Alzheimer's Disease Tests

POLICY

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.

I. Aetna considers lumbar puncture to confirm the presence of elevated phosphorylated tau (P-tau) protein and reduced beta amyloid-42 (AB42) or a low AB42/AB40 ratio as determined by the lab assay detected in cerebrospinal fluid (CSF) medically necessary for the assessment of mild cognitive impairment (MCI) when Alzheimer disease is suspected.

II. Aetna considers the following tests/measurements experimental and investigational for the diagnosis and assessment of members with Alzheimer disease and related dementias because their clinical value remains unproven for this indication (not an all-inclusive list):

- Apolipoprotein E (apoE)
- ATP-binding cassette transporter (ABCA7)
- Bcl-2 rs956572 polymorphism testing
- Beta amyloid 42 (BA-24, Aβ42) protein (plasma)
- Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1)
- Cerebrospinal fluid (CSF) chitinase enzyme activity
- Circadian rhythm analysis
- CSF golgin A4
- CSF microRNAs (e.g., hsa-miR-27a-3p)
- CSF prion protein concentration
- CSF synaptic biomarkers (e.g., GAP-43, neurogranin, SNAP-25 total, SNAP-25aa40, synaptotagmin-1)
- CSF soluble amyloid precursor proteins (sAPP) level/CSF β-secretase activity
- CSF stathmin protein level
- CSF visinin-like protein-1 (VILIP-1) level
- Cognitive event-related potentials (cognitive evoked potentials)
- DNA methylation profiling (brain tissue or peripheral blood)
- Electronystagmography (in the absence of signs of vertigo or balance disorder)
- Genetic testing (e.g., presenilin-1 gene [PSEN1], presenilin-2 gene [PSEN2], apolipoprotein E epsilon 4 allele, amyloid precursor gene, etc.)
- Genetic variation of mitochondrial DNA
- Homocysteine (serum level)
- INNO-BIA AlzBio3 immunoassay kit (a multiplex immunoassay that allows simultaneous quantification of amyloid-beta, p-tau, and t-tau)
- Insulin degrading enzyme polymorphisms
- Long-term measurement of cortisol
- Macular thickness
- Microtubule-associated protein tau (MAPT)
- MindX Blood Test - Memory/Alzheimer's
- Morphometric imaging, protein kinase C-epsilon (PKCe), and quantitative imaging of phosphorylated Erk1 and Erk2 (e.g., Discern test; NeuroDiagnostics, LLC)
- N-terminal pro-brain natriuretic peptide (NT-proBNP)
- Olfactory screening tests
- Particulate matter with an aerodynamic diameter of less than or equal to 2.5 μm (PM2.5)
- Plasma clusterin level
- Plasma lipoproteome
- Plasma prion protein concentration
- Plasma tau (including p-tau231)
- Pituitary adenylate cyclase-activating polypeptide (PACAP)
- Red blood cell omega-3 fatty acid level
- Resting state eye-closed cortical electroencephalography
- Serum ceramides
- Serum insulin-like growth factor-1 (IGF-1; also known as somatomedin C)
- Serum microRNAs
- Serum neurofilament light concentration
- Serum triglycerides
- Toxoplasma gondii
- Transforming growth factor-beta1 (TGF-β1)
- TREM2 (triggering receptor expressed on myeloid cells 2)
- Tympanometry (in the absence of hearing loss)
- Urinary AD7c-NTP (neuronal thread protein/neural thread protein)
- Videopupillography and tropicamide drop test.

See also: CPB 0071 - Positron Emission Tomography (PET)
(../1_99/0071.html), CPB0140 - Genetic Testing (../100_199/0140.html), and CPB 0181 - Evoked Potential Studies (../100_199/0181.html)
Alzheimer disease (AD) is the most common form of dementia. It is a neurologic condition characterized by loss of mental ability severe enough to interfere with normal activities of daily living, lasting at least six months and not present from birth. AD usually occurs in adulthood and is marked by a decline in cognitive functions such as remembering, reasoning and planning. The diagnosis of AD is a clinical diagnosis, focusing on the exclusion of other causes of senile dementia.

Both the tau and BA-42 molecules are components of the neurofibrillary tangles associated with AD. Levels of these molecules found in the cerebro-spinal fluid (CSF) have been investigated as a diagnostic test for AD. Additionally, there is an association between the apolipoprotein E (apoE) epsilon 4 genotype and AD. The apoE genotype consists of one of several different combinations of the 3 different alleles, which are labeled 2, 3 or 4. It has been shown that the presence of one allele (apoE4) is over-represented in patients with late onset AD.

There is inadequate data regarding the positive and negative predictive values of CSF levels of tau and BA-42 in the diagnosis of AD in patients with clinical symptoms consistent with possible AD. While the presence of an apoE4 allele may be associated with an increased risk of AD, the associated positive and negative predictive values are inadequate to validate apoE genotyping as a diagnostic test for AD. Additionally, there is no information regarding how such testing would influence the management of the patient. A high positive predictive value may not be clinically useful since the diagnosis can be made clinically.

The Agency for Health Care Policy and Research Clinical Practice Guideline addressed the diagnosis and assessment of AD and related dementias. The guideline stated that "it is not yet possible to depend on apoE genotyping for definitive guidance about diagnosis or treatment of Alzheimer's disease" (Costa et al, 1996). Furthermore, the algorithm presented in the Guideline for the recognition and initial assessment of AD did not incorporate measurement of CSF levels of the peptides tau and BA-42. The Alzheimer's Disease Guidelines Panel concluded that the role of these markers in the diagnosis and management of patients with AD are questions for further research.

Genetic tests are laboratory studies of human deoxyribonucleic acid (DNA), chromosomes, genes or gene products to diagnose the presence
Identifiable genetic mutations are rare causes of AD. Persons with early onset of AD (before age 65) may show an autosomal dominant pattern of inheritance. Nearly all of the autosomal dominant familial forms of AD are related to mutations in one of three different genes: mutations of the amyloid precursor protein (APP) gene on chromosome 21 and genes encoding presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1. However, only 2 to 10% of all persons with AD have early onset disease, and genetic mutations have been identified in 30 to 50% of these patients. In addition, although detection of genetic mutations may have prognostic significance, there is no evidence that genetic testing for AD would alter the management of patients such that clinical outcomes are improved.

The PS-1 DNA Sequencing Test, replacing the Symptomatic PS-1 Analysis and Interpretation Test (Athena Diagnostics), is used for evaluating patients with progressive dementia with onset before age 65 with a positive family history of early-onset AD. It detects sequence variations in the presenilin 1 (PS-1) gene by means of polymerase chain reaction and DNA sequencing. However, there is insufficient evidence to support its clinical value at this time.

Efforts to develop biologic markers for the presence of AD, such as tests that could be performed on samples of blood or CSF, are important research topics for confirming a suspected diagnosis of AD.

According to the Canadian Consensus Conference on Dementia (1999) and the American Academy of Neurology's practice parameter on Diagnosis of Dementia (2001), the usefulness of tests for tau, BA-42, and apoE for the diagnosis of AD has not been established. The AD-associated neuronal thread protein (AD7c-NTP) gene encodes an approximately 41 kD membrane-spanning phosphoprotein that causes apoptosis and neuritic sprouting in transfected neuronal cells. The AD7c-NTP gene is over-expressed in AD beginning early in the course of
The levels of neuronal thread protein in post-mortem brain tissue correlate with the levels measured in paired ventricular fluid samples, suggesting that the protein is secreted or released by dying cells into CSF. Recent studies have suggested that urine test for AD7c-NTP could be used to assess the risk of developing AD. de la Monte and Wands (2002) reported that elevated levels of AD7c-NTP can be detected in both CSF and urine of patients with early or moderately severe AD, and the CSF and urinary levels of AD7c-NTP correlate with the severity of dementia. The authors reported that the newest configuration of the AD7c-NTP assay, termed "7c Gold", has greater than 90 % sensitivity and specificity for detecting early AD. Munzar et al (2002) reported that the competitive enzyme-linked immuno-sorbent assay (ELISA)-format AD7C-NTP test in urine is an accurate method for determining AD7c-NTP levels in AD and could be used as a biochemical marker for AD. Additional studies are needed to validate these preliminary results and to demonstrate the impact of AD7c-NTP screening on clinical outcomes.

In a 2001 American Academy of Neurology's practice parameter for the diagnosis of dementia (Knopman et al, 2001) stated that "no laboratory tests have yet emerged that are appropriate for routine use in the clinical evaluation of patients with suspected AD". The practice parameter concluded that further research is needed to improve clinical definitions of dementia and its subtypes, as well as to determine the utility of various instruments of neuroimaging, biomarkers, and genetic testing in increasing diagnostic accuracy.

The tropicamide drop test has been proposed as a rapid, non-invasive method for the early diagnosis of AD based on the observation that patients with AD exhibit greater pupillary dilation following administration of a diluted solution (0.01 %) of the cholinergic antagonist, tropicamide. However, subsequent studies reported that pupillary response to tropicamide does not differentiate between AD patients and healthy subjects. The tropicamide drop test is associated with high individual variability in the pupillary response to topically applied drugs. Furthermore, the reliability (test-retest) of the tropicamide drop test is questionable.

Mild cognitive impairment is a transition period between physiological aging and dementia. Each year more than 12 % of individuals with mild
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cognitive impairment develop AD. In a controlled study, Eibenstein and colleagues (2005) assessed the presence of an olfactory deficit in patients with amnesic mild cognitive impairment (aMCI). A total of 29 subjects diagnosed with aMCI and a homogeneous control group of 29 subjects were enrolled in the study. Olfactory function was assessed by the Sniffin' Sticks Screening Test (SSST) and the Mini Mental State Examination, the Clinical Dementia Rating, the Geriatric Depression Scale and the Mental Deterioration Battery were used to evaluate the neurocognitive status. Individuals with aMCI showed a significant impairment of their olfactory identification compared to controls (SSST score: 8.3 +/- 2.1 versus 10.8 +/- 0.9; p < 0.001). These results suggested that olfactory tests should be part of the diagnostic armamentarium of pre-clinical dementia. They noted that a long-term follow-up might confirm the olfactory identification function as an early and reliable marker in the diagnosis of pre-clinical dementia.

Hampel and Shen (2009) noted that AD is characterized by the progressive formation of insoluble amyloid plaques and vascular deposits consisting of the amyloid beta-peptide (Abeta) in the brain. Pathological mechanisms are already active early in the pre-symptomatic stage of AD. Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), also known as beta-secretase, is one of the 2 key enzymes in APP processing; the other being gamma-secretase. The Abeta peptide results from cleavage of APP initially by BACE1 to produce the C99 fragment and releases soluble APPbeta (sAPPbeta); C99 is then further cleaved by gamma-secretase leading to the Abeta peptide. Increased BACE1 activity and elevated levels of insoluble Abeta peptide have been shown in brain tissue of patients with sporadic AD. Since the CSF is in direct contact with the extra-cellular space of the central nervous system, biochemical changes in the brain can potentially be reflected in CSF. Thus, CSF-based detection of BACE1 levels and activity might be valuable in aiding early detection and prediction, particularly in pre-clinical or even pre-symptomatic subjects who are at risk of AD. Recently, these researchers were among the first groups to quantitatively analyze the enzymatic activities and protein levels of BACE1 in the CSF. Preliminary research using recently developed BACE1 ELISAs, BACE1 enzymatic activity, sAPPbeta and total Abeta1-x ELISAs were used by examining these hypothesis driven functional candidate markers in subjects with clinically diagnosed AD and amnestic mild cognitive impairment (MCI). Two sandwich ELISAs were used and BACE1 enzymatic activities were seen by synthetic fluorescence substrate and total Abeta levels by
sandwich-ELISA. Moreover, elevated CSF levels of BACE1 protein were associated with an increased risk ratio in MCI. Interestingly, amnestic MCI subjects showed increased levels of BACE1 activity compared to healthy controls (HC) and AD patients. For total Abeta and tau, increased CSF levels were associated with a higher risk of MCI compared to HC as well. BACE1 activity was significantly correlated with BACE1 protein concentration and total Abeta levels, with Abeta being itself correlated with the BACE1 protein level. The authors concluded that currently, independent studies are ongoing to validate BACE1 and functionally associated proteins as candidate biomarkers for early detection, prediction, progression as well as for biological activity in AD.

Schmand and colleagues (2010) noted that abnormal levels of biomarkers in CSF and atrophy of medial temporal lobe (MTL) structures on magnetic resonance imaging (MRI) are being used increasingly to diagnose early AD. These investigators evaluated the claim that these biomarkers can detect pre-clinical AD before behavioral (i.e., memory) symptoms arise. They included all relevant longitudinal studies of CSF and MRI biomarkers published between January 2003 and November 2008. Subjects were not demented at baseline but some declined to MCI or to AD during follow-up. Measures of tau and beta-amyloid in CSF, MTL atrophy on MRI, and performance on delayed memory tasks were extracted from the papers or obtained from the investigators. A total of 21 MRI studies and 14 CSF studies were retrieved. The effect sizes of total tau, phosphorylated tau and amyloid beta 42 ranged from 0.91 to 1.11. The effect size of MTL atrophy was 0.75. Memory performance had an effect size of 1.06. Atrophy of MTL and memory impairment tended to increase when assessed closer to the moment of diagnosis, whereas effect sizes of CSF biomarkers tended to increase when assessed longer before the diagnosis. The authors concluded that memory impairment is a more accurate predictor of early AD than atrophy of MTL on MRI, whereas CSF abnormalities and memory impairment are about equally predictive. Consequently, the CSF and MRI biomarkers are not very sensitive to pre-clinical AD. Cerebro-spinal fluid markers remain promising, but studies with long follow-up periods in elderly subjects who are normal at baseline are needed to evaluate this promise.

In a cross-sectional study, Carlesimo et al (2010) examined the relationship between age-related memory decline and MRI hippocampal anatomical changes in a cohort of healthy individuals. A total of 76 healthy individuals (44 males and 32 females), ranging in age from 20 to
80 years, were recruited. These individuals were submitted to a 3-T MRI protocol with a whole-brain T1-weighted and diffusion-weighted scanning and a neuropsychological assessment. For each subject, these researchers calculated the volumes of the total brain (gray + white matter) and hippocampi. The segmented hippocampi defined the binary masks where mean values of mean diffusivity (MD) and fractional anisotropy (FA) were calculated. Neuropsychological evaluation included tests of verbal memory (15-min delayed recall of a 15-word list) and visuospatial memory (20-min delayed reproduction of Rey complex figure).

Hippocampal MD, but not hippocampal FA, hippocampal volume, or total brain volume, predicted performance of individuals beyond their 50s on tests of verbal as well as visuo-spatial memory. The author concluded that high mean diffusivity values in the hippocampal formation of healthy elderly individuals predict memory decline, as reflected by performance on tests of declarative verbal and visual-spatial memory.

In the present study, none of the subjects fulfilled the clinical or neuropsychological criteria for MCI. Nevertheless, in a number of subjects beyond their 50s, high MD values in the hippocampal formation were accompanied by performance scores that were not truly pathological, but fell in the lower portion of the normal range on tests of declarative memory. The relevant question is if these individuals represent a very early stage in the progression of AD, anterior to the development of an aMCI, or whether, instead, they represent the lower portion of the normal distribution formed by aged individuals who will not develop dementia. The authors stated that longitudinal studies that follow the outcome of these individuals are needed to discriminate between these 2 alternative hypotheses.

In an editorial that accompanied the afore-mentioned article, Schuff (2010) noted that these findings by Carlesimo et al raise several important issues such as (i) they imply that memory deficits in healthy individuals have a biologic underpinning without apparent tissue loss, though the processes underlying the variations in diffusivity are largely unclear, and (ii) alterations in the brain's microstructure may potentially provide new clues for a separation of normal aging from pathology. However, unless prospective studies are conducted, the issue remains open whether mean diffusivity is better as early predictor of AD than volume. It also would be premature to dismiss the value of anatomical MRI, because new developments in MRI have led to
Hooshmand et al (2010) examined the relation between serum levels of homocysteine (tHcy) and holotranscobalamin (holoTC), the active fraction of vitamin B12, and risk of incident AD in a sample of Finnish community-dwelling elderly. A dementia-free sample of 271 subjects aged 65 to 79 years derived from the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study was followed-up for 7 years to detect incident AD. The association between serum tHcy and holoTC with AD was analyzed with multiple logistic regression after adjusting for several potential confounders, including common vascular risk factors. The odds ratios (ORs) (95% confidence interval [CI]) for AD were 1.16 (1.04 to 1.31) per increase of 1 μmol/L of tHcy at baseline and 0.980 (0.965 to 0.995) for each increase of 1 pmol/L baseline holoTC. Adjustment for several potential confounders including age, sex, education, APOE ε4 allele, body mass index, Mini-Mental State Examination, smoking, stroke, and blood pressure did not alter the associations: ORs (95% CI) for AD became 1.19 (1.01 to 1.39) for tHcy and 0.977 (0.958 to 0.997) for holoTC. Adjusting for holoTC attenuated the tHcy-AD link (OR changed from 1.16 to 1.10, 95% CI: 0.96 to 1.25). The holoTC-AD relationship was less influenced by controlling for tHcy (OR changed from 0.980 to 0.984, 95% CI: 0.968 to 1.000). Addition of folate did not change any of the results. The authors concluded that these findings suggested that both tHcy and holoTC may be involved in the development of AD. The tHcy-AD link may be partly explained by serum holoTC. The authors stated that the role of holoTC in AD should be further investigated; further studies on the role of sensitive markers of B12 status in identifying individuals who are at increased risk of AD are needed.

In an editorial that accompanied the afore-mentioned study, Seshadri (2010) stated that observational studies and larger clinical trials are indicated, targeting older persons with MCI, simultaneously assessing holoTC (and B12) and plasma tHcy (and folate, methylmalonic acid). Careful examination of the evidence is needed to ascertain who is the perpetrator in the complex pathology of AD and other dementias.

Perneczky et al (2011) examined if soluble amyloid precursor proteins (sAPP) in CSF improve the identification of patients with incipient AD in a
group of patients with MCI. A cohort study with follow-up assessments of 58 patients with MCI with baseline CSF sampling was conducted: 21 patients had progressed to probable AD (MCI-AD), 27 still had MCI, 8 had reverted to normal (MCI-NAD), and 2 patients with fronto-temporal dementia (FTD) were excluded. Sixteen additional patients with FTD were included to explore the specificity of the CSF markers. Cerebrospinal fluid concentrations of sAPPα, sAPPβ, tau, and Aβ(1-42) were measured with sensitive and specific ELISAs. Associations between diagnostic status, CSF protein concentrations, and other patient characteristics were explored using multiple logistic regression analyses with stepwise variable selection. The optimal sensitivity and specificity of the best models were derived from receiver operating characteristic curves. The MCI-AD group had significantly higher sAPPβ concentrations than the MCI-NAD and the FTD groups. A combination of sAPPβ, tau, and age differentiated the MCI-AD and the MCI-NAD groups with a sensitivity of 80.0 % and a specificity of 81.0 %. The best model for the differentiation of the MCI-AD and the FTD groups included sAPPβ and tau, and showed a sensitivity of 95.2 % and a specificity of 81.2 %. Aβ(1-42) and sAPPα did not significantly contribute to the models. The authors concluded that these findings suggested that sAPPβ may be clinically useful, and superior to Aβ(1-42), in the early and differential diagnosis of incipient AD. Limitations of this study included patient recruitment at a specialized memory clinic, which may restrict the generalization of the results to the general population with incipient AD and the lack of pathologic confirmation of AD and FTD. Also, the recruitment of a modest number of patients and the relatively short follow-up period may have under-estimated the predictive value of sAPPβ. The authors stated that replications of these findings in larger multi-center studies are needed. Further studies are needed to examine the clinical value of sAPPβ for the differentiation of AD from healthy aging and from other neurodegenerative disorders, and to investigate its use as a marker for anti-amyloid treatment response.

In a case-cohort study, Schrijvers et al (2011) evaluated the potential of plasma clusterin as a biomarker of the presence, severity, and risk of AD. Plasma levels of clusterin were measured at baseline (1997 to 1999) in 60 individuals with prevalent AD, a random subcohort of 926 participants, and an additional 156 participants diagnosed with AD during follow-up until January 1, 2007 (mean [SD], 7.2 [2.3] years). Main outcome measures included prevalent AD, severity of AD measured by the Mini-Mental State Examination (MMSE) score, and the risk of developing AD.
The likelihood of prevalent AD increased with increasing plasma levels of clusterin (OR per SD increase of plasma clusterin level, 1.63; 95% CI: 1.21 to 2.20; adjusted for age, sex, education level, apolipoprotein E status, diabetes, smoking, coronary heart disease, and hypertension). Among patients with AD, higher clusterin levels were associated with more severe disease (adjusted difference in MMSE score per SD increase in clusterin levels, -1.36; 95% CI: -2.70 to -0.02; p = 0.047). Plasma clusterin levels were not related to the risk of incident AD during total follow-up (adjusted hazard ratio [HR], 1.00; 95% CI: 0.85 to 1.17; p for trend = 0.77) or within 3 years of baseline (adjusted HR, 1.09; 95% CI: 0.84 to 1.21; p for trend = 0.65). The authors concluded that plasma clusterin levels were significantly associated with baseline prevalence and severity of AD, but not with incidence of AD. They stated that increased clusterin levels do not precede development of AD and thus are not a potential early marker of subclinical disease.

Tan and colleagues (2012) stated that higher dietary intake and circulating levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have been related to a reduced risk for dementia, but the pathways underlying this association remain unclear. These investigators examined the cross-sectional relation of red blood cell (RBC) fatty acid levels to subclinical imaging and cognitive markers of dementia risk in a middle-aged to elderly community-based cohort. They related RBC DHA and EPA levels in dementia-free Framingham Study participants (n = 1,575; 854 women, age 67 +/- 9 years) to performance on cognitive tests and to volumetric brain MRI, with serial adjustments for age, sex, and education (model A, primary model), additionally for APOE ε4 and plasma homocysteine (model B), and also for physical activity and body mass index (model C), or for traditional vascular risk factors (model D).

Participants with RBC DHA levels in the lowest quartile (Q1) when compared to others (Q2-4) had lower total brain and greater white matter hyper-intensity volumes (for model A: \( \beta +/- SE = -0.49 +/- 0.19; p = 0.009 \), and \( 0.12 +/- 0.06; p = 0.049 \), respectively) with persistence of the association with total brain volume in multi-variable analyses. Participants with lower DHA and \( \omega-3 \) index (RBC DHA+EPA) levels (Q1 versus Q2-4) also had lower scores on tests of visual memory (\( \beta +/- SE = -0.47 +/- 0.18; p = 0.008 \)), executive function (\( \beta +/- SE = -0.07 +/- 0.03; p = 0.004 \)), and abstract thinking (\( \beta +/- SE = -0.52 +/- 0.18; p = 0.004 \)) in model A, the results remaining significant in all models. The authors concluded that lower RBC DHA levels are associated with smaller brain
volumes and a "vascular" pattern of cognitive impairment even in persons free of clinical dementia. Moreover, they stated that "the association between lower RBC omega-3 fatty acid levels and markers of accelerated cognitive and structural brain aging observed here should be confirmed in other populations and extended in the future to include dementia outcomes".

Tarawneh et al (2012) stated that measures of neuronal damage/dysfunction are likely good surrogates for disease progression in AD. Cerebro-spinal fluid markers of neuronal injury may offer utility in predicting disease progression and guiding prognostic and outcome assessments in therapeutic trials. Visinin-like protein-1 (VILIP-1) has demonstrated potential utility as a marker of neuronal injury. These researchers investigated the utility of VILIP-1 and VILIP-1/Aβ42 in predicting rates of cognitive decline in early AD. Individuals with a clinical diagnosis of very mild or mild AD (n = 60) and baseline CSF measures of VILIP-1, tau, p-tau181, and Aβ42 were followed longitudinally for an average of 2.6 years. Annual assessments included the Clinical Dementia Rating (CDR), CDR-sum of boxes (CDR-SB), and global composite scores. Mixed linear models assessed the ability of CSF biomarker measures to predict rates of cognitive decline over time. Baseline CSF VILIP-1 and VILIP-1/Aβ42 levels predicted rates of future decline in CDR-SB and global composite scores over the follow-up period. Individuals with CSF VILIP-1 greater than or equal to 560 pg/ml (corresponding to the upper tercile) progressed much more rapidly in CDR-SB (1.61 boxes/year; p = 0.0077) and global scores (-0.53 points/year; p = 0.0002) than individuals with lower values (0.85 boxes/year and -0.15 points/year, respectively) over the follow-up period. Cerebro-spinal fluid tau, p-tau181, tau/Aβ42, and p-tau181/Aβ42 also predicted more rapid cognitive decline in CDR-SB and global scores over time. The authors concluded that these findings suggested that CSF VILIP-1 and VILIP-1/Aβ42 predict rates of global cognitive decline similarly to tau and tau/Aβ42, and may be useful CSF surrogates for neurodegeneration in early AD. Drawbacks of this study included relatively small sample size and short follow-up period. These findings need to be validated in well-designed studies with standardized assay, larger sample size and longer durations of follow-up.

Watabe-Rudolph et al (2012) analyzed the level of novel biomarkers of DNA damage and telomere dysfunction (chitinase activity, N-acetyl-
glucosaminidase activity, stathmin, and EF-1α) in CSF of 94 patients with AD, 41 patients with non-AD dementia, and 40 control patients without dementia. Enzymatic activity of chitinase (chitotriosidase activity) and stathmin protein level were significantly increased in CSF of patients with AD and non-AD dementia compared with that of no dementia control patients. As a single marker, chitinase activity was most powerful for distinguishing patients with AD from no dementia patients with an accuracy of 85.8% using a single threshold. Discrimination was even superior to clinically standard CSF markers that showed an accuracy of 78.4% (β-amyloid) and 77.6% (tau). Combined analysis of chitinase with other markers increased the accuracy to a maximum of 91%. The biomarkers of DNA damage were also increased in CSF of patients with non-AD dementia compared with no dementia patients, and the new biomarkers improved the diagnosis of non-AD dementia as well as the discrimination of AD from non-AD dementia. The authors concluded that taken together, the findings in this study provided experimental evidence that DNA damage markers are significantly increased in AD and non-AD dementia. Moreover, they stated that prospective clinical trials are needed to evaluate whether determination of chitinase enzyme activity and stathmin protein level in CSF could improve the diagnosis and prediction of disease progression of AD.

Hudson et al (2012) noted that although several studies have described an association between AD and genetic variation of mitochondrial DNA (mtDNA), each has implicated different mtDNA variants, so the role of mtDNA in the etiology of AD remains uncertain. These researchers tested 138 mtDNA variants for association with AD in a powerful sample of 4,133 AD case patients and 1,602 matched controls from 3 Caucasian populations. Of the total population, 3,250 case patients and 1,221 elderly controls met the quality control criteria and were included in the analysis. In the largest study to-date, these investigators failed to replicate the published findings. Meta-analysis of the available data showed no evidence of an association with AD. The authors concluded that the current evidence linking common mtDNA variations with AD is not compelling.

Mielke et al (2012) examined if serum ceramides and sphingomyelins (SM) were associated with an increased risk of all-cause dementia and AD. Participants included 99 women without dementia aged 70 to 79, with baseline serum SM and ceramides, enrolled in a longitudinal
Baseline lipids, in tertiles, were examined in relation to all-cause dementia and AD using discrete time Cox proportional survival analysis. Lipids were analyzed using electrospray ionization tandem mass spectrometry. A total of 27 (27.3%) of the 99 women developed incident dementia. Of these, 18 (66.7%) were diagnosed with probable AD. Higher baseline serum ceramides, but not SM, were associated with an increased risk of AD; these relationships were stronger than with all-cause dementia. Compared to the lowest tertile, the middle and highest tertiles of ceramide d18:1-C16:0 were associated with a 10-fold (95% CI: 1.2 to 85.1) and 7.6-fold increased risk of AD (95% CI: 0.9 to 62.1), respectively. The highest tertiles of ceramide d18:1-C24:0 (HR = 5.1, 95% CI: 1.1 to 23.6) and lactosylceramide (HR = 9.8, 95% CI: 1.2 to 80.1) were also associated with risk of AD. Total and high-density lipoprotein cholesterol and triglycerides were not associated with dementia or AD. The authors concluded that results from this preliminary study suggested that particular species of serum ceramides are associated with incident AD and warrant continued examination in larger studies. Drawbacks of this study include a small sample size of women only, as well as a single baseline measurement of the biomarker.

Genetic testing has been proposed as an aid in the diagnosis of a type of Alzheimer disease (AD) known as early-onset familial AD (EOFAD). EOFAD is a rare form of AD in which onset occurs at age 30 to 60 years. Most cases are caused by mutations in one of three known genes: PSEN1, PSEN2 and APP. Testing of the APOE gene has also been proposed to predict susceptibility to early- and late-onset AD in asymptomatic individuals.

The familial form of AD is due to mutations in 3 major genes (APP gene, presenilin 1 [PSEN1] gene and presenilin 2 [PSEN2] gene). Chen et al (2012) stated that association studies of presenilin-2 (PSEN2) polymorphisms and sporadic AD have yielded inconsistent results, possibly because single studies often lack sufficient statistical power. These investigators performed a meta-analysis to evaluate the association of the 2 most extensively studied PSEN2 polymorphisms, rs8383 and 5’indel, with the risk of sporadic AD. They systematically reviewed relevant studies retrieved by Medline, Pubmed, Embase, AlzGene, and CNKI. Data were analyzed using the Stata (v11.0)
software package. The fixed effects model or random-effects model were applied depending on between-study heterogeneity. Publication bias was evaluated using Egger's test and Begg's funnel plots. Overall, the meta-analysis included 6 case-control studies for each polymorphism with 2,186 confirmed AD cases and 2,507 healthy controls in total. Analysis suggested a significant association between single-nucleotide polymorphism (SNP) rs8383 polymorphism and AD risk with no evidence of between-study heterogeneity or publication bias. In contrast, these researchers found no evidence for an association between the 5'indel polymorphism and AD risk. Further stratified analyses by apolipoprotein ε4 status or ethnicity also failed to reveal a statistically significant association between the 5'indel polymorphism of PSEN2 and AD risk.

The authors concluded that this analysis supported the hypothesis that the PSEN2 rs8383 polymorphism is associated with an enlarged risk of sporadic AD. However, they stated that larger scale association studies are needed to further validate the association of PSEN2 polymorphisms with sporadic AD risk and to define potential gene-gene interactions.

Gerrish et al (2012) noted that rare mutations in AβPP, PSEN1, and PSEN2 cause uncommon early onset forms of AD, and common variants in microtubule-associated protein tau (MAPT) are associated with risk of other neurodegenerative disorders. These researchers sought to establish whether common genetic variation in these genes confer risk to the common form of AD that occurs later in life (greater than 65 years). These investigators therefore tested single-nucleotide polymorphisms at these loci for association with late-onset AD (LOAD) in a large case-control sample consisting of 3,940 cases and 13,373 controls. Single-marker analysis did not identify any variants that reached genome-wide significance, a result that is supported by other recent genome-wide association studies. However, these investigators did observe a significant association at the MAPT locus using a gene-wide approach (p = 0.009). They also observed suggestive association between AD and the marker rs9468, which defines the H1 haplotype, an extended haplotype that spans the MAPT gene and has previously been implicated in other neurodegenerative disorders including Parkinson's disease, progressive supranuclear palsy, and corticobasal degeneration. The authors concluded that common variants at AβPP, PSEN1, and PSEN2 and MAPT are unlikely to make strong contributions to susceptibility for LOAD. However, the gene-wide effect observed at MAPT indicates a
An UpToDate review on "Gentics of Alzheimer disease" (Sherva and Kowall, 2020) states that genetic basis of AD resulted from studies of families displaying autosomal dominant inheritance of the disorder. Early studies facilitated the eventual identification of causative mutations in the following genes: amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2), which collectively account for less than 1 percent of all AD cases and 60 to 70 percent of early-onset AD. The authors state that more than 30 mutations in this gene have been described in association with AD, accounting for 10 to 15 percent of early familial AD. "Nearly all pathogenic APP mutations identified so far cluster around the three major processing sites that are relevant to the generation of amyloidogenic Aβ, increasing production of Aβ, or altering the ratio of Aβ42 to Aβ40". The PSEN1 gene has been associated with more than 150 mutation AD, accounting for up to 50 percent of early-onset familial AD. "Most mutations in PSEN1 increase the generation of the highly fibrillogenic Aβ42 species, alter the kinetics of Aβ peptide turnover, and enhance accumulation of Aβ in the brain. Blood and cerebrospinal fluid (CSF) levels of Aβ are also elevated". The PSEN2 gene is rarer, as fewer than 20 mutations of this gene have been described in early-onset familial AD. "Similar to PSEN1 mutations, PSEN2 mutations alter the cleavage activity of γ-secretase and increase the ratio of Aβ42 to Aβ40". The authors summarize by stating, "APP, PSEN1, and PSEN2 mutations account for approximately two-thirds of familial autosomal dominant AD and less than 10 percent of early-onset AD overall. Rare variants at other loci may contribute to early-onset AD risk, including some that overlap with late-onset AD (LOAD; e.g., SORL1, TREM2) and others related to the endolysosomal pathway (e.g., RUFY1, PSD2, TCIRG1, RIN3)."

Jonsson et al (2013) stated that sequence variants, including the ε4 allele of apolipoprotein E, have been associated with the risk of the common late-onset form of AD. Few rare variants affecting the risk of late-onset AD have been found. These investigators obtained the genome sequences of 2,261 Icelanders and identified sequence variants that were likely to affect protein function. They imputed these variants into the genomes of patients with AD and control participants and then tested for an association with AD. These researchers performed replication tests
using case-control series from the United States, Norway, the Netherlands, and Germany. They also tested for a genetic association with cognitive function in a population of unaffected elderly persons. A rare missense mutation (rs75932628-T) in the gene encoding the triggering receptor expressed on myeloid cells 2 (TREM2), which was predicted to result in an R47H substitution, was found to confer a significant risk of AD in Iceland (OR, 2.92; 95 % CI: 2.09 to 4.09; p = 3.42×10(-10)). The mutation had a frequency of 0.46 % in controls 85 years of age or older. These investigators observed the association in additional sample sets (OR, 2.90; 95 % CI: 2.16 to 3.91; p = 2.1×10(-12) in combined discovery and replication samples). They also found that carriers of rs75932628-T between the ages of 80 and 100 years without AD had poorer cognitive function than non-carriers (p = 0.003). The authors concluded that the findings of this study strongly implicate variant TREM2 in the pathogenesis of AD.

Guerreiro et al (2013) used genome, exome, and Sanger sequencing to analyze the genetic variability in TREM2 in a series of 1,092 patients with AD and 1,107 controls (the discovery set). These researchers then performed a meta-analysis on imputed data for the TREM2 variant rs75932628 (predicted to cause a R47H substitution) from 3 genome-wide association studies of AD and tested for the association of the variant with disease. These investigators genotyped the R47H variant in an additional 1,887 cases and 4,061 controls. They then assayed the expression of TREM2 across different regions of the human brain and identified genes that are differentially expressed in a mouse model of AD and in control mice. These researchers found significantly more variants in exon 2 of TREM2 in patients with AD than in controls in the discovery set (p = 0.02). There were 22 variant alleles in 1,092 patients with AD and 5 variant alleles in 1,107 controls (p < 0.001). The most commonly associated variant, rs75932628 (encoding R47H), showed highly significant association with AD (p < 0.001). Meta-analysis of rs75932628 genotypes imputed from genome-wide association studies confirmed this association (p = 0.002), as did direct genotyping of an additional series of 1,887 patients with AD and 4,061 controls (p < 0.001). Trem2 expression differed between control mice and a mouse model of AD. The authors concluded that heterozygous rare variants in TREM2 are associated with a significant increase in the risk of AD. Moreover, they stated that "We and others have predicted that heterozygous loss-of-function variants
may represent a substantial component of risk for common late-onset diseases. Our findings support this hypothesis, and we believe additional loss-of-function variants will be identified as risk factors for Alzheimer's disease and other late-onset complex disorders”.

In an editorial that accompanied the afore-mentioned studies by Jonsson et al (2013) as well as Guerreiro et al (2013), Neumann and Daly (2013) stated that "Pursuing the hypothesis that low-prevalence variants cause Alzheimer's disease with a moderate-to-high effect size, two groups of researchers convincingly show in the Journal that rare variants in TREM2, encoding triggering receptor expressed on myeloid cells 2 protein, cause susceptibility to late-onset Alzheimer's disease, with an odds ratio similar to that of the apolipoprotein E ε4 allele. Although the most compelling TREM2 variant (encoding a substitution of arginine by histidine at residue 47 [R47H] of the TREM2 protein) is rare, with an allelic prevalence of 0.63 % in Iceland, these findings implicate a gene and naturally arising perturbation that may generate new insights into the pathogenesis of late-onset Alzheimer's disease …. We therefore suggest that the degeneration of neurons in these diseases and in TREM2-associated Alzheimer's disease is driven by a chronic inflammatory process with dysfunction in
the microglial phagocytosis or inflammatory pathway. These studies provide a new path for experimental inquiry into the biologic roots of Alzheimer's disease”.

Zhang et al (2013) stated that the association between insulin degrading enzyme (IDE) gene polymorphisms and AD risk has been widely reported, but results were somewhat controversial. To assess the association between IDE polymorphisms and AD risk, a meta-analysis was performed. These investigators systematically reviewed relevant studies retrieved by PubMed, Embase, AlzGene, CNKI and Web of Science. Finally, 8 articles were identified for rs3758505 polymorphism and 5 for rs1832196 polymorphism. The pooled ORs were performed for all the 4 genetic models. Subgroup analysis was also performed by ethnicity. Results suggested that rs3758505 polymorphism was unlikely to be associated with genetic susceptibility of AD based on the current published studies. However, for the rs1832196 polymorphism, significant association with AD was found by the dominant model in overall and subgroup analysis. However, larger scale association studies are
necessary to further validate the association of IDE polymorphisms with sporadic AD risk and to define potential gene-gene interactions.

Caraci et al (2012) stated that an impairment of the transforming growth factor-beta1 (TGF-β1) signaling pathway has been demonstrated to be specific to the AD brain and, particularly, to the early phase of the disease. Transforming growth factor-β1 is a neurotrophic factor responsible for the initiation and maintenance of neuronal differentiation and synaptic plasticity. The deficiency of TGF-β1 signaling is associated with Aβ pathology and neurofibrillary tangle formation in AD animal models. Reduced TGF-β1 signaling seems to contribute both to microglial activation and to ectopic cell-cycle re-activation in neurons, 2 events that contribute to neurodegeneration in the AD brain. The neuroprotective features of TGF-β1 indicate the advantage of rescuing TGF-β1 signaling as a means to slow down the neurodegenerative process in AD.

Chang et al (2013) noted that the association between TGF-β1 gene polymorphisms and AD risk has been widely reported, but results were somewhat controversial and under-powered. To derive a more precise estimation of the relationship between TGF-β1 polymorphisms and AD risk, these researchers conducted a meta-analysis of all available case-control studies relating the T869C and/or C-509T polymorphisms of the TGF-β1 gene to the risk of developing AD. Eligible articles were identified by search of databases including Pub Med, Web of Science, the Chinese Biomedical Database (CBM), Chinese National Knowledge Infrastructure (CNKI) and the Wan Fang (Chinese) for the period up to March 2012. A total of 14 articles were identified, 10 with 1,657 cases and 6,971 controls for T869C polymorphism and 8 with 2,618 cases and 7,473 controls for C-509T polymorphism. The pooled ORs were performed for the allele contrasts, additive genetic model, dominant genetic model and recessive genetic model, respectively. Subgroup analysis was also performed by ethnicity. With respect to T869C and C-509T polymorphism, the combined results showed that there were no significant differences in genotype distribution between AD and control based on all studies. When stratifying for the race, there were also no statistically significant differences in genotype distribution between AD and controls. The authors concluded that the findings of this meta-analysis did not provide evidence of confirming association between the
T869C and/or C-509T polymorphisms of the TGF-β1 gene and AD.

Reitz and colleagues (2013) noted that genetic variants associated with susceptibility to LOAD are known for individuals of European ancestry, but whether the same or different variants account for the genetic risk of AD in African American individuals is unknown. Identification of disease-associated variants helps identify targets for genetic testing, prevention, and treatment. These researchers identified genetic loci associated with LO AD in African Americans. The Alzheimer Disease Genetics Consortium (ADGC) assembled multiple data sets representing a total of 5,896 African Americans (1,968 case participants, 3,928 control participants) 60 years or older that were collected between 1989 and 2011 at multiple sites. The association of AD with genotyped and imputed SNPs was assessed in case-control and in family-based data sets. Results from individual data sets were combined to perform an inverse variance-weighted meta-analysis, first with genome-wide analyses and subsequently with gene-based tests for previously reported loci. Main outcome measure was presence of AD according to standardized criteria. Genome-wide significance in fully adjusted models (sex, age, APOE genotype, population stratification) was observed for a SNP in ATP-binding cassette transporter (ABCA7) (rs115550680, allele = G; frequency, 0.09 cases and 0.06 controls; OR, 1.79 [95% CI: 1.47 to 2.12]; p = 2.2 × 10(-9)), which is in linkage disequilibrium with SNPs previously associated with AD in Europeans (0.8 < D' < 0.9). The effect size for the SNP in ABCA7 was comparable with that of the APOE ε4-determining SNP rs429358 (allele = C; frequency, 0.30 cases and 0.18 controls; OR, 2.31 [95% CI: 2.19 to 2.42]; p = 5.5 × 10(-47)). Several loci previously associated with AD but not reaching significance in genome-wide analyses were replicated in gene-based analyses accounting for linkage disequilibrium between markers and correcting for number of tests performed per gene (CR1, BIN1, EPHA1, CD33; 0.0005 < empirical p < 0.001). The authors concluded that in this meta-analysis of data from African American participants, AD was significantly associated with variants in ABCA7 and with other genes that have been associated with AD in individuals of European ancestry. Moreover, they stated that replication and functional validation of this finding is needed before this information is used in clinical settings.

Sala Frigerio et al (2013) evaluated microRNAs (miRNAs) as potential
biomarkers for AD by analyzing the expression level of miRNAs in CSF of patients with AD dementia and non-affected control subjects. Using quantitative PCR, these researchers profiled the expression level of 728 miRNAs in CSF of non-affected control subjects and patients with clinically ascertained AD dementia, and they further compared the expression level of candidate miRNAs in 37 control subjects and 35 patients with AD dementia. The level of hsa-miR-27a-3p in CSF is reduced in patients with dementia due to AD in 2 different cohorts of subjects (cohort 1: p = 0.008; cohort 2: p = 0.015; 2-tailed unpaired Welch t-test). Moreover, low levels of hsa-miR-27a-3p were accompanied by high CSF tau levels and low CSF β-amyloid levels. The authors concluded that the findings of this pilot study highlighted hsa-miR-27a-3p as a candidate biomarker for AD and provided the groundwork for further confirmation studies in larger cohorts and in other hospitals.

Roe and colleagues (2013) examined CSF AD biomarkers (β-amyloid 42 [Aβ42], tau, phosphorylated tau at threonine 181 [ptau181], tau/Aβ42, and ptau181/Aβ42) predict future decline in non-cognitive outcomes among individuals cognitively normal at baseline. Longitudinal data from participants (n = 430) who donated CSF within 1 year of a clinical assessment indicating normal cognition and were aged 50 years or older were analyzed. Mixed linear models were used to test whether baseline biomarker values predicted future decline in function (instrumental activities of daily living), weight, behavior, and mood. Clinical Dementia Rating Sum of Boxes and Mini-Mental State Examination scores were also examined. Abnormal levels of each biomarker were related to greater impairment with time in behavior (p < 0.035) and mood (p < 0.012) symptoms, and more difficulties with independent activities of daily living (p < 0.012). However, biomarker levels were unrelated to weight change with time (p > 0.115). As expected, abnormal biomarker values also predicted more rapidly changing MMSE (p < 0.041) and CDR-SB (p < 0.001) scores compared with normal values. The authors concluded that CSF biomarkers among cognitively normal individuals are associated with future decline in some, but not all, non-cognitive AD symptoms studied. Moreover, they stated that additional work is needed to determine the extent to which these findings generalize to other samples. These investigators also noted that future research should test whether the ratio of tau/Aβ42 and ptau181/Aβ42 are better predictors of decline in non-cognitive outcomes compared with individual molecular marker
Schmidt et al (2013) stated that prion protein concentration [PrP (c)] has been suggested to play a role in AD pathophysiology. Cerebrospinal fluid concentrations of PrP (c) have been shown to be reduced in AD compared with healthy controls. Furthermore, serum levels of PrP (c) have recently been reported to be associated with the cognitive status of healthy elderly subjects. These investigators hypothesized that CSF levels of PrP (c) could be associated with cognitive function of AD patients at the time of diagnosis. Alzheimer’s disease patients (n = 114) included into an observational study underwent CERAD testing and lumbar puncture at time of diagnosis/study inclusion; CSF PrP (c) was determined. Generalized linear models were fitted to assess the associations of PrP (c) plus a variety of possible confounding factors and CERAD subscale measures. No association of CSF PrP (c) and cognitive status could be established, while other factors (i.e., use of anti-psychotic drugs, use of anti-dementia drugs, female sex, pre-progression time) were related to worse cognitive function in some domains. The authors concluded that CSF PrP (c) appears not to be a useful biochemical surrogate of cognitive status in AD at the time of diagnosis.

Schmidt et al (2014) noted that recently, PrPc has been linked to AD pathogenesis; and a relation of PrPc plasma levels with cognitive status and decline of healthy elderly subjects has been reported. These researchers hypothesized baseline plasma levels of PrPc to be associated with AD progression in cognitive and functional domains. Alzheimer’s disease patients (n = 84) were included into an observational study at time of diagnosis. Baseline plasma PrPc levels were determined. Decline was assessed annually (mean follow-up time 3 years) with the aid of different standardized tests (MMSE, iADL, bADL, GDS, UPDRSIII). Multiple regression analyses were used to uncover potential associations between decline and PrPc levels. No association of PrPc and decline could be established. Presence of diabetes mellitus was linked to slower deterioration. Intake of neuroleptic drugs or memantine was associated with faster progression. The authors concluded that plasma PrPc at baseline could not be shown to be related to AD progression in this study.

Garcia-Martin et al (2014) examined the use of macular thickness as a
potential biomarker of mild AD (20 patients with mild AD and 28 age-matched control subjects). Based on their findings, these researchers proposed that the first affected area of the retina in mild AD may be the macular area. As the neurodegeneration process progresses, a significant decline in the peri-papillary retinal nerve fiber layer (RNFL) thickness becomes apparent. However, whether or not the macula is really the first area involved in early AD, or simply the first place with enough retinal ganglion cells to discern an effect deserves further investigation.

Westwood and colleagues (2014) related serum insulin-like growth factor-1 (IGF-1) to risk of AD dementia and to brain volumes in a dementia-free community sample spanning middle and older ages. Dementia-free Framingham participants from generation 1 (n = 789, age of 79 ± 4 years, 64 % women) and generation 2 (n = 2,793, age of 61 ± 9 years, 55 % women; total = 3,582, age of 65 ± 11 years, 57 % women) had serum IGF-1 measured in 1990 to 1994 and 1998 to 2001, respectively, and were followed prospectively for incident dementia and AD dementia. Brain MRI was obtained in stroke- and dementia-free survivors of both generations 1 (n = 186) and 2 (n = 1,867) during 1999 to 2005. Baseline IGF-1 was related to risk of incident dementia using Cox models and to total brain and hippocampal volumes using linear regression in multi-variable models adjusted for age, sex, APOE ε4, plasma homocysteine, waist-hip ratio, and physical activity. Mean IGF-1 levels were 144 ± 60 μg/L and 114 ± 37 μg/L in generation 1 and generation 2, respectively. These investigators observed 279 cases of incident dementia (230 AD dementia) over a mean follow-up of 7.4 ± 3.1 years. Persons with IGF-1 in the lowest quartile had a 51 % greater risk of AD dementia (HR = 1.51, 95 % CI: 1.14 to 2.00; p = 0.004). Among persons without dementia, higher IGF-1 levels were associated with greater brain volumes (β/SD increment in IGF-1 was 0.55 ± 0.24, p = 0.025; and 0.26 ± 0.06, p < 0.001, for generations 1 and 2, respectively). The authors concluded that lower serum levels of IGF-1 were associated with an increased risk of developing AD dementia and higher levels with greater brain volumes even among middle-aged community-dwelling participants free of stroke and dementia. They stated that higher levels of IGF-1 may protect against subclinical and clinical neurodegeneration. Moreover, the authors stated that this study was limited to persons of European ancestry, and therefore confirmation in independent samples and in persons of other
Danborg et al (2014) stated that microRNAs (miRNA) are biological molecules transcribed from non-protein coding regions of the genome, participating in regulating cellular processes. MiRNAs in biofluids may possess neurodegenerative disease biomarker potential for screening tests, differential diagnosis and disease progression monitoring. This systematic review clarified biomarker potential of miRNAs detected in biofluids of neurodegenerative disease patients. A total of 33 and 10 miRNAs displayed significant expression between patients with multiple sclerosis and AD, respectively, compared to healthy controls in a minimum of 2 studies.

Tan et al (2014) noted that recent findings that human serum contains stably expressed miRNAs have revealed a great potential of serum miRNA signature as disease fingerprints to diagnosis. These researchers used genome-wide serum miRNA expression analysis to investigate the value of serum miRNAs as biomarkers for the diagnosis of AD. Illumina HiSeq 2000 sequencing followed by individual quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays was used to test the difference in levels of serum miRNAs between 50 AD patients and 50 controls in the screening stages. The detected serum miRNAs then were validated by qRT-PCR in 158 patients and 155 controls. MiR-98-5p, miR-885-5p, miR-483-3p, miR-342-3p, miR-191-5p, and miR-let-7d-5p displayed significantly different expression levels in AD patients compared with controls. Among the 6 miRNAs, miR-342-3p has the best sensitivity (81.5 %) and specificity (70.1 %) and was correlated to MMSE score. This study identified 6 serum miRNAs that distinguish AD patients from healthy controls with high sensitivity and specificity. The authors concluded that serum miRNA panel (or miR-342-3p alone) may serve as a novel, non-invasive biomarker for AD. These preliminary findings need to be validated by well-designed studies.

Han et al (2014) stated that there is growing evidence that pituitary adenylate cyclase-activating polypeptide (PACAP) is associated with AD pathology in animal models, but human studies are needed. These researchers studied the brains of patients with pathologically confirmed late-onset AD and age-matched cognitively normal (CN) subjects to investigate the expression of PACAP messenger RNA (34 AD and 14 CN)
and protein (12 AD and 11 CN) in a case-control study. They reported that PACAP levels are reduced in multiple brain regions, including the entorhinal cortex, the middle temporal gyrus, the superior frontal gyrus, and the primary visual cortex. This reduction was correlated with higher amyloid burden (CERAD plaque density) in the entorhinal cortex and superior frontal gyrus but not in the primary visual cortex, a region spared in most cases of AD. PACAP expression is lower in advanced Braak stages (V and VI) than in moderate stages (III and IV). Increased PACAP levels were associated with decreased scores on the Dementia Rating Scale, a global cognitive measure. Finally, CSF levels paralleled brain levels in AD but not in Parkinson dementia or fronto-temporal dementia brains. The authors concluded that the close relationship between PACAP reduction and the severity of AD pathology suggested that down-regulation of PACAP may contribute to AD pathogenesis. Moreover, they stated that "Translation of PACAP research to human subjects may provide us with not only new early biomarkers but also exciting therapeutic options for AD. Additional studies are needed to clarify the nature of the relationship between PACAP reductions and the neuropathologic features of AD and the extent to which it might provide a target for therapeutic interventions".

In a pilot study, Marksteiner et al (2014) searched for changes in plasma levels of 27 vascular-related proteins of healthy controls, patients with MCI and AD. In a sample of 80 participants, these researchers showed that out of these 27 proteins, 6 proteins were slightly changed (up to 1.5×) in AD (alpha2-macroglobulin, apolipoprotein-A1, plasminogen activator inhibitor, RAGE, Tissue Inhibitors of Metalloproteinases-1 and Trombospondin-2) and 1 marker (serum amyloid A) was enhanced up to 6× but with a very high variance. However, N-terminal pro-brain natriuretic peptide (NT-proBNP) was significantly enhanced both in MCI and AD patients (1.9×). In a second analysis of a sample of 110 subjects including younger healthy controls, these investigators confirmed that NT-proBNP has the potential to be a stable candidate protein for both diagnosis and AD disease progression.

Schaffer et al (2015) reviewed the current diagnostic standards for AD with an emphasis on early diagnosis using the CSF biomarkers amyloid-beta, total tau (t-tau), and phosphorylated tau (p-tau) and fluorodeoxyglucose positron emission tomography (PET) imaging.
Abnormal levels of these CSF biomarkers and decreased cerebral uptake of glucose have recently been used in the early diagnosis of AD in experimental studies. These promising biomarkers can be measured using immunoassays performed in singleplex or multiplex formats. Although presently, there are no Food and Drug Administration (FDA)-approved in-vitro diagnostics (IVDs) for early detection of AD, a multiplex immunoassay measuring a panel of promising AD biomarkers in CSF may be a likely IVD candidate for the clinical AD diagnostic market. Specifically, the INNO-BIA AlzBio3 immunoassay kit, performed using bead arrays on the xMAP Luminex analyzer, allows simultaneous quantification of amyloid-beta, t-tau, and p-tau bio markers.

Rivero-Santana and colleagues (2017) stated that differential diagnosis in dementia is at present one of the main challenges both in clinical practice and research; CSF biomarkers are included in the current diagnostic criteria of AD but their clinical utility is still unclear. These investigators performed a systematic review of studies analyzing the diagnostic performance of CSF Aβ42, t-tau, and p-tau in the discrimination between AD and fronto-temporal lobar degeneration (FTLD) dementias. The following electronic databases were consulted until May 2016: Medline and PreMedline, Embase, PsycInfo, CINAHL, Cochrane Library, and CRD. For the first-time in the field, a Hierarchical Summary Receiver Operating Characteristic (HRSOC) model was applied, which avoids methodological problems of meta-analyses based on summary points of sensitivity and specificity values. They also investigated relevant confounders of CSF biomarkers' diagnostic performance such as age, disease duration, and global cognitive impairment. The p-tau/Aβ42 ratio showed the best diagnostic performance. No statistically significant effects of the confounders were observed. Nonetheless, the p-tau/Aβ42 ratio may be especially indicated for younger patients; p-tau may be preferable for less cognitively impaired patients (high MMSE scores) and the t-tau/Aβ42 ratio for more cognitively impaired patients (low MMSE scores). The authors concluded that p-tau/Aβ42 ratio has potential for being implemented in the clinical routine for the differential diagnosis between AD and FTLD. They stated that it is of utmost importance that future studies report information on confounders such as age, disease duration, and cognitive impairment, which should also stimulate understanding of the role of these factors in disease mechanisms and pathophysiology.
Bcl-2 rs956572 Polymorphism Testing

Chang and colleagues (2018) noted that AD is a complex neurodegenerative disease, and genetic differences may mediate neuronal degeneration. In humans, a single-nucleotide polymorphism in the B-cell chronic lymphocytic leukemia/lymphoma-2 (Bcl-2) gene, rs956572, has been found to significantly modulate Bcl-2 protein expression in the brain. The Bcl-2 AA genotype has been associated with reduced Bcl-2 levels and lower gray matter volume in healthy populations. These investigators hypothesized that different Bcl-2 genotype groups may modulate large-scale brain networks that determine neurobehavioral test scores. Gray matter structural covariance networks (SCNs) were constructed in 104 patients with AD using T1-weighted MRI with seed-based correlation analysis. Patients were stratified into 2 genotype groups on the basis of Bcl-2 expression (G carriers, n = 76; A homozygotes, n = 28). Four SCNs characteristic of AD were constructed from seeds in the default mode network, salience network, and executive control network, and cognitive test scores served as the major outcome factor. For the G carriers, influences of the SCNs were observed mostly in the default mode network, of which the peak clusters anchored by the posterior cingulate cortex seed determined the cognitive test scores. In contrast, genetic influences in the A homozygotes were found mainly in the executive control network, and both the dorsolateral prefrontal cortex seed and the inter-connected peak clusters were correlated with the clinical scores. Despite a small number of cases, the A homozygotes showed greater co-variance strength than the G carriers among all 4 SCNs. The authors concluded that these findings suggested that the Bcl-2 rs956572 polymorphism is associated with different strengths of structural covariance in AD that determine clinical outcomes. The greater co-variance strength in the 4 SCNs shown in the A homozygotes suggested that different Bcl-2 polymorphisms play different modulatory roles. Moreover, these researchers stated that they will investigate the biological meaning of co-variance strength in future studies with longitudinal follow-up.

The authors stated that this study had several drawbacks. Direct comparisons between the G carriers and A homozygotes showed between-group differences in NPI total scores, aggression, and sleep.
sub-scores. However, network analysis did not find any relationships linking the SCNs to any of the significant clinical results. Because the study design used a seed-based approach, it was possible that the changes were mediated by different functional networks. The use of independent component analysis may help to overcome this limitation. Another important drawback of this study was that these investigators did not include a control group, and the inclusion of a control group may have helped to elucidate whether the Bcl-2 functional polymorphisms exerted similar GM modulation patterns in healthy elderly subjects. However, associations between rs956572 functional polymorphisms and regional GM volume and functional states in healthy subjects had been reported. Given the differences in methods, sample sizes, and populations analyzed, it was difficult to compare the findings in this study directly with these reports. However, the authors found regional similarities in this study. Another potential drawback was that the seed-based analysis emphasized the SCNs that showed positive correlations with the seeds. Because the objective of this trial was to test the hypothesis of the risk of different genotype groups, anti-correlation patterns suggestive of a compensatory process in each genotype group were not explored. Because clinical significance was established in restricted nodes showing co-variance interactions, whether the seed or peak clusters imparted an equal amount of information within the whole network remained an important issue that needs to be investigated in future studies. Last, structural co-variance data could not be directly referred to as a connectivity or degenerative biomarker, although the patterns of SCN had been shown to mirror those of intrinsic connectivity patterns in healthy control subjects. The authors stated that further studies with longitudinal cohorts are needed to validate the interpretation regarding greater co-variance strength in the AA genotype and faster degeneration. In addition, to elucidate the functional effect of the A allele observed in this study, a larger sample cohort is needed in future studies so that direct comparisons of the AA and GG genotypes with equal and adequate case numbers are possible.

Cerebrospinal Fluid (CSF) β-Secretase Activity

In a cross-sectional, multi-center study, Alcolea et al (2015) examined CSF markers involved in amyloid precursor protein processing, neuronal damage, and neuro-inflammation in the pre-clinical stages of AD and participants with suspected non-Alzheimer pathology (SNAP). These
researchers collected CSF from 266 cognitively normal volunteers participating in the SIGNAL study to investigate markers involved in amyloid precursor protein processing (Aβ42, sAPPβ, β-secretase activity), neuronal damage (total-tau [t-tau], phospho-tau [p-tau]), and neuro-inflammation (YKL-40). They analyzed the relationship among biomarkers, clinical variables, and the APOE genotype, and compared biomarker levels across the pre-clinical stages of the National Institute on Aging-Alzheimer's Association classification: stage 0, 1, 2, 3, and SNAP. The median age in the whole cohort was 58.8 years (range of 39.8 to 81.6). Participants in stages 2-3 and SNAP had higher levels of YKL-40 than those in stages 0 and 1. Participants with SNAP had higher levels of sAPPβ than participants in stage 0 and 1. No differences were found between stages 0, 1, and 2-3 in sAPPβ and β-secretase activity in CSF. Age correlated with t-tau, p-tau, and YKL-40. It also correlated with Aβ42, but only in APOE ε4 carriers. Aβ42 correlated positively with t-tau, sAPPβ, and YKL-40 in participants with normal Aβ42. The authors concluded that the findings of this study suggested that inflammation in the CNS increases in normal aging and is intimately related to markers of neurodegeneration in the preclinical stages of AD and SNAP; sAPPβ and β-secretase activity are not useful diagnostic or staging markers in preclinical AD.

Furthermore, an UpToDate review on "Clinical features and diagnosis of Alzheimer disease" (Wolk and Dickerson, 2016) states that "In general, the topographic biomarkers are less specific than the molecular biomarkers but correlate better with the emergence of clinical symptoms. None of these tests is valid as a stand-alone diagnostic test, but research criteria have incorporated both molecular and topographic biomarker data into the research definitions of both symptomatic and pre-symptomatic forms of AD, anticipating that once biomarkers become more standardized they will be incorporated into clinical diagnostic algorithms for AD. At present, the use of biomarkers is limited primarily to investigational studies and clinical trials, and testing is not universally available or reimbursed by most insurers".

Cerebrospinal Fluid (CSF) Golgin A4
Kork and colleagues (2018) identified a new, as yet unknown molecule in CSF that could serve as marker for AD. These researchers immunized mice with human CSF and fused hybridomas for monoclonal antibodies and screened these antibodies for their capacity to discriminate CSF of patients with AD from CSF of controls. They then chromatographically isolated the antigen to the best discriminating antibody and identified the antigen using mass spectrometric methods. Thereafter, these investigators quantified the CSF concentration of the antigen in a new cohort of patients with AD and controls and performed immunohistochemistry of post-mortem brain tissue derived from patients with AD and controls. These researchers generated greater than 200 hybridomas and selected 1 antibody that discriminated CSF from patients with AD from that of controls. They identified golgin A4 as the antigen detected by this antibody. Golgin A4 concentration was significantly higher in CSF from patients with AD than in CSF of controls (145 [IQR 125 to 155] versus 115 [99 to 128] pg/ml, p < 0.001) and demonstrated a substantial discriminative power (area under the receiver operating characteristic curve 0.80, 95 % CI: 0.67 to 0.94). Immunohistochemistry of post-mortem brain sections from patients with AD revealed a significant accumulation of golgin A4 in granulo-vacuolar degeneration bodies (GVBs). The authors concluded that these findings supported the notion that golgin A4 could serve as a diagnostic marker in AD. Moreover, these researchers stated that for validation of this notion, prospective, multi-center diagnostic studies are needed to evaluate golgin A4 as diagnostic marker for AD. Furthermore, it has to be examined if the association of golgin A4 with GVBs is an epiphenomenon or whether golgin A4 plays a more direct role in AD, allowing it to serve as a target in therapeutic treatment strategies. This study provided Class III evidence that elevated CSF golgin A4 levels identify patients with AD.

Cerebrospinal Fluid (CSF) Phospho-Tau

An UpToDate review on "Clinical features and diagnosis of Alzheimer disease" (Wolk and Dickerson, 2019) states that "Role of biomarkers – There are several widely investigated biomarkers for the molecular and degenerative process of AD that can be supportive of a diagnosis of AD but are not yet recommended for routine diagnostic purposes. Such testing can add incremental confidence to a clinical diagnosis of AD, however, and can be useful in certain circumstances, including early-
onset dementia and atypical presentations of AD in which the differential diagnosis includes other non-amyloid neurodegenerative diseases such as FTD. Molecular biomarkers of Aβ protein deposition include low cerebrospinal fluid (CSF) Aβ42 (or Aβ42:Aβ40 ratio) and positive amyloid PET imaging using one of the amyloid PET tracers. Biomarkers of tau deposition (a key component of neurofibrillary tangles) include increased CSF total tau and phospho-tau”.

Hanes and colleagues (2020) examined if tau phosphorylated at Thr217 (p-tau T217) assay in CSF can distinguish patients with AD from patients with other dementias and healthy controls. These researchers developed and validated a novel Simoa immunoassay to detect p-tau T217 in CSF. There was a total of 190 subjects from 3 cohorts with AD (n = 77) and other neurodegenerative diseases (n = 69) as well as healthy participants (n = 44). The p-tau T217 assay (cut-off of 242 pg/ml) identified patients with AD with accuracy of 90 %, with 78 % positive predictive value (PPV), 97 % negative predictive value (NPV), 93 % sensitivity, and 88 % specificity, compared favorably with p-tau T181 ELISA (52 pg/ml), showing 78 % accuracy, 58 % PPV, 98 % NPV, 71 % specificity, and 97 % sensitivity. The assay distinguished patients with AD from age-matched healthy controls (cut-off of 163 pg/ml, 98 % sensitivity, 93 % specificity), similarly to p-tau T181 ELISA (cut-off of 60 pg/ml, 96 % sensitivity, 86 % specificity). In patients with AD, these investigators found a strong correlation between p-tau T217 and p-tau T181, total tau and β-amyloid 40, but not β-amyloid 42. The authors concluded that the findings of this study showed that p-tau T217 displayed better diagnostic accuracy than p-tau T181 suggesting that the new p-tau T217 assay has potential as an AD diagnostic test in clinical evaluation. These researchers stated that this study provided Class III evidence that a CSF immunoassay for p-tau T217 distinguishes patients with AD from patients with other dementias and healthy controls. They stated that in the future, the assay can potentially be used for diagnostic purposes as well as for patient stratification and enrichment of target populations in clinical trials for disease-modifying therapies.

The authors stated that the potential drawbacks of this study were the small sample size and reliance on data from Europe, which may limit generalizability to non-European populations. Results should be
replicated in larger cohorts ideally characterized by both amyloid and tau PET imaging and validated in routine clinical practice. In order to introduce p-tau T217 assay in clinical routine practice, the technology should undergo a structured assessment to examine its benefit in terms of clinical utility and cost-effectiveness.

Schindler et al. (2018) state that levels of amyloid β peptide 42 (Aβ42), total tau, and phosphorylated tau-181 are well-established cerebrospinal fluid (CSF) biomarkers of Alzheimer’s disease, but variability in manual plate-based assays has limited their use. The authors examined the relationship between CSF biomarkers, as measured by a novel automated immunoassay platform, and amyloid positron emission tomography (PET). CSF samples from 200 individuals underwent separate analysis for Aβ42, total tau, and phosphorylated tau-181 with automated Roche Elecsys assays. Aβ40 was measured with a commercial plate-based assay. PET with Pittsburgh Compound B was performed less than 1 year from CSF collection. The results showed that the ratios of CSF biomarkers (total tau/Aβ42, phosphorylated tau-181/Aβ42, and Aβ42/Aβ40) best discriminated Pittsburgh Compound B-positive from Pittsburgh Compound B-negative individuals. The authors concluded that CSF biomarkers and amyloid PET reflect different aspects of Alzheimer’s disease brain pathology, and therefore, less-than-perfect correspondence is expected. Automated assays are likely to increase the utility of CSF biomarkers.

An UpToDate review on "Mild cognitive impairment: Prognosis and treatment" (Petersen, 2020) states that "a number of smaller studies have examined the use of cerebrospinal fluid (CSF) markers for predicting conversion from MCI [mild cognitive impairment] to dementia". The CSF biomarkers most often found to be predictive include increased levels of tau or tau protein phosphorylated at Thr 181 and lower levels of amyloid beta 42 (Aβ42) peptide, a low ratio of Aβ42 to Aβ40 levels, and a low ratio of Aβ42 to tau levels.

Cerebrospinal Fluid (CSF) Synaptic Biomarkers (e.g., Neurogranin)

Kvartsberg and co-workers (2015) developed 3 monoclonal anti-neurogranin (NGRN) antibodies. Mass spectrometry and a novel ELISA were used to analyze CSF NGRN in 3 independent clinical cohorts including patients with AD dementia (n = 100 in total), MCI patients (n =
40) and controls (n = 80 in total). These researchers showed in 3 independent clinical cohorts a marked increase in CSF NGRN levels in AD dementia (p < 0.001 in all studies). In addition, high CSF NGRN levels at the MCI stage predicted progression to dementia due to AD with a HR of 12.8 (95% CI: 1.6 to 103.0, p = 0.02). In amyloid-positive MCI patients, high CSF NGRN correlated with a more rapid change in cognition during clinical follow-up (p = 0.03). The authors concluded that the findings of this study suggested that CSF NGRN is a novel AD biomarker that may be used to monitor synaptic degeneration, and correlates with the rate of cognitive decline in prodromal AD.

Kester and colleagues (2015) stated that NGRN appeared to be a promising novel CSF biomarker for synaptic loss; however, clinical, and especially longitudinal, data are sparse. These researchers examined the utility of NGRN, with repeated CSF sampling, for diagnosis, prognosis, and monitoring of AD. They performed a longitudinal study of consecutive patients who underwent 2 lumbar punctures between the beginning of 1995 and the end of 2010 within the memory clinic-based Amsterdam Dementia Cohort. The study included 163 patients: 37 cognitively normal participants (mean [SE] age of 64 [2] years; 38 %
female; and mean [SE] MMSE score of 28 [0.3]), 61 patients with MCI (mean [SE] age of 68 [1] years; 38 % female; and mean [SE] MMSE score of 27 [0.3]), and 65 patients with AD (mean [SE] age of 65 [1] years; 45 % female; and mean [SE] MMSE score of 22 [0.7]). The mean (SE) interval between lumbar punctures was 2.0 (0.1) years, and the mean (SE) duration of cognitive follow-up was 3.8 (0.2) years. Measurements of CSF NGRN levels were obtained in January and February 2014. Main outcome measure was levels of NGRN in CSF samples. Baseline CSF levels of NGRN in patients with AD (median level of 2,381 pg/ml [interquartile range (IQR), 1,651 to 3,416 pg/ml]) were higher than in cognitively normal participants (median level of 1,712 pg/ml [IQR, 1,206 to 2,724 pg/ml]) (p = 0.04). Baseline NGRN levels were highly correlated with total tau and tau phosphorylated at threonine 181 in all patient groups (all p < 0.001), but not with Aβ42. Baseline CSF levels of NGRN were also higher in patients with MCI who progressed to AD (median level of 2,842 pg/ml IQR, 1,882 to 3,950 pg/ml) compared with those with stable MCI (median level of 1,752 pg/ml [IQR, 1,024 to 2,438 pg/ml]) (p = .004), and they were predictive of progression from MCI to AD...
(HR, 1.8 [95% CI: 1.1 to 2.9]; stratified by tertiles). Linear mixed-model analyses demonstrated that within-person levels of NGRN increased over time in cognitively normal participants (mean [SE] level of 90 [45] pg/ml per year; \( p < 0.05 \)) but not in patients with MCI or AD. The authors concluded that neurogranin is a promising biomarker for AD because levels were elevated in patients with AD compared with cognitively normal participants and predicted progression from MCI to AD. Within-person levels of NGRN increased in cognitively normal participants but not in patients with later stage MCI or AD, which suggested that NGRN may reflect pre-symptomatic synaptic dysfunction or loss.

The authors stated that levels of NGRN in CSF were much higher in this study than in the study by Kvartsberg et al (2015), which also used an ELISA. Differences in absolute values may be a result of the different antibodies used in the 2 assays. Further studies comparing both ELISAs are needed to clarify this matter. However, the direction and order of magnitude of the variance between patient groups were the same. It is possible that differences in the calibration and/or antibodies used contributed to the absolute differences. Among the drawbacks, the cognitively normal group was a mixed group of subjects that also included patients with psychiatric disorders and temporal lobe epilepsy, which may hamper generalizability. Furthermore, the cognitively normal group was biased toward subjects who showed decline (6 progressed to MCI, and 4 to dementia), which could have even diluted the baseline effect. This bias could be due to the fact that subjects who progressed were more likely to return to the clinic for a second lumbar puncture. However, because of this follow-up, these investigators were able to evaluate change over time in NGRN levels for all stages of the AD continuum. Another drawback was that the size of the group of cognitively normal subjects was too small to reliably analyze for risk of progression with Cox proportional hazards models.

Wellington and associates (2016) evaluated the specificity of the dendritic protein NGRN in CSF from patients with a broad range of neurodegenerative diseases including a variety of dementias, tauopathies, and synucleinopathies. An optimized immunoassay was used to analyze CSF NGRN in a retrospective cohort of 331 participants with different neurodegenerative diseases, including healthy controls (n = 19), biomarker-proven AD (n = 100), genetic AD (n = 2), behavioral
variant fronto-temporal dementia (n = 20), speech variant fronto-temporal dementia (n = 21), Lewy body dementia (n = 13), Parkinson disease (n = 31), progressive supranuclear palsy (n = 46), multiple system atrophy (n = 29), as well as a heterogeneous group with non-neurodegenerative cognitive impairment (n = 50). CSF NGRN concentrations and correlations of CSF NGRN with total tau, phosphorylated tau, and β-amyloid 42 concentrations, MMSE score, and disease duration in the different groups were investigated. Median CSF NGRN concentration was higher in patients with AD compared to both controls (p < 0.001) and all other disease groups (all p < 0.001) except speech variant fronto-temporal dementia. There were no significant differences in CSF NGRN concentrations between any other neurodegenerative groups and controls. In addition, these researchers found strong correlations between NGRN and total tau (p < 0.001) and phosphorylated tau (p < 0.001). The authors concluded that these findings confirmed an increase in CSF NGRN concentration in patients with AD as previously reported and showed that this is specific to AD and not seen in a range of other neurodegenerative diseases.

This study had several drawbacks: (i) the small sample size in some groups, most notably in those with genetic AD. With this caveat, it is of interest to note that one of the highest NGRN values was observed in a PSEN1 mutation carrier (A426P, NGRN 1,162 pg/ml) – whether this reflects familial AD in general or specific mutations/mechanisms needs to be assessed in larger cohorts, (ii) the relatively high coefficients of variation of the NGRN assay (20 % to 30 %). This made it harder to interpret individual CSF NGRN concentrations in relation to fixed cut-off points, as well as to detect treatment-induced changes over time in CSF NGRN concentrations in clinical trials. Developing more precise assays for CSF NGRN is an important area for further study, (iii) while the patients with AD had their clinical diagnosis supported by biomarkers, the other diagnoses were made on clinical grounds only, as diagnostically useful biomarkers for these disorders are presently lacking. Furthermore, all controls were AD biomarker-negative. This enrichment approach allowed for a very pure control group to be defined, with the advantage that these results may reflect "real" AD versus "true" control differences; in less well characterized individuals and
in particular given that a proportion of apparently healthy controls may have pre-symptomatic AD changes, results may be less discriminatory. However, the similar CSF NGRN concentrations in the control group compared with the other non-AD groups suggested that this may not be a major issue in practice.

The authors proposed that high CSF NGRN concentrations reflect synapse degeneration in AD-affected brain regions and that CSF NGRN has potential as a diagnostic marker for AD in combination with existing CSF biomarkers. They stated that further studies are needed to test the hypothesis that CSF NGRN may have utility as a very early and potentially pre-symptomatic biomarker for AD, as a prognostic marker in the clinic, and as an outcome measure in clinical trials.

In a retrospective, cross-sectional, mono-centric study, Tible and colleagues (2020) examined the ability of a combination of synaptic CSF biomarkers to separate AD and non-AD disorders and to aid in the differential diagnosis between neurocognitive diseases. All participants explored with CSF assessments for neurocognitive decline were invited to participate. After complete clinical and imaging evaluations, a total of 243 patients were included. CSF synaptic (GAP-43, neurogranin, SNAP-25 total, SNAP-25aa40, synaptotagmin-1) and AD biomarkers were blindly quantified with ELISA or mass spectrometry. Statistical analysis compared CSF levels between the various groups of AD dementias (n = 81), MCI-AD (n = 30), other MCI (n = 49), other dementias (OD) (n = 49), and neurologic controls (n = 35) and their discriminatory powers. All synaptic biomarkers were significantly increased in patients with MCI-AD and AD-dementia compared to the other groups. All synaptic biomarkers could efficiently discriminate AD dementias from OD (AUC ≥ 0.80). All but synaptotagmin were also able to discriminate patients with MCI-AD from controls (area under the curve [AUC] ≥ 0.85) and those with AD dementias from controls (AUC ≥ 0.80). Overall, CSF SNAP-25aa40 had the highest discriminative power (AUC of 0.93 between patients with AD dementias and controls or OD, AUC of 0.90 between those with MCI-AD and controls). Higher levels were associated with 2 alleles of APOE ε4. The authors concluded that all synaptic biomarkers tested had a good discriminatory power to distinguish patients with AD abnormal CSF from those with non-AD disorders. SNAP25aa40 demonstrated the highest
power to discriminate AD CSF-positive patients from patients without AD and neurologic controls in this cohort. These researchers stated that if validated, these biomarkers could be used in clinical practice as biomarkers of synaptic alteration when the demise is still potentially reversible; as a very good diagnostic tools to differentiate early various neurodegenerative dementias; and as an indirect evaluation of the progression of synaptic pathology.

The authors stated that the main drawback of this study was that it was a retrospective, cross-sectional, mono-centric discovery cohort rather than a prospective, longitudinal, multi-centric study. As a consequence, the range of methodologic approaches has been limited, and the assessment of predictive value of these biomarkers has been restrained. In some groups, the number of APOE genotypes was too low to provide useful data for statistical analysis per group. The APOE ε2/2 genotype is extremely rare, and APOE ε4/4 is less frequent in non-AD groups. The other drawback was linked to the heterogeneity of the neurologic controls in the cohort, including cognitive complaints and psychiatric disorders. A comparison of patients with AD dementias and MCI-AD with age-matched healthy controls could be envisaged.

Circadian Rhythm Analysis

Musiek and colleagues (2018) stated that circadian rhythm disturbances occur in symptomatic AD and have been hypothesized to contribute to disease pathogenesis. However, it is unknown whether circadian changes occur during the pre-symptomatic phase of the disease. In a cross-sectional study, these researchers examined the associations between circadian function, aging, and pre-clinical AD pathology in cognitively normal adults. This trial was conducted using community volunteers from the Knight Alzheimer's Disease Research Center at Washington University in St Louis. Cognitively normal participants (n = 205) underwent 7 to 14 days of actigraphy in their home environment between 2010 and 2012, in addition to clinical assessment, amyloid imaging with Pittsburgh Compound B (PiB), and CSF biomarker collection. Data collected from 3 years before to 6 months after actigraphy were included; 16 participants were excluded owing to incomplete data collection. Circadian rhythm analysis was performed on actigraphy data using 3 methods: cosinor, non-parametric, and empirical mode decomposition. Pre-clinical AD was assessed by longitudinal
clinical assessment, amyloid imaging with PiB, and CSF biomarker collection. Data from 189 participants were included in the analyses. The mean (SD) age was 66.6 (8.3) years, and 121 participants (64 %) were women. Older age ($\beta = 0.247; p = 0.003$) and male sex ($\beta = 0.170; p = 0.04$), in the absence of amyloid pathology, were associated with a significant increase in intra-daily variability, a non-parametric measure of rest-activity rhythm fragmentation, as well as decreased amplitude by several measures. After correction for age and sex, the presence of pre-clinical amyloid plaque pathology, assessed by positive PiB imaging (mean [SD], 0.804 [0.187] for PiB negative versus 0.875 [0.178] for PiB positive; $p = 0.05$) or increasing CSF phosphorylated-tau to amyloid $\beta$42 ratio ($\beta = 0.231; p = 0.008$), was associated with increased intra-daily variability, indicating rest-activity rhythm fragmentation. The authors concluded that pre-clinical AD was associated with rest-activity rhythm fragmentation, independent of age or sex. Aging was also associated with circadian dysfunction independently of pre-clinical AD pathology, particularly in men. They stated that the presence of circadian rhythm abnormalities in the pre-clinical phase of AD suggested that circadian dysfunction could contribute to early disease pathogenesis or serve as a biomarker of pre-clinical disease.

This study had several drawbacks: The authors could not exclude the possibility that the age- or sex-related circadian changes observed in the amyloid-negative group were caused by non-amyloid pathologies, which are commonly seen in the aged brain. They did not have information on medications and co-morbidities, specifically sleep disorders. Sleep apnea in particular was not assessed, although it is common and may influence amyloid burden. Furthermore, non-circadian factors (e.g., voluntary exercise) can influence rest-activity measurements. Replication using other circadian parameters (such as core body temperature) could be considered to confirm these findings.

DNA Methylation Profiling (Brain Tissue or Peripheral Blood)

Li and colleagues (2016) stated that AD is a common aging-related neurodegenerative illness. Recently, many studies have tried to identify AD- or aging-related DNA methylation (DNAm) biomarkers from peripheral whole blood (PWB). However, the origin of PWB biomarkers is still controversial. In this study, by analyzing 2,565 DNAm profiles for PWB and brain tissue, these researchers showed that aging-related
DNAm CpGs (Age-CpGs) and AD-related DNAm CpGs (AD-CpGs) observable in PWB both mainly reflected DNAm alterations intrinsic in leukocyte subtypes rather than methylation differences introduced by the increased ratio of myeloid to lymphoid cells during aging or AD progression. The PWB Age-CpGs and AD-CpGs significantly overlapped 107 sites ($p = 2.61 \times 10^{-12}$) and 97 had significantly concordant methylation alterations in AD and aging ($p < 2.2 \times 10^{-16}$), which were significantly enriched in nervous system development, neuron differentiation and neurogenesis. More than 60.8% of these 97 concordant sites were found to be significantly correlated with age in normal peripheral CD4+ T cells and CD14+ monocytes as well as in 4 brain regions, and 44 sites were also significantly differentially methylated in different regions of AD brain tissue. The authors concluded that the PWB DNAm alterations related to both aging and AD could be exploited for identification of AD biomarkers.

Watson and associates (2016) performed a genome-wide screen of DNA methylation using the Illumina Infinium HumanMethylation450 platform on bulk tissue samples from the superior temporal gyrus of patients with AD and non-demented controls. They paired a sliding window approach with multi-variate linear regression to characterize AD-associated differentially methylated regions (DMRs). These researchers identified 479 DMRs exhibiting a strong bias for hyper-methylated changes, a subset of which were independently associated with aging. DMR intervals overlapped 475 RefSeq genes enriched for gene ontology categories with relevant roles in neuron function and development, as well as cellular metabolism, and included genes reported in AD genome-wide and epigenome-wide association studies. DMRs were enriched for brain-specific histone signatures and for binding motifs of transcription factors with roles in the brain and AD pathology. Notably, hyper-methylated DMRs preferentially overlapped poised promoter regions, marked by H3K27me3 and H3K4me3, previously shown to co-localize with aging-associated hypermethylation. Finally, the integration of DMR-associated single nucleotide polymorphisms with AD genome-wide association study risk loci and brain expression quantitative trait loci highlighted multiple potential DMRs of interest for further functional analysis. The authors characterized changes in DNA methylation in the superior temporal gyrus of patients with AD, highlighting novel loci that facilitate better characterization of
pathways and mechanisms underlying AD pathogenesis, and improve the understanding of epigenetic signatures that may contribute to the development of disease; and suggested more targeted research in this area may be needed. They stated that future challenges in the field include the development of effective strategies for integrating epigenetic and transcriptomic profiles with genetic datasets, as a means to better understand the roles of different forms of variation in AD.

Gao and co-workers (2018) noted that abnormal DNA methylation patterns have been demonstrated to be associated with the pathogenesis of AD. These researchers identified differential methylation in the superior temporal gyrus (STG) of patients with late-onset AD based on epigenome-wide DNA methylation data by bioinformatics analysis. The genome-wide DNA methylation data in the STG region of 34 patients with late-onset AD and 34 controls without dementia were recruited from the Gene Expression Omnibus database. Through systemic quality control, differentially methylated CpG sites were determined by the Student's t-test and mean methylation value differences between the 2 conditions. Hierarchical clustering analysis was applied to assess the classification performance of differentially methylated CpGs. Functional analysis was performed to examine the biological functions of the genes associated with differentially methylated CpGs. A total of 17,895 differentially methylated CpG sites were initially identified, including 11,822 hyper-methylated CpGs and 6,073 hypo-methylated CpGs. Further analysis examined 2,211 differentially methylated CpGs (covering 1,991 genes). Subjects with AD demonstrated distinctive DNA methylation patterns when compared with the controls, with a classification accuracy value of 1. Hyper-methylation was mainly detected for genes regulating the cell cycle progression, whereas hypo-methylation was observed in genes involved in transcription factor binding. The authors concluded that the findings of this study demonstrated widespread and distinctive DNA methylation alterations in late-onset AD. They stated that identification of AD-associated epigenetic biomarkers may allow for the development of novel diagnostic and therapeutic targets.

Long-Term Measurement of Cortisol

In a prospective, longitudinal study, Ennis and associates (2017) examined if cortisol dysregulation was related to AD risk. Participants were from the Baltimore Longitudinal Study of Aging (BLSA) and
submitted multiple 24-hour urine samples over an average interval of 10.56 years. Urinary free cortisol (UFC) and creatinine (Cr) were measured, and a UFC/Cr ratio was calculated to standardize UFC. To measure cortisol regulation, these researchers used within-person UFC/Cr level (i.e., within-person mean), change in UFC/Cr over time (i.e., within-person slope), and UFC/Cr variability (i.e., within-person coefficient of variation). Cox regression was used to assess whether UFC/Cr measures predicted AD risk. UFC/Cr level and UFC/Cr variability, but not UFC/Cr slope, were significant predictors of AD risk an average of 2.9 years before AD onset. Elevated UFC/Cr level and elevated UFC/Cr variability were related to a 1.31- and 1.38-times increase in AD risk, respectively. In a sensitivity analysis, increased UFC/Cr level and increased UFC/Cr variability predicted increased AD risk an average of 6 years before AD onset. The authors concluded that cortisol dysregulation as manifested by high UFC/Cr level and high UFC/Cr variability may modulate the down-stream clinical expression of AD pathology or be a pre-clinical marker of AD.

The authors noted that this study had several drawbacks: Use of 24-hour UFC provided a good measure of total daily cortisol production and excretion but obscured the cortisol circadian rhythm, which may be an important biomarker. CSF free cortisol, although difficult to collect in large longitudinal studies, would have provided an alternative measure of centrally active glucocorticoids. Few studies had correlated UFC to CSF cortisol, with one reporting a strong correlation. These researchers found significant relationships between UFC/Cr measures and AD risk using data for which the last cortisol collection occurred on average from 2.9 to 6 years before AD onset. Because AD has a prolonged prodromal period, it is difficult to distinguish causative factors from prodromal changes. The use of AD onset rather than diagnosis in this study excluded data collected during the immediate prodromal period but included data collected during the pre-clinical phase of AD, when pathology may already be evident.

MindX Blood Test - Memory / Alzheimer's

MindX Sciences offers the MindX Blood Test - Memory/Alzheimer's,
mRNA, which evaluates gene expression profiling by RNA sequencing of 24 genes using whole blood with an algorithm reported as a predictive risk score. The test is available by prescription only. The test is indicated for middle aged or elderly patients presenting signs of forgetfulness, depression, periods of high stress, and a family history of dementia. During the test, 24 biomarker genes associated with Alzheimer's Disease are sequenced to determine biomarker gene expression levels. A proprietary algorithm is applied to generate predictive risk scores. A qualified laboratory professional compiles the report that is communicated to the ordering provider detailing mortality risk and suggested medications based on the predictive scores and medication suggestions. The test report shows the memory score and the risk of AD in the first year (immediate risk) and the risk of death in future years. The report also gives a list of medications and nutraceuticals that are potentially effective in reversing this risk. These medications and nutraceuticals are ranked in the order of percentiles (MindX Sciences, 2021). MindX Sciences cite Niculescu et al (2020) published peer-reviewed research in support of blood biomarkers evaluation for memory, Alzheimer's disease.

Niculescu et al (2020) conducted longitudinal within-subject cohort studies in male and female psychiatric patients to discover blood gene expression biomarkers that track short term memory as measured by the retention measure in the Hopkins Verbal Learning Test. These biomarkers were subsequently prioritized with a convergent functional genomics approach using previous evidence in the field implicating them in Alzheimer's disease (AD). The top candidate biomarkers were then tested in an independent cohort for ability to predict state short-term memory, and trait future positive neuropsychological testing for cognitive impairment. Based on the authors studies and analyses, the biomarkers with the best overall convergent functional evidence (CFE) for relevance to memory and AD were some new genes such as RAB7A, NPC2, TGFB1, GAP43, ARSB, PER1, GUSB, and MAPT (tau). Additional top blood biomarkers include GSK3B, PTGS2, APOE, BACE1, PSEN1, and TREM2, well known genes implicated in AD by previous brain and genetic studies, in humans and animal models, which serve as reassuring de facto positive controls for their whole-genome gene expression discovery approach. Biological pathway analyses implicate LXR/RXR activation, neuroinflammation, atherosclerosis signaling, and amyloid processing. Co-directionality of expression data provide new mechanistic insights that are consistent with a compensatory/scarring scenario for brain
pathological changes. A majority of top biomarkers also have evidence for involvement in other psychiatric disorders, particularly stress, providing a molecular basis for clinical co-morbidity and for stress as an early precipitant/risk factor. Some of them are modulated by existing drugs, such as antidepressants, lithium and omega-3 fatty acids. Other drug and nutraceutical leads were identified through bioinformatic drug repurposing analyses (such as pioglitazone, levonorgestrel, salsolidine, ginkgolide A, and icarin). The authors state that their work has provided evidence for novel possible precision medicine approaches, diagnostic and therapeutic. In particular, it may lead to improved early objective assessment of state, of future risk, and to targeted preventive treatments (in essence, a risk evaluation and mitigation system) for memory disorders in general, and AD in particular, that result in decreased quality and quantity of life, at a massive cost to individuals, families and society. It also opens a novel window into disease pathophysiology, and may lead to new targets for drug development. Given the growing world-wide burden of AD, and the unsuccessful approaches to date, such new avenues should be pursued with vigor and alacrity.

Morphometric Imaging, Protein Kinase C-epsilon (PKCe), and Quantitative Imaging of Phosphorylated Erk1 and Erk2

NeuroDiagnostics, LLC. offers the Discern test which uses a small skin sample (punch biopsy) to evaluate 3 biomarkers to identify and differentiate Alzheimer's disease (AD) from other dementias. Per the manufacturer, the 3 biomarkers comprise of an AD-Index assay, a Morphometric Imaging assay and a PKC Epsilon assay, which can determine the level of synaptic loss in the brain before the onset of amyloid plaques or tangles in the earliest stages of the onset of AD (i.e., years 1-4). The skin sample is incubated for a few weeks and grown to threshold volumes of greater than 1,000,000 cells, after which it is subjected to various assay measurements for 3 separate proprietary biomarkers, each of which, independently, identifies and differentiates AD from other dementias (NeuroDiagnostics, 2020). Thus, using morphometric imaging and protein kinase C-epsilon (PKCe) concentration in response to amylodospheroid treatment by ELISA, cultured skin fibroblasts, each are reported as positive or negative for AD. In addition, the AD-Index assay is a quantitative imaging of phosphorylated ERK1 and ERK2 in response to bradykinin treatment by in situ immunofluorescence, using cultured skin fibroblasts, and are reported as a probability index for AD.
AD-index biomarker evaluation includes taking a small specimen of skin fibroblasts which is then incubated to an 80-90% confluence stage. An inflammatory agonist (a small nano-peptide that induces Erk1 and Erk2 phosphorylation in fibroblasts) stimulates the skin specimen. Quantitative imaging of the phosphorylated Erk1 and Erk2 is then used to identify and differentiate Alzheimer’s Disease (AD) from Non-AD dementia (Non-ADD) and Age-matched control (AC) specimens (NeuroDiagnostics, 2020).

Khan and Alkon (2010) evaluate early diagnostic accuracy and pathophysiologic relevance of an autopsy-confirmed Alzheimer’s disease peripheral biomarker. The authors report that the inflammatory agonist bradykinin, a small nano-peptide, that induces PKC-mediated phosphorylation of Erk1 and Erk2 in fibroblasts, was applied to punch-biopsy-obtained human skin fibroblasts. Quantitative imaging of the phosphorylated Erk1 and Erk2 bands was then used in a ratio that is mathematically configured into an AD-Biomarker Index (AD-Index). Out of 264 subjects, there were 64 autopsy examinations. Demented individuals were clinically diagnosed as AD with an overall accuracy of 78%. Among the 42 autopsy-confirmed cases for which there were also AD-Biomarker measurements, the overall accuracy of the AD-Biomarker was 98%. Among both the autopsy-confirmed and the clinically diagnosed patients, the AD-Index values were inversely correlated with the duration of disease, i.e., the time from the onset of dementia symptoms. Among the autopsy-confirmed cases, the AD-Biomarker diagnosis showed remarkably high sensitivity (97%) and specificity (100%) compared to clinical diagnosis (sensitivity: 78% and specificity: 20%). Using autopsy validation, the clinical diagnosis was only accurate at 52% level vs. the AD-Biomarker accuracy of 100% for cases with dementia not larger than 4 years of duration. Finally, application of soluble Abeta(1-42) to the fibroblasts of normal controls induced the abnormal AD-Biomarker phenotype, suggesting the pathophysiologic relevance of this AD-Biomarker measurement. The authors concluded that the AD-Biomarker, as confirmed by autopsy validation, showed significantly higher sensitivity and specificity than did clinical diagnosis, particularly at early stages of disease, and that pathophysiological relevance was demonstrated for the mechanistic basis of the AD-Biomarker measurements.

Sun and Nan (2017) state that ERK1/2 is a potent effector of neuronal death and neuroinflammation in many CNS diseases. The authors discuss the extracellular signal-regulated kinase 1/2 pathway in neurological disease. The authors summarize by stating that the link
between the ERK1/2 signaling pathway and a variety of neurological diseases, including stroke, neuro-degenerative diseases and drug addiction, demonstrates the importance of studying the ERK1/2 pathway to human health. More detailed knowledge of the physiological and pathological functions of ERK1/2 in the adult nervous system may not only provide insight for the development of new therapeutic drugs for neurological disorders but also achieve clinical benefits for patients. The authors theorize that over the next several years, additional novel therapeutic strategies that utilize ERK1/2 signaling inhibitors will likely be developed for neurological disease clinical trials.

Morphometric imaging assay biomarker evaluation includes taking the cultured skin specimen which is then stimulated with an extracellular matrix composed of an array of macromolecules, forming networks which are dysregulated in AD skin fibroblasts. Networks are rapidly formed for Age-matched control and non-AD dementia cells, but not for AD cells. According to NeuroDiagnostics, LLC, the rate and extent of network formation can be quantified and is a highly accurate diagnostic biomarker of AD that corresponds to autopsy-demonstrated pathologic hallmarks of AD – amyloid plaques and neurofibrillary tangles. Biochemical determinants of network formation are similar in many respects to synapse network formation among culture neurons (NeuroDiagnostics, 2020).

Protein kinase C epsilon (PKCε) biomarker specific antibodies are used with the cultured skin specimen to quantify relative levels of PKCε and to distinguish AD patients from non-ADD and AC patients. According to NeuroDiagnostics, LLC, AD patients demonstrate a comparative deficit in PKCε and a different response to the ab stimulus when compared to AC and Non-ADD. Patients tested must first have a clinical diagnosis of dementia (the duration of such dementia to extend from the first year of dementia forward). The intended population will range in age from 55-90 years old. Peripheral skin samples are collected at the patient's healthcare provider's facility through a standard 2-3mm skin punch biopsy taken from the bicep/forearm area. The safety and effectiveness of the AD Biomarkers has not been established for monitoring responses to therapies among patients with a clinical diagnosis of AD (NeuroDiagnostics, 2020).

Khan et al. (2015) state that in AD transgenic mice, activation of synaptogenic PKCε was found to prevent synaptotoxic amyloid-β (Aβ)-
oligomer elevation, PKCε deficits, early synaptic loss, cognitive deficits, and amyloid plaque formation. In humans, to study the role of PKCε in the pathophysiology of AD and to evaluate its possible use as an early AD-biomarker, the authors examined PKCε and Aβ in the brains of autopsy-confirmed AD patients (n = 20) and age-matched controls (AC, n = 19), and in skin fibroblast samples from AD (n = 14), non-AD dementia patients (n = 14), and AC (n = 22). Intraneuronal Aβ levels were measured immunohistochemically (using an Aβ-specific antibody) in hippocampal pyramidal cells of human autopsy brains. The authors found that the PKCε was significantly lower in the hippocampus and temporal pole areas of AD brains, whereas Aβ levels were significantly higher. The ratio of PKCε to Aβ in individual CA1 pyramidal cells was markedly lower in the autopsy AD brains versus controls. PKCε was inversely correlated with Aβ levels in controls, whereas in AD patients, PKCε showed no significant correlation with Aβ. In autopsy brains, PKCε decreased as the Braak score increased. Skin fibroblast samples from AD patients also demonstrated a deficit in PKCε compared to controls and an AD-specific change in the Aβ-oligomer effects on PKCε. The authors conclude that together, these data demonstrate that the relationship between Aβ levels and PKCε is markedly altered in AD patients' brains and skin fibroblasts, reflecting a loss of protective effect of PKCε against toxic Aβ accumulation. These changes of PKCε levels in human skin fibroblasts may provide an accurate, non-invasive peripheral AD biomarker.

The American Academy of Neurology (AAN) does not recommend routine genetic testing for dementia with Lewy bodies (DLB), Jakob-Creutzfeldt disease (CJD), or APOE genotyping for AD. There is not enough evidence to support or refute the use of other genetic markers for AD.

Per UpToDate "Clinical features and diagnosis of Alzheimer's disease" (Wolk and Dickerson, 2018) state that genetic testing is not recommended in the routine evaluation of patients with AD.

Per UpToDate "Evaluation of cognitive impairment and dementia" (Larson, 2019), the use of genetic testing for AD in patients with dementia is controversial because of the potential for both false positives and false negatives.

Particulate Matter with an Aerodynamic Diameter of Less Than or Equal to 2.5 μm (PM2.5)

Tsai and colleagues (2019) stated that air pollution is a modifiable and
preventable factor, and it is a possible risk factor for dementia. However, evidence from epidemiological studies is still limited. These researchers carried out a systematic review and meta-analysis to summarize the epidemiological evidence for long-term effects of particulate matter with an aerodynamic diameter of less than or equal to 2.5 μm (PM2.5) on dementia/AD. The inclusion criteria for eligible studies were: longitudinal cohort study design, no overlap in study population, age of study subject of greater than or equal to 50 years, detailed description of exposure assessment for PM2.5, outdoor assessment of exposure to PM2.5, usage of a clear definition of dementia/AD, and accessibility of sufficient information for meta-analysis; 6 databases were searched for eligible studies. The random-effect model was used to synthesize the associations between PM2.5 and dementia. After exclusion of all irrelevant studies, these investigators analyzed the findings of 4 cohort studies performed in Canada, Taiwan, the United Kingdom, and the United States during 2015 to 2018 among more than 12 million elderly subjects aged greater than or equal to 50 years (n = 12,119,853). The meta-analysis showed that exposure to a 10 μg/m3 increase in PM2.5 was significantly and positively associated with dementia (pooled HR = 3.26, 95% CI: 1.20 to 5.31). In subgroup analyses, exposure to a 10 μg/m3 increase in PM2.5 was found to be positively associated with AD (pooled HR = 4.82, 95% CI: 2.28 to 7.36). Analysis of current epidemiological research on PM2.5 and dementia confirmed that exposure to PM2.5 was positively associated with a higher risk for dementia. However, it is to be noted that the included studies mainly relied on claim-based diagnosis and showed large differences in methods of exposure assessment, thus, further epidemiological studies with well validated outcomes and with standardized exposure assessment models are needed to determine the relationship between PM2.5 and dementia/AD.

Plasma Lipoproteome

Li and colleagues (2018) stated that although total plasma lipoproteome consists of proteins that have shown promises as biomarkers that can identify AD, effect sizes are modest. These investigators provided initial proof-of-concept that the plasma lipoproteome more likely differ between AD cases and controls when measured in individual plasma lipoprotein fractions than when measured as total in immuno-depleted plasma. These researchers first developed a targeted proteomics method based on selected reaction monitoring (SRM) and liquid chromatography and
tandem mass spectrometry for measurement of 120 tryptic peptides from 79 proteins that are commonly present in plasma lipoproteins. Then in a proof-of-concept case-control study of 5 AD cases and 5 sex- and age-matched controls, these researchers applied the targeted proteomic method and performed relatively quantification of 120 tryptic peptides in plasma lipoprotein fractions (fractionated by sequential gradient ultracentrifugation) and in immuno-depleted plasma (of albumin and IgG).

Unadjusted p values from 2-sample t-tests and overall fold change was used to evaluate a peptide relative difference between AD cases and controls, with lower p values (< 0.05) or greater fold differences (> 1.05 or < 0.95) suggestive of greater peptide/protein differences. Within-day and between-days technical precisions (mean % CV [SD] of all SRM transitions) of the targeted proteomic method were 3.95 % (2.65) and 9.31 % (5.59), respectively. Between-days technical precisions (mean % CV [SD]) of the entire plasma lipoproteomic workflow including plasma lipoprotein fractionation was 27.90 % (14.61); 10 tryptic peptides that belonged to 5 proteins in plasma lipoproteins had unadjusted p values < 0.05, compared to no peptides in immuno-depleted plasma. Furthermore, 27, 32, 17, and 20 tryptic peptides in VLDL, IDL, LDL and HDL, demonstrated overall peptide fold differences greater than 1.05 or less than 0.95, compared to only 6 tryptic peptides in immuno-depleted plasma. The overall comparisons, therefore, suggested greater peptide/protein differences in plasma lipoproteome when measured in individual plasma lipoproteins than as total in immuno-depleted plasma. Specifically, protein complement C3’s peptide IHWESASLLR, had unadjusted p values of 0.00007, 0.00012, and 0.0006 and overall 1.25, 1.17, 1.14-fold changes in VLDL, IDL, and LDL, respectively. After positive False Discovery Rate (pFDR) adjustment, the complement C3 peptide IHWESASLLR in VLDL remained statistically different (adjusted p value < 0.05). The authors concluded that these findings may warrant future studies to examine plasma lipoproteome when measured in individual plasma lipoprotein fractions for AD diagnosis.

Plasma Tau

Mattsson and colleagues (2016) examined if plasma tau is altered in AD and whether it is related to changes in cognition, CSF biomarkers of AD pathology (including β-amyloid [Aβ] and tau), brain atrophy, and brain metabolism. This was a study of plasma tau in prospectively followed patients with AD (n = 179), patients with mild cognitive impairment (n = 195), and cognitive healthy controls (n = 189) from the Alzheimer's
Alzheimer's Disease Tests - Medical Clinical Policy Bulletins | Aetna Disease Neuroimaging Initiative (ADNI) and cross-sectionally studied patients with AD (n = 61), MCI (n = 212), and subjective cognitive decline (n = 174) and controls (n = 274) from the Biomarkers for Identifying Neurodegenerative Disorders Early and Reliably (BioFINDER) study at Lund University, Sweden. A total of 1,284 participants were studied.

Associations were tested between plasma tau and diagnosis, CSF biomarkers, MRI measures, 18fluorodeoxyglucose-PET, and cognition. Higher plasma tau was associated with AD dementia, higher CSF tau, and lower CSF Aβ42, but the correlations were weak and differed between ADNI and BioFINDER. Longitudinal analysis in ADNI showed significant associations between plasma tau and worse cognition, more atrophy, and more hypo-metabolism during follow-up. The authors concluded that plasma tau partly reflected AD pathology, but the overlap between normal aging and AD was large, especially in patients without dementia. They stated that despite group-level differences, these results did not support plasma tau as an AD biomarker in individual people; future studies may test longitudinal plasma tau measurements in AD.

The authors noted that while this study was the largest published on plasma tau, but there were some drawbacks: (i) ADNI and BioFINDER samples were handled with different protocols and analyzed at different laboratories with different kit lots, which may have contributed to the varying results. Some ADNI participants had plasma tau below the lower limit of quantification of the assay (14 CNs, 11 patients with MCI, 10 patients with AD). These measurements were uncertain but they were included because excluding them would have biased the data toward higher plasma tau (excluding them did not change the main results, data not shown); (ii) these researchers used only 1 plasma tau assay. It is possible that other assays capture tau fragments that are less sensitive to peripheral degradation and more likely to reflect AD pathology, and (iii) these investigators did not co-vary for co-pathologies that may affect plasma tau levels.

Ashton and colleagues (2021) noted that the quantification of phosphorylated tau in biofluids, either CSF or plasma, has shown great promise in detecting AD pathophysiology. Tau phosphorylated at threonine 231 (p-tau231) is one such biomarker in CSF; however, its usefulness as a blood biomarker is currently unknown. These researchers developed an ultrasensitive single molecule array (Simoa) for the quantification of plasma p-tau231 that was validated in 4 independent cohorts (n = 588) in different settings, including the full AD continuum and
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non-AD neurodegenerative disorders. Plasma p-tau231 was able to identify patients with AD and differentiate them from amyloid-β negative cognitively unimpaired (CU) older adults with high accuracy (AUC = 0.92 to 0.94). Plasma p-tau231 also distinguished AD patients from patients with non-AD neurodegenerative disorders (AUC = 0.93), as well as from amyloid-β negative MCI patients (AUC = 0.89). In a neuropathology cohort, plasma p-tau231 in samples taken on average of 4.2 years prior to post-mortem very accurately identified AD neuropathology in comparison to non-AD neurodegenerative disorders (AUC = 0.99), this is despite all patients being given an AD dementia diagnosis during life. Plasma p-tau231 was highly correlated with CSF p-tau231, tau pathology as assessed by [18F]MK-6240 PET, and brain amyloidosis by [18F]AZD469 PET. Remarkably, the inflection point of plasma p-tau231, increasing as a function of continuous [18F]AZD469 amyloid-β PET standardized uptake value ratio, was shown to be earlier than standard thresholds of amyloid-β PET positivity and the increase of plasma p-tau181. In addition, plasma p-tau231 was significantly increased in amyloid-β PET quartiles 2-4, whereas CSF p-tau217 and plasma p-tau181 increased only at quartiles 3-4 and 4, respectively. Finally, plasma p-tau231 differentiated individuals across the entire Braak stage spectrum, including Braak staging from Braak 0 through Braak I-II, which was not observed for plasma p-tau181. The authors concluded that this novel plasma p-tau231 assay identified the clinical stages of AD and neuropathology equally well as plasma p-tau181, but increases earlier, already with subtle amyloid-β deposition, prior to the threshold for amyloid-β PET positivity has been attained, and also in response to early brain tau deposition. Therefore, these investigators stated that plasma p-tau231 is a promising novel biomarker of emerging AD pathology with the potential to facilitate clinical trials to identify vulnerable populations below PET threshold of amyloid-β positivity or apparent entorhinal tau deposition.

The authors stated that a main drawback of this study was that they could not directly compare plasma p-tau231 with plasma p-tau217. However, using CSF p-tau217 as a substitute for plasma p-tau217 (which is very likely a stronger predictor of disease pathophysiology) they were confident in their conclusions. Although these findings showed that plasma p-tau231 assay can identify AD in a primary care setting, the primary care cohort had no confirmatory biomarkers, of individuals with MCI and identification of pre-clinical AD in CU adults. Given the early increases in p-tau231, these assessments would have reduced the
Furthermore, the use of cross-sectional Aβ PET Centiloids as a proxy of time in the disease was employed and it was not guaranteed that a greater Aβ PET SUVR was indicative of more advanced disease state. Lastly, to definitively and fully describe the ordinal sequence of plasma p-tau biomarkers, from pre-clinical to symptomatic phases of the disease, large-scale longitudinal studies with multiple time-points are needed.

**Resting State Eye-Closed Cortical Electroencephalography**

Babiloni and co-workers (2018) tested the hypothesis that cortical sources of resting state eyes-closed electroencephalographic (rsEEG) rhythms reveal different abnormalities in cortical neural synchronization in groups of patients with mild cognitive impairment due to AD (ADMCI) and dementia with Lewy bodies (DLBMcI) as compared to cognitively normal elderly (Nold) subjects. Clinical and rsEEG data in 30 ADMCI, 23 DLBMcI, and 30 Nold subjects were available in an international archive. Age, gender, and education were carefully matched in the 3 groups. The MMSE score was matched between the ADMCI and DLBMcI groups.

Individual alpha frequency peak (IAF) was used to determine the delta, theta, alpha1, alpha2, and alpha3 frequency band ranges. Fixed beta1, beta2, and gamma bands were also considered. eLORETA estimated the rsEEG cortical sources. Receiver operating characteristic curve (ROCC) classified these sources across individuals. Compared to Nold, IAF showed marked slowing in DLBMcI and moderate in ADMCI. Furthermore, the posterior alpha 2 and alpha 3 source activities were more abnormal in the ADMCI than the DLBMcI group, while widespread delta source activities were more abnormal in the DLBMcI than the ADMCI group. The posterior delta and alpha sources correlated with the MMSE score and correctly classified the Nold and MCI individuals (area under the ROCC > 0.85). The authors concluded that the ADMCI and DLBMcI patients showed different features of cortical neural synchronization at delta and alpha frequencies underpinning brain arousal and vigilance in the quiet wakefulness. They stated that future prospective cross-validation studies are needed to test the clinical validity of these rsEEG markers.

**Serum Neurofilament Light Concentration**

In a cross-sectional study, Weston and colleagues (2017) examined if serum neurofilament light (NFL) concentration is increased in familial AD
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(FAD), both pre- and post-symptom onset, and whether it is associated with markers of disease stage and severity. These investigators recruited 48 individuals from families with PSEN1 or APP mutations to this trial – 18 had symptomatic AD and 30 were asymptomatic but at 50 % risk of carrying a mutation. Serum NfL was measured using an ultrasensitive immunoassay on the single molecule array (Simoa) platform. Cognitive testing and MRI were performed; 33 participants had serial MRI, allowing calculation of atrophy rates. Genetic testing established mutation status. A generalized least squares regression model was used to compare serum NfL among symptomatic mutation carriers, pre-symptomatic carriers, and non-carriers, adjusting for age and sex. Spearman coefficients assessed associations between serum NfL and the following: estimated years to/from symptom onset (EYO), cognitive measures, and MRI measures of atrophy. A total of 19 of the asymptomatic participants were mutation carriers (mean EYO -9.6); 11 were non-carriers. Compared with non-carriers, serum NfL concentration was higher in both symptomatic (p < 0.0001) and pre-symptomatic mutation carriers (p = 0.007). Across all mutation carriers, serum NfL correlated with EYO (p = 0.81, p < 0.0001) and multiple cognitive and imaging measures, including MMSE (p = -0.62, p = 0.0001), Clinical Dementia Rating Scale sum of boxes (p = 0.79, p < 0.0001), baseline brain volume (p = -0.62, p = 0.0002), and whole-brain atrophy rate (p = 0.53, p = 0.01). The authors concluded that serum NfL concentration was increased in FAD prior to symptom onset and correlated with measures of disease stage and severity. They stated that serum NfL may thus be a feasible biomarker of early AD-related neurodegeneration; these findings supported the further investigation of serum NfL as an easily accessible biomarker of early AD-related neurodegeneration.

These investigators stated that while these findings were encouraging, there were a number of issues regarding the utility of NfL as a biomarker of early AD. While as a group the pre-symptomatic carriers had higher mean NfL than non-carriers, there was a degree of overlap in observed values. The utility of serum NfL to diagnose pre-symptomatic AD at the individual level therefore remains uncertain and needs re-assessment in independent cohorts. The changes in serum NfL through the course of the disease were analyzed in cross-sectional data only, so it was also not known whether serum NfL tracks progression at an individual level.

Furthermore, while these results supported the use of serum NfL as a marker of neurodegeneration in AD, NfL is not a specific marker to AD and has been shown to increase in a number of other conditions, including HIV-associated dementia, progressive supranuclear palsy,
therefore be that serum NfL will be most useful for identifying and tracking AD-related neurodegeneration when combined with a test to confirm underlying AD molecular pathology, e.g., CSF tau/Aβ1-42 or amyloid PET. The authors also noted that this study had several drawbacks – the sample size was not large (n = 48), owing primarily to the relative rarity of FAD mutations. However, this remains one of the largest single-center FAD cohorts yet reported. For a number of participants, not all cognitive and imaging assessments were completed. However, minimal changes were seen when re-running the analyses to include only those participants who had completed all assessments. These researchers estimated the age when each mutation carrier would be expected to develop symptoms based on parental age at onset, which was closely associated with actual age at onset; however, this remained a proxy measure, and it is only with longitudinal follow-up that age at onset can be confirmed.

In an editorial that accompanied the afore-mentioned study, Mielke and Otto (2017) stated that “The results by Weston et al on serum NfL as a blood-based marker of AD severity and disease progression are promising. The next question is how to translate this research for use at the clinical level. In addition to the extensive approval process for a clinically approved laboratory panel, other steps are needed. First, studies suggest that other plasma measures (e.g., total tau10) may also be non-specific markers of neurodegeneration. A comparison of these potential markers is needed to determine which one, or a combination, is most clinically useful. Second, there is a need to understand what serum NfL and other potential markers look like in the population – their range; associations with age, race, sex, and co-morbidities; and intra-individual variation. Understanding these aspects will help fast track serum NfL and other potential blood-based markers into useful clinical practice in both rural and urban settings”.

**Toxoplasma Gondii**

Nayeri Chegeni and colleagues (2019) noted that toxoplasmosis is a major public health concern due to neurotropic nature and role in the development of mental and behavioral disorders. Alzheimer's disease is an important nervous disease that results in the reduction of the amount of beta-amyloid plaque deposition and irreversible loss of neurons in the brain. Although a few studies had evaluated the association between AD and toxoplasmosis, the present study as a systematic review and meta-
analysis of published studies examined the possible association between Toxoplasma gondii (T. gondii) and AD. A systematic literature search was carried out using 7 electronic databases from the inception to November 25, 2018 with no restriction of language that examined toxoplasmosis (as an exposure) and AD (as a disease). The random effect model was used to determine the total OR and total p-value. A total of 8 studies involving 3,239 subjects (360 patients and 2,879 controls) met the eligibility criteria. Then, 8 articles were used for meta-analysis with respect to inclusion and exclusion criteria. The results of the meta-analysis (random effect model) showed a common OR of 1.53 (95% CI: 1.07 to 2.18). Despite the fact that there was no evidence of publication bias (p = 0.079) using formal statistical test, the visual inspection of the funnel graph suggested that the observed effect was fueled mainly by 3 studies with large effects (and large standard errors). Moreover, the file-drawer effect (i.e., publishing mainly studies with positive results) might play a role in the phenomenon. The authors concluded that the findings of this meta-analytic study suggested that T. gondii can be considered a risk factor for the development of AD and exacerbation of its symptoms. However, the number of published relevant studies was still relatively low, and the risk of the presence of publication bias was relatively high. Thus, these researchers stated that the investigation of the clinically important question of the possible association between toxoplasmosis and AD definitely deserves further attention.

CPT Codes/ HCPCS Codes/ICD-10 Codes
Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by “+”

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT codes covered if selection criteria are met:</td>
<td></td>
</tr>
<tr>
<td><strong>Amyloid precursor protein (APP) genetic testing, phosphorylated tau at threonine 181, tau/Aβ42, ptau181/Aβ42, CSF total tau (t-tau), CSF phosphorylated tau at Thr217 (p-tau T217)-hyphen no specific code</strong></td>
<td></td>
</tr>
<tr>
<td>62270</td>
<td>Spinal puncture, lumbar, diagnostic</td>
</tr>
<tr>
<td>70551</td>
<td>Magnetic resonance (e.g., proton) imaging, brain (including brain stem); without contrast material</td>
</tr>
<tr>
<td>70552</td>
<td>with contrast material(s)</td>
</tr>
<tr>
<td>70553</td>
<td>without contrast material, followed by contrast material(s) and further sequences</td>
</tr>
<tr>
<td>81405</td>
<td>Molecular pathology procedure, Level 6 full gene sequence PSEN1 (presenilin 1) (e.g., Alzheimer disease)</td>
</tr>
</tbody>
</table>

CPT codes not covered for indications listed in the CPB:
### Alzheimer's Disease Tests

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 rs956572 polymorphism testing, Resting state eye-closed cortical electroencephalography, serum neurofilament light concentration, Cerebrospinal fluid (CSF) golgin A4 testing, plasma lipoproteome, particulate matter with an aerodynamic diameter of less than or equal to 2.5μm (PM2.5)</td>
<td>-no specific code</td>
</tr>
<tr>
<td>0206U</td>
<td>Neurology (Alzheimer disease); cell aggregation using morphometric imaging and protein kinase C-epsilon (PKCe) concentration in response to amylospheroid treatment by ELISA, cultured skin fibroblasts, each reported as positive or negative for Alzheimer disease</td>
</tr>
<tr>
<td>+0207U</td>
<td>Disease quantitative imaging of phosphorylated ERK1 and ERK2 in response to bradykinin treatment by in situ immunofluorescence, using cultured skin fibroblasts, reported as a probability index for Alzheimer disease</td>
</tr>
<tr>
<td>0289U</td>
<td>Neurology (Alzheimer disease), mRNA, gene expression profiling by RNA sequencing of 24 genes, whole blood, algorithm reported as predictive risk score</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
</tr>
<tr>
<td>81406</td>
<td>Molecular pathology procedure, Level 7 (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)</td>
</tr>
<tr>
<td>82172</td>
<td>Apolipoprotein, each</td>
</tr>
<tr>
<td>82530</td>
<td>Cortisol; free [long-term measurement of cortisol]</td>
</tr>
<tr>
<td>82533</td>
<td>Cortisol; total [long-term measurement of cortisol]</td>
</tr>
<tr>
<td>83090</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified [ATP-binding cassette transporter (ABCA7) test, cerebrospinal fluid microRNAs, cerebrospinal fluid prion protein concentration, plasma prion protein concentration or transforming growth factor-beta1, simultaneous quantification of amyloid-beta, p-tau, t-tau, CSF neurogranin, and plasma tau] [cerebrospinal fluid (CSF) synaptic biomarkers (e.g., GAP-43, SNAP-25 total, SNAP-25aa40, synaptotagmin-1)] [plasma p-tau231]</td>
</tr>
<tr>
<td>83880</td>
<td>Natriuretic peptide</td>
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<tr>
<td>84305</td>
<td>Somatomedin</td>
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<tr>
<td>84478</td>
<td>Triglycerides</td>
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<tr>
<td>86777</td>
<td>Antibody; Toxoplasma</td>
</tr>
<tr>
<td>86778</td>
<td>Antibody; Toxoplasma, IgM</td>
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</tbody>
</table>
Basic vestibular evaluation, includes spontaneous nystagmus test with eccentric gaze fixation nystagmus, with recording, positional nystagmus test, minimum of 4 positions, with recording, optokinetic nystagmus test, bidirectional foveal and peripheral stimulation, with recording, and oscillating tracking test, with recording.

Vestibular function tests, with recording (e.g., ENG, PENG), and medical diagnostic evaluation [in the absence of signs of vertigo or balance disorder]

Tympanometry and reflex threshold measurements

Tympanometry (impedance testing) [in the absence of hearing loss]

Acoustic reflex testing [in the absence of hearing loss]

Acoustic immittance testing, includes tympanometry (impedance testing), acoustic reflex threshold testing, and acoustic reflex decay testing

Brain imaging, positron emission tomography (PET); metabolic evaluation

Molecular cytogenetics

DNA analysis for APOE epsilon 4 allele for susceptibility to Alzheimer's disease

Florbetapir f18, diagnostic, per study dose, up to 10 millicuries

Positron emission tomography radiopharmaceutical, diagnostic, for non-tumor identification, not otherwise classified

Flutemetamol F18, diagnostic, per study dose, up to 5 millicuries

Alzheimer's disease

Mild cognitive impairment, so stated

Cerebral amyloid angiopathy

Other abnormal findings on diagnostic imaging of central nervous system

Abnormal brain scan

Dementia in other diseases classified elsewhere without behavioral disturbance [Alzheimer related dementias]

Dementia in other diseases classified elsewhere with behavioral disturbance [Alzheimer related dementias]

Personality and behavioral disorders due to known physiological condition
The above policy is based on the following references:


23. Felician O, Sandson TA. The neurobiology and pharmacotherapy


36. Han P, Liang W, Baxter LC, et al. Pituitary adenylate cyclase-


49. Larson EB. Evaluation of cognitive impairment and dementia. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed May 2019.


73. Petersen RC. Mild cognitive impairment: Prognosis and treatment. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2020.


110. Wolk DA, Dickerson BC. Clinical features and diagnosis of Alzheimer disease. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed November 2018.

Videopupillography/Tropicamide Drop Test


Amendment to
Aetna Clinical Policy Bulletin Number: 0349 Alzheimer's Disease:
Experimental Tests

There are no amendments for Medicaid.

revised 02/09/2022