sci.* **2007**, *1096*, 184–195. [[CrossRef](#)] [[PubMed](#)]
189. Katayama, T.; Imaizumi, K.; Sato, N.; Miyoshi, K.; Kudo, T.; Hitomi, J.; Morihara, T.; Yoneda, T.; Gomi, F.; Mori, Y.; et al. Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. *Nat. Cell Biol.* **1999**, *1*, 479–485. [[CrossRef](#)] [[PubMed](#)]
190. Area-Gomez, E.; Del Carmen Lara Castillo, M.; Tambini, M.D.; Guardia-Laguarta, C.; de Groof, A.J.; Madra, M.; Ikenouchi, J.; Umeda, M.; Bird, T.D.; Sturley, S.L.; et al. Upregulated function of mitochondria-associated er membranes in Alzheimer disease. *EMBO J.* **2012**, *31*, 4106–4123. [[CrossRef](#)] [[PubMed](#)]
191. Hedskog, L.; Pinho, C.M.; Filadi, R.; Ronnback, A.; Hertwig, L.; Wiehager, B.; Larssen, P.; Gellhaar, S.; Sandebring, A.; Westerlund, M.; et al. Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer’s disease and related models. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 7916–7921. [[CrossRef](#)] [[PubMed](#)]
192. Hartl, F.U.; Bracher, A.; Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. *Nature* **2011**, *475*, 324–332. [[CrossRef](#)] [[PubMed](#)]
193. Brehme, M.; Voisine, C.; Rolland, T.; Wachi, S.; Soper, J.H.; Zhu, Y.; Orton, K.; Villella, A.; Garza, D.; Vidal, M.; et al. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* **2014**, *9*, 1135–1150. [[CrossRef](#)] [[PubMed](#)]
194. Baird, N.A.; Douglas, P.M.; Simic, M.S.; Grant, A.R.; Moresco, J.J.; Wolff, S.C.; Yates, J.R.; Manning, G.; Dillin, A. Hsf-1-mediated cytoskeletal integrity determines thermotolerance and life span. *Science* **2014**, *346*, 360–363. [[CrossRef](#)] [[PubMed](#)]
195. Brandvold, K.R.; Morimoto, R.I. The chemical biology of molecular chaperones-implications for modulation of proteostasis. *J. Mol. Biol.* **2015**, *427*, 2931–2947. [[CrossRef](#)] [[PubMed](#)]
196. Duncan, E.J.; Cheetham, M.E.; Chapple, J.P.; van der Spuy, J. The role of hsp70 and its co-chaperones in protein misfolding, aggregation and disease. *Subcell. Biochem.* **2015**, *78*, 243–273. [[PubMed](#)]
197. Pratt, W.B.; Gestwicki, J.E.; Osawa, Y.; Lieberman, A.P. Targeting hsp90/hsp70-based protein quality control for treatment of adult onset neurodegenerative diseases. *Annu. Rev. Pharmacol. Toxicol.* **2015**, *55*, 353–371. [[CrossRef](#)] [[PubMed](#)]
198. Agarraberes, F.A.; Dice, J.F. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. *J. Cell Sci.* **2001**, *114*, 2491–2499. [[PubMed](#)]
199. Hoshino, T.; Muraio, N.; Namba, T.; Takehara, M.; Adachi, H.; Katsuno, M.; Sobue, G.; Matsushima, T.; Suzuki, T.; Mizushima, T. Suppression of Alzheimer’s disease-related phenotypes by expression of heat shock protein 70 in mice. *J. Neurosci.* **2011**, *31*, 5225–5234. [[CrossRef](#)] [[PubMed](#)]
200. Wilhelmus, M.M.M.; Boelens, W.C.; Otte-Holler, I.; Kamps, B.; Kusters, B.; Maat-Schieman, M.L.C.; Waal, R.M.W.; Verbeek, M.M. Small heat shock protein hspb8: Its distribution in Alzheimer’s disease brains and its inhibition of amyloid-beta protein aggregation and cerebrovascular amyloid-beta toxicity. *Acta Neuropathol.* **2006**, *111*, 139–149. [[CrossRef](#)] [[PubMed](#)]
201. Walther, D.M.; Kasturi, P.; Zheng, M.; Pinkert, S.; Vecchi, G.; Ciryam, P.; Morimoto, R.I.; Dobson, C.M.; Vendruscolo, M.; Mann, M.; et al. Widespread proteome remodeling and aggregation in aging c-elegans. *Cell* **2015**, *161*, 919–932. [[CrossRef](#)] [[PubMed](#)]
202. Knowles, T.P.J.; Vendruscolo, M.; Dobson, C.M. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 384–396. [[CrossRef](#)] [[PubMed](#)]

203. Gidalevitz, T.; Stevens, F.; Argon, Y. Orchestration of secretory protein folding by er chaperones. *BBA-Mol. Cell Res.* **2013**, *1833*, 2410–2424. [[CrossRef](#)] [[PubMed](#)]
204. Jansen, G.; Maattanen, P.; Denisov, A.Y.; Scarffe, L.; Schade, B.; Balghi, H.; Dejgaard, K.; Chen, L.Y.; Muller, W.J.; Gehring, K.; et al. An interaction map of endoplasmic reticulum chaperones and foldases. *Mol. Cell. Proteom.* **2012**, *11*, 710–723. [[CrossRef](#)] [[PubMed](#)]
205. Penke, B.; Fülöp, L.; Szűcs, M.; Frecska, E. The role of sigma-1 receptor, an intracellular chaperone in neurodegenerative diseases. *Curr. Neuropharmacol.* **2017**. [[CrossRef](#)] [[PubMed](#)]
206. Leigh, P.N.; Whitwell, H.; Garofalo, O.; Buller, J.; Swash, M.; Martin, J.E.; Gallo, J.M.; Weller, R.O.; Anderton, B.H. Ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic-lateral-sclerosis-morphology, distribution, and specificity. *Brain* **1991**, *114*, 775–788. [[CrossRef](#)] [[PubMed](#)]
207. Johnston, J.A.; Ward, C.L.; Kopito, R.R. Aggresomes: A cellular response to misfolded proteins. *J. Cell Biol.* **1998**, *143*, 1883–1898. [[CrossRef](#)] [[PubMed](#)]
208. Kopito, R.R. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* **2000**, *10*, 524–530. [[CrossRef](#)]
209. Kaganovich, D.; Kopito, R.; Frydman, J. Misfolded proteins partition between two distinct quality control compartments. *Nature* **2008**, *454*, 1088–1095. [[CrossRef](#)] [[PubMed](#)]
210. Tanaka, K.; Matsuda, N. Proteostasis and neurodegeneration: The roles of proteasomal degradation and autophagy. *BBA-Mol. Cell Res.* **2014**, *1843*, 197–204. [[CrossRef](#)] [[PubMed](#)]
211. Wang, J.S.; Maldonado, M.A. The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. *Cell. Mol. Immunol.* **2006**, *3*, 255–261. [[PubMed](#)]
212. Mabb, A.M.; Ehlers, M.D. Ubiquitination in postsynaptic function and plasticity. *Annu Rev. Cell Dev. Biol.* **2010**, *26*, 179–210. [[CrossRef](#)] [[PubMed](#)]
213. Jankowska, E.; Stoj, J.; Karpowicz, P.; Osmulski, P.A.; Gaczynska, M. The proteasome in health and disease. *Curr. Pharm. Des.* **2013**, *19*, 1010–1028. [[PubMed](#)]
214. Gadhve, K.; Bolshette, N.; Ahire, A.; Pardeshi, R.; Thakur, K.; Trandafir, C.; Istrate, A.; Ahmed, S.; Lahkar, M.; Muresanu, D.F.; et al. The ubiquitin proteasomal system: A potential target for the management of Alzheimer’s disease. *J. Cell. Mol. Med.* **2016**, *20*, 1392–1407. [[CrossRef](#)] [[PubMed](#)]
215. Ciechanover, A.; Kwon, Y.T. Degradation of misfolded proteins in neurodegenerative diseases: Therapeutic targets and strategies. *Exp. Mol. Med.* **2015**, *47*, e147. [[CrossRef](#)] [[PubMed](#)]
216. Ryu, K.Y.; Sinnar, S.A.; Reinholdt, L.G.; Vaccari, S.; Hall, S.; Garcia, M.A.; Zaitseva, T.S.; Bouley, D.M.; Boekelheide, K.; Handel, M.A.; et al. The mouse polyubiquitin gene ubb is essential for meiotic progression. *Mol. Cell. Biol.* **2008**, *28*, 1136–1146. [[CrossRef](#)] [[PubMed](#)]
217. Vilchez, D.; Saez, I.; Dillin, A. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nat. Commun.* **2014**, *5*, 5659. [[CrossRef](#)] [[PubMed](#)]
218. Galluzzi, L.; Baehrecke, E.H.; Ballabio, A.; Boya, P.; Bravo-San Pedro, J.M.; Cecconi, F.; Choi, A.M.; Chu, C.T.; Codogno, P.; Colombo, M.I.; et al. Molecular definitions of autophagy and related processes. *EMBO J.* **2017**, *36*, 1811–1836. [[CrossRef](#)] [[PubMed](#)]
219. Ravikumar, B.; Sarkar, S.; Davies, J.E.; Futter, M.; Garcia-Arencibia, M.; Green-Thompson, Z.W.; Jimenez-Sanchez, M.; Korolchuk, V.I.; Lichtenberg, M.; Luo, S.Q.; et al. Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol. Rev.* **2010**, *90*, 1383–1435. [[CrossRef](#)] [[PubMed](#)]
220. Nixon, R.A. The role of autophagy in neurodegenerative disease. *Nat. Med.* **2013**, *19*, 983–997. [[CrossRef](#)] [[PubMed](#)]
221. Wolfe, D.M.; Lee, J.H.; Kumar, A.; Lee, S.; Orenstein, S.J.; Nixon, R.A. Autophagy failure in Alzheimer’s disease and the role of defective lysosomal acidification. *Eur. J. Neurosci.* **2013**, *37*, 1949–1961. [[CrossRef](#)] [[PubMed](#)]
222. Whyte, L.S.; Lau, A.A.; Hemsley, K.M.; Hopwood, J.J.; Sargeant, T.J. Endo-lysosomal and autophagic dysfunction: A driving factor in Alzheimer’s disease? *J. Neurochem.* **2017**, *140*, 703–717. [[CrossRef](#)] [[PubMed](#)]
223. Moreau, K.; Fleming, A.; Imarisio, S.; Ramirez, A.L.; Mercer, J.L.; Jimenez-Sanchez, M.; Bento, C.F.; Puri, C.; Zavodszky, E.; Siddiqi, F.; et al. Picalm modulates autophagy activity and tau accumulation. *Nat. Commun.* **2014**, *5*. [[CrossRef](#)] [[PubMed](#)]
224. Nixon, R.A.; Wegiel, J.; Kumar, A.; Yu, W.H.; Peterhoff, C.; Cataldo, A.; Cuervo, A.M. Extensive involvement of autophagy in Alzheimer disease: An immuno-electron microscopy study. *J. Neuropathol. Exp. Neurol.* **2005**, *64*, 113–122. [[CrossRef](#)] [[PubMed](#)]

225. Boland, B.; Kumar, A.; Lee, S.; Platt, F.M.; Wegiel, J.; Yu, W.H.; Nixon, R.A. Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *J. Neurosci.* **2008**, *28*, 6926–6937. [[CrossRef](#)] [[PubMed](#)]
226. Khalil, H.; Tazi, M.; Caution, K.; Ahmed, A.; Kanneganti, A.; Assani, K.; Kopp, B.; Marsh, C.; Dakhallah, D.; Amer, A.O. Aging is associated with hypermethylation of autophagy genes in macrophages. *Epigenetics* **2016**, *11*, 381–388. [[CrossRef](#)] [[PubMed](#)]
227. Chen, S.; Jing, Y.Y.; Kang, X.; Yang, L.; Wang, D.L.; Zhang, W.; Zhang, L.; Chen, P.; Chang, J.F.; Yang, X.M.; et al. Histone h2b monoubiquitination is a critical epigenetic switch for the regulation of autophagy. *Nucleic Acids Res.* **2017**, *45*, 1144–1158. [[CrossRef](#)] [[PubMed](#)]
228. Fullgrabe, J.; Klionsky, D.J.; Joseph, B. The return of the nucleus: Transcriptional and epigenetic control of autophagy. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 65–74. [[CrossRef](#)] [[PubMed](#)]
229. Friedman, L.G.; Qureshi, Y.H.; Yu, W.H. Promoting autophagic clearance: Viable therapeutic targets in Alzheimer's disease. *Neurotherapeutics* **2015**, *12*, 94–108. [[CrossRef](#)] [[PubMed](#)]
230. Zlokovic, B.V. Clearing amyloid through the blood-brain barrier. *J. Neurochem.* **2004**, *89*, 807–811. [[CrossRef](#)] [[PubMed](#)]
231. De la Torre, J.C. Vascular risk factor detection and control may prevent Alzheimer's disease. *Ageing Res. Rev.* **2010**, *9*, 218–225. [[CrossRef](#)] [[PubMed](#)]
232. Zenaro, E.; Piacentino, G.; Constantin, G. The blood-brain barrier in Alzheimer's disease. *Neurobiol. Dis.* **2016**, 41–56. [[CrossRef](#)] [[PubMed](#)]
233. Di Marco, L.Y.; Venneri, A.; Farkas, E.; Evans, P.C.; Marzo, A.; Frangi, A.F. Vascular dysfunction in the pathogenesis of Alzheimer's disease—A review of endothelium-mediated mechanisms and ensuing vicious circles. *Neurobiol. Dis.* **2015**, *82*, 593–606. [[CrossRef](#)] [[PubMed](#)]
234. Banks, W.A. From blood-brain barrier to blood-brain interface: New opportunities for cns drug delivery. *Nat. Rev. Drug Discov.* **2016**, *15*, 275–292. [[CrossRef](#)] [[PubMed](#)]
235. Alafuzoff, I.; Adolfsson, R.; Grundke-Iqbal, I.; Winblad, B. Blood-brain barrier in Alzheimer dementia and in non-demented elderly. An immunocytochemical study. *Acta Neuropathol.* **1987**, *73*, 160–166. [[CrossRef](#)] [[PubMed](#)]
236. Kress, B.T.; Iliff, J.J.; Xia, M.S.; Wang, M.H.; Wei, H.L.S.; Zeppenfeld, D.; Xie, L.L.; Kang, H.Y.; Xu, Q.W.; Liew, J.A.; et al. Impairment of paravascular clearance pathways in the aging brain. *Ann. Neurol.* **2014**, *76*, 845–861. [[CrossRef](#)] [[PubMed](#)]
237. Zlokovic, B.V. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* **2005**, *28*, 202–208. [[CrossRef](#)] [[PubMed](#)]
238. Silverberg, G.D.; Messier, A.A.; Miller, M.C.; Machan, J.T.; Majmudar, S.S.; Stopa, E.G.; Donahue, J.E.; Johanson, C.E. Amyloid efflux transporter expression at the blood-brain barrier declines in normal aging. *J. Neuropathol. Exp. Neurol.* **2010**, *69*, 1034–1043. [[CrossRef](#)] [[PubMed](#)]
239. Wijesuriya, H.C.; Bullock, J.Y.; Faull, R.L.M.; Hladky, S.B.; Barrand, M.A. Abc efflux transporters in brain vasculature of Alzheimer's subjects. *Brain Res.* **2010**, *1358*, 228–238. [[CrossRef](#)] [[PubMed](#)]
240. Banks, W.A.; Kumar, V.B.; Farr, S.A.; Nakaoke, R.; Robinson, S.M.; Morley, J.E. Impairments in brain-to-blood transport of amyloid-beta and reabsorption of cerebrospinal fluid in an animal model of Alzheimer's disease are reversed by antisense directed against amyloid-beta protein precursor. *J. Alzheimers Dis.* **2011**, *23*, 599–605. [[PubMed](#)]
241. Sengillo, J.D.; Winkler, E.A.; Walker, C.T.; Sullivan, J.S.; Johnson, M.; Zlokovic, B.V. Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* **2013**, *23*, 303–310. [[CrossRef](#)] [[PubMed](#)]
242. Talbot, K.; Wang, H.Y.; Kazi, H.; Han, L.Y.; Bakshi, K.P.; Stucky, A.; Fuino, R.L.; Kawaguchi, K.R.; Samoyedny, A.J.; Wilson, R.S.; et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with igf-1 resistance, irs-1 dysregulation, and cognitive decline. *J. Clin. Investig.* **2012**, *122*, 1316–1338. [[CrossRef](#)] [[PubMed](#)]
243. Reger, M.A.; Watson, G.S.; Green, P.S.; Baker, L.D.; Cholerton, B.; Fishel, M.A.; Plymate, S.R.; Cherrier, M.M.; Schellenberg, G.D.; Frey, W.H.; et al. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J. Alzheimers Dis.* **2008**, *13*, 323–331. [[CrossRef](#)] [[PubMed](#)]

244. McGeer, P.L.; McGeer, E.G. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: Implications for therapy. *Acta Neuropathol.* **2013**, *126*, 479–497. [[CrossRef](#)] [[PubMed](#)]
245. Takata, K.; Kitamura, Y.; Saeki, M.; Terada, M.; Kagitani, S.; Kitamura, R.; Fujikawa, Y.; Maelicke, A.; Tomimoto, H.; Taniguchi, T.; et al. Galantamine-induced amyloid-beta clearance mediated via stimulation of microglial nicotinic acetylcholine receptors. *J. Biol. Chem.* **2010**, *285*, 40180–40191. [[CrossRef](#)] [[PubMed](#)]
246. Go, M.; Kou, J.H.; Lim, J.E.; Yang, J.L.; Fukuchi, K.I. Microglial response to Ips increases in wild-type mice during aging but diminishes in an Alzheimer’s mouse model: Implication of tlr4 signaling in disease progression. *Biochem. Biophys. Res. Commun.* **2016**, *479*, 331–337. [[CrossRef](#)] [[PubMed](#)]
247. Sheedy, F.J.; Grebe, A.; Rayner, K.J.; Kalantari, P.; Ramkhalawon, B.; Carpenter, S.B.; Becker, C.E.; Ediriweera, H.N.; Mullick, A.E.; Golenbock, D.T.; et al. Cd36 coordinates nlrp3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nat. Immunol.* **2013**, *14*, 812–820. [[CrossRef](#)] [[PubMed](#)]
248. Lenart, N.; Brough, D.; Denes, A. Inflammasomes link vascular disease with neuroinflammation and brain disorders. *J. Cereb. Blood Flow Metab.* **2016**, *36*, 1668–1685. [[CrossRef](#)] [[PubMed](#)]
249. Matcovitch-Natan, O.; Winter, D.R.; Giladi, A.; Aguilar, S.V.; Spinrad, A.; Sarrazin, S.; Ben-Yehuda, H.; David, E.; Gonzalez, F.Z.; Perrin, P.; et al. Microglia development follows a stepwise program to regulate brain homeostasis. *Science* **2016**, *353*. [[CrossRef](#)] [[PubMed](#)]
250. Ransohoff, R.M.; El Khoury, J. Microglia in health and disease. *CSH Perspect Biol.* **2016**, *8*, a020560. [[CrossRef](#)] [[PubMed](#)]
251. Hong, S.; Beja-Glasser, V.F.; Nfonoyim, B.M.; Frouin, A.; Li, S.M.; Ramakrishnan, S.; Merry, K.M.; Shi, Q.Q.; Rosenthal, A.; Barres, B.A.; et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* **2016**, *352*, 712–716. [[CrossRef](#)] [[PubMed](#)]
252. Elali, A.; Rivest, S. Microglia in Alzheimer’s disease: A multifaceted relationship. *Brain Behav. Immun.* **2016**, *55*, 138–150. [[CrossRef](#)] [[PubMed](#)]
253. McDade, E.; Bateman, R.J. Stop Alzheimer’s before it starts. *Nature* **2017**, *547*, 153–155. [[CrossRef](#)] [[PubMed](#)]
254. Bachurin, S.O.; Bovina, E.V.; Ustyugov, A.A. Drugs in clinical trials for Alzheimer’s disease: The major trends. *Med. Res. Rev.* **2017**, *37*, 1186–1225. [[CrossRef](#)] [[PubMed](#)]
255. Baumgart, M.; Snyder, H.M.; Carrillo, M.C.; Fazio, S.; Kim, H.; Johns, H. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. *Alzheimers Dement.* **2015**, *11*, 718–726. [[CrossRef](#)] [[PubMed](#)]
256. Cummings, J.; Aisen, P.S.; DuBois, B.; Frolich, L.; Jack, C.R., Jr.; Jones, R.W.; Morris, J.C.; Raskin, J.; Dowsett, S.A.; Scheltens, P. Drug development in Alzheimer’s disease: The path to 2025. *Alzheimers Res. Ther.* **2016**, *8*, 39. [[CrossRef](#)] [[PubMed](#)]
257. Cazarim, M.D.; Moriguti, J.C.; Ogunjimi, A.T.; Pereira, L.R.L. Perspectives for treating Alzheimer’s disease: A review on promising pharmacological substances. *Sao Paulo Med. J.* **2016**, *134*, 342–354. [[CrossRef](#)] [[PubMed](#)]
258. Parsons, C.G.; Rammes, G. Preclinical to phase 2 amyloid beta (a) peptide modulators under investigation for Alzheimer’s disease. *Expert Opin. Inv. Drug* **2017**, *26*, 579–591. [[CrossRef](#)] [[PubMed](#)]

