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Review - Part of the Special Issue: Alzheimer's Disease - Amyloid, Tau and Beyond

Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers

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ABSTRACT

The global prevalence of dementia is as high as 24 million, and has been predicted to quadruple by the year 2050. In the US alone, Alzheimer disease (AD) – the most frequent cause of dementia characterized by a progressive decline in cognitive function in particular the memory domain – causes estimated health-care costs of $ 172 billion per year. Key neuropathological hallmarks of the AD brain are diffuse and neuritic extracellular amyloid plaques – often surrounded by dystrophic neurites – and intracellular neurofibrillary tangles. These pathological changes are frequently accompanied by reactive microgliosis and loss of neurons, white matter and synapses. The etiological mechanisms underlying these neuropathological changes remain unclear, but are probably caused by both environmental and genetic factors. In this review article, we provide an overview of the epidemiology of AD, review the biomarkers that may be used for risk assessment and in diagnosis, and give suggestions for future research.

Contents

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1. Introduction

The global prevalence of dementia, which is characterized by progressive deterioration in cognition, function and behavior, places a considerable burden on society. Currently, the prevalence is estimated to amount to 24 million and predicted to quadruple by the year 2050. In the US alone, Alzheimer disease (AD) – the most frequent cause of dementia – is associated with estimated health-care costs of $172 billion per year [1].

The key pathological changes observed in AD brain tissue are amyloid-β (Aβ) peptide deposited extracellularly in diffuse and neuritic plaques, and hyperphosphorylated tau (p-tau) protein, a microtubule assembly protein accumulating intracellularly as neurofibrillary tangles (NFTs). Additional changes include reactive microgliosis and widespread loss of neurons, white matter and synapses. The exact mechanisms leading to these changes remain to be determined.

2. Diagnostic criteria

Since their proposal in 1984, the key classification for the diagnosis of AD has been the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria [2]. These criteria combine clinical and neuropathological patterns and assign diagnoses of "possible", "probable" and "definite AD" [2]. The AD spectrum is now recognized to be broader than was previously thought and is acknowledged to include pathological changes other than amyloid plaques and NFTs (see below). Correspondingly, the NINCDS–ADRDA criteria are again under review. The biomarkers incorporated in the updated criteria are expected to increase the diagnostic specificity. In 1999, an intermediate state between normal cognition and dementia has been defined as "mild cognitive impairment (MCI)". In particular in clinical settings MCI has proved a useful label to define people who are at risk of developing AD.

3. Prevalence and incidence

By 2005, 24.2 million people worldwide had dementia and 4.6 million new cases were arising every year [3]. Approximately 70% of these cases were attributed to AD. Among regional populations of 60 year-olds, those from North America and Western Europe are believed to exhibit the highest prevalence and incidence rate of dementia, followed by those from Latin America and China and its western-Pacific neighbors (Fig. 1a and b) [3]. For all these populations, the incidence rate for dementia increases exponentially with age, with the most pronounced increase occurring through the 7th and 8th decades of life. Similar patterns are observed for the prevalence and incidence of AD. There is evidence that in western societies prevalence and increase display a cohort effect with later-born individuals having a lower risk than those born earlier in the past century [4–7] (Fig. 2).

4. Genetic epidemiology of AD

Based on its age of onset, AD is classified into early onset AD (EOAD, onset <65 years) accounting for 1–5% of all cases, and late-onset AD (LOAD, onset ≥65 years) accounting for >95% of affecteds. While clinically indistinguishable from LOAD, EOAD is generally associated with a more rapid rate of progression and a Mendelian pattern of inheritance. Three genes (*APP*, *PSEN1* and *PSEN2*) which all encode proteins involved in APP breakdown and Aβ generation, have been firmly implicated in the pathophysiology of EOAD. AD-linked mutations in these three genes exhibit high penetrance (>85%), are mostly autosomal dominantly inherited, and lead with certainty to Aβ aggregation and early-onset disease. Consequently, they are considered 'diagnostic biomarkers' of the disease.

In contrast, the genes involved in LOAD increase disease risk in a non-Mendelian fashion. First-degree relatives of patients with LOAD have twice the expected life-time risk of people without an AD-affected first-degree relative. In addition, LOAD occurs more frequently in monozygotic than in dizygotic co-twins, suggesting a substantial genetic contribution of ~60–80% to this disorder.

For more than a decade, only one genetic risk factor, the *APOE*ε4 allele, located on chromosome 19q13, was an unequivocally established "susceptibility" gene in non-Hispanic Whites of European ancestry. APOE is a lipid-binding protein and is expressed in humans as three common isoforms coded for by three alleles, APOEε2, ε3, and ε4. A single *APOE-ε4* allele is associated with a 2- to 3-fold increased risk, having two copies is associated with a five-fold or more increase [8]. In addition, each

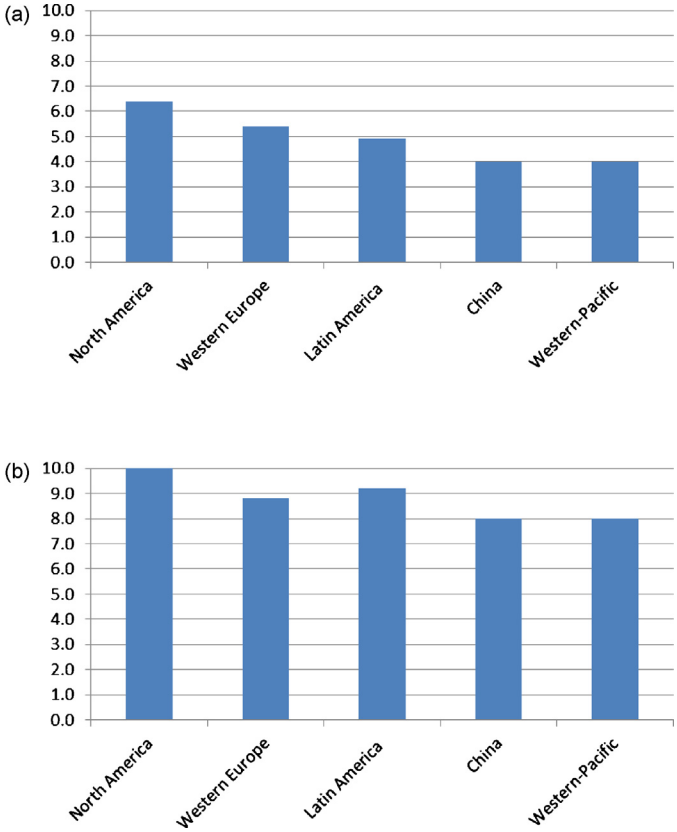

Fig. 1. (a) Global prevalence of dementia (%) [3]. (b) Incidence rates (per 1000 individuals in the population) [3].

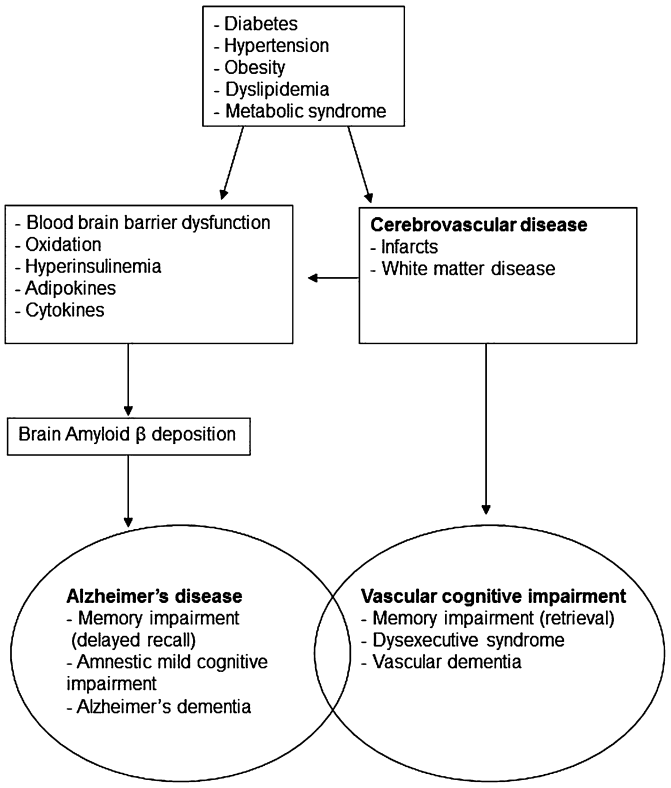

Fig. 2. Potential mechanisms linking vascular risk factors and cognitive impairment.

inherited APOEε4 allele lowers the age-at-onset by 6–7 years [9–16]. APOEε4 is also associated with lower cognitive performance, in particular the memory domain, is associated with mild cognitive impairment (MCI) [17–20] and with progression from MCI to dementia [17–27]. While the population attributable risk for APOEε4 is estimated at 20–50% [28], the presence of ε4 is neither necessary nor sufficient for developing the disease [29]. In ethnic groups other than non-Hispanic Whites the association between APOE and LOAD was largely inconsistent across studies.

4.1. Findings from genome-wide association studies (GWAS)

In the beginning of the century, thousands of candidate-gene-based association studies aiming to identify additional susceptibility loci were performed but only one gene, the sortilin-related receptor (*SORL1*) which is implicated in intracellular trafficking of APP, could be consistently replicated in independent datasets and implicated in the disease. The main reasons for these inconsistencies between studies are sample heterogeneity with differences in linkage disequilibrium (LD) patterns and allele frequencies, and small sample sizes leading to limited power to detect small or moderate effect sizes. In the past five years, technological advances in high-throughput genome-wide arrays allowed the hypothesis-free simultaneous examination of thousands to millions of polymorphisms across the genome, and large international collaborative efforts capitalizing on this technology have significantly advanced the knowledge on the genetic underpinnings of LOAD and pathways involved by identifying more than 20 novel risk loci.

The major GWAS studies contributing to this gained knowledge are summarized in Table 1. Most were performed in non-Hispanic Whites of European ancestry. The first set of studies identified *CLU*, *PICALM*, *CR1* and *BIN1* as susceptibility loci [30–32]. CLU, also known as apolipoprotein J (ApoJ), is a lipoprotein highly expressed in both the periphery and the brain [33]. Like ApoE, it is involved in lipid transport [34]. Clu is also hypothesized to act as an extracellular chaperone that influences Aβ-aggregation and receptor-mediated Aβ clearance by endocytosis [33]. Unlike APOE, there are no known coding variants that account for the observed genetic association to CLU, suggesting that genetic variation in expression levels may be responsible for the altered risk for LOAD [35]. *BIN1* (amphiphysin II) is a member of the Bin1/amphiphysin/RVS167 (BAR) family of genes that have been involved in diverse cellular processes, including actin dynamics, membrane trafficking and clathrin-mediated endocytosis [36] which affect APP processing and Aβ production or Aβ clearance from brain. PICALM is also involved in clathrin-mediated endocytosis and recruits clathrin and adaptor protein complex 2 to sites of vesicle assembly [37]. CR1 is a cell-surface receptor that is part of the complement system. It has binding sites for complement factors C3b and C4b and is involved in clearing immune-complexes containing these two proteins. Since Aβ oligomers can bind C3b, CR1 may participate in the clearance of Aβ. CR1 may also play a role in neuroinflammation, which is a prominent feature in AD [38]. Interestingly, Clu may play a role in this process as an inhibitor [39]. In summary, this first set of GWAS identified loci mainly clustering in three pathways, namely immune response, APP processing and lipid metabolism and endocytosis.

The second set of large GWAS studies identified additional susceptibility genes (*CD33*, *MS4A4A/MS4A4E/MS4A6E cluster*, *ABCA7*, *CD2AP* and *EPHA1*) [40,41]. In line with the pathways identified by the first set of GWAS, all of these five loci are likely involved in the immune system while the ABCA7 is in addition

Table 1
Major GWAS studies performed.

Study	Ethnic group	Sample size	Genes identified outside *APOE* region
[31]	Caucasian	Stage 1: 2032 AD cases; 5328 controls Stage 2: 3978 AD cases; 3297 controls	*CLU*, *CR1*
[30]	Caucasian	Stage 1: 3941 AD cases; 7848 controls Stage 2: 2023 AD cases; 2340 controls	*CLU*, *PICALM*
[32]	Caucasian	Stage 1: 3006 AD cases; 4642 controls Stage 2: 2032 AD cases; 5328 controls Stage 3: 3333 AD cases; 6995 controls	*BIN1*, *XOC3L2/BLOC1S3/MARK4*, *CLU*, *PICALM*
[41]	Caucasian	Stage 1: 8309 AD cases; 7366 controls Stage 2: 3531 AD cases; 3565 controls	*MS4A4A*, *CD2AP*, *CD33* and *EPHA1*, *CR1*, *CLU*, *BIN1*, *PICALM*
[40]	Caucasian	Stage 1: 6688 AD cases; 13,685 controls Stage 2: 4896 AD cases; 4903 controls Stage 3: 8286 AD cases; 21,258 controls	*ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33*, *CD2AP*
[54]	Caucasian	Stage 1: 17,008 AD cases; 37,646 controls Stage 2: 8572 AD cases; 11,312 controls	*CR1*, *CD33*, *BIN1*, *CD2AP*, *CLU*, *EPHA1*, *PICALM*, *MS4,ABCA7HLA-DRB5/HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4/RIN3*, *DSG2*
[59]	Caribbean Hispanic	549 AD cases; 544 controls	*CLU*, *PICALM*, *BIN1*, *CUGBP2*, loci on 2p25.1; 3q25.2; 7p21.1; 10q23.1
[60]	African American	1968 AD cases; 3928 controls	*ABCA7*, *intergenic locus on 5q35.2*

involved in lipid metabolism and APP processing. The *CD33* gene encodes a protein that is a member of a family of cell surface immune receptors that bind extracellular sialylated glycans and signal via a cytoplasmic domain called the immunoreceptor tyrosine inhibitory motif [42,43]. CD33 has primarily been studied in the peripheral immune system where it is expressed on myeloid progenitors and monocytes and also in the brain. In the periphery, CD33 appears to inhibit proliferation of myeloid cells [44]. The *MS4A4A/MS4A4E/MS4A6E* locus is part of a cluster of 15 *MS4A* genes on chromosome 11 and encodes proteins with multiple membrane-spanning domains that were initially identified by their homology to CD20, a B-lymphocyte cell surface molecule. Little is known about the function of MS4A4A gene products; however, like CD33, MS4A4A is expressed on myeloid cells and monocytes and likely has an immune-related function. *EPHA1* encodes a member of the ephrin family of cell surface receptors which interact with ephrin ligands on adjacent cells to modulate cell adhesion, migration, and axon guidance and synapse formation and plasticity. While there is a substantial body of research on the function of ephrin receptors in general, little is known about the EPHA1 gene product. Like other ephrin receptors, it regulates cell morphology and motility [45] and early work implicated this receptor in regulating vascular morphogenesis and angiogenesis [46]. EPHA1 knockout in mouse results in abnormal tail and reproductive tract development [47], but no effects on the brain. Consistent with this notion, in mouse, expression is restricted to epithelial tissue. In humans, EPHA1 is expressed by CD4-positive T lymphocytes [48], monocytes [49], intestinal epithelium, and colon. Combined with the lack of evidence for brain expression this may suggest that, like CD33, CR1, and MS4A4/MS4A6E, the role of the EPHA1 gene product in AD may be mediated though the immune system. The CD2 associated protein gene (CD2AP) encodes a scaffolding protein that binds directly to actin [50], nephrin and other proteins involved in cytoskeletal organization. In the immune system, CD2AP is required for synapse formation [51] in a process that involves clathrin-dependent actin polymerization. *ABCA7* is an integral transmembrane ATP-binding cassette transporter belonging to the ABC family proteins that mediate the biogenesis of high-density lipoprotein with cellular lipid and helical apolipoproteins [52]. It binds APOA-I and functions in apolipoprotein-mediated phospholipid and cholesterol efflux from cells [53]. In addition, *ABCA7* affects the transport of other important proteins, including amyloid precursor protein [53], through the cell membrane and is involved in host defense through effects on phagocytosis by macrophages of apoptotic cells [52].

Finally, the largest GWAS to date, performed by the International Genomics of Alzheimer's Project (IGAP), combined all non-Hispanic White datasets from the four individual consortia and performed a mega-metaanalysis that included 74,046 subjects [54]. In addition to *APOE*, *CR1*, *BIN1*, *CD2AP*, *EPHA1*, *CLU*, *MS4A6A*, *PICALM*, *ABCA7* and *CD33* this GWAS identified 12 additional susceptibility loci at genome-wide significance (*HLA-DRB5/HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4/RIN3*, *DSG2*, *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2*, *CASS4*) and an additional 13 candidate loci with *p*-values between $p = 7.4 \times 10^{-7}$ and $p = 6.6 \times 10^{-8}$ (intergenic locus, *HS3ST1*, *SQSTM1*, *TREML2*, *NDUFAF6*, *ECHDC3*, *AP2A2*, *ADAMTS20*, *IGH*, *SPPL2A*, *TRIP4*, *SCIMP*, *ACE*). As described above, the *SORL1* (sortilin-related receptor, L (DLR class) 1) had previously been demonstrated to modulate trafficking and processing of APP in a candidate gene approach [55,56]. With the exception of *CD33* and *DSG2*, all loci could be validated in a replication stage. Failure to replicate *DSG2* encoding desmoglein2 – a calcium-binding transmembrane glycoprotein component of desmosomes in vertebrate epithelial cells – was expected as evidence for this locus in the discovery stage was based on a single SNP and was not supported by any SNP in LD. Out of the 12 novel loci reaching genome-wide significance, most cluster in the specific pathways identified by the earlier GWAS, i.e. immune response (*HLA-DRB5/DRB1*, *INPP5D*, *MEF2C*), APP processing (*SORL1*, *CASS4*), Tau pathology (*CASS4*, *FERMT2*), cell migration (*PTK2B*) and lipid transport and endocytosis (*SORL1*) strongly reinforcing the importance of these pathways in LOAD etiology. In addition, consistent with the notion of a complex disease, the findings of this study further suggest the existence of additional pathways. One of these may be hippocampal synaptic function: MEF2C limits excessive synapse formation during activity-dependent refinement of synaptic connectivity and thus may facilitate hippocampal-dependent learning and memory [57]. Mutations and deletions at this locus have also been are associated with mental retardation, stereotypic movements, epilepsy, and cerebral malformation. The protein encoded by *PTK2B* is involved in induction of long-term potentiation in the hippocampal CA1 (cornu ammonis 1) region, a central process in the formation of memories. *PTK2B* is only ~130 kb away from *CLU*. However, the fact that the two most strongly associated SNPs at these loci are not in LD and that there is a recombination hotspot between these loci suggests that both associations are independent. Another pathway suggested by the signals in *CELF1*, *NME8* and *CASS4* may be cytoskeletal function and axonal transport.

In these GWAS performed in non-Hispanic Whites of European ancestry, the most strongly associated SNPs at each locus other than APOE demonstrated population attributable fractions (PAFs) between 1.0% and 8.0% with effect sizes ranging from ORs of 1.16 to 1.20, i.e. much smaller than for *APOE* [58]. In the largest GWAS performed to date in Caribbean Hispanics [59] associations in *CLU*, *PICALM*, and *BIN1* were replicated and several additional loci on 2p25.1, 3q25.2, 7p21.1 and 10q23.1 – which could be replicated in an independent cohort of non-Hispanic Whites of European ancestry from the National Institute on Aging Late-Onset Alzheimer's Disease Family Study (NIA-LOAD) – were observed. Finally, in the largest GWAS of African Americans performed, Reitz et al. [60] identified *ABCA7* as a major susceptibility locus in this ethnic group. Interestingly, in contrast to all GWAS loci identified in Caucasians, the *ABCA7* locus had in African Americans an effect size as strong as that of APOEe4 (i.e. a 70–80% increase in risk compared to a 10–20% increase in risk through the GWAS loci observed in Whites). Although this finding may represent a winner's curse (i.e. inflation of the estimated effect in a discovery set in relation to follow-up studies) and needs to be confirmed by independent studies in African Americans and functional methods, this finding may have major implications for developing targets for genetic testing, prevention and treatment in this ethnic group if proven true. In addition, this study confirmed *APOE* as a susceptibility gene in this ethnic group, which had been prior to this study inconsistent across studies.

5. Non-genetic risk and protective factors

5.1. Cerebrovascular disease

Cerebrovascular changes such as hemorrhagic infarcts, small and large ischemic cortical infarcts, vasculopathies and white matter changes increase the risk of dementia but the specific underlying mecahnisms remain unclear. Infarcts or white matter hyperintensies may lead directly to the damage of brain regions that are important in memory function, such as the thalamus and the thalamo-cortical projections. However, they may also increase the deposition of Aβ, which in turn can lead to cognitive decline or could induce inflammatory responses impairing cognitive function. Finally, hypoperfusion may lead to overexpression of cyclin-dependent kinase 5 (CDK5), a serine–threonine kinase critical to synapse formation and synaptic plasticity [61]. Aberrant CDK5

activation is associated with neuronal apoptosis and death [62]. This kinase may also be involved in the abnormal phosphorylation of tau, contributing to the formation of NFTs [63], and might be a key protein linking NFT pathology to amyloid plaques.

5.2. Blood pressure

Data from cross-sectional studies and longitudinal studies relating blood pressure levels measured in late life with cognitive decline and dementia remain inconsistent [64–67]. To some extent, these controveries can be attributed to differences in study design, specifically variation in the time between measurement of blood pressure and assessment of cognitive abilities and in the age at which these parameters were measured. However, data from observational studies exploring the association between elevated levels of blood pressure in mid-life (40–60 years of age) and late-life cognitive impairment have proved to be relatively consistent across cohorts suggesting that elevated blood pressure in mid-life does increase the risk of later-life cognitive impairment, dementia and AD [68–71].

Hypertension may increase the risk of AD through an effect on the vascular integrity of the blood–brain barrier (BBB), resulting in protein extravasation into brain tissue [72]. In turn, protein extravasation can lead to cell damage, a reduction in neuronal or synaptic function, apoptosis, and an increase in Aβ accumulation resulting in cognitive impairment [73]. With increasing age, the effect of elevated blood pressure on AD risk diminishes and may even become inverted, with an increase in blood pressure showing a protective effect. This observation may be explained by the fact that following the onset of AD, blood pressure begins to decrease, possibly as a result of vessel stiffening, weight loss and changes in the autonomic regulation of blood flow. The randomized, placebo-controlled trials (RCTs) evaluating the benefit of antihypertensive treatments in patients with cognitive impairment were inconsistent [74–80].

5.3. Type 2 diabetes

In observational studies, type 2 diabetes (T2D) has been found to nearly double the risk of AD [81,82]. The mechanisms linking T2D and LOAD are not clear but may include cerebrovascular and noncerebrovascular mechanisms [83]. T2D is a risk factor for stroke and is accompanied by other vascular risk factors including hypertension and dyslipidemia [84]. The observation from pathology studies that T2D is associated with infarcts but not AD pathology in persons with clinical LOAD [85] suggests that the presence of infacrtcs – which in turn decreases the thresehold of amyloid necessary to cause cognitive impairment – may represent the main mechanism linking T2D to LOAD.

Noncerebrovascular mechanisms potentially linking T2D and LOAD include hyperinsulinemia and advanced products of glycosylation. Hyperinsulinemia precedes and may accompany T2D [86]. Insulin can cross the blood–brain barrier, and peripheral insulin infusion in the elderly increases 42-amino-acid β-amyloid (Aβ42) levels in the CSF [87], a surrogate marker of Aβ clearance in the brain and an indirect marker of LOAD risk. There are insulin receptors in the brain including the hippocampus and entorhinal cortex, structures affected early in LOAD. Insulin-degrading enzyme (IDE) has been linked to clearance of Aβ in the brain, and insulin and Aβ are both competing substrates for IDE [88]. Insulin in the brain can increase the deposition of Aβ and tau protein phosphorylation, which are central to the pathogenesis of LOAD [89]. Peripheral hyperinsulinemia may downregulate insulin uptake in the blood–brain barrier due to saturation over physiological levels. This may result in reduction of insulin levels in the brain and downregulation of expression of IDE and reduction in IDE-mediated amyloid reduction. This observation has been used to support the use of rosiglitazone, an insulin sensitizer, and intranasal insulin in the treatment of LOAD. In a T2D environment, diabetic animal and human tissues contain increased advanced products of glycosylation and upregulation of their receptor. Increased expression of this receptor is observed in LOAD.

5.4. Body weight

Prospective studies have linked both low and high body weight to an increased risk of cognitive impairment and AD and suggesting a U-shaped relationship [90–92] that is dependent on the age at which body weight is measured and seems to be driven by central obesity [93]. In addition, there is evidence for reverse causation in the years preceding dementia onset caused by loss of body weight due to malnutrition during the prodromal phase of dementia [90].

5.5. Plasma lipid levels

While most cross-sectional studies and observational studies relating dyslipidemia in late life with cognitive impairment or AD are inconsistent [94–96], studies that explored the association between lipids measured in mid-life and risk of incident AD mostly indicate a harmful effect. The latter findings are supported by genetic linkage and association studies that have clearly identified several genes involved in cholesterol metabolism or transport as AD susceptibility genes, including apolipoprotein E (APOE), apolipoprotein J (APOJ, CLU), ATP-binding cassette subfamily A member 7 (ABCA7), and sortilin-related receptor (SORL1). Functional cell biology studies further support a critical involvement of lipid raft cholesterol in the modulation of Aβ precursor protein processing by β-secretase and γ-secretase resulting in altered Aβ production. However, conflicting evidence comes from epidemiological studies showing no or controversial association between dyslipidemia and AD risk, randomized clinical trials observing no beneficial effect of statin therapy, and cell biology studies suggesting that there is little exchange between circulating and brain cholesterol, that increased membrane cholesterol level is protective by inhibiting loss of membrane integrity through amyloid cytotoxicity, and that cellular cholesterol inhibits colocalization of β-secretase 1 and Aβ precursor protein in nonraft membrane domains, thereby increasing generation of plasmin, an Aβ-degrading enzyme.

5.6. Metabolic syndrome

Various studies have assessed the relationship between metabolic syndrome as a whole and the risk of AD or cognitive decline. Most of these investigations demonstrated a positive association between the presence of this syndrome and cognitive dysfunction [97–99].

5.7. Smoking

Case–control studies have largely suggested that smoking lowers the risk of AD [100,101], whereas prospective studies have shown that smoking increases this risk [102–104] or has no effect on the probability of developing this disease [105,106]. A meta-analysis that examined the relationship between smoking and AD while accounting for tobacco-industry affiliation found that the combined results of 18 crossectional studies without industry affiliation yielded no association [107]. By contrast, data from eight crossectional studies with tobacco-industry affiliation suggested that smoking protected against AD. Fourteen cohort studies without tobacco-industry affiliation yielded a significant increase

in the risk of AD with smoking. Smoking may affect the risk of AD via several mechanisms. It may increase the generation of free radicals, leading to high oxidative stress, or affect the inflammatory immune system, leading to activation of phagocytes and further oxidative damage [108]. Second, it may affect AD risk by promoting cerebrovascular disease. However, there is also evidence for a protective effect, suggesting that nicotine induces increases in nicotinic acetylcholine receptors (nAChR) thereby counterbalancing the loss of nAChR observed in AD that leads to cholinergic deficits.

5.8. Traumatic brain injury

Retrospective studies [109–111] suggested that individuals with a history of traumatic brain injury (TBI) had a higher risk of dementia than individuals with no history of such injury. Two meta-analyses [112,113] demonstrated that among patients with TBI, the risk of dementia was higher in men than in women. While prospective studies of the relationship between TBI and AD have proved inconsistent [114–116], postmortem and experimental studies support a link between these conditions [117]. Evidence also exists that after human brain injury, the extent of Aβ pathology and tau pathology increases in brain tissue, cerebrospinal fluid (CSF) Aβ levels are elevated and APP is overproduced [118].

6. Protective non-genetic factors

6.1. Diet

There is evidence that consumption of a Mediterranean diet, which is characterized by a high intake of plant foods and fish, with olive oil as the primary source of monounsaturated fat, a low intake of red meat and poultry and a moderate intake of wine, is associated with a reduced incidence of AD and MCI [119,120] independent of the levels of physical activity [121] and vascular comorbidity [122]. Diets high in fish, fruit and vegetables are high in antioxidants and polyunsaturated fatty acids (PUFAs). Reactive oxygen species are clearly associated with neuronal damage in AD but whether this association is a primary or secondary event in the neurotoxic process remains unclear. Aβ deposition leads to a decrease in cerebral iron and copper concentrations, resulting in oxidative stress and neuronal damage [123]. In vitro studies suggest that vitamin E reduces Aβ-associated lipid peroxidation and apoptosis [124]. In addition, carotenes and vitamin C protect against lipid peroxidation [125], and vitamin C reduces the formation of nitrosamines and may affect catecholamine synthesis [126,127]. It is also possible that intake of antioxidants reduces AD risk through decreasing the risk of cerebrovascular disease [128]. Besides reducing oxidative stress, PUFAs have favorable effects on neuronal and vascular functions and inflammatory processes [129,130]. Observational population-based studies individually assessing the impact of vitamins E and C or PUFAs were inconclusive [131–141], and most RCTs examining the effects of antioxidant or PUFA supplementation have found no association with cognitive performance [142–147].

6.2. Physical activity

Epidemiological and experimental data suggest that physical exercise may promote brain health. Conflicting results have, however, emerged from cross-sectional and longitudinal observational studies that have examined the relationship between exercise levels and cognitive decline or dementia: while some studies have indicated that physical activity has a beneficial effect on brain health, other studies have shown no association between these variables [148–152]. RCTs exploring the effect of exercise on cognitive function in healthy elderly individuals have yielded conflicting results [153–156]. Physical activity could affect cognition through an increase in cerebral blood flow, oxygen extraction and glucose utilization, as well as activation of growth factors promoting structural brain changes, such as an increase in capillary density. In addition, rodent studies suggest that physical activity decreases the rate of amyloid plaque formation [157].

6.3. Intellectual activity

Several subsequent prospective studies and RCTs suggest that people, at both young [158] and old [149] ages, who engage in cognitively stimulating activities, such as learning, reading or playing games, are less likely to develop dementia than individuals who did not engage in these activities. The benefit of cognitive training seems to be domain specific and more pronounced in persons without memory impairment, however.

7. Biomarkers

Biomarkers are useful for the determination of disease risk but are also invaluable in establishing a diagnosis. While the autosomal dominantly inherited mutations are definite markers of the disease, the additional biomarkers, that have been identified and include various measurements from CSF, blood and neuroimaging, only contribute to increasing the specificity of diagnosis.

7.1. Plasma biomarkers

Plasma biomarkers of AD comprise only small or lipophilic proteins and proteins carried by transporters able to cross the BBB. Under physiological conditions there is a steady-state level of brain Aβ that is balanced by the production and deposition of Aβ in the brain and the peripheral production by platelets. As a consequence, in cognitively healthy individuals, brain Aβ levels are reflected by plasma Aβ concentrations. In patients with dementia, in whom Aβ is deposited in amyloid plaques, the relationship between brain and plasma Aβ levels is not clear. In familial AD [159,160] and Down syndrome with *APP* triplication [161], total Aβ levels and $A\beta_{1-42}$ levels in plasma are elevated. In sporadic AD, studies exploring the usefulness of plasma Aβ as a risk biomarker are controversial [162,163] likely due to variability in the timing of sample collection across studies, the use of different antibodies to detect Aβ, and a lack of validation of plasma Aβ as a risk biomarker for AD. There is evidence that elevated plasma $A\beta_{1-42}$ is an antecedent risk factor for sporadic AD, while decreasing levels or a decline in the $A\beta_{1-42}/A\beta_{1-40}$ ratio indicate disease onset. Several other molecules that have been investigated as plasma biomarkers for AD risk such as cholesterol, homocysteine or inflammation-related proteins including C-reactive protein, IL-1β, TNF, IL-6 and transformating growth factor β were inconsistent across studies.

7.2. CSF biomarkers

Due to the free transport of proteins between the brain and the CSF, levels of $A\beta_{1-42}$, total tau (t-tau) and p-tau in CSF reflect the metabolic processes in the brain and can be used to aid the accurate diagnosis of AD at an early stage of disease [164]. In MCI or AD CSF levels of $A\beta_{1-42}$ are decreased while t-tau or p-tau are increased compared to cognitively normal individuals [165]. The combined evaluation of $A\beta_{1-37}$, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$ and $A\beta_{1-42}$ may further increase sensitivity and specificity in predicting progression from MCI to AD [166]. The association between CSF biomarkers and the concentrations of deposited amyloid (in plaques) and NFTs in the brain remains unclear. It has been

Table 2
Additional CSF biomarkers assessed for prediction of Alzheimer's disease and cognitive impairment.

Biomarker
24S-Hydroxycholesterol
Albumin
Amyloid-β
Angiotensinogen
Apolipoprotein AI
Apolipoprotein AII
Apolipoprotein E
Complement component C3a
Complement component C4a
Cystatin C
Cystatin C, 8 amino acid amino-terminal truncation
Immunoglobulin heavy chain
N-acetyllactosamine
Neuronal pentraxin-1
Prostaglandin-H_2 D-isomerase
Retinol-binding protein
Thioredoxin
Transthyretin
Vascular growth factor
α-1-Antitrypsin
α-1β Glycoprotein
β-2-Microglobulin
β-Fibrinogen
β-Secretase

proposed that Aβ deposition in amyloid plaques may lead to a reduction of soluble Aβ in the brain and CSF [167]. Some studies suggested a positive correlation between p-tau CSF levels and NFT concentration in people with AD [168] but other studies did not observe such correlations [169,170]. Reasons for inconsistencies between studies may be differences in factors influencing CSF tau or Aβ levels. For example, carrying an *APOE ε4* allele or higher age accelerate the deposition of $A\beta_{1-42}$ in brain and lower $A\beta_{1-42}$ levels in CSF [171]. Moreover, CSF APOE levels positively correlate with t-tau and 24S-hydroxycholesterol (24S-OHC) CSF levels in patients with cognitive impairment [172]. A large-scale meta-analysis of 14 studies exploring biomarkers for preclinical AD suggested that the sensitivity of $A\beta_{1-42}$, t-tau and p-tau is modest (effect sizes: 0.91–1.11) [173]. Several additional CSF biomarkers have been explored but were inconsistent across studies (Table 2) leaving their predictive value unclear.

7.3. Imaging biomarkers

7.3.1. Structural MRI

On structural MRI, LOAD is characterized by atropy in the medial temporal lobe in particlar in the (para)hippocampus and the amygdala. In EOAD, brain atrophy may be spread more posteriorly and may involve the posterior cortex [174], occipital lobes, posterior cingulate and precuneus [175]. Atrophy in the hippocampus and entorhinal cortex is associated with a decline in memory function, progression of memory impairment [176] and an increased risk of AD [177]. In addition, white matter changes may appear. However, none of these changes are specific to AD occurring also in various other neurodegenerative disorders as well as normal aging. Nevertheless, several studies have suggested certain structural MRI biomarkers possess some degree of discriminative diagnostic power. There is evidence that among patients with amnestic MCI, those converting to LOAD show greater atrophy in the hippocampus and the inferior and middle temporal gyri than those who do not convert to AD [178]. Evidence also exists that atrophy in the corpus callosum (particularly the anterior region) may help to distinguish AD from frontotemporal dementia, in which the posterior area shows greater atrophy [179]. Diffusion tensor imaging (DTI) allows to identify reductions in white matter integrity in several brain areas of persons with AD [180,181,182] and MCI [182,183,184] compared to non-demented individuals suggesting that these changes occur early in the disease process. In line with this notion, preclinical and presymptomatic carriers of familial AD mutations display enhanced white matter degradation compared to non-carriers [185]. Arterial spin labeling (ASL)-MRI allows examination of functional cerebral perfusion deficits using MRI. ASL-MRI studies have revealed reduced cerebral blood flow in AD patients compared to non-demented persons [186–188], can discriminate MCI from AD [188], and can predict cognitive decline and progression from MCI to AD [189].

7.3.2. Functional MRI

Functional MRI (fMRI) visualizes neuronal activity during rest or a task activating specific brain regions. The most common method used measures alterations in blood flow based on changes in deoxyhemoglobin concentration (BOLD-fMRI) [190]. Several studies have demonstrated a decreased BOLD signal in the medial temporal lobe, parietal lobe and hippocampal areas of persons with AD compared to controls during a cognitive task [191–193]. In addition, some studies have observed different task-associated neuronal activity patterns in patients with MCI and healthy controls [174].

As there is disrupted connectivity in the default mode network of AD brains compared to healthy controls [194,195], resting state fMRI can been used to investigate functional connectivity deficits in AD. In line with this notion, studies have shown that also resting fMRI may differentiate AD from MCI and MCI from controls [196]. In a direct comparison of resting state versus task-associated fMRI in individuals at risk for AD, the resting default network analyses provided more robust and sensitive data [197]. BOLD signals are dependent on several anatomical, physiological and imaging parameters, and can be interpreted qualitatively or semiquantitatively. As a result, interindividual and intra-individual variability limits the use in the differential diagnosis of dementia-causing disorders. Nevertheless, recent advances in fMRI have allowed intrinsic functional networks in the human brain to be defined. The study of cognitive–behavioral function in the early stages of neurodegenerative disorders may allow the identification of the neuroanatomical networks affected by these diseases, and may assist in the differential diagnosis of the various disorders that underlie dementia. BOLD levels may also have utility for assessing treatment effects in pharmacological fMRI studies. Several investigations have demonstrated increased brain activation during task performance following the administration of acetylcholinesterase inhibitors (AChEIs) [198,199]. A recent resting state fMRI study revealed AChEI-induced improvement of functional connectivity in the hippocampus which correlated with cognitive improvement [200]. Increased BOLD activation with novel therapeutics may signal potential efficacy.

7.4. Positron emission tomography (PET) and single photon emission computed tomography (SPECT)

PET and single-photon emission CT (SPECT) have been extensively evaluated as diagnostic tools for dementia, and both techniques have shown good diagnostic and prognostic capabilities. Several positron emission tomography (PET) ligands targeting amyloid, tau, or metabolic activity have been investigated. An early radiotracer shown to bind both to amyloid plaques and NFT [201] is 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile (FDDNP). FDDNP visualizes protein aggregates in limbic regions including the hippocampus and

amygdala, correlates with cognitive deficits in AD [201,202], and differentiates between persons with AD, MCI and without cognitive impairment [203]. Small signal differences in the cortex, however, may reduce its utility [202].

The first amyloid specific imaging probe that was developed is the Pittsburgh compound B (PIB). PIB binds selectively to cortical and striatal Aβ plaques, shows a strong positive correlation with AD diagnosis, fibrillary amyloid plaques at autopsy [204–206] and is inversely correlated with CSF Aβ42 levels in the presence of clinical AD [207]. However, there is also substantial PIB retention in some non-demented individuals [206] and longitudinal studies need to clarify whether this retention represents preclinical AD. Florbetaben (^{18}F-BAY94-9172) and Florbetapir (^{18}F AV-45) are novel amyloid imaging agents with similar binding profiles but longer half-life than PIB that can also differentiate AD from controls and other dementias [206,208] with strong sensitivity and specificity [197,209].

Cerebral metabolism, as measured by ^{18}F-fluorodeoxyglucose (FDG)-PET imaging is decreased in AD. Several studies have demonstrated reductions in regional (e.g. parietal) cerebral glucose metabolism in MCI and AD compared to healthy controls [210,211]. FDG-PET measures are strongly related to cognitive deficits [207,211] and may further be able to predict progression from MCI to AD [212,213]. It has a high sensitivity (94%) but a low specificity (73–78%) for the diagnosis of dementia [214]. Combining analyses of FDG-PET and ASL-MRI data differentiates AD patients from controls more effectively than either technique alone [215].

Finally, molecular imaging probes indicative of microglial activation have been developed for use in PET imaging. Most of these radioligands bind to the 18 kDa translocator protein (TSPO), also known as the peripheral benzodiazepine receptor, which reflects neuroinflammatory processes [216]. The most frequently employed PET probe, ^{11}C-(R)-PK11195, displays increased retention in AD [217] and MCI patients [218] versus aged non-demented individuals.

Many SPECT tracers target dopamine transporters or receptors [219]. These ligands discriminate AD from DLB with high sensitivity and specificity and have also been employed to demonstrate that reduced striatal uptake corresponds to reduced nigral neuronal number but not Aβ, tau, or α-synuclein pathology [220]. Other radiotracers developed for SPECT imaging, such as ^{123}I-quinuclidinyl benzilate (^{123}I-QNB), target muscarinic acetylcholine (ACh) receptors and reflect cholinergic system integrity. AD patients display reduced ^{123}I-QNB uptake compared to controls, indicating decreased cholinergic function. Probes that target vesicular ACh transporters (^{123}I-IBVM) and nicotinic ACh receptors (^{123}I-5IA-85380) display similar reductions in cholinergic activity in AD compared to age-matched controls [221]. These tracers may be able to monitor disease progression during clinical trials of symptomatic drugs that target cholinergic neurons and may have a role in documenting neuronal preservation in trials of neuroprotective agents.

A recent study [222] has estimated the diagnostic and prognostic accuracy of different imaging markers and pertinent metrics, and the amount and source of variance among them. This study suggests that the diagnostic and prognostic accuracy of imaging AD biomarkers is at least as dependent on how the biomarker is measured as on the type of biomarker itself. While acknowledging that imaging biomarkers capture different neurobiological constructs (brain amyloidosis, neuronal injury at the molecular level, and neuronal injury at the gross structural level), this observation provides empirical support to current efforts aimed at developing standard operating procedures (SOPs) for AD biomarkers in the diagnostic routine and in clinical trials.

8. Conclusions

Substantial progress has been made over the past few decades in understanding AD. In particlaur findings from genetic studies have pointed to specific mechanistic pathways including APP metabolism, immune response, inflammation, lipid metabolism and intracellular trafficking/endocytosis. If confirmed, these findings have the potential to open new avenues for genetic testing, prevention and treatment. However, before this gained knowledge can applied in clinical settings several issues must be addressed. First, the specific causative variants in the known susceptibility genes as well as genes not yet identified must be mapped. Currently, large-scale whole exome and whole genome sequencing efforts are under way aiming to identify causative common and rare variants associated with LOAD. These efforts include whites and Caribbean Hispanics, and although variants identified by this effort will need to be functionally confirmed, these studies hold the promise to lead to a more accurate understanding of the genetic risk factors in these ethnic groups and refinement of risk estimates and diagnostic and predictive testing protocols specific for individual ethnic groups. Similar efforts are needed for African Americans and additional ethnic groups that have a high prevalence of LOAD but have been widely neglected by genomic LOAD research. Second, it has to be clarified through which exact meachanism these and other – not yet identified – pathways lead to the disease and how pathways interact. Monotherapy is not likely to be sufficiently effective in a complex disease, and a more detailed risk profile would provide clues for a better multitargeted interventional strategy. Third, to achieve effective prevention and treatment strategies the early identification of the disease process (before its clinical expression) must be improved; this includes the development of standard operating procedures for AD biomarkers in the diagnostic routine. Effectively screening of those at clearly definable risk in whom progression from cognitively normal function or MCI to AD would be likely and where an intervention would stop a specific pathology progressing is essential.

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