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**Topical issue on:**
*LIPIDS & BRAIN IV: LIPIDS IN ALZHEIMER'S DISEASE*
LIPIDS & BRAIN IV : LES LIPIDES DANS LA MALADIE D'ALZHEIMER

REVIEW OPEN ACCESS

Topical Issue

# Small molecule biomarkers in Alzheimer's disease

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**Abstract –** Alzheimer's disease (AD) is a progressive neurodegenerative disease which affects a growing number of people as the population ages worldwide. Alzheimer's Disease International estimated that more than 100 million people will be living with dementia by 2050. At present there are no disease-modifying therapies and research has expanded to the −omic sciences with scientists aiming to get a holistic view of the disease using systems medicine. Metabolomics and Lipidomics give a snap-shot of the metabolism. As analyzing the brain *in vivo* is difficult, the metabolic information of the periphery has potential to unravel mechanisms that have not been considered, such as those that link the brain to the liver and the gut or other organs. With that in mind we have produced a mini-review, to record a number of studies in the field and the molecular pathways that have been flagged in animal and human models of AD. Human studies deal with cohorts in the order of the hundreds due to the difficulty of organizing AD studies, however it is possible that these first pilots point towards important mechanisms. The trend in these small studies is the involvement of many organs and pathways. Some findings, that have been reproduced, are ceramides being increased, phospholipids and neurotransmitters depleted and sterols being found depleted too. Initial findings point to an important role to lipid homeostasis in AD, this is not surprising as the brain's main constituents are water and lipids.

**Keywords:** Alzheimer's disease / metabolomics / lipidomics / biomarker

**Résumé – Biomarqueurs à petites molécules dans la maladie d'Alzheimer.** La maladie d'Alzheimer est une maladie neurodégénérative progressive qui affecte un nombre croissant de personnes en raison du vieillissement de la population observé dans le monde entier. La fédération internationale d'associations Alzheimer's disease International estime que plus de 100 millions de personnes vivront avec cette démence d'ici à 2050. Il n'existe actuellement aucun traitement de la maladie et la recherche s'est élargie aux sciences -omiques avec l'objectif scientifique d'obtenir une approche globale de la maladie en utilisant une médecine des systèmes. La métabolomique et la lipidomique donnent un aperçu du métabolisme. L'analyse du cerveau *in vivo* s'avérant difficile, l'information métabolique de la périphérie possède le potentiel de démêler des mécanismes qui n'ont pas été pris en compte, tels que ceux reliant le cerveau au foie et à l'intestin ou à d'autres organes. Dans cet esprit, nous proposons une mini-revue, afin de lister un certain nombre d'études relevant de ce champ et les voies moléculaires qui ont été signalées chez les modèles animaux et humains de la maladie d'Alzheimer. Les études humaines traitent des cohortes de quelque centaines d'individus en raison de la difficulté à organiser des études sur cette pathologie, mais il est possible que ces premiers pilotes pointent vers des mécanismes importants. La tendance dans ces petites études est l'implication de nombreux organes et voies. Certaines conclusions, qui ont été reproduites, indiquent que les céramides sont augmentées, les phospholipides et les neurotransmetteurs appauvris et les stérols également épuisés. Les premiers résultats indiquent un important rôle de l'homéostasie des lipides dans la maladie d'Alzheimer, ce qui ne semble guère surprenant puisque les principaux constituants du cerveau sont l'eau et les lipides.

**Mots clés :** maladie d'Alzheimer / métabolomique / lipidomique / biomarqueur

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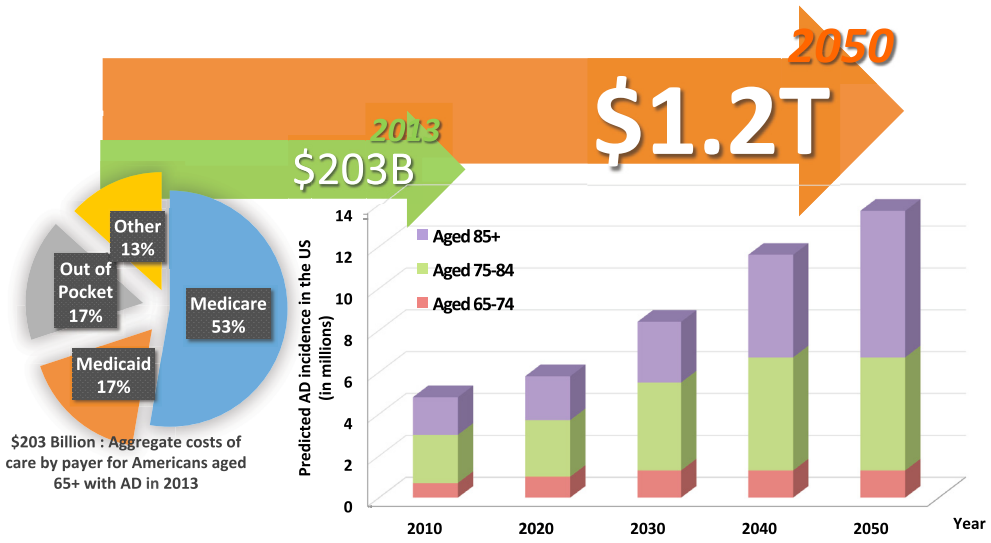


**Fig. 1.** Alzheimer's projection on incidence cost of Alzheimer's care. Pie chart shows the breakdown of aggregate cost of care in the US. Data drawn from (Alzheimer's, 2015, Hebert *et al.*, 2013, Thies *et al.*, 2013).

## 1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized with memory impairment and personality behavior changes, caused by gradual brain cell apoptosis. It is the most common form of dementia accounting for 50% to 70% of cases. Other dementias include Lewy body disease (LBD), frontotemporal dementia (FTD), vascular dementia (VAD) and Parkinson's' disease (PD). In some cases, patients can be diagnosed with a combination of two or more dementias.

AD can present in two forms, early-onset AD (EOAD) and late-onset AD (LOAD). This report will mainly focus on LOAD. Incidence of LOAD normally occurs in people of 65 or older, it has no clear familial pattern and it is considered sporadic (sporadic AD). EOAD occurs before the age of 65 (mean age of 46 years old, but can appear as early as 30s) (Ryman *et al.*, 2014) and it accounts for less than 1% of all AD cases. EOAD is largely hereditary and genetic mutation is the biggest risk factor. EOAD has association with these deterministic variations and it can be called autosomal dominant AD (ADAD, also termed familial AD).

Currently AD is the 6$^{th}$ leading cause of death and the 3$^{rd}$ leading cause of death among people aged 65 or older in the United States (US) (James *et al.*, 2014). Despite this, there is currently no treatment for the disease. The estimated incidence of AD is only likely to increase due to the rapid increase in number of elders. Current estimates suggest AD incidence will increase from 5.4 million (2016) to 13.7 million (2050) in the US (Hebert *et al.*, 2013; Alzheimer's, 2015).

The progress of AD is relatively slow; people often live on average 4 to 6 years after diagnosis and some can live as long as 20 years. People with AD require partial or full assistance and patients lose ability to take care of their other chronic health problems, such as diabetes or heart disease. All of these factors contribute in making AD an expensive disease. In the US, the disease was reported to cost $226 billion in 2012, and this cost is predicted to increase to $1.2 trillion by 2050 (Alzheimer's, 2015).

Despite these facts (summarized in Fig. 1), the reality is that the disease mechanism is not comprehensively understood; definite AD diagnosis can only be made on post-mortem brains, and there is no AD prevention or treatment therapies. Currently, just a few drugs are available for AD patients and they are only able to slow the progression of symptoms. Therefore, it is vitally important to determine the biochemistry behind AD, and develop tools for AD diagnosis, treatment and prevention.

## 2 Current markers of AD

The AD diagnosis criteria was set up by NINDS-ADRA in 1984 and was reported to have average sensitivity and specificity of 81% and 70% respectively (Knopman *et al.*, 2001). This meant that some individuals were identified to having AD when in fact they were non-AD dementia. A recent guideline set up by NIA-AA in 2011 recommended measurements of Aβ and tau (brain and CSF) as diagnostic adjuncts to achieve better AD diagnostic accuracy. These criteria also defined individuals in pre-clinical AD and/or MCI stages (McKhann *et al.*, 2011). As the result, the following tests can be employed to aid AD diagnosis (Tab. 1) and are often termed AD biomarkers.

A biomarker is defined by the U.S. National Institutes of Health Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic process, or pharmacologic responses to a therapeutic intervention" (Atkinson *et al.*, 2001). Biomarkers allow clinicians to evaluate the state of an individual's health; confirm disease onset and progression; or gauge whether a treatment is working or not. They must display high sensitivity and specificity, while ease-of use is also an important factor to be defined as biomarkers. (Desai and Grossberg, 2005; Hampel

**Table 1.** Classification of biomarkers used in the differential diagnosis of AD. These markers are recommended by NIA-AA.

| Biomarkers for AD | Pathophysiological correlate |
|---|---|
| Neuropsychological | |
| Tests for episodic memory /delayed recall | Hippocampal amnesia due to AD |
| Cerebrospinal fluid | |
| Aβ42 | Neuronal Aβ plaque accumulation |
| Total-tau | Neuronal degeneration |
| Phosphorylated tau | Intra neuronal NFT |
| Amyloid Imaging | |
| Aβ PET | Neuronal Aβ plaque accumulation |
| Structural neuroimaging | |
| Magnetic resonance imaging (MRI) | Neuronal injury marker |
| Computed tomography (CT) | Neuronal injury marker |
| Functional neuroimaging | |
| FDG-PET | Glucose hypometabolism |

*et al.*, 2010; Humpel, 2011). Examples include body temperature for fever, blood pressure for the risk of stroke, cholesterol values for coronary and vascular diseases, and C-reactive protein (CRP) for inflammation.

As shown in Table 1, AD biomarkers are mainly based on measurements of Aβ and tau and allow relatively high AD diagnosis accuracy (Harris *et al.*, 2015). In particular, CSF $A\beta_{42}$, total- and phosphor- tau measurements can detect individuals in preclinical stage of AD, and therefore have an ability of predicting the risk of progression to AD. However, these markers are the subject of debate for a number of reasons. PET imaging is expensive while lumbar puncture for CSF collection is invasive. Most importantly, DMT drugs directed toward attenuating the Aβ and tau pathways have all failed to show positive results during clinical trials (Cummings *et al.*, 2016). As a result, focus in AD research has shifted towards early intervention and prevention of AD. Also, the scientific community believe that pathology other than amyloid and tau needs to be targeted (Dong *et al.*, 2012; Reitz, 2012) as well as a need to discover new biomarkers which would recognise individuals in the asymptomatic (preclinical) stage.

A test with biomarkers would benefit AD patients in a number of ways, it would distinguish AD from other types of dementia and allow more accurate treatment to be initiated (Humpel, 2011). It can potentially identify novel mechanistic pathways of the disease and consequently set the path for the discovery of a disease-modifying drug. Conversely, biomarkers would allow the correct selection of people in a preclinical stage of AD for clinical trials and help with prevention therapies (Fiandaca *et al.*, 2014).

Finding biomarkers of Alzheimer's disease is a priority in the medical research field, especially biomarkers for the preclinical and clinical stages of AD. This has involved assays of blood (Hye *et al.*, 2014; Mapstone *et al.*, 2014), skin (Khan and Alkon, 2010), urine (Ghanbari *et al.*, 1998), hair (Son *et al.*, 2016), odour (Kimball *et al.*, 2016a), and olfactory deficits (Tabert *et al.*, 2005) with candidates being proteins, genes, clinical symptoms or small metabolites. Of these, peripheral blood has been the most extensively interrogated, as it is accessible by minimally invasive means and offers a relatively inexpensive substrate for analysis. Also crucially, blood is comprised of a liquid component (plasma or serum) and a wide variety of cells (mononuclear leukocytes, erythrocytes and platelets). The liquid component is often used for proteins or small molecules profiling; Ribonucleic Acid (RNA) assay can be obtained from cells (and serum and plasma exosomes). This particular nature of blood therefore represents a unique opportunity for biomarker discovery and development.

For discovery of novel blood-based biomarkers of AD, a wide array of technological platforms have been applied in recent years. For example, application of Polymerase Chain Reaction (PCR) has proposed circulating microRNAs (Kumar *et al.*, 2013; Tan *et al.*, 2014); the most promising result showed a panel of 12 microRNAs which differentiated AD from controls with accuracy of 93% (Leidinger *et al.*, 2013). Transcriptomic tests have also been applied on blood; a signature consisting of 170 probe sets has been identified to discriminate AD from controls with sensitivity and specificity of 81.3% and 67.1% respectively (Fehlbaum-Beurdeley *et al.*, 2012).

A wide number of studies have also focused on blood-based proteomic biomarkers of AD, Aβ being the initial focus. Cross-sectional studies comparing levels of blood Aβ in AD have not shown consistent results; negative (Blennow *et al.*, 2010; Toledo *et al.*, 2013; Snyder *et al.*, 2014; Janelidze *et al.*, 2016) as well as positive (Lopez *et al.*, 2008; Mayeux *et al.*, 2003; Lambert *et al.*, 2009) test results have been reported. Several other proteins have also been proposed; Ray *et al.* described a panel of 18 proteins being able to predict AD from controls with 90% accuracy (Ray *et al.*, 2007a) while glycan signatures have shown to discriminate with a sensitivity and specificity of 89.3% and 79.1% respectively (Lundstrom *et al.*, 2014). Recently, O'Bryant *et al.*, (2016) established a 21 protein panel as an AD discriminator in n = 1329 study; the sensitivity and specificity were 63% and 98% respectively. Other groups have also looked at potential preclinical AD biosignatures. A panel of 25 proteins was found to predict conversion of AD from controls with sensitivity of 80% and specificity of 91% (O'Bryant *et al.*, 2010) while 10 plasma proteins were found to predict progression to AD from MCI (accuracy 87%) (Hye *et al.*, 2014). Another promising cross-sectional study showed 18 proteins being able to predict conversion from symptomatic MCI to AD with accuracy of 91% (Ray *et al.*, 2007b); successive validation studies failed to replicate (Soares *et al.*, 2009; Marksteiner *et al.*, 2011).

## 3 Lipidomics and metabolomics

The last decades have seen much advancement in analytical technologies such as nuclear magnetic resonance (NMR) and high-resolution tandem mass spectrometry (MS). One of the many applications of this technology is the accurate detection and fingerprinting or profiling of small molecule metabolites (< 1500 Da). The field where as many small

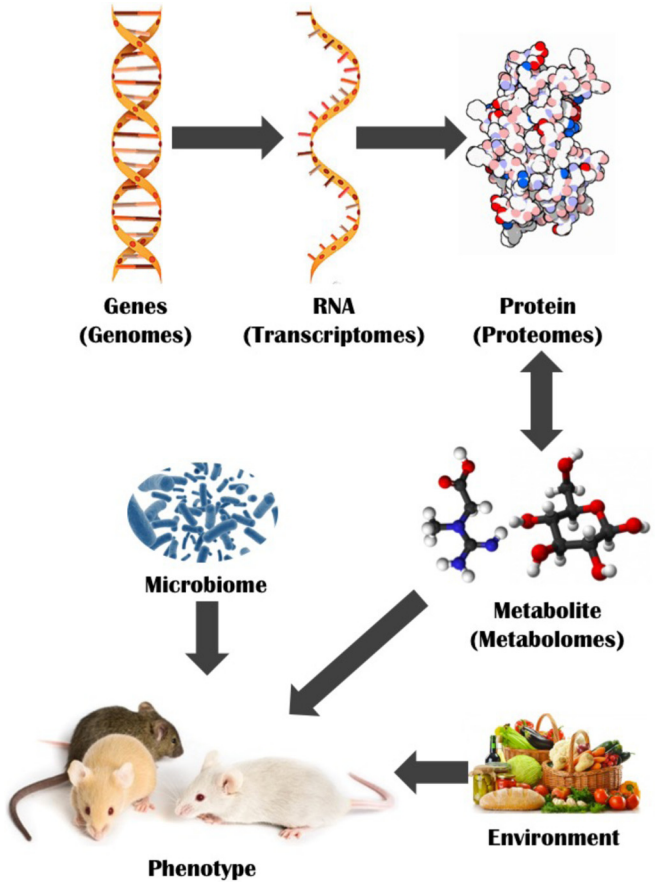


**Fig. 2.** A schematic diagram of a systems approach to biology. The diagram shows that metabolites are the terminal downstream products of genomes, transciptomes, and proteomes in the whole organisms.

molecules are measured is often termed as 'metabolomics' or sometimes 'metabonomics'.

Metabolomics involves a comprehensive and simultaneous systematic profiling of metabolite concentrations and their fluctuation that reflects in response of living systems to pathophysiological stimuli and/or genetic modifications and the surrounding environment (Beckonert *et al.*, 2007; Want *et al.*, 2010; Wilson, 2011). Metabonomics is another term often used interchangeably with metabolomics as the analytical and modeling procedures are the same. Metabolomics can be categorized into two distinct approaches, classically it is non-targeted (or untargeted), but it is also possible to analyse a chosen panel of metabolites and call it targeted metabolomics. The non-targeted approach involves quantification/relative-quantification of as many as possible metabolites in a biological system. Targeted approach is where chemically characterized and biochemically annotated metabolites are quantified using specific methodologies designed with a target in mind (Roberts *et al.*, 2012).

Metabolomics has a number of inherent advantages compared to the use of other profiling techniques such as genomics and proteomics. Since metabolites are the terminal downstream product of the genome, monitoring the perturbations in a pool of metabolites could reflect underlying disease pathology and further, disease prognosis and diagnosis (Fig. 2). Also, metabolomics can react very fast to stimuli or to change, therefore it is capable of viewing the most current view of the system's biology. Through evolution, metabolic pathways have been conserved, which means metabolic pathways are similar in rodent and humans. Therefore, metabolic signatures identified in mechanistic and therapeutic studies for animal models can in theory be translated into human studies (Holmes and Nicholson, 2007). Moreover, metabolomics is relatively inexpensive and time efficient when compared to genomics or proteomics, and can be applied to variety of easily accessible biofluids such as plasma, CSF, urine, and peripheral tissues, thus highlighting the clinical utility of the approach (Nicholson *et al.*, 2007).

The overall size of the metabolome remains elusive in metabolomics field, but it is estimated to range from a few thousands to hundreds of thousands of small molecules (Wishart *et al.*, 2009; Psychogios *et al.*, 2011). Recent advancements in analytical technologies have allowed measurements of dynamic changes in many molecules in biological samples simultaneously. These technologies include high-resolution NMR and MS coupled with chromatographic techniques. However, no analytical techniques is available for the analysis of the entire metabolome content. Often a range of criteria such as speed, cost, properties of the analytes, sensitivity and the choice of biofluid needs to be considered.

## 4 Lipid metabolism

Implication of altered lipid metabolism in AD pathogenesis was first suggested by Alois Alzheimer (Sparks *et al.*, 1994; Foley, 2010), but this hypothesis has been gaining acceptability only in recent years (Proitsi *et al.*, 2014; Djelti *et al.*, 2015), after the discovery of ε4 allele of the apolipoprotein E (ApoE) gene being the strongest LOAD genetic risk factor (Corder *et al.*, 1993; Bertram and Tanzi, 2008). Implication of lipids in AD is perhaps logical considering all enzymes involved in amyloidogenic pathway (APP, β-secretase and γ-secretase) are transmembrane proteins. A perturbed lipid bilayer composition and organization will impact on trafficking and/or proteolytic activity of these transmembrane proteins, and thus APP processing and Aβ production (Hartmann *et al.*, 2007).

Of all lipid classes, high cholesterol levels in brain (cholesterol homeostasis) has been implicated with AD the most. Cholesterol has an essential role in the brain, helping to maintain neuronal functions such as neurotransmitter release and synaptic plasticity (Koudinov and Koudinova, 2001; Linetti *et al.*, 2010). However, when cholesterol is in excess in the brain (*de novo*), it is esterified into cholesteryl esters by acyl-cholesterol-acyltransferase-1 (ACAT1), and accumulation of this intracellular cholesteryl ester can enhance Aβ release (Puglielli *et al.*, 2001; Di Paolo and Kim, 2011). Supporting this hypothesis, pharmacological inhibition of ACAT1 has been found to reduce both Aβ production and cholesteryl esters (Bhattacharyya and Kovacs, 2010; Hutter-Paier *et al.*, 2010). Additionally, cholesterol is known to modulate β-secretase and γ-secretase activities directly, with a rich environment of cholesterol having been found to accelerate Aβ deposition (Simons *et al.*, 1998; Wahrle *et al.*, 2002; Vetrivel and Thinakaran, 2010).

Supporting these biochemical studies are case-control studies where statins, cholesterol-lowering drugs, have been found associating with lower risk of AD development (Jick *et al.*, 2000; Wolozin *et al.*, 2007). Statin has also shown to promote ectodomain (domain of a membrane protein that extends into extracellular space) shedding of APP through the α-secretase pathway (non-amyloidogenic pathway), inhibiting Aβ production in a cholesterol-independent manner (Pedrini *et al.*, 2005). However, retrospective clinical trials involving a prospective cohort have shown conflicting results, Wolozin *et al.* (2007) reported lower incidence of the dementia while Arvanitakis *et al.* (2008) showed no relationship between statin medication and AD incidence, cognitive decline and AD pathologies. Additionally, epidemiological studies testing associations between low-density lipoprotein (LDL) cholesterol (often referred as 'bad' cholesterol) and AD have also produced mixed results. While increased levels of total cholesterol levels have been found in post-mortem AD brains (Kuo *et al.*, 1998) and associated with higher AD risk (Kivipelto *et al.*, 2002; Whitmer *et al.*, 2005), no relationship (Tan *et al.*, 2003) or the opposite relationship (Reitz *et al.*, 2004; Mielke *et al.*, 2005; Snowden *et al.*, 2017) have also been reported.

In brain, ApoE is the predominant apolipoprotein (Liu *et al.*, 2013). Interestingly, ApoE is also involved in Aβ clearance (Kim *et al.*, 2009). The activity of ApoE is greatly dependent on its lipidation level, regulated by adenosine triphosphate binding cassette A1 (ABCA1). Immunochemical studies have demonstrated that the lack of ABCA1 results in deficient lipidation of ApoE and consequently leads to accumulation of amyloid plaques burden, indicating poorly lipidated ApoE to be associated with amyloidogenic pathway (Hirsch-Reinshagen *et al.*, 2005; Koldamova *et al.*, 2005; Wahrle *et al.*, 2005). Additionally, the effect of ApoE seems to be isoform-dependent; ε4 allele of ApoE results in the highest Aβ deposition (ε4 > ε3 > ε2) (Sepehrnia *et al.*, 1989; Farrer *et al.*, 1997; Kok *et al.*, 2009). However, the mechanism by which ApoE alleles differentially modulate the amyloidogenic pathway is less clear.

In addition to cholesterol, sphingolipids have been found to play a role in Aβ production. Sphingolipids include ceramide, sphingomyeline and glycosphingolipids, and are believed to play important roles in signal transmission and cell recognition (Ohanian and Ohanian, 2001; Hannun and Obeid, 2002). Ceramide, a central component in sphingolipid metabolism, has been suggested to facilitate the regulation of BACE-medicated processing of APP by prolonging the half-life of BACE (Puglielli *et al.*, 2003). Furthermore, these lipid species have been found to mediate oxidative stress-induced neuronal apoptosis, independently of its role in BACE activity regulation (Spiegel and Milstien, 1995; Spiegel and Milstien, 2002; Cutler *et al.*, 2004; He *et al.*, 2010). They have shown to activate enzymes involved in cell apoptosis raging from protein kinase (Lozano *et al.*, 1994), cathepsin D (Heinrich *et al.*, 1999) and serine/threonine protein phosphatase (Dobrowsky *et al.*, 1993). These findings are supported by retrospective epidemiologic studies where high ceramide levels have been found in AD blood (Han XL *et al.*, 2011), CSF (Satoi *et al.*, 2005) and brain (Filippov *et al.*, 2012), while higher serum ceramide levels have also been associated with AD incidence (Mielke *et al.*, 2012). These findings suggest potential of ceramide-based therapeutic treatments for delaying AD development. Inhibition of sphingomyelinase (SMase), a catalyst involved in sphingomyeline to ceramide conversion, has been suggested as a therapeutic agent. In addition, neutral SMase (N-SMase) knout out mice has shown lower activation of pro-inflammatory enzymes and subsequently greater protection of neurons (Jana and Pahan, 2010). Dinkins *et al.*, (2014) have also demonstrated that by inhibiting N-SMase activity they observed lower brain ceramide levels and $A\beta_{42}$ burden.

Phospholipids are another family of lipid species to be associated with AD along with enzymes involved in phospholipid metabolism. A number of case-control studies have reported abnormal phospholipids levels in AD individuals, namely, lower levels for phosphatidylinositol (PI) (Prasad *et al.*, 1998; Stokes and Hawthorne, 1987; Pettegrew *et al.*, 2001), for phosphatidylethanolamine (PE) (Nitsch *et al.*, 1992; Guan *et al.*, 1999; Prasad *et al.*, 1998) and for phosphatidylcholine (PC) (Nitsch *et al.*, 1992; Wells *et al.*, 1995; Prasad *et al.*, 1998; Guan *et al.*, 1999) in AD brain and in AD blood (Mapstone *et al.*, 2014; Whiley *et al.*, 2014) while they were found increased in AD CSF (Mulder *et al.*, 2003; Walter *et al.*, 2004). Phospholipids are a major component of the cell membrane and play important roles in cellular functions, such as extracellular receptor signalling, intercellular second messengers and cellular pressure regulation (Lutjohann *et al.*, 2012).

## 5 Applications of lipidomics/metabolomics in AD

Although metabolomics is a relatively young 'omic' field, in recent years, an extensive number of studies have applied this approach to establish differences in metabolite levels between cognitively normal (CN) individuals and AD patients. Various biological matrices have been investigated; CSF is very useful due to its composition reflecting in part brain metabolic production. Non-invasive samples such as blood (serum or plasma), urine and saliva have also been analysed with the aim to identify more accessible biomarkers translatable to the clinical practice. Brain is also another source but the focus mainly has been the *in situ* characterization of neuropathology. Animal model and human AD studies utilizing metabolomics will be discussed in the following section.

### 5.1 Animal models of AD

Metabolomic techniques have been utilized in animal models of AD. With regards to the animal models, it is important to note that the commonly employed transgenic (Tg) animal models are based on over-expression of APP ($APP_{Tg2576}$, $APP_{V717F}$ and CRND8 transgenic lines). Some models also co-express the mutated human presenilin 1 (PSEN1) or presenilin 2 (PSEN2) allowing accelerated amyloid deposition (APP × PS1 or APP × PS2 double transgenic models respectively). TASTPM is another double transgenic mouse model carrying two mutations associated with APP and PSEN1. SAMP8 is another model which has

been frequently used as this model displays a phenotype of accelerated aging and thus age-associated increase in hippocampal Aβ and age-associated behavioural impairments. These animal models are more closely associated with familial form of AD rather than LOAD, but they can still offer an opportunity to investigate the early pathological disease mechanisms.

The earliest studies on animal models were approached by Magnetic Resonance Spectroscopy (MRS) and investigated neurochemical profiles in cerebrum samples. Examination on cerebral cortex (Dedeoglu *et al.*, 2004), cortex and hippocampus (Marjanska *et al.*, 2005), and frontal cortex (von Kienlin *et al.*, 2005) showed a decrease in the levels of N-acetylaspartate (NAA) and glutamate in the brains where amyloid toxicity was widespread. In addition, these metabolites have been found to correlate (negatively) with brain amyloid burden (von Kienlin *et al.*, 2005). Studies utilizing different analytical platforms have also reported decreased NAA and glutamate levels; these include studies utilizing NMR on APP transgenic mouse hippocampus, cortex, frontal cortex, midbrain and cerebellum (Salek *et al.*, 2010), and GC-MS metabolomics on PS1 transgenic hippocampus (Trushina *et al.*, 2012). Glutamate is a primary excitatory neurotransmitter and its decreased levels may reflect a loss of glutamatergic neurons or decreased glutamate activity (Lin *et al.*, 2003). Biological function of NAA in the brain is not well understood, but it is commonly regarded as a marker of neuronal density and integrity (Schuff *et al.*, 1997). A diagnostic test based on the decreased levels of NAA and glutamate in APP × PS2 mice brains was able to classify 'AD like' mice from normal mice with 92% sensitivity and 82% specificity (von Kienlin *et al.*, 2005). In addition to decreased levels of NAA and glutamate, these studies have also reported myo-inositol (m-In) content elevated in APP and PS1 mouse cerebrums (Marjanska *et al.*, 2005; Woo *et al.*, 2010; Forster *et al.*, 2012). M-In is predominantly expressed in glial cells compared to neurons; its levels are considered to be indicative of osmotic stress and astroglosis (Castillo *et al.*, 2000). However, other mouse model studies have shown contradicting results (Salek *et al.*, 2010; Trushina *et al.*, 2012).

Assays using NMR on plasma and liver samples from APP × PS1 transgenic mice showed significant changes in levels of lipids, nucleotides and energy-related metabolites, suggesting occurrence of oxidative stress and impaired energy metabolism (Wu *et al.*, 2016). The impaired energy metabolism was also visible when serum samples were subjected to NMR, glucose, citrate, 3-hydroxybutyrate, pyruvate were found to be decreased (Liang *et al.*, 2008). Moreover, analysis on urine samples from APP mice revealed significant metabolic alterations (increased levels of 3-hydroxykynurenine, homogentisate and allantoin) indicative of oxidative stress (Fukuhara *et al.*, 2013).

While NMR or MRS platforms are ideal for detecting highly abundant metabolites (particularly those involved in energy metabolism), MS based metabolomics allows to look at a wider range of the metabolome network. Direct MS analysis on APP transgenic mouse cortex and cerebellum showed lower sulfatide levels (Cheng *et al.*, 2010), while perturbations in homeostasis of lipids, energy management, and metabolism of amino acids and nucleotides were visible on APPxPS1 hippocampus and cortex (Gonzalez-Dominguez *et al.*, 2015h). Employing ultra high resolution MS instruments, Lin *et al.*, (2013) were able to observe over-production of eicosanoids indicating neuroinflammation on hippocampal tissues (Lin *et al.*, 2013), and enhanced biosynthesis of amino acid and amino acid derivatives in cerebellum (Lin *et al.*, 2014). Blood metabolomics using MS has identified numerous disturbed pathways in serum samples from APPxPS1 mouse, including perturbed phospholipid homeostasis, energy-related failures, hyperammonemia, inflammation (increased eicosanoids), among others (Gonzalez-Dominguez *et al.*, 2015c). Peripheral organs from APPxPS1 Tg mouse models have included liver, kidneys, spleen and thymus (Gonzalez-Dominguez *et al.*, 2015e). The findings demonstrated significant impairments in energy metabolism, lipid homeostasis, oxidative stress and amino acids metabolism. These findings were largely consistent with those found from Tg mouse brain tissue samples (Lin *et al.*, 2014; Lin *et al.*, 2013; Gonzalez-Dominguez *et al.*, 2015h). These findings highlighted the systemic nature of AD as metabolite dysregulation was seen in multi-organs, not just the brain.

Utilizing MS coupled with chromatography allows detection of a broader network of metabolic pathways when compared to NMR or direct MS approaches. Whole brain metabolic profiles from APPxPS1 revealed perturbations related to amino acid metabolism and steroid biosynthesis (Hu *et al.*, 2012). Impairment in sterol metabolic pathway has also been visible in terms of increased cholesterol and cholesterol ester levels on whole brain (Sharman *et al.*, 2010; Tajima *et al.*, 2013) and hippocampal tissues (Wang HL *et al.*, 2014). Lipid analysis on APPxPS1 Tg mouse whole brain samples showed elevated levels of lysophophatidylcholines (LysoPC), free fatty acids, monoacylglycerols and phosphatidic acids (Piro *et al.*, 2012). The study was also able to identify significant perturbations in brain eicosanoids (Piro *et al.*, 2012), in line with another study (Lin *et al.*, 2013). Further inactivation of monoacylglycerol lipase has been found to reverse the perturbated metabolism of eicosanoids and reduce neuroinflammation (Piro *et al.*, 2012). LC-MS techniques have also been applied for analysing hydrophilic metabolites in urine samples from CRND8 transgenic mice. Significant changes were observed in urinary levels of multiple families of metabolites; these included amino acids, fatty acids and neurotransmitter conjugates (Peng *et al.*, 2014; Tang *et al.*, 2016). Altered levels of these metabolites have been identified in brain and blood samples as well (Dedeoglu *et al.*, 2004; Liang *et al.*, 2008; Salek *et al.*, 2010; Lin *et al.*, 2013), suggesting whole body response to AD.

González-Domínguez *et al.* has employed a combination of GC-/LC- MS platforms and using the APPxPS1 mouse model has shown numerous alterations in the homeostasis of lipids, amino acids, nucleotides, energy-related metabolites and some other compounds in the whole organism, including in serum (Gonzalez-Dominguez *et al.*, 2015d), liver and kidney (Gonzalez-Dominguez *et al.*, 2015f), spleen and thymus (Gonzalez-Dominguez *et al.*, 2015i), and various regions of the brain (Gonzalez-Dominguez *et al.*, 2014d). Many of these impairments could also be detected in APP mouse hippocampus when the same methodology was applied (Trushina *et al.*, 2012).

Summary of metabolomics studies conducted in animal models of AD can be seen in Table 2.

**Table 2.** Summary of metabolomics studies conducted in animal models of AD.

| Animal model | Sample | Platform | Findings | Ref. |
|---|---|---|---|---|
| $APP_{Tg2576}$(n = 9)<br>WT(n = 10) | Frontal cortex | NMR | ↑Taurine;<br>↓N-acetyl-aspartate, glutamate, glutathione | (Dedeoglu *et al.*, 2004) |
| APPxPS1(n = 7–8)<br>WT(n = 5–9) | Whole brain | NMR | ↑Myo-inositol, choline, glycerophosphocholine;<br>↓succinate | (Forster *et al.*, 2012) |
| CRND8<br>(n = 5/5 young/old)<br>WT<br>(n = 4/8 young/old) | Cortex, frontal cortex, cerebellum, hippocampus, striatum, pons, midbrain, olfactory bulb | NMR | ↑Lactate, aspartate, glycine, alanine, leucine, isoleucine, valine, free fatty acids;<br>↓N-acetyl-aspartate, glutamate, glutamine, taurine, γ-aminobutyric acid, choline, phosphocholine, creatine, phosphocreatine, succinate | (Salek *et al.*, 2010) |
| APP × PS1(n = 10)<br>WT(n = 10) | Cortex(frontal, occipital, parietal, temporal), thalamus, hippocampus | NMR | ↑Myo-inositol, scyllo-inositol;<br>↓N-acetyl-aspartate | (Woo *et al.*, 2010) |
| $APP_{Tg2576}$(n = 39)<br>WT(n = 44) | Frontal cortex, rhinal cortex, hippocampus, cerebellum, midbrain | NMR | ↑Creatine, taurine;<br>↓glutamate, N-acetyl-aspartate, myo-inositol, phosphocholine, γ-aminobutyric acid | (Lalande *et al.*, 2014) |
| APP × PS1(n = 6)<br>WT(n = 6) | Whole brain | NMR | ↓N-acetyl-aspartata, γ-aminobutyric acid, creatine, ascorbic acid | (Graham *et al.*, 2013b) |
| APP × PS1(n = 6)<br>WT(n = 6) | Plasma | NMR | ↓Glutamate, glutamine, methionine, acetate, citrate | (Graham *et al.*, 2013b) |
| APP × PS1(n = 27)<br>WT(n = 33) | Plasma | NMR | ↑Lipoproteins, triglycerides | (Wu *et al.*, 2016) |
| APP × PS1(n = 27)<br>WT(n = 33) | Liver | NMR | ↑Unsaturated fatty acids, 3-hydroxybutyrate, aspartate, glutamine, uridine diphosphate, uridine monophosphate, adenosine monophosphate; ↓alanine, lactate, betaine | (Wu *et al.*, 2016) |
| SAMP8(n = 5)<br>SAMR1(n = 5) | Serum | NMR | ↑Lactate, saturated fatty acids, alanine, methionine, glutamine, VLDL;<br>↓inosine, glucose, polyunsaturated fatty acids, choline, phosphocholine, HDL, LDL, 3-hydroxy-butyrate, citrate, pyruvate | (Liang *et al.*, 2008) |
| $APP_{Tg2576}$(n = 3–5)<br>WT(n = 3–5) | Urine | NMR | ↑3-hydroxykynurenine, homogentisate, allantoin, acetate, trans-aconitate, tyrosine, hippurate, citrate, urea;<br>↓trigonelline, 2-oxoglutarate, dimethylamine, trimethylamine, threonine, 1-methylnicotinamide | (Fukuhara *et al.*, 2013) |
| $APP_{V717F}$,<br>$APP_{V717F}$ × APOE<br>$APP_{sw}$<br>$APP_{sw}$ × APOE<br>WT | Cortex, cerebellum | MS | ↓Sulfatides | (Cheng *et al.*, 2010) |
| APP × PS1(n = 30)<br>WT(n = 30) | Serum | MS | ↑SFA-PLs, eicosanoids, triglycerides, diglycerides, choline, GPE, inosine;<br>↓PUFA-PLs, LPLs, cholesteryl esters, free fatty acids, urea, serine, valine, threonine, pyroglutamate, creatine, phosphoethanolamine, histidine, carnitine, glucose, tyrosine, tryptophan | (Gonzalez-Dominguez *et al.*, 2015c) |

**Table 2.** (continued).

| Animal model | Sample | Platform | Findings | Ref. |
|---|---|---|---|---|
| APP × PS1(n = 30)<br>WT(n = 30) | Hippocampus, cortex, cerebellum, olfactory bulb | MS | ↑LPLs, PL(18:0/20:4), 22:5PLs, 22:4-PLs, propionyl-carnitine, free fatty acids, eicosanoids, uracil, hypoxanthine, xanthine, FAPy-adenine, adenosine, inosine, acetate, propionate, pyruvate, alanine, choline, valine, G3P, phosphocholine, N-acetyl-spermidine, GPC, GPI;<br>↓PUFA-PLs, acyl-carnitines, steroids, adenine, UMP, AMP, urea, aspartate, dopamine, tyrosine, homocarnosine | (Gonzalez-Dominguez *et al.*, 2015h) |
| APP × PS1(n = 30)<br>WT(n = 30) | Liver, kidney, spleen, thymus | MS | Alterations in the metabolism of phospholipids, fatty acids, acyl-carnitines, eicosanoids, acyl-glycerols, steroids, amino acids, nucleotides, energy-related metabolites(changes depending on the tissue) | (Gonzalez-Dominguez *et al.*, 2015e) |
| APP × PS1(n = 8–9)<br>WT(n = 8–9) | Brain, plasma | MS | Longitudinal perturbations(6–18 months) in metabolism of phospholipids, amino acids, polyamines and acyl-carnitines | (Pan *et al.*, 2016) |
| APP × PS1 × IL4 (n = 7)<br>APP × PS1(n = 7)<br>WT(n = 7) | Serum | MS | ↓Urea, histamine, threonine, aspartate, urocanic acid, dopamine, citrulline, tyrosine;<br>↑1-methyl-histamine, eicosanoids | (Gonzalez-Dominguez *et al.*, 2015g) |
| CRND8(n = 6)<br>WT(n = 6) | Hippocampus | MS | Alterations in the homeostasis of arachidonic acid, glucose metabolism and fatty acid β-oxidation | (Lin *et al.*, 2013) |
| CRND8(n = 6)<br>WT(n = 6) | Cerebellum | MS | ↑Eicosanoids, amino acids and derivatives, xanthosine, xanthine, urate;<br>↓inosine, guanosine | (Lin *et al.*, 2014) |
| SAMP8<br>n = 5 (2 months)<br>n = 6 (7 months)<br>n = 7 (12 months) | Hippocampus | GC-MS | ↑Cholesterol, oleic acid, phosphoglyceride, N-acetyl-aspartate;<br>↓alanine, serine, glycine, aspartate, glutamate, γ-aminobutyric acid, malic acid, butanedioic acid, fumaric acid, citric acid, pyroglutamic acid, urea, lactic acid | (Wang HL *et al.*, 2014) |
| TASTPM(n = 16)<br>WT(n = 5) | Whole brain | GC-MS | ↑D-fructose, valine, serine, threonine;<br>↑zymosterol | (Hu *et al.*, 2012) |
| TASTPM(n = 16)<br>WT(n = 5) | Plasma | GC-MS | ↑D-glucose, d-galactose, linoleica cid, arachidonic acid, palmitic acid, gluconic acid | (Hu *et al.*, 2012) |
| $APP_{Tg2576}$(n = 15)<br>WT(n = 17);<br>CRND8(n = 9)<br>WT(n = 9);<br>$APP_{V717I}$(n = 10)<br>WT(n = 12) | Urine | GC-MS | ↑Phenylacetone;<br>↓3-methylcyclopentanone, 4-methyl-6-hepten-3-one, 6-hydroxy-6-methyl-3-heptanone | (Kimball *et al.*, 2016b) |
| CRND8(n = 12)<br>WT(n = 12) | Urine | RP-MS | ↑Methionine, desaminotyrosine, phenylacetylglycine, dihydrouracil, ureidopropionic acid, thiocysteine;<br>↓choline, taurine, N1-acetyl-spermidine, 5-hydroxy-indoleacetic acid, hydroxyphenylglycine | (Peng *et al.*, 2014) |
| CRND8<br>(n = 18/12,12/18 weeks)<br>WT(n = 12/12, 12/18 weeks) | Urine | HILIC-MS | Fifty-five differential metabolites involved in the homeostasis of amino acids(*e.g.* tryptophan, tyrosine, phenylalanine, lysine), fatty acids, purines and pyrimidines, ascorbate, and others | (Tang *et al.*, 2016) |

**Table 2.** (continued).

| Animal model | Sample | Platform | Findings | Ref. |
|---|---|---|---|---|
| APP × PS1(n = 30)<br>WT(n = 30) | Serum | RP-MS/<br>GC-MS | Alterations in the metabolism of phospholipids, sphingolipids, fatty acids, cholesterol and bile acids, energy-related metabolites and amino acids | (Gonzalez-Dominguez *et al.*, 2015d) |
| APP × PS1(n = 30)<br>WT(n = 30) | Hippocampus, cortex, striatum, cerebellum, olfactory bulb | RP-MS/<br>GC-MS | Alterations in the metabolism of phospholipids, sphingolipids, energy-related metabolites, amino acids and nucleotides | (Gonzalez-Dominguez *et al.*, 2014d) |
| APP × PS1(n = 30)<br>WT(n = 30) | Liver, kidney | RP-MS/<br>GC-MS | Alterations in the metabolism of phospholipids, sphingolipids, fatty acids, acyl-carnitines, cholesterol and bile acids, energy-related metabolites and amino acids | (Gonzalez-Dominguez *et al.*, 2015f) |
| APP × PS1(n = 30)<br>WT(n = 30) | Spleen, thymus | RP-MS/<br>GC-MS | Alterations in the metabolism of phospholipids, sphingolipids, fatty acids, acyl-carnitines, energy-related metabolites, amino acids and purines | (Gonzalez-Dominguez *et al.*, 2015i) |
| $APP_{Tg2576}$(n = 3)<br>PS1(n = 3)<br>APP × PS1(n = 6)<br>WT(n = 6) | Hippocampus | RP-MS/<br>GC-MS | Alterations in nucleotide, TCA cycle, energy transfer, carbohydrate, neurotransmitter and amino acid metabolic pathways | (Trushina *et al.*, 2012) |

### 5.2 Metabolomics in AD patients

While animal models represent at best familial AD, not sporadic forms such as LOAD which have multifactorial origins, human studies can also be challenging because of multifactorial issues, such as disease severity and comorbidities, together with the difficulty of organizing big cohorts to correct appropriately for this variation.

#### 5.2.1 Brain

The earliest metabolomics studies on AD focused on post-mortem brain samples, utilizing MRS as an analytical platform. *In vitro* analyses were performed in order to quantify the content of some important neurochemicals in different brain regions. These studies commonly identified reduced levels of N-acetyl-asparate, glutamate and glutamine, together with increased myo-inositol content (Klunk *et al.*, 1992; Miller *et al.*, 1993; Mohanakrishnan *et al.*, 1995; Shonk *et al.*, 1995; Mohanakrishnan *et al.*, 1997). Shonk *et al.*, (1995) evaluated these metabolites as putative neuronal markers, and found that the ratio of myo-inositol and N-acetyl-aspartate was able to distinguish AD from normality with 83% sensitivity and 98% specificity. It is interesting to note that these changes were also identified in the brains of adult AD-like mouse in the presence of amyloid burden (Dedeoglu *et al.*, 2004; Marjanska *et al.*, 2005; von Kienlin *et al.*, 2005; Salek *et al.*, 2010) as well as in the brains of young APPxPS1 Tg mouse in the absence of amyloid plaques (Wengenack *et al.*, 2000; Marjanska *et al.*, 2005).

More recently, non-targeted approaches utilizing NMR technique have been employed. Graham *et al.*, (2014) were able to identify increased alanine and taurine levels in the neocortex region of AD brains; ratio of these two metabolites was found to discriminate AD from controls with Receiver Operation Characteristic (ROC) area under curve (AUC) value of 76%. Further when cortical profiles between AD and amyotrophic lateral sclerosis (ALS) were compared using the same methodology, alanine, glutamate and glutamine levels were found to be increased in AD, highlighting these abnormalities to be AD specific (Botosoa *et al.*, 2012).

Numerous studies have applied DIMS due to its high-throughput capability. In 2001, Han *et al.*, (2001) reported a decrease in plasmalogen content in cerebellar white matter at a very mild stage of ADs, a trend which was also later seen in AD grey matter (Wood *et al.*, 2015). In the following year, Han *et al.*, (2002) attempted to reproduce the finding of decreased plasmalogen levels in AD brain, but was unable to observe the change at a significant level. Instead the group observed depletion in sulfatide levels in white matter and grey matter regions (Han *et al.*, 2002), a finding that was later seen in a mouse model study (Cheng *et al.*, 2010). Sulfatide is known to mediate diverse biological processes including myelination, signal transduction, neuronal plasticity and cell morphogenesis (Vos *et al.*, 1994; Merrill *et al.*, 1997), suggesting sulfatide loss may lead to neuronal dysfunction and AD pathogenesis (Han *et al.*, 2002). Interestingly, depletion of brain sulfatide level was also observed in pre-clinical AD (Cheng *et al.*, 2010) and mild AD (Wood *et al.*, 2015), suggesting that sulfatide deficiency is possibly among the early events of AD development.

More hydrophilic brain metabolites have also been found to be associated to AD pathology. Recently, Xu *et al.*, (2016) employed GC-MS to compare profiles of 9 AD brains to 9 control brains, in 9 different brain regions. The group detected 55 metabolites differentiating the two cohorts; these 55 metabolites were mainly amino acid and nucleoside, highlighting presence of widespread metabolic perturbations in AD brain (Xu *et al.*, 2016). Utilizing the same analytical technique on 43 brain samples, Snowden *et al.*, (2017) showed

dysregulation in unsaturated fatty acid metabolism in the brains for patients with varying degrees of AD pathology.

LC-MS has also been employed to compare metabolic profiles of AD brains to those of control brains, a recent study has found a significant increases in the levels of spermine and spermidine were identified in AD brains, suggesting involvement of an abnormal brain expression of the polyamine pathway in AD pathogenesis (Inoue *et al.*, 2013). More recently, a targeted metabolomics approach has been utilized where glucose level was measured from 14 control, 14 AD and 15 asymptomatic AD brains (An *et al.*, 2017). The study showed higher brain glucose levels associating with AD pathology and the expression of AD symptoms, further highlighting glucose hypometaolism in AD brain (An *et al.*, 2017).

### 5.2.2 CSF

CSF is an ideal matrix because of its direct contact with the extracellular space in the brain, thus directly reflecting the brain's metabolic production. Kaddurah-Daouk *et al.*, (2011) examined levels of metabolites involved in key neurotransmitter pathways and oxidative stress using 30 post-mortem ventricular CSF samples (15 AD *vs* 15 CN). For this purpose, a metabolic platform based on LCECA with pentane sulfonic acid as ion-paring agent was employed. The study observed depletion of norephinephrine, methionine, α-tocopherol and 3-methoxytyramine levels and increased levels of 5-hydroxytryptophan in AD (Kaddurah-Daouk *et al.*, 2011). However, it is important to note that this study utilized post-mortem CSF samples (Kaddurah-Daouk *et al.*, 2011). Along with brain samples discussed in the earlier section, post-mortem samples generally imply the severe-phase of AD at the time of collection. Additionally, metabolite levels in post-mortem tissue/biofluid samples may be impacted by the death process (as well as post-mortem intervals) (Trushina and Mielke, 2014).

NMR-based metabolomics to CSF samples have demonstrated great utility in differentiating AD from CN (Jukarainen *et al.*, 2008; Kork *et al.*, 2009; Laakso *et al.*, 2015). One of these studies quantified 31 metabolites from 76 AD *vs* 45CN CSF samples and found that these metabolites were able to classify the samples correctly with an accuracy of 85.5% (Laakso *et al.*, 2015). LCECA has also been employed; Kaddurah-Daouk *et al.*, (2013) looked into metabolic profiles of 50 AD, 36 MCI and 15CN CSF samples and was able to reveal elevated levels of methionine and 5-hydroxyindoleacetic acid in AD and MCI compared to CN samples. These metabolites were also found to be related to CSF $A\beta_{42}$, indicating the perturbations in pathways involving methionine and 5-hydroxyindoleacetic acid could be associated with amyloidgenic pathway (Kaddurah-Daouk *et al.*, 2013).

Multi-platforms based on the combination of complementary chromatographic science prior to MS detection have also been employed (Czech *et al.*, 2012; Ibanez *et al.*, 2013; Trushina *et al.*, 2013a). Utilizing RP and HILIC, Trushina *et al.*, (2013a) analysed 15 AD, 15 MCI and 15CN CSF samples, and found approximately 30% of the metabolic pathways altered in MCI compared to CN, and 60% in AD compared to CN. This finding showed that the number of affected pathways increases with disease severity.

Another multiplatform available for CSF metabolic profiling is the combination of GC-MS and LC-MS methodology. Using this technique, Czech *et al.*, (2002) were able to establish differences in catercholamines and steroids in the CSF samples from 51CN, 53 mild AD and 26 moderate AD participants. The group also found increased cortisol levels associating with the AD progression, while combination of cysteine and various amino acids provided predictive models with sensitivity and specificity above 80% (Czech *et al.*, 2012).

A recent study employed CE-MS to monitor perturbations in the polar metabolome associated with AD progression by analysing CSF from subjects with different cognitive status, AD, MCI that progressed to AD within 2 years, MCI that remained stable after 2 years, and subjective cognitive impairment (Ibanez *et al.*, 2012). Initially, ten metabolites including choline, valine, arginine, suberylglycerin, carnitine, creatine, serine and histidine were identified from 73 samples with classification accuracy of 90.1% for the four groups. The group then tested the classification model into 12 test samples, initially blinded to diagnosis, and 83% of the samples were correctly classified, indicating these polar CSF metabolites could potentially be utilized as a diagnostic tool (Ibanez *et al.*, 2012). The same group employed the combination of orthogonal RP-MS and HILIC-MS on the same sample cohorts (Ibanez *et al.*, 2013). From statistical modelling, 17 metabolites were identified showing strong association with cognitive status related to AD, and classification model based on these 17 metabolites was able to predict the development of AD with an accuracy of 98.7%, and sensitivity and specificity values above 95%. The model included altered level of histidine (Ibanez *et al.*, 2013), which was also present their previous work (Ibanez *et al.*, 2012).

### 5.2.3 Blood

Blood biomarkers would be ideal for their implementation in the clinical practice. CSF metabolites may be more reflective of brain changes but CSF collection is invasive, making it unsuitable for screening purposes. As a result, it is no surprise that most of the metabolic profiling studies have focused on blood-based biomarkers.

González-Domínguez *et al.*, (2014b, 2014c, 2014e, 2015a) studied serum lipid profiles by utilizing various lipid extraction and DI-MS, each technique focusing on different classes of blood metabolites. The group has been able to detect abnormal overproduction of several molecular species:

- glycerides indicating membrane breakdown;
- prostaglandins, imidazole and histidine indicating oxidative stress;
- glutamine, glutamate and dopamine indicating impaired neurotransmission systems.

Other groups also employed DIMS for profiling of blood metabolites and found altered levels of long-chain sphingomyelines and ceramides (Han X *et al.*, 2011), plasmalogen and diglycerdies (Wood *et al.*, 2016), and phospholipids (Klavins *et al.*, 2015).

Focusing on lipid profiles, Sato *et al.*, (2012) was able to find a considerable reduction in circulating demosterol levels in AD plasma, both in untargeted and targeted analyses. Other plasma lipids classes reported to have close relationships with AD included bile acids (Greenberg *et al.*, 2009), LysoPCs and

**Table 3.** Summary of metabolomics studies conducted in AD human samples.

| Sample Size | Sample Source | Platform | Findings | Ref. |
|---|---|---|---|---|
| AD(n = 22)<br>CN(n = 18) | Serum | DIMS | ↓PUFA-PCs;<br>↑SFA-PCs | (Gonzalez-Dominguez *et al.*, 2012) |
| AD(n = 22)<br>CN(n = 18) | Serum | DIMS | ↓PUFA-PLs, valine, glutamine, N-acetyl-glutamine, glutamate, histidine, arginine, dopamine, carnitine, creatine;<br>↑SFA-PLs, free fatty acids, eicosanoids, triglycerides, choline, glycerophosphocholine, glucose | (Gonzalez-Dominguez *et al.*, 2014e) |
| AD(n = 22)<br>CN(n = 18) | Serum | DIMS | ↓PUFA-PLs, oleamide, histidine, arginine, imidazole, taurine, guanidine, putrescine;<br>↑eicosanoids, diglycerides, kynurenine | (Gonzalez-Dominguez *et al.*, 2014c) |
| AD(n = 19)<br>CN(n = 17) | Serum | DIMS | ↓PUFA-PLs, LPLs, plasmalogens;<br>↑SFA-PLs | (Gonzalez-Dominguez *et al.*, 2014b) |
| AD(n = 30)<br>CN(n = 30) | Serum | DIMS | ↓Fatty acid amides, urea, creatine, malate, taurine, dopamine, serotonin;<br>↑ceramides, diglycerides, free fatty acids, alanine, picolinic acid | (Gonzalez-Dominguez *et al.*, 2015a) |
| AD(n = 26)<br>CN(n = 26) | Plasma | DIMS | ↓Long-chain sphingomyelins;<br>↑ceramides | (Han XL *et al.*, 2011) |
| AD(n = 90)<br>MCI(n = 77)<br>CN(n = 51) | Serum | DIMS | ↓Plasmalogens;<br>↑diglycerides | (Wood *et al.*, 2016) |
| AD(n = 43)<br>MCI(n = 33)<br>CN(n = 35) | Plasma | DIMS | ↓Phosphatidylcholines;<br>↑lyso-phosphatidylcholines | (Klavins *et al.*, 2015) |
| AD(n = 23)<br>CN(n = 21) | Serum | GC-MS | ↓Valine, urea, aspartate, pyroglutamate, glutamine, phenylalanine, asparagine, ornithine, pipecolate, histidine, tyrosine, palmitic acid, urate, tryptophan, stearic acid, cysteine;<br>↑lactate, α-ketoglutarate, isocitrate, glucose, oleic acid, adenosine, cholesterol | (Gonzalez-Dominguez *et al.*, 2015b) |
| AD(n = 37<br>CN(n = 46) | Serum | GC-MS | ↑Glutamate | (Orešič *et al.*, 2018) |
| AD(n = 10)<br>MCI(n = 10)<br>CN(n = 10) | Plasma | RP-MS | ↓Lysophospholipid (18:1) | (Sato *et al.*, 2010) |
| AD(n = 10)<br>CN(n = 10) | Plasma | RP-MS | ↓Demosterol | (Sato *et al.*, 2012) |
| AD(n = 16)<br>MCI(n = 12)<br>CN(n = 10) | Plasma | RP-MS | ↑1-(9E-hexadecenoyl)-sn-glycero-3-phosphocholine, d-glucosaminide, glycocholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid | (Greenberg *et al.*, 2009) |
| AD(n = 20)<br>CN(n = 20) | Plasma | RP-MS | ↓Lysophosphatidylcholines, tryptophan, phytosphingosine, dihydrosphingosine, hexadecasphinganine | (Li *et al.*, 2010a) |
| AD(n = 36)<br>MCI(n = 48)<br>CN(n = 40) | Plasma | RP-MS | ↓Long chain cholesteryl esters | (Proitsi *et al.*, 2015) |
| AD(n = 205)<br>CN(n = 207) | Plasma | RP-MS | ↓Phosphatidylcholines<br>↑Ceramide | (Kim *et al.*, 2016) |
| AD(n = 52)<br>MCI(n = 62)<br>CN(n = 59) | Plasma | RP-MS | ↓Phosphatidylcholines | (Whiley *et al.*, 2014) |

**Table 3.** (continued).

| Sample Size | Sample Source | Platform | Findings | Ref. |
|---|---|---|---|---|
| AD/MCI (n = 35) Converters (n = 18) CN(n = 53) | Plasma | RP-MS | ↓Phosphatidylcholines, lyso-phospholipids, serotonin, phenylalanine, proline, lysine, taurine, acyl-carnitines, malate; ↑glycoursodeoxycholic acid, proline-asparagine | (Mapstone *et al.*, 2014) |
| AD(n = 75) MCI(n = 17) CN(n = 45) | Serum | RP-MS | ↓PUFA-PLs, PUFA-SMs, sulfatides, monoglycerides, oleamide, histidine pregnenolone sulfate; ↑SFA-PLs, SFA-SMs, ceramides, acyl-carnitines, phenyl-acetyl-glutamine | (Raúl *et al.*, 2016) |
| AD(n = 148) CN(n = 152) | Plasma | RP-MS | ↓Cholesteryl esters, triglycerides; ↑phosphatidylcholine | (Proitsi *et al.*, 2017) |
| AD(n = 175) MCI(n = 356) CN(n = 189) | Serum | RP-MS | ↓Acyl-carnitines, phosphatidylcholines, sphingomyelins; ↑valine, α-aminoadipic acid | (Toledo *et al.*, 2017) |
| AD(n = 660) MCI(n = 583) | Serum | RP-MS | ↑Sphinganine-1-phosphate, 7-ketocholesterol, 3-methoxytyrosine, deoxyribose-5-phosphate, p-phenyllactic acid, lyso-phosphatidylcholine; ↓phenylalanine, ornithine, glutamate | (Liang *et al.*, 2016b) |
| AD(n = 46) CN(n = 37) | Serum | RP-MS | ↓1-methylinosine, 16-bromo-9-hexadecenoic acid, lysophosphatidylcholines, dihydrosphingosine, N-acetyl-glutamine, 1α, 25-dihydroxy-2α-(3-hydroxypropoxy) vitamin D3; ↑palmitic amide, monoiodothyronine, antrimethyltridecanoic acid, PGE2α dimethyl amine, (6R)-vitamin D3 6, 19-(4-phenyl-1, 2, 4-triazoline-3, 5-dione) | (Cui *et al.*, 2014) |
| AD_MCI (n = 19) MCI(n = 16) CN(n = 37) | Plasma | HILIC-MS | ↓4-amino-butanal, γ-aminobutyric acid, ornithine, N-acetyl-putrescine; ↑creatine, arginine, methylthioadenosine, N-acetyl-spermidine, N-diacetyl-spermine, putrescine, spermidine, spermine | (Graham *et al.*, 2015) |
| AD(n = 42) MCI(n = 14) CN(n = 37) | Serum | CE-MS | ↓Creatine, asparagine, methionine, histidine, carnitine, N-acetyl-spermidine, valeryl-carnitine; ↑choline, creatinine, dimethylarginine, homocysteinyl-cysteine, acyl-carnitines, peptides | (Gonzalez-Dominguez *et al.*, 2014a) |
| AD(n = 47) MCI(n = 143) CN(n = 46) | Serum | RP-MS/ GC-MS | Baseline: ↓plasmalogens, phosphatidylcholines, sphingomyelins, sterols; ↑histamine Progression: ↑2, 4-dihydroxybutanoic acid | (Orešič *et al.*, 2011) |
| AD(n = 57) MCI(n = 58) CN(n = 57) | Plasma | RP-MS/ GC-MS | ↓Fatty acids, dimethylglycine, glutamate, uridine, glyceraldehyde, butanedioic acid, 2-butenedioic acid, 5-oxoproline, taurine, hypotaurine, malate; ↑glutamine, 2-aminoadipic acid, proline, cysteine, cytidine, thymine, hypoxanthine, carbohydrates, citrate | (Wang G *et al.*, 2014) |
| AD(n = 30) MCI(n = 30) CN(n = 30) | Plasma | RP-MS/ HILIC-MS | ↓Sphingomyelins | (Armirotti *et al.*, 2014) |

**Table 3.** (continued).

| Sample Size | Sample Source | Platform | Findings | Ref. |
|---|---|---|---|---|
| AD(n = 15)<br>MCI(n = 15)<br>CN(n = 15) | Plasma | RP-MS/<br>HILIC-MS | Changes in more than 150 metabolites (amino acids, energy-related metabolites, lipids, neurotransmitters) | (Trushina *et al.*, 2013b) |
| AD(n = 3)<br>FTD(n = 4)<br>LBD(n = 3)<br>CN(n = 9) | Serum | CE-MS | ↑β-alanine, creatinine, hydroxyproline, glutamine, isocitrate, cytidine | (Tsuruoka *et al.*, 2013) |
| AD(n = 12)<br>CN(n = 5) | Whole brain | MRS | ↓N-acetyl-aspartate, γ-aminobutyric acid; ↑ glutamate | (Klunk *et al.*, 1992) |
| AD(n = 13)<br>CN(n = 4) | Temporoparietal cortex | MRS | ↓N-acetyl-aspartate, creatine, γ-aminobutyric acid | (Mohanakrishnan *et al.*, 1995) |
| AD(n = 13)<br>CN(n = 4) | Hippocampus, cerebellum | MRS | ↓N-acetyl-aspartate, γ-aminobutyric acid | (Mohanakrishnan *et al.*, 1997) |
| AD(n = 8)<br>ALS(n = 11) | Frontal cortex | NMR | ↑Alanine, acetate, glutamate, glutamine; ↓lactate, creatine | (Botosoa *et al.*, 2012) |
| AD(n = 15)<br>CN(n = 15) | Neocortex | NMR | ↑Alanine, taurine | (Graham *et al.*, 2014) |
| AD(n = 24)<br>CN(n = 6) | Superior frontal cortex, superior temporal cortex, inferior parietal cortex, cerebellum | DIMS | ↓Plasmalogens | (Han *et al.*, 2001) |
| AD(n = 17)<br>CN(n = 5) | Middle frontal gyrus, superior temporal gyrus, inferior parietal lobule, hippocampus, subiculum, entorhinal cortex | DIMS | ↓Sulfatides;<br>↑ceramides | (Han *et al.*, 2002) |
| AD(n = 6)<br>CN(n = 8) | Superior frontal gyrus | DIMS | ↓Sulfatides | (Cheng *et al.*, 2013) |
| AD(n = 34)<br>MCI(n = 19)<br>CN(n = 28) | Frontal cortex grey matter, frontal cortex white matter | DIMS | ↓Plasmalogens, phosphatidylethanolamines, docosahexaenoic acid, phosphatidylethanolamines, sulfatides; ↑monoglycerides, diglycerides ; ↑monoglycerides, diglycerides, VLCFA 26:0 | (Wood *et al.*, 2015) |
| AD(n = 9)<br>CN(n = 9) | Hippocampus, entorhinal cortex, middle-temporal gyrus, sensory cortex, motor cortex, cingulate gyrus, cerebellum | GC-MS | Changes in 55 metabolites, including energy related metabolites (glucose metabolism, TCA), amino acids, nucleosides, and others | (Xu *et al.*, 2016) |
| AD(n = 10)<br>CN(n = 10) | Frontal lobe, parietal lobe, occipital lobe | RP-MS | ↑Spermidine, spermine, N-acetyl-spermidine, N-acetyl-spermine, putrescine | (Inoue *et al.*, 2013) |
| ADI–II(n = 7)<br>ADIII–IV(n = 4)<br>ADV–VI(n = 5)<br>CN(n = 4) | Entorhinal cortex | RP-MS | ↓dGMP, glycine, xanthosine, inosine diphosphate, deoxyguanosine; ↑guanine | (Ansoleaga *et al.*, 2015) |
| AD(n = 15)<br>CN(n = 15) | Neocortex | HILIC-MS | Seventy-six unidentified discriminant signals | (Graham *et al.*, 2013a) |
| AD(n = 21)<br>CN(n = 19) | Frontal cortex | RP-MS/<br>HILIC-MS | Alterations in the metabolism of phospholipids and six metabolic pathways of the central metabolism: Alanine, Aspartate, and Glutamate Metabolism; Arginine and Proline Metabolism; Cysteine and Methionine Metabolism; Glycine, Serine, and Threonine Metabolism; Purine Metabolism; Pantothenate and CoA Biosynthesis | (Paglia *et al.*, 2016) |
| AD(n = 14)<br>asyAD(n = 15)<br>CN(n = 14) | Middle frontal gyrus<br>Inferior temporal gyrus<br>Cerebellum | RP-MS | ↓Glucose | (An *et al.*, 2017) |

**Table 3.** (continued).

| Sample Size | Sample Source | Platform | Findings | Ref. |
|---|---|---|---|---|
| AD(n = 14)<br>asyAD(n = 15)<br>CN(n = 14) | Middle frontal gyrus<br>Inferior temporal Gyrus<br>Cerebellum | GC-MS | ↑Cholestenol, docosahexaenoic acid, carbamic acid, hexanedioic acid, ascorbate, gluconic acid, cysteine, L-DOPA, allantoin, coumaric acid, adenine, deoxyflurouridine, arginine, GABA;<br>↓cholesterol, linoleic acid, methyl-heptadecadiynoic acid, oleic acid, palmitic acid, dimethylglycine, guanidobutanoate, aminobutanal, aspartate, fumaric acid, linolenic acid, indoleacetic acid, eicosapentaenoic acid, hypoxanthine, oxoarginine, methyl-stearate, octadecanal | (Snowden *et al.*, 2017) |
| AD(n = 15)<br>CN(n = 15) | Post-mortem CSF | LCECA | ↓Norepinephrine, α-tocopherols, 3-methoxytyramine, ascorbate;<br>↑5-hydroxytryptophan, methoxy-hydroxyphenyl glycol | (Kaddurah-Daouk *et al.*, 2011) |
| AD(n = 34)<br>MCI(n = 19)<br>CN(n = 28) | CSF | MS | CSF: ↓docosahexaenoic | (Wood *et al.*, 2015) |
| AD(n = 20)<br>CN(n = 27) | CSF | NMR | Specific resonances in AD (unidentified) | (Kork *et al.*, 2009) |
| AD(n = 10)<br>CN(n = 34) | CSF | NMR | ↑Creatinine | (Jukarainen *et al.*, 2008) |
| AD(n = 76)<br>early_AD (n = 26)<br>MCI(n = 33)<br>CN(n = 45) | CSF | NMR | Phenylalanine, glutamate, lactate, acetate, α-hydroxyisovalerate, α-hydroxybutyrate | (Laakso *et al.*, 2015) |
| AD(n = 50)<br>CN(n = 50) | CSF | DIMS | ↑Sphingomyelins, phosphatidylcholines | (Koal *et al.*, 2015) |
| AD(n = 17)<br>CN(n = 17) | CSF | RP-MS | Fifty-three unidentified discriminant signals | (Myint *et al.*, 2009) |
| AD(n = 40)<br>MCI(n = 36)<br>CN(n = 38) | CSF | RP-MS | ↑Methionine, 5-hydroxyindoleacetic acid, vanillylmandelic acid, xanthosine, glutathione, hypoxanthine | (Kaddurah-Daouk *et al.*, 2013) |
| AD(n = 23)<br>AD_MCI(n = 9)<br>sMCI(n = 22)<br>CN(n = 19) | CSF | CE-MS | ↓Arginine, suberylglycine, carnitine, histidine;<br>↑choline, valine, tripeptide, dimethylarginine, creatine, serine | (Ibanez *et al.*, 2012) |
| AD(n = 79)<br>CN(n = 51) | CSF | RP-MS/<br>GC-MS | ↓Uridine;<br>↑cysteine, tyrosine, phenylalanine, methionine, serine, pyruvate, taurine, creatinine, cortisol, dopamine | (Czech *et al.*, 2012) |
| AD(n = 40)<br>CN(n = 38) | CSF | RP-MS/<br>GC-MS | Two unidentified discriminant signals | (Motsinger-Reif *et al.*, 2013) |
| AD(n = 21),<br>cMCI(n = 12)<br>sMCI(n = 21)<br>CN(n = 21) | CSF | RP-MS/<br>HILIC-MS | Changes in levels of uracil, xanthine, uridine, tyrosyl-serine, methylsalsolinol, nonanoylglycine, dopamine-quinone, caproic acid, vanylglycol, histidine, pipecolic acid, hydroxyphophinyl-piruvate, creatinine, taurine, C16-sphingosine-1-phosphate, tryptophan, 5′-methylthioadenosine | (Ibanez *et al.*, 2013) |

**Table 3.** (continued).

| Sample Size | Sample Source | Platform | Findings | Ref. |
|---|---|---|---|---|
| AD(n = 15)<br>MCI(n = 15)<br>CN(n = 15) | CSF | RP-MS/<br>HILIC-MS | Changes in more than 150 metabolites (amino acids, energy-related metabolites, lipids, neurotransmitters) | (Trushina *et al.*, 2013a) |
| AD(n = 256)<br>CN(n = 218) | Saliva | RP-MS | ↓Inosine, 3-dehydrocarnitine, hypoxanthine;<br>↑sphinganine-1-phosphate, ornithine, p-phenyllactic acid | (Liang *et al.*, 2015) |
| AD(n = 660)<br>MCI(n = 583) | Saliva | RP-MS | ↓Inosine, 3-dehydrocarnitine, hypoxanthine;<br>↑cytidine, sphinganine-1-phosphate, ornithine, p-phenyllactic acid, pyroglutamate, glutamate, tryptophan | (Liang *et al.*, 2016a) |
| MCI(n = 20)<br>CN(n = 20) | Saliva | RP-MS | ↓Taurine, peptides, 1, 8-diazacyclotetradecane-2, 9-dione, 4-(hydroxylamino)-N, N-dimethylaniline;<br>↑2-amino-heptanoic acid/l-alanine-n-butyl ester/N-methyl-isoleucine | (Zheng *et al.*, 2012) |
| AD(n = 3)<br>FTD(n = 4)<br>LBD(n = 3)<br>CN(n = 9) | Saliva | CE-MS | ↓Arginine, tyrosine | (Tsuruoka *et al.*, 2013) |
| AD(n = 46)<br>CN(n = 37) | Urine | RP-MS | ↓Acetyl-carnitine, estra-1, 3, 5(10), 7-tetraene-3, 17α-diol, p-cresol glucuronide, etiocholanolone glucuronide ;<br>↑N-acryloylglycine, argininosuccinic acid, dethiobiotin, isobutyryl-l-carnitine, 2-hydroxy-N-(2-hydroxyethyl)-N, N-dimethyl-1-dodecanaminium, l-aspartyl-4-phosphate, l-glutamine, 5-l-glutamylglycine, azelaic acid, aminopterin, cytidine | (Cui *et al.*, 2014) |
| AD(n = 31)<br>MCI(n = 15)<br>CN(n = 36) | Hair | GC-MS | ↑7β-hydroxycholesterol | (Son *et al.*, 2016) |

phytosphingosine (sphingolipid precursors) (Li *et al.*, 2010b), among others (Mapstone *et al.*, 2014). Utilizing *in-vial* dual extraction protocol (Whiley *et al.*, 2012; Godzien *et al.*, 2013), abnormal levels of long chain cholesteryl esters/triglycerides (Proitsi *et al.*, 2015; Proitsi *et al.*, 2017) and omega-3 containing PC molecules (Whiley *et al.*, 2014; Kim *et al.*, 2016) have been shown in two separate study cohorts. Additionally, increased plasma levels of phosphorylethanolamine (PE) and PC species have been found to associate with brain amyloid burden (Voyle *et al.*, 2016).

Focusing on more hydrophilic blood metabolites, a study involving 75 AD, 17 MCI and 45CN serum samples revealed accumulation of acylcarnitine in relation to mitochondrial dysfunction, and lower levels of oleamide and monoglycerides caused by impaired endocannabionoid system (Gonzalez-Dominguez *et al.*, 2016). A study involving much bigger cohorts of 660 AD and 583 MCI serum samples found increased levels of sphinganine-1-phosphate and 7-ketocheolsterol; the combination of these metabolites predicting AD conversion with accuracy value of 95% (Liang *et al.*, 2016b). These two metabolites have also been found to be strong AD discriminators from MCI and CN in saliva samples (Liang *et al.*, 2015, 2016a).

HILIC-MS has shown disturbance in 22 biochemical pathways, including homeostasis of polyamines and arginine metabolism (Graham *et al.*, 2015), while CE-MS revealed the presence of oxidative stress and defects in energy metabolism (Tsuruoka *et al.*, 2013; Gonzalez-Dominguez *et al.*, 2014a). Utilizing a RP-MS/HILIC-MS multi-platform, Trushina *et al.*, (2013b) identified 154 metabolites with significant changes in AD *vs* CN subjects (Trushina *et al.*, 2013b) while Armirotti *et al.*, (2014) has employed RP-MS/HILIC-MS multi-platform to find abnormally high levels of sphingomyelins in AD blood. Wang G *et al.*, (2014) looked at profiles of 57 AD, 58 MCI and 57CN plasma samples in 2014 and identified a panel of 6 metabolites discriminating AD from CN with ROC AUC of 1, and a panel of 5 metabolites discriminating MCI from CN with ROC AUC of 0.998. In a prospective study with aim to determine serum metabolomic profiles associating with progression and diagnosis of

AD, Orešič *et al.*, (2011) has utilized GC-MS/RP-MS to compare blood metabolic profiles of stable MCIs and converting MCIs. A panel of 3 metabolites (lower levels of one PC, one carboxylic acid and 2-4-dihydroxybutanoic acid molecules) was identified to be associated with progressive MCI; its AD conversion predictive value (ROC AUC) was 0.77 (Orešič *et al.*, 2011).

Most recently, Toledo *et al.* employed a targeted approach and quantified 187 serum metabolites from 199CN, 356 MCI and 175 AD participants. (Toledo *et al.*, 2017). The study was able to reveal abnormal changes in sphingomyleins and PC levels related to early stages of AD, as well as abnormal changes in acylcarnitines and several amines levels related to later symptomatic stages of AD. The group hypothesized that dysregulation in lipid metabolism during early stages of AD leads to breakdown in lipid membranes which consequently results in disruption in energy metabolism seen during later stages of AD. In another study where targeted approach was employed, a comparison between 38 AD and 46 control serum metabolic profiles showed an increase glutamate levels in AD (Orešič *et al.*, 2018).

### 5.2.4 Saliva, urine and hair

Saliva, urine and hair metabolites have been studied due to their ease-to-collect. Tsuruoka *et al.*, (2013) analysed saliva metabolic profiles to complement serum metabolic profiles by employing CE-MS. Abnormal levels of arginine and tyrosine in AD saliva samples were found when compared to CN samples as well as FTD and LBD samples, highlighting the abnormal levels of arginine and tyrosine as specific to AD (Tsuruoka *et al.*, 2013). In a study involving urine samples, Cui *et al.*, (2014) employed RP-MS and discovered impaired metabolic pathways involving L-glutamine and 5-L-glutamylglycine in AD. The study then utilized these two metabolites to test their AD predictive abilities, and the corresponding ROC AUC values were 0.709 and 0.700 respectively (Cui *et al.*, 2014).

In more recent years, sterol profiles of hair have been exploited as a possible source of AD markers due to hair being able to reflect biochemical stress at the local or at systemic levels (Slominski and Wortsman, 2000; Slominski *et al.*, 2012). Son *et al.*, (2016) analysed 31 AD, 15 MCI and 36CN hair samples by employing GC-MS and discovered an increased amount of 7β-hydroxycholesterol as a possible AD predictive biomarker. Ratio of 7β-hydroxycholesterol to cholesterol was found to be able to discriminate MCI *vs* CN and AD *vs* CN with ROC AUC values of 0.750 and 0.729 respectively (Son *et al.*, 2016).

Summary of metabolomics studies conducted on samples from AD participants can be seen in Table 3.

## 6 Conclusion

Overall, an extensive number of studies have applied metabolomics in order to identify:

- perturbations in metabolic pathways that reflect changes associated with AD pathology;
- a panel of metabolites which would potentially be used as an AD diagnostic tool.

Taking together the findings, it seems clear that multiple metabolic alterations could be occurring during progression to AD. These include lipids, metabolites involved in neurotransmitter systems and energy metabolism.

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