Novel blood biomarkers that correlate with cognitive performance and hippocampal volumetry– potential for early diagnosis of Alzheimer's disease.

Fred Hudd[‡§], Anna Shiel[‡§] Matthew Harris[‡], Paul Bowdler, Bryony Wood[*], Demi Tsivos[*], Alfie Wearn[*], Michael Knight,[*], Risto Kauppinen[†], Elizabeth Coulthard[*], Paul White[‡] and Myra Elizabeth Conway[‡].

[‡]Faculty of Health and Life Sciences, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY, UK.

[*]Dementia Research Group, Faculty of Medicine and Dentistry, University of Bristol, Bristol, BS16 1LE, UK.

[†]Clinical Research and Imaging Centre (CRICBristol), University of Bristol, 60 St Michael's Hill, Bristol, BS2 8DX.

[§]Both authors contributed equally to the authorship of this manuscript.

Running title: Biomarkers for Alzheimer's disease

Address correspondence: Myra E. Conway, Faculty of Health and Life Sciences, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY, UK

Tel: 0044 117 328 3552

Fax: 0044 117 328 2904

email: myra.conway@uwe.ac.uk.

ABSTRACT

Background: Differential diagnosis of people presenting with mild cognitive impairment (MCI) that will progress to Alzheimer's disease (AD) remains clinically challenging. Current criteria used to define AD include a series of neuropsychological assessments together with relevant imaging analysis such as magnetic resonance imaging (MRI). The clinical sensitivity and specificity of these assessments would be improved by the concommitant use of novel serum biomarkers. The branched chain aminotransferase proteins (BCAT) are potential candidates as they are significantly elevated in AD brain, correlate with Braak Stage and may have a role in AD pathology.

Objective: In this hypothesis-driven project, we aimed to establish if serum BCAT and its metabolites are significantly altered in AD participants and assess their role as markers of disease pathology.

Methods: Serum amino acids were measured using a triple quadrupole mass spectrometer for tandem mass spectroscopy together with BCAT levels using Western blot analysis, coupled with neuropsychological assessments and MRI.

Results: We present data supporting a substantive mutually correlated system between BCAT and glutamate, neuropsychological tests, and MRI for the diagnosis of AD. These three domains, individually, and in combination, show good utility in discriminating between groups. Our model indicates that BCAT and Glutamate accurately distinguish between control and AD participants and in combination with the neuropsychological assessment, MoCA, improved the overall sensitivity to 1.00 and specificity to 0.978.

Conclusion: These finding indicate that BCAT and Glutamate have potential to improve the clinical utility and predictive power of existing methods of AD assessment and hold promise as early indicators of disease pathology.

KEYWORDS: BCAT, glutamate, AD, cognitive assessment, biomarkers.

INTRODUCTION

Alzheimer's disease (AD) is an age-related disease of the central nervous system associated with progressive cognitive impairment, memory loss and emotional disturbance. An estimated 46.8 million people worldwide have dementia and AD reflects 60-70% of cases [1]. The pathological process is characterised by the accumulation of extracellular amyloid plaques and intracellular aggregation of neurofibrillary tangles containing hyperphosphorylated tau, resulting in extensive neuronal deficits [2]. Although there is currently no cure for AD disease-modifying treatments are emerging that not only target Aβ accumulation and the tau pathway [3,4] but also neuroinflammation [5,6,7] and nutritional pathways [8,9]. The inadequacy of recent drugs to provide a cure could, in part be due to their late-stage administration and/or insensitive methods of detecting cognitive changes highlighting the clinical need for robust diagnostics [10].

Currently, the criteria used to define the cognitive wellbeing of an individual are defined by the National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), which recognise that the biological changes that occur in AD span several decades. New biomarkers will need to discriminate between disease stages including (a) pre-symptomatic disease, (b) prodromal AD i.e. those with symptomatic pre-dementia AD and (c) AD or atypical forms of AD [11]. Individuals presenting with memory impairment undergo a battery of cognitive tests and imaging analysis such as magnetic resonance imaging (MRI), to rule out other causes including tumours [12,13]. Using these approaches and with expert clinical experience, clinical criteria alone produce an 80% positive predictive value and a 60% negative predictive value for a diagnosis of AD. Recent advances in imaging such as MRI [14], amyloid-positron emission tomography (PET) [15,16] and single-photon emission computed tomography (SPECT) [17], allow for detection of brain atrophy, abnormalities in brain metabolism and hypoperfusion, respectively. However, these analyses are expensive and not widely available. Biomarkers in cerebrospinal fluid (CSF), including amyloid (such as $A\beta_{1-42}$), total

tau and p-tau or using PET scanning have credible sensitivity and specificity in predicting the conversion from MCI to AD but have yet to be adopted in a routine clinical setting due to expense, lack of expertise and relevant hardware [18-21].

Together with the development of CSF biomarkers, there has been a drive to develop blood-based biomarkers, which are less invasive with potential for a wider clinical utility. Numerous candidates have emerged including plasma $A\beta_{1-42}$ and the ratio of $A\beta_{1-42}/A\beta_{1-40}$ [22], plasma phospholipids [23], plasma proteins [24-27], microRNA expression profiling [28] and multi-analyte platforms targeting neuroinflammatory changes [29,30] and protein panels [31]. In an effort to target multiple affected pathways a number of research groups have also used proteomics or metabolomics platforms to predict AD onset [32]. These assays, combined with high throughput mass spectroscopy analysis, offer exciting new opportunities to better diagnose cognitive impairment. However, there are still considerable challenges for current blood-based biomarkers which includes lack of reproducibility between studies (most likely due to a lack of assay development or validation), an inconsistent approach to study design that spans from sample collection through to reporting and biomarker specificity.

In this report, we present a novel blood-based biomarker, the branched chain aminotransferase (BCAT) protein and its metabolite, glutamate that has the potential to predict AD progression. This hypothesis-driven research is based on the role of the BCAT proteins in regulating brain glutamate [33], the major neurotransmitter responsible for learning and memory. The BCAT proteins catalyse the reversible transamination of the branched chain amino acids (BCAAs) leucine, isoleucine and valine to their respective branched chain α-keto acids and glutamate. The BCAAs are easily transported across the blood brain barrier (BBB) and compete with the aromatic amino acids (AAA), phenylalanine, tyrosine and tryptophan for the same transporter. The BCAAs and AAA are precursors to the major neurotransmitters in the brain including glutamate, dopamine, norepinephrine and serotonin, where their plasma levels are intrinsically linked to cognition. There are two main isoforms of BCAT, a ubiquitous mitochondrial isoform, BCATm and a largely neuronal-specific cytosolic isoform, BCATc [33].

The cytosolic isoform is expressed in the soma and proximal dendrites of glutamatergic and GABAergic neuronal cells, supporting its role in contributing to the storage and metabolic pools of glutamate [34]. The mitochondrial form was shown to be expressed in the brain vasculature, particularly in endothelial cells, where a supporting role for astrocytic regulation of glutamate uptake has been proposed. These proteins were found to be significantly increased in the hippocampal, frontal and temporal region of AD brain, where BCATm correlated with Braak stage, indicating a link with disease severity [35]. In other studies, it has been shown that a mutation in the BCAT2 gene results in the clinical presentation of individuals with mild-cognitive impairment (MCI) and brain lesions together with hyperleucinaemia and hypervalinaemia, supporting a role for BCAT in sustaining optimal cognitive function [36].

In this study, we show for the first time that perturbations in BCAT/glutamate metabolism observed in AD brain is reflected in serum. We present data that the amino acid, glutamate, a product of BCAT metabolism, is found to be significantly increased in serum AD. These analytes also show correlation with several neuropsychological tests and structural MRI analysis, indicating that the BCAT protein and glutamate show promise as early diagnostic biomarkers. Therefore, because BCAT is intrinsically linked with metabolic pathways that are perturbed in AD it has the advantage over other blood markers in that it is associated with AD disease progression. Moreover, we have assessed whether the predictive power of BCAT as a blood biomarker of AD could be increased by combining with neuropsychology and neuroimaging scores, offering a combined assessment of AD pathology.

MATERIALS AND METHODS

Materials

Rabbit polyclonal antibodies to BCATc and BCATm were purchased from Insight Biotechnology (Peterborough, UK). The specificity of the BCATc and BCATm antibodies has been validated for western blotting [35, 36]. Chemiluminescent substrate was purchased from Millipore (Watford, UK) and rabbit polyclonal antibody to transferrin from Abcam (Cambridge, UK).

Participants

The study was approved by North Somerset and South Bristol Research Ethics Committee, Bristol, UK. All experiments were carried out in accordance with ethical and NHS governance guidelines and approvals. University of Bristol agree in principle to anonymised data sharing with external researchers and each data request is assessed individually. Participants with the capacity to consent were recruited from the following local and national volunteer registers: Join Dementia Research, BRACE charity, Avon and Wiltshire Mental Health Partnership Trust, ReMemBr group research database and North Bristol NHS Trust. A total of 118 participants were recruited, where 71 were healthy aged individuals and 30 people had a recent diagnosis of MCI (both amnesic and multi-domain MCI) and 17 people with a diagnosis of probable AD. MCI was diagnosed at the Dementia Research Clinic and other local memory services using standard criteria and, for inclusion, all MCI had to have had memory complaints and/or score more than 1 SD below expected for age on a test of memory. Inclusion criteria: All participants had capacity to consent to research and were either drug-free or on stable treatment. In summary: 1) Patients diagnosed with Alzheimer's disease with a Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA) score of greater than 10/30 and have memory deficits demonstrated on at least one of their clinical cognitive assessments. 2) Patients diagnosed with MCI who have either symptoms of amnesia or >1 SD impairment below expected for age performance in episodic memory clinical cognitive tests. 3) Healthy

participants age-matched to patients above. Exclusion criteria 1) patients with dementia likely to be of a different aetiology; 2) participants taking medication likely to interfere significantly with cognitive function (except cholinesterase inhibitor and memantine used routinely in dementia) 3) participants with physical limitations such that they will not be able to carry out the requirements of the study; 4) participants with a history of significant claustrophobia or previous panic or claustrophobia while in an MRI scanner; 5) participants with significant other neurological or psychiatric illnesses likely to interfere with performance on the task. All participants underwent a screening interview to assess demographic factors thought to impact on cognitive decline (IQ, history of head trauma, epilepsy, smoking and alcohol intake) (Table 1). There was no significant difference between AD, MCI and control groups with respect to most parameters, however, there was a significant difference with respect to years of education and IQ score. For blood analysis a number of people were unable to provide a sample, therefore a total of 46 controls, 17 MCI and 13 AD were recruited for this aspect of the study.

ApoE genotyping

Genomic DNA was extracted from 300 µL of whole blood using UltraClean® Blood DNA isolation Non-Spin Kit (Cambio) and detected using ultraviolet spectrophotometry (Thermo Scientific™ NanoDrop™, Loughborough, UK). ApoE genotyping was performed using a PCD-RFLP method, previously described by Hixson and Vernier, 1990 [37,38]. Briefly, DNA was amplified by PCR using oligonucleotide primers (Eurofins Genomics; upstream primer: 5'-TCC AAG GAG CTG CAG GCG GCG CA -3', downstream primer: 5'- ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A - 3'). ApoE was amplified using a PTC-200 Peltier Thermal Cycler at 94°C for 12 minutes followed by 48 cycles at 94°C for 1 minute, 65°C for 1 minute and a final extension of 75°C for 1 minute. The PCR product was visualised on a 2.5% ethidium bromide agarose gel at 227 bp before overnight digestion with *Hha*I in a total volume of 50 µL at 37°C. Digestion products were visualised using a 3.5% Metaphor agarose gel (Lonza).

Western blot analysis of serum samples

The protein concentration of serum samples from 46 controls, 17 MCI and 15 AD blood samples were calculated using the Schaffner and Weissmann method [39]. Protein samples (10 µg) were separated on a NuPAGE® Novex® 4-12% Bis-Tris 1 mm gel with 1x NuPAGE® MES SDS Running buffer for 1 hour at 150 volts. The proteins were then transferred to a PVDF membrane overnight at 50 volts in transfer buffer (20% methanol, 25 mM Tris, 190 mM glycine, pH 8.3). The membrane was washed in TBST (0.1% Tween, 200 mM NaCl, 2 mM Tris, pH 7.5) and blocked with 5% BSA in TBST for 1 hour. Primary antibody was prepared in 5% BSA (1/1000 for BCATc and BCATm) and added for 1 hour before several TBST washes and 1 hour incubation with HRP-linked secondary antibody (1/2000). After further TBST washes, positive bands were visualised using chemiluminescent HRP substrate and an Odyssey® Fc Imaging System (LI-COR Biosciences). Integrated area densitometry was carried out using Image Studio Ver 5.2 and the results were measured in relation to total protein. For re-probing, membranes were incubated with 1 M NaOH for 8 minutes and washed in TBST before treatment from the blocking step onwards.

Analysis of the branched chain and aromatic amino acid profiles using LC-MS/MS

Aliquots of 100 µL of serum were combined with 50 µL of deuterium labelled internal standard and prepared to a final volume of 500 µL with acetonitrile containing 0.1% formic acid. The solution was vortexed and centrifuged at 3,200 x g for 10 minutes. An aliquot of 50 µL of supernatant was transferred to sample vials for analysis and evaporated under a stream of nitrogen gas until dry. Dried sample residue was reconstituted with 200 µL of 0.1% formic acid. Chromatographic separation was performed using an Agilent 1260 Series LC system. This was coupled with an Agilent 6460 triple quadrupole mass spectrometer for tandem mass spectroscopy (Agilent JetStream electrospray ionisation) and multiple reaction monitoring. Samples were injected onto an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm x 150 mm, 5 µm) maintained at 20°C. The following elution gradient included Mobile phase A = 0.1%

formic acid and mobile phase B = 100% acetonitrile. Gradient elution was used to separate each amino acid. The amino acids and their internal standards were monitored and their peak area ratio relative each other calculated allowing for the correction of transition overlap between neutral leucine and isoleucine.

Cognitive Assessment

Participant carried out a battery of tasks designed to probe hippocampal function as well as tests of wider cognitive function. Tests that were performed as part of the experimental procedure included: 1) Montreal cognitive assessment (MoCA, as a global cognitive screen). 2) Paired Associate Learning from CANTAB as a pattern/spatial memory task widely used and thought to be a "hippocampal" task - used as an early marker of AD. 3) Adapted verbal learning task (similar to Hopkins Verbal Learning Task) testing memory over 30 minutes, 24 hours, 2, 7 and 14 days [40].

MRI

All participants were scanned with Siemens Magnetom Skyra 3T MRI system using a 32Ch head coil. The MRI protocol was as follows: 3D T1-weighted MRI was collected with MPRAGE: coronal, TR 2200 ms, TE 2.42 ms, TI 900 ms, flip angle 9°, resolution 0.34 x 0.34 x 1.60 mm^3 (after 2-fold interpolation in-plane), matrix size 540 x 640 x 144 (after 2-fold interpolation in-plane), GRAPPA factor 2, time 5:25. Quantitative T2 images were acquired with a multi-contrast spin-echo sequence: coronal, TR 4500, TE 12 ms, number of echoes 10, echo spacing 12 ms, resolution 0.34 x 0.34 x 1.7 mm^3 (after 2-fold interpolation in-plane, and inclusive of 15% slice gap), matrix size 540 x 640, 34 slices, GRAPPA factor 2, time 11:07.

MRI volumetric analyses

Total brain, gray matter, white matter and CSF space volumes were determined using FSL routines (http://www.fmrib.ac.uk/fsl). Morphometry of hippocampus and its sub-regions were determined from T2 images using the manual protocol by Wood et al (2015) [41]. This protocol

provides volumes for 6 subfields as follows: CA1, CA2, CA3, dentate gyrus, subiculum, hippocampal and entorhinal.

Statistical Analysis

The data was screened for its accuracy and validity and for any potentially influential or outlying observations which may otherwise unduly impact results. A between-participants analysis of variance for a one-way design, with a post-hoc application of Tukey's HSD pairwise test was used to assess for differences between Control/MCI/AD for BCATc, BCATm, amino acids, brain sub-volumes, and the cognitive data. Residuals under each ANOVA were examined to ensure they did not grossly deviate from the assumption of errors being independent identically distributed normal deviates. Pearson's correlation coefficient was used to assess the strength and direction of correlations among quantitative measures.

MoCA scores were used in a linear discriminant analysis to develop a baseline model to discriminate between Control/MCI/AD. We sought to extend this baseline model to determine whether the inclusion of BCATc, BCATm, the amino acids and brain sub-volumes would improve the discrimination between groups. Accuracy of discrimination was determined using leave-one-out cross-validation. To account for the putative ordering of outcome (Control/MCI/AD) we further used MoCA scores in an ordinal logistic regression model. We sought to extend this baseline ordinal logistic regression model to determine whether the inclusion of BCATc, BCATm, the amino acids and brain sub-volumes would improve the predictive capability of the baseline model. This modelling utilising linear discriminant analysis and ordinal logistic regression using MoCA as a baseline model was repeated using Dentate Gyrus volume in replace of MoCA. We further sought to investigate the utility of the blood markers in isolation and repeated the modelling with BCATm/BCATc as the baseline marker Optimal sensitivity and specificity for predicting MCI or AD, and for AD was determined using the ordinal logistic regression model through the maximisation of Youden's Index. Leave one accuracy was also included, which is a standard cross-validation technique where (i) an

observation temporarily held out (ii) the model is developed with the hold out observation not included in the model building (iii) the model is used to predict the holdout observation. This process is completed for each and every observation. The resulting percentage accuracy is a better and less optimistic indicator of predictive accuracy of applying the model to new unseen data.

RESULTS

Clinical data and ApoE Genotyping

Clinical data for each participant was determined at baseline and summarised in Table 1. There was a significant difference between groups (Control/MCI/AD) with respect to the age they left school, years of education and reports of head injury. Overall, the majority of patients (49%) presented with ApoE 3/3 genotype, considered a 'neutral' genotype, with 9% patients with e4/4, linked to AD and the remainder of the patients were heterozygotes. There were no participants with the e2/2 genotype, which shows a protective effect against AD [36]. Interestingly, only 1 participant that was determined to have probable AD was shown to have the e4/4 genotype with 4 other showing a heterozygote pattern. However, there were a number of participants that were designated controls that did carry the e4/4 genotype. Moreover, in some control patients, in particular with the ApoE4 genotype (where ApoE4 carriers have a 40% greater risk of developing AD) levels of their BCAT proteins were increased, indicating that these patients show common emerging profiles similar to those with MCI or AD. We hypothesise that in this instance these biomarkers may have scope as predictors of preclinical AD.

Increased BCAT expression in AD serum reflect AD brain patterns

Blood analysis revealed that the BCAT proteins were increased in patients with AD compared to healthy controls ($p < 0.0001$ and $p=0.0003$, BCATc (Figure 1A) and BCATm (Figure 1B), respectively) and clearly distinguished between groups (Table 2). The data show that in some MCI cases their blood profiles reflected changes similar to that observed for AD (Figure 1A, lane 1 (AD) and lane 3 (MCI), respectively). Other MCI cases did not reflect this profile and shared typical biochemical characteristics like controls (Figure 1A, lane 4, 5 (MCI) relative to the control lanes). Table 2 summarizes mean scores on BCAT by participant group and details the results from a one-way analysis of variance and a post hoc application of Tukey's honestly significant differences test for each measure. Mean levels for BCATc and BCATm significantly differ between at least two patient groups ($p < 0.05$). In the sample, there is a significant

difference in the level of the BCAT proteins between control and MCI ($p=0.0121$ and $p=0.0053$, for BCATc and BCATm, respectively) and control and AD ($p < 0.0001$ and $p=0.0025$, BCATc and BCATm, respectively).

Increased levels of glutamate in AD serum differentiates between groups

The blood amino acids, in particular the branched chain amino acids (BCAAs), glutamate and the aromatic amino acids (AAAs) showed an altered amino acid profile in the plasma from AD patients relative to controls. In particular, there was an increase in the concentration of glutamate from control to MCI to AD (Table 3). Table 3 summarises mean levels and within group variation for the amino acid levels. In the sample, mean levels of Isoleucine, Leucine, Valine and Tyrosine significantly differ between conditions. In the control compared with MCI a pairwise comparison showed there was a significant difference between the BCAAs Isoleucine ($p=0.0077$), Leucine ($p=0.0141$), and valine ($p=0.0063$), indicating that these amino acid profiles may be early predictors of preclinical AD (Figure 2).

Differentiation between participant groups

The hippocampus of AD patient displays marked atrophy compared to control (Figure 3). The MRI data indicate that while reduction of total hippocampal volume is evident in both MCI and mild AD, the volume reduction concerns primarily CA1, dentate gyrus and subiculum and that tissue loss in these sub-regions is uneven. As described we have administered versatile cognitive neuropsychology tests to the same participants to gain understanding of functional consequences of the described morphometric changes in the hippocampus and its sub-regions (Table 4). The Montreal Cognitive Assessment test (MoCA), Paired Associative Learning, and HVLT verbal learning test all discriminate between AD, MCI and healthy older groups. Table 4 summarises mean scores on cognitive assessments by patient group and summarises the results from a one-way analysis of variance and a post hoc application of Tukey's honestly significant differences test for each measure. In each instance, sample means levels demonstrate significantly poorer cognitive ability in MCI compared to control,

and significantly poorer cognitive ability in AD compared to MCI. The F-statistic indicates MoCA scores have the greatest within sample discriminatory effects.

Mean levels for CA1, Dentate Gyrus, Subiculum, Hippocampal, and Entorhinal monotonically decrease with progression from Control, to MCI, to AD, and these trends are statistically significant (Table 5). In all cases for CA1, CA2, Dentate Gyrus, Subiculum and Hippocampal, the mean levels in Control are significantly higher than MCI, and AD ($p < 0.05$), but with no significant difference between MCI and AD ($p > 0.05$).

BCATm and BCATc are significantly correlated, $r = 0.851$, $p < 0.001$, and are significantly correlated with MoCA, r = -0.317, p = 0.006, and r = -0.388, p = 0.001, respectively. Glutamate is not significantly correlated with BCATm ($r = 0.096$, $p = 0.442$). However, Glutamate is significantly correlated with BCATc ($r = 0.303$, $p = 0.013$) and is significantly correlated with MoCA ($r = -0.329$, $p = 0.006$). CA1, CA2, Dentate gyrus, Subiculum, Hippocampal and Entorhinal volumes are all significantly correlated with MoCA as shown in Table 6. Leucine, Isoleucine, Valine, Phenylalanine and Tyrosine form a mutually correlated system (r >0.85, p < 0.001). However, these amino acids are not correlated with MoCA (p > 0.2 in all cases), and Leucine, Isoleucine and Valine are each significantly correlated with BCATm and BCATc.

Multivariate discriminant analysis shows that MoCA scores by themselves have good and significant discriminant powers between Control/MCI/AD (classification accuracy 81.8%; leave one-out-cross validated 81.8%). This baseline discriminant model is significantly improved with the inclusion of any one of BCATc, BCATm, Glutamate, Valine, Leucine, Isoleucine, Phenylalanine, or Tyrosine (as shown in Table 7), however, this baseline discriminant model is not improved with the inclusion of any of the brain volume measures. Likewise, ordinal logistic regression shows that MoCA scores by themselves have good predictive powers for Control/MCI/AD This baseline ordinal logistic regression model is significantly improved by the inclusion of any of, BCATc (p = 0.001), Glutamate (p = 0.035), Leucine (p = 0.019), Valine (p = 0.008), Phenylalanine (p = 0.030), Isoleucine (p = 0.044), or Tyrosine (p = 0.005).The

inclusion of BCATm is not deemed to be statistically significant ($p = 0.054$) in this sample. The inclusion of the Dentate Gyrus in this MoCA baseline model did not significantly improve the model ($p = 0.297$).

Multivariate discriminant analysis shows that Dentate Gyrus sub-volume has some significant discriminant powers between Control/MCI/AD (classification accuracy 61.6%; leave one-out-cross validated 61.6%). This baseline discriminant model is significantly improved with the inclusion of any one of BCATm, Glutamate, and Leucine. Likewise, ordinal logistic regression shows that Dentate Gyrus volume is significantly related to outcome. Further, this baseline ordinal logistic regression model is significantly improved by the inclusion of any of, BCATm ($p = 0.011$), Glutamate ($p = 0.003$), Leucine ($p = 0.045$), Valine ($p = 0.048$) but with levels of predictive accuracy notably lower when compared with the models using MoCA.

For blood markers only, BCATc, BCATm and Glutamate in combination and individually show good discriminatory powers (Table 7). For MoCA alone, sensitivity and specificity were 0.947 and 0.900 with respect to AD. This ordinal logistic regression model was significantly improved when combined with BCATc or the amino acids (Table 7). Of note is the measure of BCATc with MoCA which improved sensitivity for AD to 1.00 and specificity to 0.902, compared with sensitivity of 0.917, and specificity of 0.969 for BCATm and MoCA in combination. In contrast, the MoCA models were not greatly improved by the inclusion of the Dentate Gyrus subfield volume.

DISCUSSION

Biomarkers have a wide range of applications in clinical research, drug discovery and clinical diagnosis. While dementia biomarkers in body fluids are highly promising, justifying population-based studies, prediction value could be hugely increased by combining with other assessment modalities. Our data suggest that the predictive power of BCAT or glutamate is increased by either structural information from MRI of vulnerable brain structures [14] and/or neuropsychological tests [11]. Here, in this collaborative study, across three disciplines, we address the potential of BCAT and glutamate to predict AD with our main goal of working towards the early diagnosis of AD.

The key aspects of the current guidelines recognize that AD progresses on a spectrum with three stages, an early, preclinical stage with no symptoms, a middle stage of MCI and a final stage marked by symptoms of dementia [11]. The potential use of biomarkers as indicators of underlying AD pathology has been recognized formally in diagnostics criteria [11]. However, in these guidelines the recommendation is that the use of biomarkers is still focused on research rather than in a clinical setting. In our study, blood analysis revealed that the BCAT proteins were increased in patients with AD compared to healthy controls and clearly distinguished between groups (Figure 1 and Table 2). Previous studies by our group have shown that the levels of the BCAT proteins, which regulate BCAA and glutamate metabolism, are increased in the hippocampus, frontal and temporal cortex in AD relative to matched control brains [35]. As discussed, the upregulation of BCATm correlated with Braak stage, suggesting that the level of this enzyme is directly related to the progression of the disease [35]. Interestingly, there is no significant difference between mean BCAT levels in MCI compared to the more severe AD participants. This argues that BCAT may reflect early pathological changes in the brain supporting our findings in brain tissue. This analysis correlated with the MoCA score and with various neuropsychological tests and several indicators of hippocampal volume reduction, suggesting that BCAT is a valid measure of AD pathogenesis (Figure 4 and Table 7).

Studies from cell and animal models suggest that increased levels of BCATs may, at least initially, be neuroprotective or when considering glutamatergic signalling compensatory [35]. Using a rat model of brain injury, upregulation of BCATc was clearly evident in neurons that had normal morphology, despite the fact that overall levels correlated with the extent of cell death [42]. A potential underlying mechanism could be related to regulation via the brain-derived neurotrophic factor (BDNF), important in mediating neuronal survival [43]. In response to BDNF treatment, mRNA fingerprinting showed that BCATc was significantly upregulated [44]. In a separate study, the authors demonstrated that upregulation of BCATc mRNA in adult BNDF transgenic mice was particular to the parietal cortex, the hilus, and CA3 hippocampal subfield [45]. Our group previously showed that in AD brain similar regional upregulation of BCAT was observed and in general the neurons that were immunopositive for BCATc in the human brain tissue also appeared morphologically intact [35].

However, sustained increased BCAT expression could increase the production of glutamate and exacerbate neuronal excitotoxicity, potentially contributing to cell death. Here, we show that there is a significant increase in the levels of serum glutamate that monotonically increased across the three groups (Figure 3A). Interestingly, proton magnetic resonance (^{1}H MRS) assessment of metabolites such as glutamate showed a decrease in hippocampal brain glutamate between control, MCI and AD participants [46]. Glutamatergic neurons located in the hippocampus, frontal, temporal and parietal cortex have been reported to be severely affected in AD relative to similar neurons in the motor and sensory cortex and considered a possible early event in the pathogenesis of the disease [47,48]. A recent study has indicated that measures of the glutamatergic system i.e. reduced glutamatergic presynaptic bouton density, correlated with MCI more so than Aβ or Tau [49]. More important and potentially relevant to the current study was that this study reported an increase in glutamatergic synapses in individuals with MCI. The authors interpreted this increase as a compensatory mechanism to counteract the effects of pre-existing synaptotoxicity to improve transmission

probability. Therefore, whilst a neuroprotective has been proposed for increased BCAT, its increased expression may also reflect a compensatory mechanism. The glutamate/glutamine cycle has received much attention as several aspects of this cycle are perturbed in AD brain including 1) Reduced levels of VGLUT1 and 2 in the prefrontal cortex of individuals with AD [50] 2) Aβ peptides accumulate more in VGLUT1/2-containing terminals than in non-VGLUT terminals indicating a preferential targeting of these type of neurons [51] 3) Synaptic glutamate transporter defect, an early sign in disease pathology [52] 4) Low levels of glutamate synthetase in astrocytes [53,54]. This extensive evidence together with our data supports a role for BCAT and glutamate as early indicators of disease pathology.

Levels of BCAAs were tested because excess or limiting dietary BCAA causes neurological dysfunction, reducing cognitive function, however, the mechanisms underlying these links are not entirely understood and require further investigation. Several studies have reported the amino acid profiles in the CSF and plasma of individuals with MCI and AD, generating a mixed data set with wide variability possibly due to study design, methodologies or instrumentation [55]. We show here that levels of leucine, isoleucine and valine independently and together are lower in Control relative to MCI and AD participants. Based on pairwise comparisons there is a significant increase in the BCAAs in MCI relative to Control subjects, where monitoring a change over time holds promise as a predictor of AD. A genome-wide association study (GWAS) identified several genetic variants that are associated with increased BCAA levels [52]. Using Mendelian randomization (MR) Larsson and Markus suggested that individuals with a genetic predisposition to raised plasma isoleucine were positively associated with AD [53]. More recently, the BCAAs have been shown to have a predictive value for the development of new-onset diabetes up to 12 years after the baseline examination, which incidentally has a 50% increased risk of developing AD [56,57]. Conversely, in other conditions such as liver disease [58], traumatic brain injury (TBI) [59,60] and sepsis [61], levels of blood BCAAs are low. In TBI, where brain glutamate is low, the plasma concentrations of the BCAAs are also decreased, whereas the concentrations of the AAA's (phenylalanine,

tryptophan and tyrosine) are increased [57]. In patients recovering from TBI, BCAA supplementation has demonstrated a therapeutic benefit with improved cognition. Similar reports have been highlighted when treating chronic hepatic encephalopathy and sepsis. In patients with liver disease, supplementation with BCAAs reduces ascites [62], hepatic encephalopathy [63] and malnutrition [64]. In Huntington's disease, plasma BCAAs are significantly decreased [65], and correlate with disease severity over time [66]. These clinical presentations highlight how sub-optimal or chronically high levels of BCAAs affect brain function and how the BCAT metabolic proteins and activity are important to cognitive wellbeing. It is important to note that whilst alterations in BCAT and BCAA metabolism are associated with various disorders, further studies are required to better determine the optimal level required for cognitive well-being, which will better inform clinical practice.

In a recent case report, a patient presented with hypervalinaemia, hyperleucine-isoleucinemia, headaches, and MCI with brain white matter lesions [36]. Traditionally, one would relate increased levels of blood BCAAs with Maple Syrup Urine disease, an autosomal recessive disorder, caused by a deficiency of the branched-chain α-keto acid dehydrogenase complex (BCKDC) [67]. The patient was found to have two heterogeneous BCAT2 mutations, including c.509G-A and c.790G-A, suggesting that when BCAT2 is mutated, BCAA metabolism is perturbed, cognition is impaired and brain lesions develop. When the patient was treated with Vitamin B6 the symptoms subsided and the lesions decreased. This is particularly relevant to AD as a study that used vitamin B supplementation over 2 years showed how accelerated brain atrophy in elderly subjects with MCI was slowed by 30%, which corresponded to a decrease of 31.7% in total homocysteine levels [68]. In their report the authors assumed that the reduction was directly related to the homocysteine pathway, however, BCAT is also a vitamin B-dependent enzyme and may also be important in this mechanism. We propose that BCAT is upregulated in a neuro-protective manner that responds to nutritional or redox changes in the cell. However, as with most biological systems, an imbalance in metabolism could generate secondary consequences, such as increased glutamate production leading to

neuronal toxicity, a recognised contributing factor to AD pathology. If BCATis oxidised it could assume a moonlighting neurotoxic role [69]. Together both the BCAT proteins and their metabolites hold promise as markers of AD pathology and may also serve as targets for future therapy-related approaches.

In the current study, serum analyses were conducted together with advanced MRI imaging and neuropsychology tests to detect alteration in the hippocampus in MCI and AD participants. The MRI data have indicated that while reduction of total hippocampal volume is evident in both MCI and AD brains, the volume reduction concerns primarily CA1, dentate gyrus and subiculum. We have administered versatile cognitive neuropsychology tests to the same participants to gain understanding of functional consequences of the above described morphometric changes in the hippocampus and its sub-regions. Here, we show that the morphological changes detected by MRI analysis correlates with MoCA (assessment of long-term memory) (Table 6) indicating that both these measures can be used in assessment of control compared with AD participants. The sensitivity and specificity values reported in table 7 show that baseline models utilising either MoCA or Dentate Gyrus are improved by incorporating the BCAT proteins or the amino acids. Diagnostically the most clinically relevant assays to date include measurement of CSF $A\beta_{1-42}$ tTau, pTau and the Aβ/total tau index (ATI). Using combinations of these biomarkers the clinical sensitivity and specificity can vary between studies and can reach values up to 90% and 85%, respectively [for review see 70]. However, current studies measuring these parameters in blood have not generated substantial data in support of their diagnostic use. Other studies favour multi-analyte approaches such as the Luminex-based platform that identified 30 protein markers in discriminating AD from controls (AUC=0.95) [71] indicating that a panel approach has potential to better delineate between participant groups.

In conclusion, using this proof of concept model we have shown that measurement of BCATc, BCATm and the amino acids increased the sensitivity and specificity of MoCA analysis in correctly identifying participants free of AD pathology (90%) and those with the disease to

100% accuracy. This analysis indicates that there is a high degree of information overlap between MoCA, the MRI measures, and the amino acid profiles. However, BCAT and the amino acids significantly improve the predictive potential of MoCA and significantly improve the predictive potential of the MRI measures. These findings, coupled with the underpinning of extant literature, indicate that the combined modalities show diagnostic potential subject to the caveats of single centre cross-sectional exploratory studies warranting the need for a larger or longitudinal study.

ACKNOWLEDGEMENTS

This study was supported by The Free Masons Charity in collaboration with BRACE (Bristol Research into Alzheimer's disease and Care of the Elderly) awarded to M.E.C. at the University of the west of England.

CONFLICT OF INTEREST/DISCLOSURE STATEMENT

The authors have no conflict of interest to report.

REFERENCES

[1] Prince, M., Comas-Herrera, A., Knapp, M., Guerchet, M., and Karagiannidou, M., (2016) Alzheimer's disease International World Alzheimer report. Alzheimer's disease International, London.

[2] Armstrong RA. (2009) The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. *Folia Neuropathol.* **47**(4):289-99. Review.

[3] Kurnellas MP, Rothbard JB, Steinman L. (2015) Self-Assembling Peptides Form Immune Suppressive Amyloid Fibrils Effective in Autoimmune Encephalomyelitis. *Curr Top Behav Neurosci.* **26**:221-32.

[4] Noble W, Pooler AM, Hanger DP. (2011) Advances in tau-based drug discovery. *Expert Opin Drug Discov.* **6**(8):797-810.

[5] Ferrera P, Arias C. (2005) Differential effects of COX inhibitors against beta-amyloid-induced neurotoxicity in human neuroblastoma cells. *Neurochem Int.* **47**(8):589-96.

[6] Gasparini L, Ongini E, Wenk G. (2004) Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: old and new mechanisms of action. *J Neurochem.* **91**(3):521-36. Review.

[7] Reitz C. (2012) Alzheimer's disease and the amyloid cascade hypothesis: a critical review. *Int J Alzheimers Dis.* **2012**:369808.

[8] Gustafson DR, Clare Morris M, Scarmeas N, Shah RC, Sijben J, Yaffe K, Zhu X. (2015) New Perspectives on Alzheimer's Disease and Nutrition. *J Alzheimers Dis.* **46**(4):1111-27.

[9] Shah RC. (2011) Medical foods for Alzheimer's disease. *Drugs Aging.* **28**(6):421-8.

[10] Cummings J. (2012) Alzheimer's disease diagnostic criteria: practical applications. *Alzheimers Res Ther.* **4**(5):35.

[11] Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, Delacourte A, Galasko D, Gauthier S, Jicha G, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Stern Y, Visser PJ, Scheltens P. (2007) Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol*. **6**(8):734-46. Review.

[12] Sutphen CL, Fagan AM, Holtzman DM. (2014) Progress update: fluid and imaging biomarkers in Alzheimer's disease. *Biol Psychiatry.* **75**(7):520-6.

[13] Viola KL, Sbarboro J, Sureka R, De M, Bicca MA, Wang J, Vasavada S, Satpathy S, Wu S, Joshi H, Velasco PT, MacRenaris K, Waters EA, Lu C, Phan J, Lacor P, Prasad P, Dravid VP, Klein WL. (2015) Towards non-invasive diagnostic imaging of early-stage Alzheimer's disease. *Nat Nanotechnol*. **10**(1):91-8.

[14] Willette AA, Calhoun VD, Egan JM, Kapogiannis D. (2014) Alzheimer′s Disease Neuroimaging Initiative. Prognostic classification of mild cognitive impairment and Alzheimer's disease: MRI independent component analysis. *Psychiatry Res.* **224**(2):81-8.

[15] Thal DR, Attems J, Ewers M. (2014) Spreading of amyloid, tau, and microvascular pathology in Alzheimer's disease: findings from neuropathological and neuroimaging studies. *J Alzheimers Dis*. **42** Suppl 4:S421-9.

[16] Ito H, Shimada H, Shinotoh H, Takano H, Sasaki T, Nogami T, Suzuki M, Nagashima T, Takahata K, Seki C, Kodaka F, Eguchi Y, Fujiwara H, Kimura Y, Hirano S, Ikoma Y, Higuchi M, Kawamura K, Fukumura T, Böö ÉL, Farde L, Suhara T. (2014) Quantitative Analysis of Amyloid Deposition in Alzheimer Disease Using PET and the Radiotracer ^{11}C-AZD2184. *J Nucl Med*. **55**(6):932-8.

[17] O'Brien JT, Firbank MJ, Davison C, Barnett N, Bamford C, Donaldson C, Olsen K, Herholz K, Williams D, Lloyd J. (2014) 18F-FDG PET and perfusion SPECT in the diagnosis of Alzheimer and Lewy body dementias. *J Nucl Med*. **55**(12):1959-65.

[18] Kauwe JS, Jacquart S, Chakraverty S, Wang J, Mayo K, Fagan AM, Holtzman DM, Morris JC, Goate AM. (2007) Extreme cerebrospinal fluid amyloid beta levels identify family with late-onset Alzheimer's disease presenilin 1 mutation. *Ann Neurol.* **61**(5):446-53.

[19] Cruchaga C, Kauwe JS, Harari O, Jin SC, Cai Y, Karch CM, Benitez BA, Jeng AT, Skorupa T, Carrell D, Bertelsen S, Bailey M, McKean D, Shulman JM, De Jager PL, Chibnik L, Bennett DA, Arnold SE, Harold D, Sims R, Gerrish A, Williams J, Van Deerlin VM, Lee VM, Shaw LM, Trojanowski JQ, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD, Peskind ER, Galasko D, Fagan AM, Holtzman DM, Morris JC; (2013) GERAD Consortium; Alzheimer's Disease Neuroimaging Initiative (ADNI); Alzheimer Disease Genetic Consortium (ADGC), Goate AM. GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. *Neuron*. **78**(2):256-68.

[20] Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol*. **9**(1):119-28.

[21] Shen Y, Wang H, Sun Q, Yao H, Keegan AP, Mullan M, Wilson J, Lista S, Leyhe T, Laske C, Rujescu D, Levey A, Wallin A, Blennow K, Li R, Hampel H. (2018) Increased plasma Beta-Secretase 1 may predict conversion to Alzheimer's Disease Dementia in individuals with Mild Cognitive Impairment. *Biol Psychiatry*. **83**(5):447-455.

[22] Galozzi S, Marcus K, Barkovits K. (2015) Amyloid-β as a biomarker for Alzheimer's disease: quantification methods in body fluids. *Expert Rev Proteomics*. **12**(4):343-54.

[23] Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, MacArthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, Nazar MD, Rich SA, Berlau DJ, Peltz CB, Tan MT, Kawas CH, Federoff HJ. (2014) Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med*. **20**(4):415-8.

[24] Hye A, Riddoch-Contreras J, Baird AL, Ashton NJ, Bazenet C, Leung R, Westman E, Simmons A, Dobson R, Sattlecker M, Lupton M, Lunnon K, Keohane A, Ward M, Pike I, Zucht HD, Pepin D, Zheng W, Tunnicliffe A, Richardson J, Gauthier S, Soininen H, Kłoszewska I, Mecocci P, Tsolaki M, Vellas B, Lovestone S. (2014) Plasma proteins predict conversion to dementia from prodromal disease. *Alzheimers Dement*. **10**(6):799-807.

[25] Thambisetty M, Simmons A, Velayudhan L, Hye A, Campbell J, Zhang Y, Wahlund LO, Westman E, Kinsey A, Güntert A, Proitsi P, Powell J, Causevic M, Killick R, Lunnon K, Lynham S, Broadstock M, Choudhry F, Howlett DR, Williams RJ, Sharp SI, Mitchelmore C, Tunnard C, Leung R, Foy C, O'Brien D, Breen G, Furney SJ, Ward M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Hodges A, Murphy DG, Parkins S, Richardson JC, Resnick SM, Ferrucci L, Wong DF, Zhou Y, Muehlboeck S, Evans A, Francis PT, Spenger C, Lovestone S. (2010) Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Arch Gen Psychiatry*. **67**(7):739-48.

[26] IJsselstijn L, Dekker LJ, Koudstaal PJ, Hofman A, Sillevis Smitt PA, Breteler MM, Luider TM. (2011) Serum clusterin levels are not increased in presymptomatic Alzheimer's disease. *J Proteome Res*. **10**(4):2006-10.

[27] Schrijvers EM, Koudstaal PJ, Hofman A, Breteler MM. (2011) Plasma clusterin and the risk of Alzheimer disease. *JAMA.* **305**(13):1322-6.

[28] Tan L, Yu JT, Tan MS, Liu QY, Wang HF, Zhang W, Jiang T, Tan L. (2014) Genome-wide serum microRNA expression profiling identifies serum biomarkers for Alzheimer's disease. *J Alzheimers Dis*. **40**(4):1017-27.

[29] Kim E, Jung YS, Kim H, Kim JS, Park M, Jeong J, Lee SK, Yoon HG, Hwang GS, Namkoong K. (2014) Metabolomic signatures in peripheral blood associated with Alzheimer's disease amyloid-β-induced neuroinflammation. *J Alzheimers Dis.* **42**(2):421-33.

[30] Restrepo L, Stafford P, Johnston SA. (2013) Feasibility of an early Alzheimer's disease immunosignature diagnostic test. *J Neuroimmunol* **254**(1-2):154-60.

[31] Hye A, Lynham S, Thambisetty M, Causevic M, Campbell J, Byers HL, Hooper C, Rijsdijk F, Tabrizi SJ, Banner S, Shaw CE, Foy C, Poppe M, Archer N, Hamilton G, Powell J, Brown RG, Sham P, Ward M, Lovestone S. Proteome-based plasma biomarkers for Alzheimer's disease. Brain. 2006 Nov;129(Pt 11):3042-50. [32] Lista S, Faltraco F, Prvulovic D, Hampel H. (2013) Blood and plasma-based proteomic biomarker research in Alzheimer's disease. *Prog Neurobiol*. 101-102:1-17.

[33] Conway, ME, and Hutson, SM (2015) The Cytosolic and Mitochondrial Branched Chain Aminotransferase. Branched Chain Amino Acids in Clinical Nutrition. Springer New York, 25-40.

[34] Hull J, Hindy ME, Kehoe PG, Chalmers K, Love S, Conway ME. (2012) Distribution of the branched chain aminotransferase proteins in the human brain and their role in glutamate regulation. *J Neurochem.* **123**(6):997-1009.

[35] Hull J, Patel V, El Hindy M, Lee C, Odeleye E, Hezwani M, Love S, Kehoe P, Chalmers K, Conway M. (2015) Regional Increase in the Expression of the BCAT Proteins in Alzheimer's Disease Brain: Implications in Glutamate Toxicity. *J Alzheimers Dis.* **45**(3):891-905.

[36] Wang XL, Li CJ, Xing Y, Yang YH, Jia JP. (2015) Hypervalinemia and hyperleucine-isoleucinemia caused by mutations in the branched-chain-amino-acid aminotransferase gene. *J Inherit Metab Dis*. **38**(5):855-61.

[37] Hixson JE, Vernier DT. (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res.* **31**(3):545-8.

[38] Calero O, Hortigüela R, Bullido MJ, Calero M. (2009) Apolipoprotein E genotyping method by real time PCR, a fast and cost-effective alternative to the TaqMan and FRET assays. *J Neurosci Methods.* **183**(2):238-40.

[39] Schaffner W, Weissmann C. (1973) A rapid, sensitive, and specific method for the determination of protein in dilute solution. *Anal Biochem*. **56**(2):502-14.

[40] Dillon SE, Tsivos D, Knight M, McCann B, Pennington C, Shiel AI, Conway ME, Newson MA, Kauppinen RA, Coulthard EJ. (2017) The impact of ageing reveals distinct roles for human dentate gyrus and CA3 in pattern separation and object recognition memory. *Sci Rep*. **7**(1):14069.

[41] Wood, B., Knight, M.J., Tsivos, D., Oliver, R., Coulthard, E., Kauppinen, R.A. (2015) Magnetic resonance scanning and segmentation procedure at 3T for volumetry of human hippocampal subfields. *Biomed Spectr Imag* **4** 197-208.

[42] Kholodilov N, Neystat M, Oo T, Hutson S Burke R (2000) Upregulation of cytosolic branched chain aminotransferase in substantia nigra following developmental striatal target injury. *Mol Brain Res* **75**:281-286.

[43] Caleo M, Menna E, Chierzi S, Cenni MC, Maffei L (2000) Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system. *Curr Biol* **10**, 1155-1161.

[44] Madeddu F, Naska S, Menna E, Chiellini C, Sweatt AJ,Hutson SM, Benzi L, Maffei M, Maffei L, Bozzi Y (2004) Intraocular delivery of BDNF following visual cortex lesion upregulates cytosolic branched chain aminotransferase (BCATc) in the rat dorsal lateral geniculate nucleus. *Eur J Neurosci* **20**, 580-586.

[45] Castellano S, Macchi F, Scali M, Huang JZ, Bozzi Y (2006) Cytosolic branched chain aminotransferase (BCATc) mRNA is up-regulated in restricted brain areas of BDNF transgenic mice. *Brain Res* **1108**, 12-18.

[46] Rupsingh, R., Borrie, M. Smith, M., Wells, J.L. Bartha, R. (2011) Reduced hippocampal glutamate in Alzheimer disease, *Neurobiol Aging* **32** 802-810.

[47] Francis PT. (2003) Glutamatergic systems in Alzheimer's disease. *Int J Geriatr Psychiatry.* 18(Suppl 1):S15-21. Review.

[48] Revett TJ, Baker GB, Jhamandas J, Kar S. (2013) Glutamate system, amyloid ß peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology. J Psychiatry Neurosci. **38**(1):6-23.

[49] Bell KF, Bennett DA, Cuello AC. (2007) Paradoxical upregulation of glutamatergic presynaptic boutons during mild cognitive impairment. *J Neurosci*. **27**(40):10810-7.

[50] Kashani A, Lepicard E, Poirel O, Videau C, David JP, Fallet-Bianco C, Simon A, Delacourte A, Giros B, Epelbaum J, Betancur C, El Mestikawy S. (2008) Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease. *Neurobiol Aging.* **29**(11):1619-30.

[51] Sokolow S, Luu SH, Nandy K, Miller CA, Vinters HV, Poon WW, Gylys KH. (2012) Preferential accumulation of amyloid-beta in presynaptic glutamatergic terminals (VGluT1 and VGluT2) in Alzheimer's disease cortex*. Neurobiol Dis*. **45**(1):381-7.

[52] Cummings DM, Liu W, Portelius E, Bayram S, Yasvoina M, Ho SH, Smits H, Ali SS, Steinberg R, Pegasiou CM, James OT, Matarin M, Richardson JC, Zetterberg H, Blennow K, Hardy JA, Salih DA, Edwards FA. (2015) First effects of rising amyloid-β in transgenic mouse brain: synaptic transmission and gene expression. *Brain.* **138**(Pt 7):1992-2004.

[53] Yeh CY, Verkhratsky A, Terzieva S, Rodríguez JJ. (2013) Glutamine synthetase in astrocytes from entorhinal cortex of the triple transgenic animal model of Alzheimer's disease is not affected by pathological progression. *Biogerontology.* **14**(6):777-87.

[54] Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ. (2011) Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? *Mol Neurodegener.* **6**:55.

[55] Griffin JW, Bradshaw PC. (2017) Amino Acid Catabolism in Alzheimer's Disease Brain: Friend or Foe? *Oxid Med Cell Longev*. 5472792.

[56] Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, Schmidt AF, Imamura F, Stewart ID, Perry JR, Marney L, Koulman A, Karoly ED, Forouhi NG, Sjögren RJ, Näslund E, Zierath JR, Krook A, Savage DB, Griffin JL, Chaturvedi N, Hingorani AD, Khaw KT, Barroso I, McCarthy MI, O'Rahilly S, Wareham NJ, Langenberg C. (2016) Genetic Predisposition to an Impaired Metabolism of the Branched-Chain Amino Acids and Risk of Type 2 Diabetes: A Mendelian Randomisation Analysis. *PLoS Med*. **13**(11):e1002179.

[57] Larsson SC, Markus HS. (2017) Branched-chain amino acids and Alzheimer's disease: a Mendelian randomization analysis. *Sci Rep*. **7**(1):13604.

[58] Itou M, Kawaguchi T, Taniguchi E, Oku Y, Fukushima N, Ando E, Oriishi T, Uchida Y, Otsuka M, Tanaka S, Iwasaki S, Torii M, Yoshida K, Adachi Y, Suga M, Yoshiyama M, Ibi R, Akiyama Y, Takakura M, Mitsuyama K, Tsuruta O and Sata M. (2009) Branched-chain amino acid supplements reduced ascites and increased the quality of life in a patient with liver cirrhosis: A case report. *Molecular Medicine Reports*. **2**(6):977-81.

[59] Vuille-Dit-Bille RN, Ha-Huy R and Stover JF. (2012) Changes in plasma phenylalanine, isoleucine, leucine, and valine are associated with significant changes in intracranial pressure and jugular venous oxygen saturation in patients with severe traumatic brain injury. *Amino Acids.* **43**(3):1287-96.

[60] Jeter CB, Hergenroeder GW, Ward NH, Moore AN and Dash PK. (2013) Human mild traumatic brain injury decreases circulating branched-chain amino acids and their metabolite levels. *Journal of Neurotrauma*. **30**(8):671-679

[61] Mattick JSA, Kamisoglu K, Ierapetritou MG, Androulakis IP, Berthiaume F. (2013) Branched-chain amino acid supplementation: impact on signaling and relevance to critical illness. *Wiley Interdiscip Rev Syst Biol Med.* **5**(4):449-460.

[62] Sato S, Watanabe A, Muto Y, Suzuki K, Kato A, Moriwaki H, Kato M, Nakamura T; LIV-EN Study Group. (2005) Clinical comparison of branched-chain amino acid (l-Leucine, l-Isoleucine, l-Valine) granules and oral nutrition for hepatic insufficiency in patients with decompensated liver cirrhosis (LIV-EN study). *Hepatol Res.* **31**(4):232-40.

[63] Fukuda K, Yao H, Murai K, Ibayashi S, Fujishima M. (1999) Long-term treatment of portosystemic encephalopathy with oral branched-chain amino acids--a case report. *Fukuoka Igaku Zasshi*. **90**(12):464-9.

[64] Habu D, Nishiguchi S, Nakatani S, Kawamura E, Lee C, Enomoto M, Tamori A, Takeda T, Tanaka T, Shiomi S. (2003) Effect of oral supplementation with branched-chain amino acid granules on serum albumin level in the early stage of cirrhosis: a randomized pilot trial. *Hepatol Res*. **25**(3):312-318.

[65] Mochel, F., Benaich, S., Rabier, D. and Durr, A. (2011) Validation of plasma branched chain amino acids as biomarkers in Huntington disease. *Archives of Neurology*. **68**(2):265-7.

[66] Mochel, F., Charles, P., Seguin, F., Barritault, J., Coussieu, C., Perin, L., Le Bouc, Y., Gervais, C., Carcelain, G., Vassault, A., Feingold, J., Rabier, D. and Durr, A. (2007) Early energy deficit in Huntington disease: identification of a plasma biomarker traceable during disease progression. *PLoS One*. **2**(7):e647.

[67]. Chuang JL, Chuang DT. (2000) Diagnosis and mutational analysis of maple syrup urine disease using cell cultures. *Methods Enzymol.* **324**:413-23.

[68] Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, Agacinski G, Oulhaj A, Bradley KM, Jacoby R, Refsum H. (2010) Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. *PLoS One*. **5**(9):e12244.

[69] Conway, M. E. and Lee, C. (2015) The redox switch that regulates molecular chaperones. *Biomolecular Concepts*, **6** (4). pp. 269-284.

[70] Lewczuk P, Riederer P, O'Bryant SE, Verbeek MM, Dubois B, Visser PJ, Jellinger KA, Engelborghs S, Ramirez A, Parnetti L, Jack CR Jr, Teunissen CE, Hampel H, Lleó A, Jessen F, Glodzik L, de Leon MJ, Fagan AM, Molinuevo JL, Jansen WJ, Winblad B, Shaw LM, Andreasson U, Otto M, Mollenhauer B, Wiltfang J, Turner MR, Zerr I, Handels R, Thompson AG, Johansson G, Ermann N, Trojanowski JQ, Karaca I, Wagner H, Oeckl P, van Waalwijk van Doorn L, Bjerke M, Kapogiannis D, Kuiperij HB, Farotti L, Li Y, Gordon BA, Epelbaum S, Vos SJB, Klijn CJM, Van Nostrand WE, Minguillon C, Schmitz M, Gallo C, Lopez Mato A, Thibaut F, Lista S, Alcolea D, Zetterberg H, Blennow K, Kornhuber J; Members of the WFSBP Task Force Working on this Topic: Peter Riederer, Carla Gallo, Dimitrios Kapogiannis, Andrea Lopez Mato, Florence Thibaut. (2018) Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry. *World J Biol Psychiatry*. **19**(4):244-328.

[71] O'Bryant SE, Xiao G, Barber R, Reisch J, Hall J, Cullum CM, Doody R, Fairchild T, Adams P, Wilhelmsen K, Diaz-Arrastia R; (2011) Texas Alzheimer's Research and Care Consortium. A blood-based algorithm for the detection of Alzheimer's disease. *Dement Geriatr Cogn Disord*. **32**(1):55-62.

Table 1. Clinical presentation and ApoE genotyping of participants.

	CONTROL n (%)	MCI n (%)	ADD n (%)	*p-value*
Gender (female)[§]	28 (56)	10 (46)	11 (69)	0.3603
Age, median IQR[*]	70 (7.5)	74.5 (12.25)	75.5 (15)	0.0279
ApoE, E4 allele[§]	4 (9)	2 (13)	1 (7)	0.9143
Years of education, median IQR[*]	15 (5)	13 (3)	12.5 (5.5)	0.0070
Age left school, median IQR[*]	17 (2)	16 (2)	16.5 (3)	0.0308
IQs score median (IQR) [*]	116.4 (16)	110.7 (13.1)	122.4 (17.5)	0.1093
History of smoking[¥]	24 (48)	11 (50)	9(56.3)	0.9500
Alcohol units per week, median (IQR) [*]	3.5 (9.25)	2 (11.5)	3 (8.25)	0.7389
Family history of dementia Yes [§]	26 (52)	6 (28)	9 (57)	0.1414
Loss of conscious (Yes) [§]	5 (10)	2 (9)	2 (13)	0.9400
Head injury (Yes) [§]	7 (14)	9 (41)	3 (19)	0.0364
Epilepsy (Yes) [*]	1 (2)	0 (0)	1 (6.25)	0.3930

[§]**Chi squared**

[*]**Kruskal-Wallis**

[¥]**Fisher's exact test**

Table 2. BCATc and BCATm (mean ± standard deviation) summarised by participant group.

	Control (n = 45)	MCI (n = 16)	AD (n = 11)	ANOVA F[§] (2, 69)	p	Pairwise Comparison		
						Control vs MCI	Control vs AD	MCI vs AD
BCATc	0.0062 ± 0.0072	0.0213 ± 0.0276	0.0373 ± 0.0275	15.45	<0.0001	0.0121	<0.0001	0.5870
BCATm	0.0077 ± 0.0073	0.0252 ± 0.0315	0.0294 ± 0.0257	9.189	0.0003	0.0053	0.0025	0.8310

[§]One-way ANOVA F-statistic, and p-values from applying Tukey's HSD to all pairwise comparisons.

Table 3. Amino acid levels (μmol/L) in serum of AD, MCI and control participants.

Amino Acids	Control (n = 42)	MCI (n = 17)	AD (n = 11)	ANOVA F (2,67)	p	Pairwise Comparison [a]		
						Control vs MCI	Control vs AD	MCI vs AD
Glutamate	0.8436 ± 0.5192	1.791 ± 1.308	2.370 ± 2.054	10.57	0.0001	0.0103	0.0003	0.3649
Isoleucine	0.6056 ± 0.2861	0.993 ± 0.573	0.734 ± 0.634	4.837	0.0109	0.0077	0.6554	0.2792
Leucine	1.1650 ± 0.5998	1.874 ± 0.978	1.631 ± 1.369	4.620	0.0132	0.0141	0.2476	0.7420
Phenylalanine	0.5356 ± 0.2926	0.773 ± 0.416	0.599 ± 0.435	2.814	0.0670	0.0530	0.8535	0.4044
Tyrosine	0.6735 ± 0.332	1.031 ± 0.468	1.112 ± 1.065	4.477	0.0150	0.0607	0.0490	0.9209
Valine	2.0280 ± 1.0340	3.323 ± 1.824	2.763 ± 1.940	5.347	0.0070	0.0063	0.2833	0.5669

*One-way ANOVA F-statistic, and p-values from applying Tukey's HSD to all pairwise comparisons.

Table 4. Cognitive data (mean ± standard deviation) summarised by participant group.

	Control (n = 50)	MCI (n = 22)	AD (n = 16)	ANOVA F[§] (2, 85)	p	Pairwise Comparison		
						Control vs MCI	Control vs AD	MCI vs AD
MoCA*	27.26 ± 1.780	22.27 ± 2.84	16.44 ± 5.04	90.77	<0.0001	<0.0001	<0.0001	<0.0001
	Control (n = 47)	**MCI (n = 19)**	**AD (n = 12)**	**ANOVA F (2, 75)**	**p**	**Pairwise Comparison**		
						Control vs MCI	**Control vs AD**	**MCI vs AD**
PAL[¥] Mean Reaction Time	2148 ± 454.1	3264 ± 1267	5774 ± 3341	29.73	<0.0001	0.0176	<0.0001	<0.0001
PAL[¥] Total Accuracy	0.695 ± 0.115	0.542 ± 0.108	0.349 ± 0.102	49.80	<0.0001	<0.0001	<0.0001	<0.0001
	Control (n = 50)	**MCI (n = 22)**	**AD (n = 14)**	**ANOVA F (2, 83)**	**p**	**Pairwise Comparison**		
						Control vs MCI	**Control vs AD**	**MCI vs AD**
HVLT[¥] 20 Minute Delay	9.26 ± 2.489	4.364 ± 3.170	1.000 ± 1.414	69.27	<0.0001	<0.0001	<0.0001	0.0007
	Control (n = 41)	**MCI (n = 17)**	**AD (n = 6)**	**ANOVA F (2, 61)**	**p**	**Pairwise Comparison**		
						Control vs MCI	**Control vs AD**	**MCI vs AD**
HVLT 24 Hour Delay	8.976 ± 2.779	3.529 ± 3.204	0 ± 0	42.06	<0.0001	<0.0001	<0.0001	0.0260

*MoCA the Montreal Cognitive Assessment Test

[¥]PAL paired Associate Learning Task

[ζ]HVLT Hopkins verbal learning test (20-minute delay and 24-hour delay)

[§]One-way ANOVA F-statistic, and p-values from applying Tukey's HSD to all pairwise comparisons.

Table 5. Subfield volumes for AD, MCI and control participants.

	Control (n = 44)	MCI (n = 18)	AD (n = 11)	ANOVA F (2, 70)	p	Pairwise Comparison		
						Control vs MCI	Control vs AD	MCI vs AD
CA1	1.713 ± 0.2401	1.410 ± 0.3711	1.370 ± 0.4200	9.486	0.0002	0.0021	0.0040	0.9367
CA2	0.020 ± 0.0051	0.016 ± 0.0050	0.015 ± 0.0050	5.578	0.0057	0.0228	0.0343	0.9621
CA3	0.076 ± 0.0160	0.081 ± 0.0150	0.082 ± 0.0310	0.670	0.5146	0.6734	0.5992	0.9709
Dentate Gyrus	1.087 ± 0.1186	0.955 ± 0.1830	0.886 ± 0.1890	10.62	<0.0001	0.0063	0.0004	0.4493
Subiculum	0.483 ± 0.0631	0.425 ± 0.1060	0.389 ± 0.0718	8.353	0.0006	0.0242	0.0015	0.4344
Hippocampal	3.623 ± 0.3848	3.141 ± 0.6780	3.019 ± 0.7129	9.015	0.0003	0.0046	0.0031	0.8158
Entorhinal	0.579 ± 0.0878	0.529 ±0.1210	0.433 ± 0.1288	9.084	0.0003	0.1941	0.0002	0.0477

Table 6. Correlation analysis with MoCA, BCATm and BCATc

Correlations with MoCA Measure	r	p	Measure	r	p
CA1	0.398	< 0.001	**Leucine**	-0.108	0.375
CA2	0.255	0.029	**Isoleucine**	-0.054	0.662
CA3	-0.234	0.042	**Valine**	-0.089	0.467
Dentate Gyrus	0.420	< 0.001	**Glutamate**	-0.329	0.006
Sub total volume	0.490	< 0.001	**Phenylalanine**	0.011	0.930
Hippocampal	0.400	< 0.001	**Tyrosine**	-0.143	0.240
Entorhinal	0.455	< 0.001			
Correlations with BCATm **Measure**	**r**	**p**	**Measure**	**r**	**p**
CA1	-0.333	0.008	**Leucine**	0.412	0.001
CA2	-0.027	0.832	**Isoleucine**	0.415	0.001
CA3	0.120	0.353	**Valine**	0.457	0.001
Dentate Gyrus	-0.262	0.040	**Glutamate**	0.096	0.442
Sub total volume	-0.209	0.102	**Phenylalanine**	0.356	0.003
Hippocampal	-0.298	0.019	**Tyrosine**	0.356	0.003
Entorhinal	-0.116	0.369			
Correlations with BCATc **Measure**	**r**	**p**	**Measure**	**r**	**p**
CA1	-0.298	0.019	**Leucine**	0.454	<0.001
CA2	-0.058	0.652	**Isoleucine**	0.441	<0.001
CA3	0.056	0.667	**Valine**	0.493	<0.001
Dentate Gyrus	-0.246	0.054	**Glutamate**	0.303	0.013
Sub total volume	-0.192	0.136	**Phenylalanine**	0.385	0.001
Hippocampal	-0.258	0.043	**Tyrosine**	0.415	0.001
Entorhinal	-0.161	0.211			

Table 7 Percentage accuracy under leave one-out-out (L-O-O) cross-validation of baseline linear discriminant model (BCATm, BCATc, MoCA or Dentate Gyrus) and extended model, along with p-value for inclusion of additional parameter in the baseline ordinal regression model. Optimal sensitivity and specificity for MCI and AD combined, and AD only using the ordinal logistic regression model.

	Discriminant Analysis		Logistic regression	MCI or AD	AD
	Accuracy	L-O-O Accuracy	p-value	Sensitivity and Specificity	Sensitivity and Specificity
BCATm	64.4	61.1		0.750; .622	0.750; .705
BCATm + BCATc	65.8	64.4	0.007	0.786; .733	0.917; .770
BCATm + Glutamate	69.7	66.7	0.001	0.704; .718	0.727; .691
BCATm + Valine	65.2	59.1	0.282	0.667; .692	0.727; .600
BCATm + Leucine	62.1	54.5	0.207	0.741; .692	0.636; .600
BCATm + Isoleucine	63.6	59.1	0.506	0.667; .667	0.636; .655
BCATm + Phenylalanine	62.1	56.1	0.710	0.630; .667	0.636; .640
BCATm + Tyrosine	60.6	53.0	0.088	0.630; .667	0.636; .618
BCATc	64.4	64.4		0.750; .796	0.833; .754
BCATm + Glutamate	63.6	59.1	0.007	0.704; .785	0.818; .782
BCATm + Valine	62.1	60.6	0.696	0.704; .744	0.727; .764
BCATm + Leucine	62.1	62.1	0.526	0.704; .744	0.727; .755
BCATm + Isoleucine	66.7	65.2	0.968	0.741; .718	0.727; .764

BCATm + Phenylalanine	59.1	59.1	0.876	0.741; .718	0.727; .764
BCATm + Tyrosine	62.1	60.6	0.212	0.704; .718	0.727; .764
MoCA	81.8	81.8		0.900; .895	0.947; .900
MoCA + Dentate Gyrus	78.1	78.1	0.297	0.897; .909	0.818; 0.903
MoCA + BCATc	93.2	89.0	0.001	1.000; .978	1.000; .902
MoCA + BCATm	89.0	86.3	0.054	0.964; .966	0.917; .969
MoCA + Glutamate	88.4	85.4	0.035	0.926; .976	0.909; .879
MoCA + Valine	87.0	84.1	0.008	0.976; .967	0.818; .914
MoCA + Leucine	89.9	84.1	0.019	0.963; .976	0.727; .948
MoCA + Isoleucine	87.0	85.5	0.044	0.963; .976	0.818; .845
MoCA + Phenylalanine	88.4	85.5	0.030	0.963; .929	0.909; .845
MoCA + Tyrosine	87.0	85.5	0.005	0.963; .952	0.818; 862
Dentate Gyrus	61.6	61.6		0.655; 727	0.727; .774
Dentate Gyrus + BCATc	71.0	71.0	0.002	0.826; .795	0.800; .846
Dentate Gyrus + BCATm	72.6	66.1	0.020	0.897; .708	0.887; .800
Dentate Gyrus + Glutamate	63.9	55.7	0.005	0.864; .795	0.778; .788
Dentate Gyrus + Valine	63.9	60.7	0.069	0.818; .744	0.778; .750

Dentate Gyrus + Leucine	63.9	60.7	0.065	0.818; .821	0.778; .750
Dentate Gyrus + Isoleucine	63.9	63.9	0.151	0.773; .769	0.778; .750
Dentate Gyrus + Phenylalanine	59.0	57.4	0.259	0.773; .718	0.778; .769
Dentate Gyrus + Tyrosine	60.7	52.5	0.096	0.818; .718	0.778; .769
Dentate Gyrus + MoCA	78.1	78.1	<.001	0.897; .919	0.818; .919

FIGURE LEGENDS

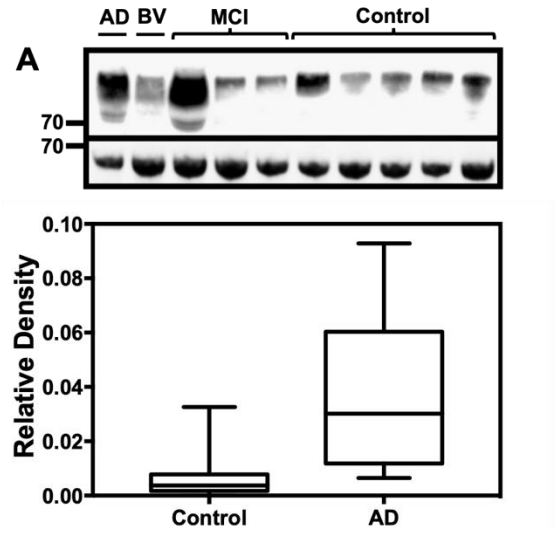
FIGURE 1. Levels of serum BCATc and BCATm for control, MCI and AD participants. The protein concentration of serum samples were calculated using the Schaffner and Weissmann method [39] and separated on a NuPAGE® Novex® 4-12% Bis-Tris followed by Western blot analysis using antibodies specific for BCATc and BCATm (1/1000 dilution). Positive bands were visualised using chemiluminescent HRP substrate and an Odyssey® Fc Imaging System (LI-COR Biosciences). Integrated area densitometry was carried out using ImageJ Ver 1.49 and the results were measured in relation to the relative density of albumin and the total protein. **Panel A:** Sample Western blot analysis of BCATc from control, MCI and AD participants together with albumin loading control. Box plot showing the relative density of serum BCATc for control (n = 45; median 0.003745, range 0.0000532 – 0.03241) and AD participants (n = 11; median 0.03018, range 0.006469 – 0.09285). **Panel B:** Sample western blot analysis of BCATm from control, MCI and AD particpants together with albumin loading control. Box plot showing the relative density of serum BCATm for control (n = 45; median 0.004928, range 0.0003564 – 0.03642) and AD participants (n = 11; median 0.01922, range 0.004722 – 0.08177).

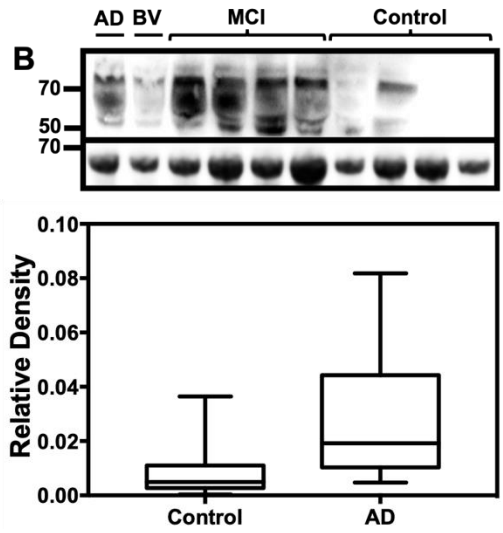
FIGURE 2. Amino acid profiles of control, MCI and AD participants. Amino acid concentrations were calculated relative to deuterium labelled internal standards and normailsed against total protein concentration. **Panel A:** Box plot showing serum glutamate concentration for control (n = 42; median 0.6667, range 0.07649 – 2.171), MCI (n = 17; median 0.9547, range 0.2818 – 4.341) and AD participants (n = 11; median 2.212, range 0.2203 – 7.211)**. Panel B:** Box plot showing serum isoleucine concentration for control (n = 42; median 0.6291, range 0.08 – 1.728), MCI (n = 17; median 0.9008, range 0.387 – 2.651) and AD participants (n = 11; median 0.5541, range 0.242 – 2.508). **Panel C:** Box plot showing serum leucine concentration for control (n = 42; median 1.174, range 0.1649 – 3.762), MCI (n = 17; median 1.703, range 0.8577 – 4.769) and AD participants (n = 11; median, range 0.5615 – 5.429). **Panel D:** Box plot showing serum phenylalanine concentration for control (n = 42;

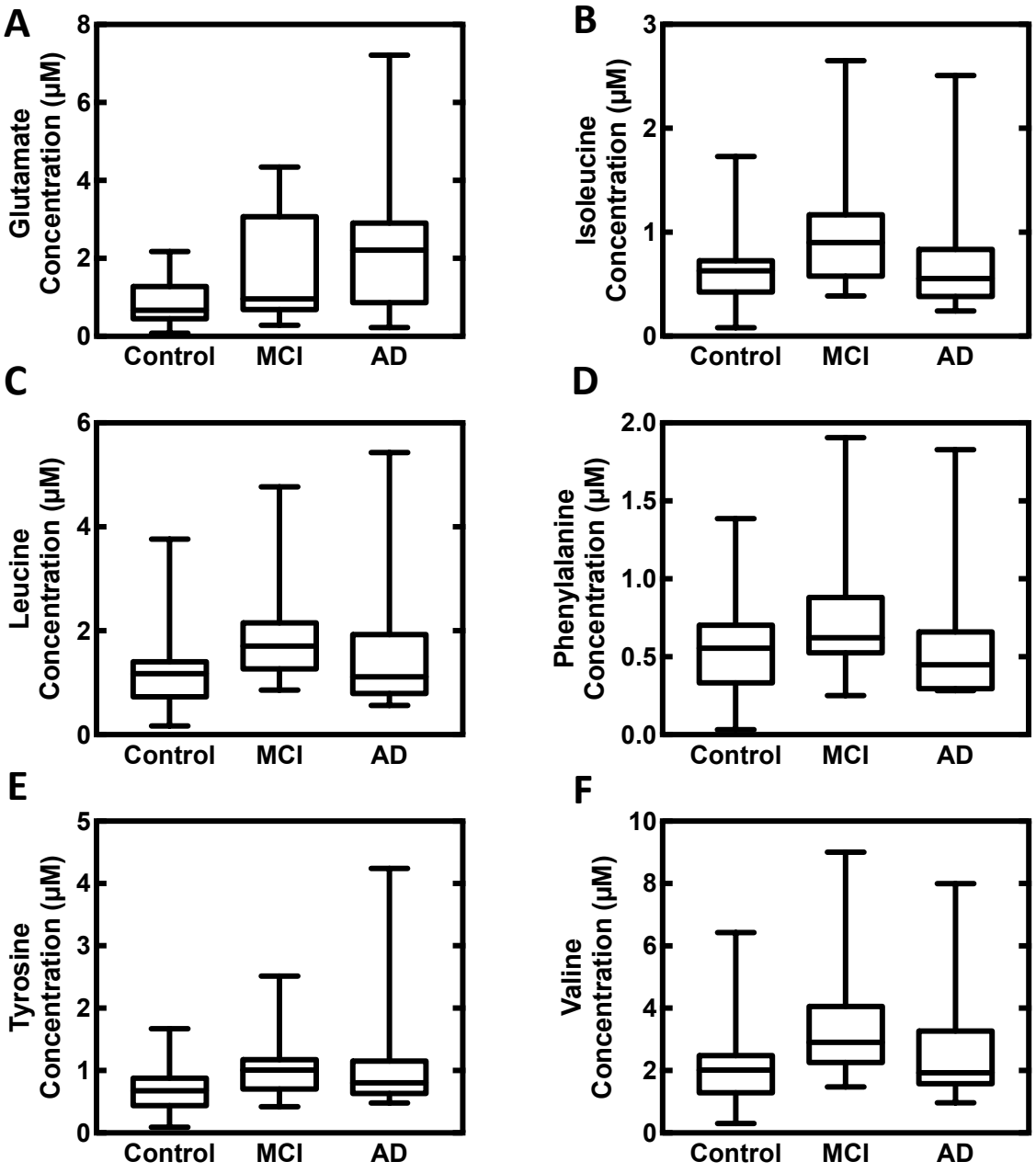
median 0.5552, range 0.03266 – 1.386), MCI (n = 17; median 0.6218, range 0.2508 – 1.905) and AD participants (n = 11; median 0.4479, range 0.2827 – 1.828). **Panel E:** Box plot showing serum tyrosine concentration for control (n = 42; median 0.6735, range 0.09193 – 1.67), MCI (n = 17; median 1.009, range 0.4194– 2.515) and AD participants (n = 11; median 0.8049, range 0.4791 – 4.242). **Panel F:** Box plot showing serum valine concentration for control (n = 42; median 2.019, range 0.3036 – 6.422), MCI (n = 17; median 2.901, range 1.473 – 9.005) and AD participants (n = 11; median 1.928, range 0.9637 – 7.994).

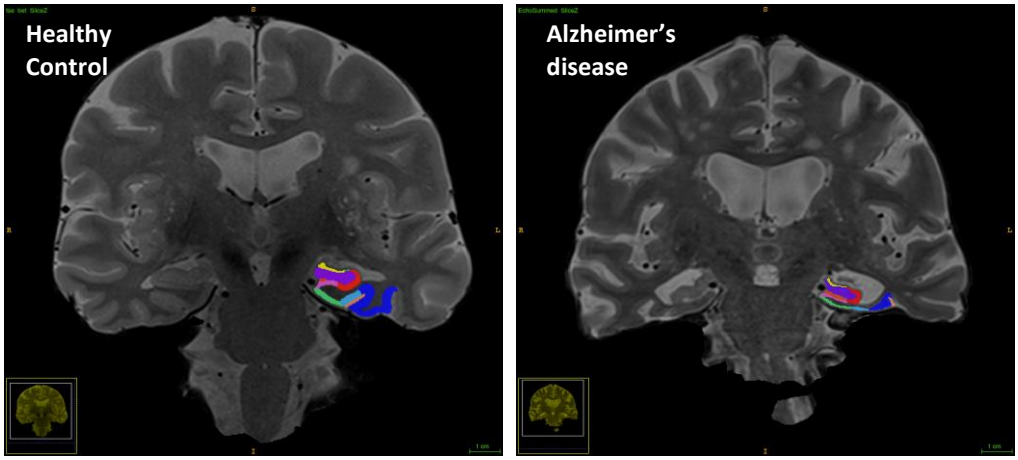
FIGURE 3: T2-weighted coronal MRI images of healthy elderly control (left) and AD patient (right). The Hippocampus of AD patient displays marked atrophy compared to control. Left Hippocampus and surrounding cortices is masked for each subject using ASHS protocol: Red=CA1; Purple=DG; Yellow=CA3; Lime Green=CA2; Pink=Subiculum; Pale Green=Entorhinal Cortex; Light Blue=BA35; Dark Blue=BA36; Orange=Misc; Beige=Collateral Sulcus. (N=73)

FIGURE 4: Plot of estimated probability of MCI against estimated probability of AD. Estimated probabilities derived from the fitted ordinal logistic regression model with MoCA and BCATc as predictor variables (N=72).


AD
BV
MCI
Control
A
70
70
Relative Density
0.10
0.08
0.06
0.04
0.02
0.00
Control
AD


AD
BV
MCI
Control
B
70
50
70
Relative Density
0.10
0.08
0.06
0.04
0.02
0.00
Control
AD


A
Glutamate Concentration (μM)
0
2
4
6
8
Control
MCI
AD
B
Isoleucine Concentration (μM)
0
1
2
3
Control
MCI
AD
C
Leucine Concentration (μM)
0
2
4
6
Control
MCI
AD
D
Phenylalanine Concentration (μM)
0.0
0.5
1.0
1.5
2.0
Control
MCI
AD
E
Tyrosine Concentration (μM)
0
1
2
3
4
5
Control
MCI
AD
F
Valine Concentration (μM)
0
2
4
6
8
10
Control
MCI
AD


Healthy
Control
Alzheimer's
disease