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# Alzheimer's disease–like pathology has transient effects on the brain and blood metabolome

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ABSTRACT

The pathogenesis of Alzheimer's disease (AD) is complex involving multiple contributing factors. The extent to which AD pathology affects the metabolome is still not understood nor is it known how disturbances change as the disease progresses. For the first time, we have profiled longitudinally (6, 8, 10, 12, and 18 months) both the brain and plasma metabolome of APPswe/PS1deltaE9 double transgenic and wild-type mice. A total of 187 metabolites were quantified using a targeted metabolomic methodology. Multivariate statistical analysis produced models that distinguished APPswe/PS1deltaE9 from wild-type mice at 8, 10, and 12 months. Metabolic pathway analysis found perturbed polyamine metabolism in both brain and blood plasma. There were other disturbances in essential amino acids, branched-chain amino acids, and also in the neurotransmitter serotonin. Pronounced imbalances in phospholipid and acylcarnitine homeostasis were evident in 2 age groups. AD-like pathology, therefore, affects greatly on both the brain and blood metabolomes, although there appears to be a clear temporal sequence whereby changes to brain metabolites precede those in blood.



## 1. Introduction

Dementia mainly affects the elderly, with the prevalence doubling every 5 years over the age of 65 (Prince et al., 2014). Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder and the most common form of dementia, accounting for 60%–80% of all dementia cases (Prince et al., 2014). AD is clinically characterized by progressive memory loss, mood changes, problems with communication and reasoning, and eventual loss of independent living. Familial AD, often associated with an earlier onset (<65 years of age), is an autosomal dominant form of AD caused by mutations in the genes encoding amyloid precursor protein (APP) and presenilins 1 and 2 (PS1 and PS2) leading to the subsequent accumulation of amyloid β (Aβ) (Borchelt et al., 1997; Jankowsky et al., 2004; Selkoe, 2001; Selkoe and Schenk, 2003). AD is characterized by the pathologic accumulation of extracellular Aβ and abnormally phosphorylated tau filaments in neurons that lead to senile plaques and neurofibrillary tangles, respectively (Blennow et al., 2006; Selkoe, 2004; Skovronsky et al., 2006). Transgenic mouse models containing mutations in the human APP and/or PS1 genes are widely used in experimental studies to investigate the pathophysiological role of Aβ in early-onset AD patients. The APPswe/PS1deltaE9 (APP/PS1) strain is one such example that has been extensively characterized and utilized. These mice develop Aβ plaques at 5–6 months of age, although production of Aβ has been shown to occur as early as 3 months in the form of both Aβ(1–40) and (1–42) (Volianskis et al., 2010). APP/PS1 mice display progressive age-related impairments in memory that appear as early as 7 months of age (Volianskis et al., 2010; Xiong et al., 2011). In behavioral tests, the mice show deficits in measuring spatial navigation and reference learning (Xiong et al., 2011). Although APP/PS1 mice do not model all facets of human AD, they do enable longitudinal investigations not normally possible in people in a clinical environment.

Metabolomics is the scientific investigation of chemical processes involving metabolites. Metabolomic techniques can

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comprehensively and simultaneously help to measure disturbances in metabolic pathways that reflect changes downstream from genomic, transcriptomic and proteomic systems in a high-throughput manner (Beckonert et al., 2007; Fiehn, 2002). It holds considerable potential as a discovery platform for identifying not only novel diagnostic biomarkers for AD but also many other neurodegenerative diseases. Metabolomic studies have previously been undertaken in APP/PS1 mice (Chen et al., 2012; Gonzalez-Dominguez et al., 2014a, 2014b, 2015b; Graham et al., 2013b; Marjanska et al., 2005; Trushina et al., 2012; Yao et al., 2009); however, most of these studies (including our own, Graham et al., 2013b) suffer from limitations commonly befalling many metabolomic investigations conducted to date. The present study was designed having noted earlier approaches to undertake a more robust metabolomic evaluation of this important model of AD. Many previous studies had inadequate consideration of the optimal experimental design, a common arguable limitation being the use of arbitrary sample sizes without formal statistical power calculations. Another common limitation was a cross-sectional approach examining a single time point, therefore providing only the narrowest of windows through which to view and obtain reliable biological information. Most previous studies were also restricted to 1 sample type in isolation and did not examine whether biochemical alterations were more widespread. Finally, all potential sources of biological variation (i.e., potential confounders) were not always minimized in the experimental design, such as considering the gender of animals which can have a strong influence on the metabolome (Dunn et al., 2015; Graham et al., 2013a; Qiao et al., 2011). The present study undertook a targeted and quantitative methodology with optimal sample size precalculated to achieve 100% statistical power. A total of 187 prenominated metabolites were measured in both brain and blood samples from female animals, and this included amino acids, biogenic amines, phospholipids, and acylcarnitines.

Earlier metabolomic studies have revealed a number of biochemical disturbances in APP/PS1 mice. Previous studies using in vivo proton magnetic resonance spectroscopy found decreases in *N*-acetylasparatate (NAA) and glutamate and an increase in myo-inositol concentrations in APP/PS1 mice (Chen et al., 2012; Marjanska et al., 2005). Glycolytic pathways involving the Kreb's cycle, and neurotransmitter and amino acid metabolism, were found to be significantly affected in APP/PS1 mouse brain (Trushina et al., 2012). Furthermore, $^{1}$H nuclear magnetic resonance metabolomic studies found altered ascorbate, creatine, γ-aminobutyric acid, and NAA in APP/PS1 mouse brain and altered acetate, citrate, glutamine, and methionine in blood plasma (Graham et al., 2013b). A recent study applying gas chromatography-mass spectrometry and ultra-performance liquid chromatography-mass spectrometry investigated the metabolic perturbations in 5 brain regions of APP/PS1 mice at 6 months of age (Gonzalez-Dominguez et al., 2014a, 2014b). Region-specific alterations were observed for some metabolites associated with abnormal fatty acid composition of phospholipids and sphingomyelins (SPHs) or differential regulation of neurotransmitter amino acids (e.g., glutamate, glycine, serine, and NAA). Disturbances in phospholipids, energy deficiencies, altered homeostasis of amino acid, and oxidative stress in APP/PS1 mouse spleen and thymus were also observed (Gonzalez-Dominguez et al., 2015b). One study employing high-performance liquid chromatography coupled to an evaporative light-scattering detector compared the cortical levels of cholesterol and phospholipid subclasses at ages 4 and 9 months (Yao et al., 2009), and found that membrane lipids of APP/PS1 mice including cholesterol and phospholipid were significantly decreased at 9 months (Yao et al., 2009). Among phospholipid subclasses, phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine (PC) were selectively reduced (Yao et al., 2009). Despite the fact that metabolomic studies have pinpointed some metabolites affected by the development of AD-like pathology, the findings are often conflicting, fragmented, and incongruent. The aim of this study was to longitudinally study the profile of predefined metabolites in an important and widely used transgenic AD model over much of its life span and to monitor disturbances close to the initial pathologic insult and those that arise within the blood circulation.

## 2. Materials and methods

### 2.1. Brain tissue and plasma from APP/PS1 mouse

Founder APP/PS1 male mice were initially obtained from the Jackson laboratory (USA) and bred at the Ulster University. Heterozygous males were bred with wild-type (WT) C57/Bl6 females bought locally (Harlan, UK). APP/PS1and WT mice were housed under identical conditions and fed the same rodent maintenance diet (14% fat, 32% protein, and 54% carbohydrate, total energy of 3.0 kcal/g; Harlan).

APP/PS1 mice are a transgenic C57BL/6J mouse model co-expressing the Swedish mutation (K595N/M596L) and the deltaE9 PS1 exon deletion (mutated human PS1) (Lalonde et al., 2005). Offspring were tail snipped and genotyped using PCR. PCR used primers specific for the APP sequence (forward: GAATTCCGACATGACTCAGG and reverse: GTTCTGCTGCATCTTGGACA). Mice not expressing the transgene were used as WT controls. For this study, female APP/PS1dE9 mice, aged 6, 8, 10, 12, and 18 months, and age-matched WT female C57BL/6 littermate controls ($n = 8–9$) were used. Mice were fasted for 16 hours, and blood samples were collected into heparinized tubes, centrifuged for 30 seconds at 13,000 × *g*, and the resulting plasma were stored at −80 °C before metabolomic investigations. Whole mouse brain was also collected in non-fasted mice deeply anaesthetized with pentobarbital. Tissue was snap frozen in liquid nitrogen and stored at −80 °C until further use.

### 2.2. Brain tissue extraction

Mouse brain samples were collected into individual tubes to avoid cross-contamination, then lyophilized and cryogenically milled to a fine dry powder. Powdered postmortem brain tissue (25 ± 0.5 mg) was extracted in 300 μL in a solvent (85% ethanol and 15% phosphate-buffered saline buffer) previously optimized for brain metabolite profiling (Urban et al., 2010). The samples were sonicated (5 minutes), vortexed (30 seconds), and centrifuged (10,000 × *g*, 4 °C, 5 minutes), and the supernatant was retained for analysis.

### 2.3. Targeted metabolomics

Quantitative mass spectrometry–based metabolomic profiling was performed using the Biocrates AbsoluteIDQ p180 (Biocrates, Life Science AG, Innsbruck, Austria), as previously described (Nkuipou-Kenfack et al., 2014; Roemisch-Margl et al., 2012). The AbsoluteIDQ p180 kit provides simultaneous quantification of amino acids, acylcarnitines, SPHs, PCs, hexose (glucose), and biogenic amines in many biological samples. The samples were processed according to the manufacturer's instructions and analyzed on a triple-quadrupole mass spectrometer (Xevo TQ-MS, Waters Corporation, Milford, CT, USA). The data were recorded in a 96-well format, and 7 calibration standards were integrated in the kit. Human EDTA plasma samples spiked with standard metabolites were used as quality control samples to assess reproducibility of the assay. Briefly, 10 μL of mouse plasma samples and 10 μL of postmortem brain extract (prepared as described earlier) were used for the targeted metabolomic analysis. The amino acids and