REVIEW

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Metabolomics-based promising candidate biomarkers and pathways in Alzheimer's disease

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Received November 14, 2014, accepted December 13, 2014

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Pharmazie 70: 277–282 (2015) *doi: 10.1691/ph.2015.4859*

Pathologically, loss of synapses and neurons, extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) are observed in the brains of patients with Alzheimer's disease (AD). These features are associated with changes Aβ (amyloid β) 40, Aβ42, total tau and phosphorylated tau (p-tau), which are as definitely biomarkers for severe AD state. However, biomarkers for effectively diagnosing AD in the pre-clinical state for directing therapeutic strategies are lacking. Metabolic profiling as a powerful tool to identify new biomarkers is receiving increasing attention in AD. This review will focus on metabolomics-based detection of promising candidate biomarkers and pathways in AD to facilitate the discovery of new medicines and disease pathways.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease involving complex progressive conditions that are loss of memory, impairment of cognition, behavioral disorder, and completely losing ability of self-sufficiency in the final stage (Li et al. 2014). AD is the most frequent form of dementia in the elderly with prevalence of 25∼50% over the age of 85 (Hong-Qi et al. 2012). In China impacts of AD get more broaden as older population increases, which gives a great economic burden on both public and private payers, and reduces life qualities of AD patients.

AD is characterized by amyloid-β (Aβ) aggregation with plaque development, and the hyperphosphorylation and aggregation of tau protein with formation of tangles (Ballard et al. 2011; Yan et al. 2014). The current diagnostic criteria are used to identify patients with AD who have overt dementia. Actually, these noticeable clinical symptoms are observed 20-30 years later than those pathologic changes in the brain. These pathologic changes in the brain have begun to accrue in many "cognitively normal" elderly individuals. However, there is no clinical method to diagnose AD definitively before symptoms become apparent. In addition, there is also no method to determine whether mild cognitive impairment (MCI) individuals will progress to AD or not, because approximately 11% of MCI individuals progress to apparent dementia after a 3-year follow-up period (Petersen et al. 1999). Early diagnosis of MCI individual and therapeutic intervention at this stage will effectively slow disease progression and reduce the cost burden. There is a growing need for biomarker identification of AD in preclinical stages when there is no significant brain damage.

Imaging techniques like structural magnetic resonance imaging (MRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), provide information on the regional distribution of changes (Hampel et al. 2010; Perrin et al. 2009). Biochemical biomarkers include: 1) core biomarkers that are the fundamental pathogenic factors in AD such as Aβ40, Aβ42, total tau and phosphorylated tau (p-tau); 2) downstream biomarkers that reflect secondary pathologic changes such as axonal degeneration (Panza et al. 2014). Up to now, Aβ40, Aβ42, total tau and p-tau are the important elements of the AD progression and have emerged as currently leading diagnostic and prognostic biomarkers in brain tissues, cerebrospinal fluid (CSF) and plasma/serum. However, it has been described that plasma Aβ40 and Aβ42 cannot reflect Aβ processing in the brain (Irizarry 2004; Vanderstichele et al. 2000).

There is increasing evidence suggesting that inflammation and oxidative stress also contribute to the pathological changes. Because by-products of inflammation and oxidative stress mediate toxicity in AD to induce loss of synaptic integrity and progressive neurodegeneration (Doty et al. 2014). Polyunsaturated fatty acids (targets for free radical attack), isoprostanes, and 8-hydroxy-guanine (representing oxidized RNA), have been reported to be changed in AD (Montine and Morrow 2005; Pratico et al. 1998; Weidner et al. 2011). Both inflammatory cells and their secreted products are involved in AD. For example, α-1-antichymotrypsin (ACT), α-2-macroglobulin (α2 M), activated factors of the classical pathway of complement, and cytokines such as IL-1β and TNF-α, contribute to the pathophysiology of AD (Blacker et al. 1998; Emmerling et al. 2000; Eriksson et al. 1995; Rubio-Perez and Morillas-Ruiz 2012). Indeed, although many potential candidate biomarkers were identified, some results showed no consistence or had no follow-up studies. Thus, more promising biomarkers have to be identified by simple, noninvasive and reliable methods.

Metabolomics, or metabonomics, offer powerful tools to study perturbations of the metabolic network by identifying numerous small molecules in biochemical processes. Metabolomics presents an accurate biochemical phenotype in different states of the living system. Metabolomic profiling reflects changes of

gene expression or environmental factors under disease states, and different life style and diet in humans or other animals under healthy states. Metabolomic profiling is performed in body fluids, plasma/serum, different tissues, and cerebrospinal fluid (CSF). Samples mostly used in AD are urine, CSF, plasma/serum and brain tissues. Common technologies used for metabolomics include ^{1}H NMR spectroscopy and mass spectrometry (MS). MS methods are always connected with separation techniques such as liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE), or ultra-high-pressure LC (UPLC) for a pre-separation. Metabolites generated from MS or NMR are analyzed by different statistical methods according to different purposes. Principal components analysis (PCA) using Pareto-scaled data is performed to reduce the dimensionality of the data and to reveal any clustering of study groups in an unsupervised manner, which provides a summary or overview of all observation or samples in the data table. Partial least squares (PLS) and orthogonal-PLS (OPLS) represent two modeling methods to construct a predictive model to camper and evaluate diseases condition or/and progression based on the differential metabolites accountable for disease.

Although several MRI, PET-based markers for AD are currently available, they are less useful for identifying and monitoring the effect of drugs on the neurodegenerative process, as well as identifying alternative disease mechanisms and associated biomarkers that can help to diagnose AD in the preclinical and early clinical stages (i.e., MCI). Thus, biomarkers that clearly discriminate between different states of disease and between remission and flare-ups would be enormously useful for clinical practice. The biomarkers that are important to support diagnosis and to provide an insight into the underlying pathophysiological mechanisms of AD, are far from clear at present. Here, we review the current status of biomarker candidates AD based on metabolomics analysis.

2. Metabolomics-based lipid profiling in plasma/serum of AD patients

Lipid-mediated signaling involves a large number of physiological processes, including metabolic disorders, cardiovascular diseases, and neurodegenerative disorders such as AD (Lemaitre et al. 2014; Reed 2011; Sahebkar et al. 2014). Since the ε4 allele of the apolipoprotein E (ApoE) gene was identified as the strongest known genetic risk factor for sporadic AD, a direct link of lipid metabolism and AD was established (Corder et al. 1993). ApoE is a component of lipoproteins in plasma and brain, and acts as crossroads between the lipidome and dementia. ApoE encodes a ∼34 kDa protein that is mostly secreted by glial cells (>95%) and serves as a mediator that affects the uptake of lipoprotein particles in the brain via the low density lipoprotein receptor related protein (LRP) and the very low-density family lipoprotein receptor (Krieger and Herz 1994). ApoE plays direct or indirect roles in the amyloid metabolism which are supported by the evidence that ApoE binds Aβ and results in a toxic accumulation of the Aβ. Recently, Garai et al. (2014) found that ApoE primarily bonded to oligomers and also affected their growth that led to the nuclei required for fibril growth (Garai et al. 2014). However, the results of linking between tau and ApoE are discrepant (Morris et al. 2010; Shafaati et al. 2007).

Many researches using targeted experimental design have provided growing evidence that lipid molecules such as cholesterol and phosphoinositides are related to AD pathogenesis (Gamba et al. 2012; Martins et al. 2009; Mulder et al. 2003). However, lipid metabolism pathways are complex and their perturbations are not only observed in AD but in many diseases, especially in cardiovascular diseases with high incidence in the elderly. In addition, AD patients are always characterized as losing weight, which results in lower lipid levels. Therefore, lipid profiling based on metabolomics analysis would be a promising method to detect the specificity of a lipid class as accurate biomarkers involved in AD pathogenesis.

Using LC/MC method, levels of desmosterol and desmosterol/cholesterol ratio in the plasma of AD patients have been described to be significantly decreased compared with healthy elder controls (Sato et al. 2012). Because LC/MS method does not need derivatization and allows a good separation from another endogenous desmosterol isomer, LC/MS method is better than GC-MS for desmosterol identification. The authors further confirmed that desmosterol/cholesterol ratio in CSF of same patients correlated well with its plasma ratio. Additionally, changes of the desmosterol/cholesterol ratio were not found in neuronal disease patients' plasma and CSF, suggesting the specific association of desmosterol with Alzheimer's disease and/or with cognitive decline. Although desmosterol/cholesterol ratio can be expected to predict the AD progress, in this study plasma levels of cholesterol showed no significant difference between healthy elder controls and AD. Conversely, it is thought that both the generation and clearance of Aβ are regulated by cholesterol, and drugs that inhibit cholesterol synthesis can lower Aβ in animal models of AD (Puglielli et al. 2003). Cholesterol accumulation has been observed in senile plaques of AD patients, as well as in transgenic APP (SW) mice (Mori et al. 2001). The quantity of free cholesterol per senile plaque was similar to the quantity of Aβ peptide (Panchal et al. 2010).

However, more recent studies focused on the specificity of lipid subclasses associated with AD severity rather than an overall lipid flux. Although identified biomarkers are different, these biomarkers were shown to affect the same pathways, including cholesterol biosynthesis and metabolism, sphingolipids transport, lipid metabolism and other phospholipid and plasmalogen pathways.

A global version of possible factors triggering membrane breakdown were exhibited in AD patients by identifying changes of phosphatidylcholines, phosphatidylethanolamines, plasmenylcholines, plasmenylethanolamines and different classes of lysophospholipids (Gonzalez-Dominguez et al. 2014). Lysophospholipids are transiently expressed by phospholipase A2 and rapidly acylated with acyl-CoA in the deacylation-reacylation cycle (Farooqui et al. 2000). In AD patients, phospholipase A2 was significantly decreased (-35 to -53%) in parietal and temporal cortices of patients with AD, while the activities of lysophospholipid acyltransferase were increased by approximately 50-70% in the same brain areas (Ross et al. 1998), which suggested that a lysophospholipid metabolism disorder is involved in the pathology of AD.

Alteration of phosphatidylcholines were also investigated using MS and NMR methods; three phosphatidylcholine molecules (phosphatidylcholine 16:0/20:5, 16:0/22:6, and 18:0/22:6) were significantly diminished in AD patients (Whiley et al. 2014). Phosphatidylcholines are a class of 1,2-diacylglycerophospholipids that are an essential component of cell membranes (Frisardi et al. 2011). The type of fatty acid linked to the phosphatidylcholine molecular moiety include polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA), which determines the levels of phosphatidylcholines. In AD patients, polyunsaturated fatty acid (PUFA) phosphatidylcholines decreased and SFA phosphatidylcholines increased (Whiley et al. 2014). Actually, there are studies that reported reduced PUFA-containing phospholipids reduced both in brain and plasma from patients with AD (Cunnane et al. 2012; Martin et al. 2010), and particularly in phosphatidylcholine species. The degree of saturation in membrane lipids affects the cell membrane fluidity, permeability and charge. Phosphatidylcholines

also play as components of lipoprotein and interact with ApoE as part of the HDL group involved in cholesterol transport. In another study using ultra UPLC coupled to MS and comprehensive two-dimensional GC coupled to time-of-flight MS also identified phosphatidylcholines, which were diminished in the serum of AD patients (Oresic et al. 2011). In addition to phosphatidylcholines, ether phospholipids, sphingomyelins and sterols were also described to be reduced in the serum of AD patients (Oresic et al. 2011).

As phosphatidylcholine is a precursor for sphingomyelin, it is understandable that both phosphatidylcholines and sphingomyelins are diminished in AD patients. Sphingomyelin species are important cellular membrane constituents which interact with cholesterol in construction, metabolism and transport, and which are abundant in lipid rafts. However, sphingomyelin levels in AD patients showed differences according to time to onset of memory impairment and sample to different tissues. For example, elevated sphingomyelin levels were found in AD brain using ^{31}P NMR, which correlated with neuropathological hallmarks of the disease and measures of cognitive decline (Pettegrew et al. 2001). Conversely, levels of sphingomyelins were not significantly different in brain tissue samples from any of the three AD patient groups (different stages of the disease progression include mild, moderate, and severe) compared with controls (Cutler et al. 2004). The conflicting results could be due to the detection methods used. However, metabolomics-based methods consistently showed lower levels of sphingomyelins during memory impairment. Because sphingomyelin is a major source of ceramide generated from sphingomyelin by sphingomyelinases, changes of sphingomyelins are always associated with changes of ceramides. Recent studies showed that in addition to lower levels of sphingolipids in early AD, levels of two ceramide species (N16:0 and N21:0) were significantly higher in early AD. Ratios of ceramide to sphingomyelin species containing identical fatty acyl chains differed significantly between AD patients and controls (Han et al. 2011).

^{1}H NMR spectroscopy was used to analyze lipoprotein particles, various low-molecular-weight metabolites, and also individual lipid molecules together with their degree of (poly)(un)saturation in MCI (Tukiainen et al. 2008). These results showed that a low relative amount of ω-3 would be more indicative of high risk for AD than low serum ω-3 or polyunsaturated fatty acid (PUFA) concentration. The results also revealed that the low level of sphingomyelin phosphatidylcholine correlated with high levels of total cholesterol in MCI patients.

3. Metabolites profiling in cerebrospinal fluid of AD patients

As the composition of CSF is related to metabolite production in the brain, CSF has become a preferred sample to investigate central nervous system disorders. Research efforts have in general been focused on lipid metabolites in serum of AD patients, but most studies in CSF of AD primarily focused on amino acids and related metabolites.

Using a nontargeted metabolomic approach based on CE-MS, Ibañez et al. (2012) examined metabolic differences in CSF samples to investigate different cognitive status related to AD progression. Choline, dimethylarginine, arginine, valine, proline, serine, histidine, creatine, carnitine, and suberylglycine were identified as possible disease progression biomarkers. These biomarkers were confirmed using a blind small test, the results showed that diagnostic accuracy reached 83% and the predictive values obtained were higher than those reported with classical CSF AD biomarkers (Aβ42 and tau) (Ibanez et al. 2012). In this study, choline was found to be increased in AD patients. Choline is involved in the metabolism many biochemical molecules, such as betaine, methionine, and phospholipid biosynthesis, acting as a precursor of the neurotransmitter acetylcholine. A role for choline in the development of AD has been described. A report showed that choline and its metabolites, such as glycerophosphocholine and phosphocholine, elevated in AD patients compared with age-matched healthy controls (Walter et al. 2004). Free amino acids valine, serine, histidine, and arginine showed diverse patterns in different status of AD. Although many of free amino acids were in good agreement with previously published studies, diverse patterns in different status of AD made it hard to predict and diagnose disease.

LC electrochemical array platform was used to identify biomarkers in AD and MCI patients (Kaddurah-Daouk et al. 2013). In AD patients, methionine (MET), 5-hydroxyindoleacetic acid (5-HIAA), vanillylmandelic acid, xanthosine and glutathione were elevated. Whereas, MCI patients had elevated 5-HIAA, MET, hypoxanthine and other metabolites *versus* controls. The results suggested that MCI and AD were not only associated with an overlapping pattern of perturbations, but could be distinguished by different biomarkers. RP/UHPLC–MS and HILIC/UHPLC–MS methods were also used to investigate CSF samples from patients with different AD stages (Ibanez et al. 2013). They found that uracil, taurine, nonanolglycine, caproic acid and sphingosine-phosphate continuously increased in the MCI-AD patients, and they decreased dramatically in AD patients. As for tryptophan and pipecolicacid, they decreased in MCI-AD patients, but increased in AD patients. Creatinine consistently decreased, while histidine and tyrosil-serine consistently increased from MCI-AD status to AD status. These biomarkers are involved in many pathways including DNA and RNA synthesis, mitochondria function, energy metabolism, oxidative stress. Some of these biomarkers were changed in agreement with previous studies, but uridine was different from recent work. Czech et al. (2012) observed that uridine decreased in the CSF of AD patients, which were thought to be related to reduced synaptic plasticity and neuronal deficits. As uridine is one of the four basic components of RNA and upon digestion of foods containing RNA, uridine is released from RNA and is absorbed intact in the gut. Therefore, an important effect of diet on these results cannot be ruled out. In addition to uridine, the authors also found that acetylcholine, norepinephrine, catecholamines, cysteine were changed in the CSF of AD patients (Czech et al. 2012). Changes of norepinephrine, norepinephrine concentration in CSF increased in this observation, were a reflection of compensatory effects. However, the concentration of norepinephrine at the site of locus coeruleus neuronal projections in the frontal and temporal cortex was substantially lowered (Reinikainen et al. 1990).

Studies using samples from *post-mortem* ventricular CSF (15 AD and 15 nondemented subjects with autopsy confirmed diagnoses) have shown that metabolites related to tyrosine, tryptophan, purine, and tocopherol pathways were altered in patients with AD(Kaddurah-Daouk et al. 2011). Although *post-mortem* and *ante-mortem* neurochemical studies in AD limited its application for diagnosing diseases because of characteristics of the progressive development in AD, the metabolites identified could be used to analyze differences in biochemical pathway which in turn could yield candidate biomarkers for testing in future.

4. Metabolic profiling of AD in animals

Animal models are essential for the understanding of disease pathophysiology and progression, and are widely used in preclinical pharmaceutical discovery. Metabolomics can enhance

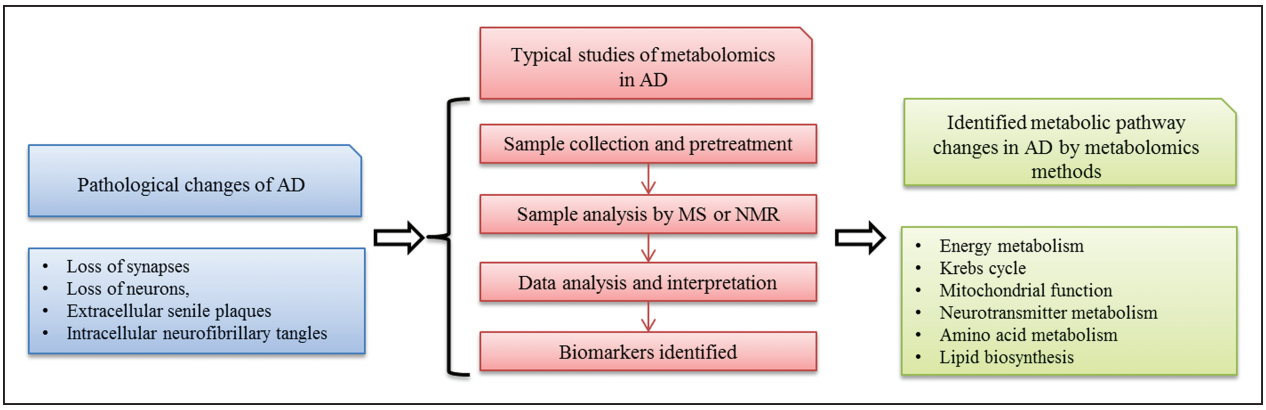

Fig. 1: Summary of metabolomics in AD.

the understanding the pharmacological effects of drugs. The endogenous metabolites and metabolic pathways identified in animal models can lead to the discovery of novel drug targets. There are several reports so far to show metabolites changes in AD using anmial models.

NMR-based metabolomics analysis has been used to study urinary metabolites in transgenic mice that expressed mutant tau and APP (Fukuhara et al. 2013).Urine samples were collected when transgenic mice were at the age of 2, 10, and 15 months, which represented prior to onset, early/middle, and late stages of AD, respectively. In this study, 3-hydroxykynurenine, homogentisate and allantoin were described to play a role in the development of AD. These metabolites are involved in the oxidative stress pathway by producing reactive oxygen species (ROS) or being oxidized by ROS, respectively. The fact that oxidative stress plays an important role in AD pathogenesis has been widely studied and confirmed. The increasing evidence suggested that oxidative stress increases neuron degeneration and death in AD patients (Padurariu et al. 2013). However, because tissue injury itself can induce ROS generation which also can secondarily lead to neuron death, it is not known whether this is a primary or a secondary event. Studies using metabolomics method confirmed that oxidative stress mechanisms played their roles in the pathogenesis of AD by using urine samples. It was also shown that oxidative stress happened at a very early period in the progression of AD by identifying 3-hydroxykynurenine, homogentisate and allantoin in AD mice.

Transgenic CRND8 mice, which encoded a mutant form of the amyloid precursor protein 695 were used to address the metabolic signatures in the hippocampus of the Aβ with MS method (Lin et al. 2013). These mice developed extracellular Aβ deposits 2∼3 months. The authors found that eicosanoids in arachidonic acid metabolism, fatty acid beta-oxidation disorders as well as disturbed glucose metabolism were highlighted as metabolic traits of Aβ toxicity in transgenic CRND8 mice. In this study, arachidonic acid metabolism dominated the metabolic perturbation in hippocampal tissues of transgenic CRND8 mice, the results indicated that Aβ toxicity functioned neuroinflammation in hippocampal tissue. New theranostic opportunities might be offered by characterization of altered arachidonic acid metabolism for AD.

Recent MS studies have provided evidence for the role of mitochondrial dysfunction in the early stages of AD (Trushina et al. 2012). Transgenic mice were exhibited mitochondrial trafficking inhibition. 15 most important metabolites were identified, which are linked to mitochondria and energy metabolism, such as inorganic phosphates (Pi), creatinine, NAA, AMP, adenosine, malonic acid, IMP, adenine, b-alanine, lactic acid, ATP, and glycerol. Metabolic pathway analysis revealed that nucleotide metabolism, mitochondrial Krebs cycle, carbohydrate, and amino acid metabolic pathways were altered in all three familiar AD mouse models, which was in agreement with studies conducted in patients and AD mice. Metabolomic signatures of mitochondrial stress and altered energy metabolism indicated alterations in nucleotide, Krebs cycle, energy transfer, carbohydrate, neurotransmitter, and amino acid metabolic pathways. Biochemical studies demonstrate alterations in the activity of mitochondrial enzymes involved in Krebs cycle and electron transport chains in *post-mortem* AD brains (Atamna and Frey 2007; Bubber et al. 2005).

5. Discussion and perspectives

Metabolic profiling of biofluids from AD patients represents a powerful approach for the detection of biomarkers and underlying mechanisms of AD. The most common samples for AD detection are CSF and plasma. These investigated samples hold different information on AD. Metabolic profiling of plasma/serum get us more focused on lipid metabolism, while in CSF energy metabolism, Krebs cycle, mitochondrial function, neurotransmitter and amino acid metabolism, and lipid biosynthesis are involved. A recent study using CSF and plasma from the same individuals in relationship to AD progression showed that signatures in CSF and plasma have significant overlap, and pathways were identified that were specifically affected in either plasma or CSF (Trushina et al. 2013).

Theoretically, metabolomics-based biomarkers in AD patients are useful for distinguishing AD from other dementia diseases, diagnosing AD in pre-clinical states, monitoring pharmacological effects of a drug to shorten the drug developing time, facilitating to discover novel mechanisms of AD, and accurately reflecting the side effects of drugs. However, as a promising method in drug development, the current application of metabolomics in AD remains limited. Indeed, although there is a lot of research on potential candidate biomarkers in AD based on metabolomics, follow-up studies are either lacking or have failed to confirm by repeatability of results in different research laboratories.

References

Atamna H, Frey WH (2007) Mechanisms of mitochondrial dysfunction and energy deficiency in Alzheimer's disease. Mitochondrion 7: 297–310.

Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. Lancet 377: 1019–1031.

Blacker D, Wilcox MA, Laird NM, Rodes L, Horvath SM, Go RC, Perry R, Watson B, Jr., Bassett SS, McInnis MG, Albert MS, Hyman BT, Tanzi RE (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. Nat Genet 19: 357–360.

Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE (2005) Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. Ann Neurol 57: 695–703.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261: 921–923.

Cunnane SC, Schneider JA, Tangney C, Tremblay-Mercier J, Fortier M, Bennett DA, Morris MC (2012) Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 29: 691–697.

Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci USA 101: 2070–2075.

Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, Hampel H, Kastler J, Senn H. Metabolite profiling of Alzheimer's disease cerebrospinal fluid (2012) PLoS One 7: e31501.

Doty KR, Guillot-Sestier MV, Town T (2014) The role of the immune system in neurodegenerative disorders: Adaptive or maladaptive? Brain Res doi: 10.1016/j.brainres.2014.09.008.

Emmerling MR, Watson MD, Raby CA, Spiegel K (2000) The role of complement in Alzheimer's disease pathology. Biochim Biophys Acta 1502: 158–171.

Eriksson S, Janciauskiene S, Lannfelt L (1995) Alpha 1-antichymotrypsin regulates Alzheimer beta-amyloid peptide fibril formation. Proc Natl Acad Sci USA 92: 2313–2317.

Farooqui AA, Horrocks LA, Farooqui T (2000) Deacylation and reacylation of neural membrane glycerophospholipids. J Mol Neurosci 14: 123–135.

Frisardi V, Panza F, Seripa D, Farooqui T, Farooqui AA (2011) Glycerophospholipids and glycerophospholipid-derived lipid mediators: a complex meshwork in Alzheimer's disease pathology. Prog Lipid Res 50: 313–330.

Fukuhara K, Ohno A, Ota Y, Senoo Y, Maekawa K, Okuda H, Kurihara M, Okuno A, Niida S, Saito Y, Takikawa O (2013) NMR-based metabolomics of urine in a mouse model of Alzheimer's disease: identification of oxidative stress biomarkers. J Clin Biochem Nutr 52: 133–138.

Gamba P, Testa G, Sottero B, Gargiulo S, Poli G, Leonarduzzi G (2012) The link between altered cholesterol metabolism and Alzheimer's disease. Ann N Y Acad Sci 1259: 54–64.

Garai K, Verghese PB, Baban B, Holtzman DM, Frieden C (2014) The binding of apoE to oligomers and fibrils of amyloid-beta alters the kinetics of amyloid aggregation. Biochemistry 53: 6323–6331.

Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL (2014) Combination of metabolomic and phospholipid-profiling approaches for the study of Alzheimer's disease. J Proteomics 104: 37–47.

Hampel H, Frank R, Broich K, Teipel SJ, Katz RG, Hardy J, Herholz K, Bokde AL, Jessen F, Hoessler YC, Sanhai WR, Zetterberg H, Woodcock J, Blennow K (2010) Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives. Nat Rev Drug Discov 9: 560–574.

Han X, Rozen S, Boyle SH, Hellegers C, Cheng H, Burke JR, Welsh-Bohmer KA, Doraiswamy PM, Kaddurah-Daouk R (2011) Metabolomics in early Alzheimer's disease: identification of altered plasma sphingolipidome using shotgun lipidomics. PLoS One 6: e21643.

Hong-Qi Y, Zhi-Kun S, Sheng-Di C (2012) Current advances in the treatment of Alzheimer's disease: focused on considerations targeting Abeta and tau. Transl Neurodegener 1: 21.

Ibanez C, Simo C, Barupal DK, Fiehn O, Kivipelto M, Cedazo-Minguez A, Cifuentes A (2013) A new metabolomic workflow for early detection of Alzheimer's disease. J Chromatogr A 1302: 65–71.

Ibanez C, Simo C, Martin-Alvarez PJ, Kivipelto M, Winblad B, Cedazo-Minguez A, Cifuentes A (2012) Toward a predictive model of Alzheimer's disease progression using capillary electrophoresis-mass spectrometry metabolomics. Anal Chem 84: 8532–8540.

Irizarry MC (2004) Biomarkers of Alzheimer disease in plasma. NeuroRx 1: 226–234.

Kaddurah-Daouk R, Rozen S, Matson W, Han X, Hulette CM, Burke JR, Doraiswamy PM, Welsh-Bohmer KA (2011) Metabolomic changes in autopsy-confirmed Alzheimer's disease. Alzheimers Dement 7: 309–317.

Kaddurah-Daouk R, Zhu H, Sharma S, Bogdanov M, Rozen SG, Matson W, Oki NO, Motsinger-Reif AA, Churchill E, Lei Z, Appleby D, Kling MA, Trojanowski JQ, Doraiswamy PM, Arnold SE (2013). Alterations in metabolic pathways and networks in Alzheimer's disease. Transl Psychiatry 3: e244.

Krieger M, Herz J (1994) Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). Annu Rev Biochem 63: 601–637.

Lemaitre RN, Johnson CO, Hesselson S, Sotoodehnia N, McKnight B, Sitlani CM, Rea TD, King IB, Kwok PY, Mak A, Li G, Brody J, Larson E, Mozaffarian D, Psaty BM, Huertas-Vazquez A, Tardif JC, Albert CM, Lyytikainen LP, Arking DE, Kaab S, Huikuri HV, Krijthe BP, Eijgelsheim M, Wang YA, Reinier K, Lehtimaki T, Pulit SL, Brugada R, Muller-Nurasyid M, Newton-Cheh CH, Karhunen PJ, Stricker BH, Goyette P, Rotter JI, Chugh SS, Chakravarti A, Jouven X, Siscovick DS (2014) Common variation in fatty acid metabolic genes and risk of incident sudden cardiac arrest. Heart Rhythm 11: 471–477.

Li M, Guo K, Ikehara S (2014) Stem cell treatment for Alzheimer's disease. Int J Mol Sci 15: 19226–19238.

Lin S, Liu H, Kanawati B, Liu L, Dong J, Li M, Huang J, Schmitt-Kopplin P, Cai Z (2013) Hippocampal metabolomics using ultrahigh-resolution mass spectrometry reveals neuroinflammation from Alzheimer's disease in CRND8 mice. Anal Bioanal Chem 405: 5105–5117.

Martin V, Fabelo N, Santpere G, Puig B, Marin R, Ferrer I, Diaz M (2010) Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex. J Alzheimers Dis 19: 489–502.

Martins IJ, Berger T, Sharman MJ, Verdile G, Fuller SJ, Martins RN (2009) Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. J Neurochem 111: 1275–1308.

Montine TJ, Morrow JD (2005) Fatty acid oxidation in the pathogenesis of Alzheimer's disease. Am J Pathol 166: 1283–1289.

Mori T, Paris D, Town T, Rojiani AM, Sparks DL, Delledonne A, Crawford F, Abdullah LI, Humphrey JA, Dickson DW, Mullan MJ (2001) Cholesterol accumulates in senile plaques of Alzheimer disease patients and in transgenic APP(SW) mice. J Neuropathol Exp Neurol 60: 778–785.

Morris JC, Roe CM, Xiong C, Fagan AM, Goate AM, Holtzman DM, Mintun MA (2010) APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol 67: 122–131.

Mulder C, Wahlund LO, Teerlink T, Blomberg M, Veerhuis R, van Kamp GJ, Scheltens P, Scheffer PG. (2003) Decreased lysophosphatidylcholine/phosphatidylcholine ratio in cerebrospinal fluid in Alzheimer's disease. J Neural Transm 110: 949–955.

Oresic M, Hyotylainen T, Herukka SK, Sysi-Aho M, Mattila I, Seppanan-Laakso T, Julkunen V, Gopalacharyulu PV, Hallikainen M, Koikkalainen J, Kivipelto M, Helisalmi S, Lotjonen J, Soininen H (2011) Metabolome in progression to Alzheimer's disease. Transl Psychiatry 1: e57.

Padurariu M, Ciobica A, Lefter R, Serban IL, Stefanescu C, Chirita R (2013) The oxidative stress hypothesis in Alzheimer's disease. Psychiatr Danub 25: 401–409.

Panchal M, Loeper J, Cossec JC, Perruchini C, Lazar A, Pompon D, Duyckaerts C (2010) Enrichment of cholesterol in microdissected Alzheimer's disease senile plaques as assessed by mass spectrometry. J Lipid Res 51: 598–605.

Panza F, Solfrizzi V, Imbimbo BP, Tortelli R, Santamato A, Logroscino G (2014) Amyloid-based immunotherapy for Alzheimer's disease in the time of prevention trials: the way forward. Expert Rev Clin Immunol 10: 405–419.

Perrin RJ, Fagan AM, Holtzman DM (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature 461: 916–922.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E (1999) Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 56: 303–308.

Pettegrew JW, Panchalingam K, Hamilton RL, McClure RJ (2001) Brain membrane phospholipid alterations in Alzheimer's disease. Neurochem Res 26: 771–782.

Pratico D, V MYL, Trojanowski JQ, Rokach J, Fitzgerald GA (1998) Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation *in vivo*. FASEB J 12: 1777–1783.

Puglielli L, Tanzi RE, Kovacs DM (2003) Alzheimer's disease: the cholesterol connection. Nat Neurosci 6: 345–351.

Reed TT (2011) Lipid peroxidation and neurodegenerative disease. Free Radic Biol Med 51: 1302–1319.

Reinikainen KJ, Soininen H, Riekkinen PJ (1990) Neurotransmitter changes in Alzheimer's disease: implications to diagnostics and therapy. J Neurosci Res 27: 576–586.

Ross BM, Moszczynska A, Erlich J, Kish SJ (1998) Phospholipid-metabolizing enzymes in Alzheimer's disease: increased lysophospholipid acyltransferase activity and decreased phospholipase A2 activity. J Neurochem 70: 786–793.

Rubio-Perez JM, Morillas-Ruiz JM (2012) A review: inflammatory process in Alzheimer's disease, role of cytokines. Sci World J 2012: 756357.

Sahebkar A, Chew GT, Watts GF (2014) Recent advances in pharmacotherapy for hypertriglyceridemia. Prog Lipid Res 56C: 47–66.

Sato Y, Suzuki I, Nakamura T, Bernier F, Aoshima K, Oda Y (2012) Identification of a new plasma biomarker of Alzheimer's disease using metabolomics technology. J Lipid Res 53: 567–576.

Shafaati M, Solomon A, Kivipelto M, Bjorkhem I, Leoni V (2007) Levels of ApoE in cerebrospinal fluid are correlated with Tau and 24S-hydroxycholesterol in patients with cognitive disorders. Neurosci Lett 425: 78–82.

Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC (2013) Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One 8: e63644.

Trushina E, Nemutlu E, Zhang S, Christensen T, Camp J, Mesa J, Siddiqui A, Tamura Y, Sesaki H, Wengenack TM, Dzeja PP, Poduslo JF (2012) Defects in mitochondrial dynamics and metabolomic signatures of evolving energetic stress in mouse models of familial Alzheimer's disease. PLoS One. 7: e32737.

Tukiainen T, Tynkkynen T, Makinen VP, Jylanki P, Kangas A, Hokkanen J, Vehtari A, Grohn O, Hallikainen M, Soininen H, Kivipelto M, Groop PH, Kaski K, Laatikainen R, Soininen P, Pirttila T, Ala-Korpela M (2008) A multi-metabolite analysis of serum by 1H NMR spectroscopy: early systemic signs of Alzheimer's disease. Biochem Biophys Res Commun 375: 356–361.

Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E (2000) Standardization of measurement of beta-amyloid(1–42) in cerebrospinal fluid and plasma. Amyloid 7: 245–258.

Walter A, Korth U, Hilgert M, Hartmann J, Weichel O, Fassbender K, Schmitt A, Klein J (2004) Glycerophosphocholine is elevated in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging 25: 1299–1303.

Weidner AM, Bradley MA, Beckett TL, Niedowicz DM, Dowling AL, Matveev SV, LeVine H, 3rd, Lovell MA, Murphy MP (2011) RNA oxidation adducts 8-OHG and 8-OHA change with Abeta42 levels in late-stage Alzheimer's disease. PLoS One 6: e24930.

Whiley L, Sen A, Heaton J, Proitsi P, Garcia-Gomez D, Leung R, Smith N, Thambisetty M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Lovestone S, Legido-Quigley C (2014) Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. Neurobiol Aging 35: 271–278.

Yan S, Li Z, Li H, Arancio O, Zhang W (2014) Notoginsenoside R1 increases neuronal excitability and ameliorates synaptic and memory dysfunction following amyloid elevation. Sci Rep 4: 6352.