



Targeted lipidomics distinguishes patient subgroups in mild cognitive impairment (MCI) and late onset Alzheimer's disease (LOAD)



Paul L. Wood^{a,*}, Victoria A. Locke^a, Patrick Herling^a, Angelina Passaro^b, Giovanni B. Vigna^b, Stefano Volpato^b, Giuseppe Valacchi^c, Carlo Cervellati^d, Giovanni Zuliani^b

^a *Metabolomics Unit, Dept. of Physiology and Pharmacology, DeBusk College of Osteopathic Medicine, Lincoln Memorial University, 6965 Cumberland Gap Pkwy., Harrogate, TN 37752, United States*

^b *Medical Science Dept., Cardiopulmonary and Internal Medicine, University of Ferrara, Ferrara, Italy*

^c *Department of Life Sciences and Biotechnology, University of Ferrara, Via Luigi Borsari 46, 44121, Ferrara, Italy*

^d *Department of Biomedical and Specialist Surgical Sciences, Section of Medical Biochemistry, Molecular Biology and Genetics, University of Ferrara, via Borsari 46, 44121 Ferrara, Italy*

ARTICLE INFO

Article history:

Received 15 September 2015

Received in revised form 3 November 2015

Accepted 11 November 2015

Available online 14 November 2015

Keywords:

MCI

AD

Plasmalogens

DAG

Patient stratification

ABSTRACT

Background: Diverse research approaches support the concept that a clinical diagnosis of Late-Onset Alzheimer's Disease (LOAD) does not distinguish between subpopulations with differing neuropathologies, including dementia patients with amyloid deposition and dementia patients without amyloid deposition but with cortical thinning. Mild cognitive impairment (MCI) is generally considered the prodromal phase for LOAD, however, while a number of studies have attempted to define plasma biomarkers for the conversion of MCI to LOAD, these studies have not taken into account the heterogeneity of patient cohorts within a clinical phenotype.

Methods: Studies of MCI and LOAD in several laboratories have demonstrated decrements in ethanolamine plasmalogen levels in plasma and brain and increased levels of diacylglycerols in plasma and brain. To further extend these studies and to address the issue of heterogeneity in MCI and LOAD patient groups we investigated the levels of diacylglycerols and ethanolamine plasmalogens in larger cohorts of patients utilizing, high-resolution (0.2 to 2 ppm mass error) mass spectrometry.

Results: For the first time, our lipidomics data clearly stratify both MCI and LOAD subjects into 3 different patient cohorts within each clinical diagnosis. These include i) patients with lower circulating ethanolamine plasmalogen levels; ii) patients with augmented plasma diacylglycerol levels; and iii) patients with neither of these lipid alterations.

Conclusions: These represent the first serum biochemical data to stratify MCI and LOAD patients, advancing efforts to biochemically define patient heterogeneity in cognitive disorders.

General significance: Lipidomics offers a new approach for identifying biomarkers and biological targets in cognitive disorders.

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1. Introduction

Since cognitive testing lacks the sensitivity required to detect the pre-symptomatic stages of LOAD, researchers continue to search for biomarkers of pre-MCI, MCI, and LOAD [1]. However, there currently are no validated biomarkers for the early detection of MCI or LOAD [2]. In the search for biomarkers of cognitive dysfunction it is essential for researchers to address the complex issue of patient heterogeneity in clinically defined MCI and LOAD patient populations [3–5]. Lipidomics is a powerful analytical platform that is being utilized to address this

complexity [6–17]. While a number of lipid alterations have been reported in LOAD, significant discrepancies between laboratories need to be resolved and a number of reported lipid changes require further validation. This literature has been reviewed a number of times but is restricted to LOAD with minimal available lipidomics data for MCI subjects [1,6,14–15,18–19]. However, previous studies of LOAD have consistently demonstrated decrements in ethanolamine plasmalogen (PIsEs) levels in plasma [7–11] and brain [11–16] and increased levels of diacylglycerols (DAGs) in plasma [10–11,17] and brain [11–15]. Pilot studies of MCI plasma and brain also have detected elevated levels of DAGs [10–11], suggesting a role early in the disease process. To extend these observations we performed a targeted high-resolution mass spectrometric analysis of plasma DAGs and PIsEs in larger cohorts of MCI and LOAD subjects.

* Corresponding author.

E-mail address: paul.wood@lmunet.edu (P.L. Wood).

2. Materials and methods

2.1. Study participants

This study conforms to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was conducted accordingly to the guidelines for Good Clinical Practice (European Medicines Agency). Signed informed consent which was written in compliance with local and national ethical guidelines was obtained from each patient or from relatives or a legal guardian prior to patient inclusion in the study [20]. Cognitive function was evaluated with the Mini-Mental State Exam (MMSE) and the Babcock Story Recall Test (BSRT) [20–23]. Serum was collected and stored at -80 °C for biochemical analyses.

2.2. Study participants: elderly controls (Table 1)

Elderly controls with no evidence of cognitive decline were included in the study.

2.3. Study participants: mild cognitive impairment (MCI, Table 1)

The diagnosis of MCI was made by trained geriatricians based on the presence of short/long-term memory impairment, with/without impairment in other single or multiple cognitive domains, in an individual who didn't meet the standardized criteria for dementia [22]. MMSE along with a battery of neuropsychological tests was used to evaluate the degree of cognitive impairment [23]. Patients with MCI due to known causes (e.g. depression, extensive white matter pathology, vitamin B12 deficiency) were excluded. Subjects affected by severe congestive heart failure (New York Heart Association class III–IV), severe liver or kidney disease, severe chronic obstructive pulmonary disease, and cancer, and those taking NSAIDs, antibiotics or steroids at the time of recruitment were also excluded. Only patients still independent in the activities of daily living (ADLs) were included in the study [20].

2.4. Study participants: late onset Alzheimer's Disease (LOAD, Table 1)

A diagnosis of LOAD was made by trained geriatricians according to the NINCDS-ADRDA criteria [22]. All patients (controls, MCI, and LOAD) underwent a brain Computer Tomography (CT) scan at baseline performed with a third-generation scanner at 10 mm thickness (SOMATOM HQ, Siemens Healthcare, Milan, Italy). The CT information was used to support the clinical diagnosis and to diagnose possible brain pathologies associated with secondary cognitive impairment.

2.5. Lipid extraction and analysis

Lipids were extracted from 100 µL of serum with methy-tert-butyl ether and methanol containing [²H₈]arachidonic acid, [¹³C₁₈]stearic acid, [¹³C₃]DAG 36:2, [²H₃₁]phosphatidylethanolamine 34:1, and bromocriptine as internal standards [10–11,22]. Extracts were dried by centrifugal vacuum evaporation and dissolved in isopropanol:methanol:chloroform (4:2:1) containing 15 mM ammonium acetate. Constant infusion lipidomics (5 µL per min) were performed utilizing high-resolution

(140,000 at 200 amu; 0.2–2 ppm mass error) data acquisition [24–25] on an orbitrap mass spectrometer (Thermo Q Exactive). The spray voltage was 3.3 kV, the sheath gas was 10, the capillary temperature was 320 °C, and the S lens RF was 50. Washes (500 µL) with methanol followed by hexane/ethyl acetate (3:2), between samples, were used to minimize ghost effects. In positive ion electrospray ionization (ESI), the ammonium adducts of diacylglycerols (DAG) were quantitated. The cation of bromocriptine was used to monitor for potential mass axis drift. Relative DAG levels were obtained by determining the ratio of the endogenous DAG peak area to the peak area of 1 nanomole of the internal standard [¹³C₃]DAG 36:2. In negative ion ESI, the anions of phosphatidylethanolamines (PtdE) were quantitated. The anion of bromocriptine was used to monitor for potential mass axis drift. Relative PtdE levels were obtained by determining the ratio of the endogenous PtdE peak area to the peak area of 1 nanomole of the internal standard [²H₃₁]PtdE 34:1.

2.6. Statistical analysis

R values (ratio of endogenous lipid peak area to the peak area of an appropriate internal standard) were calculated. Data are presented as mean ± SEM. Data were analyzed with the Kruskal–Wallis test, followed by the Dunn's t test to compare MCI and LOAD groups to the controls [10–11].

3. Results and discussion

3.1. Diacylglycerols (DAGs)

For the current targeted lipidomics study we stratified the MCI and AD patient groups (Table 1) based upon their Mini-Mental State Exam (MMSE) score as we have previously reported [10]. In addition, the Babcock Story Recall Test (BSRT) [20–21] was utilized to support the MMSE scores with regard to evaluation of decline in cognitive function for individual patients (Table 2). Utilizing this stratified design our targeted lipidomics analyses detected a number of DAGs that were elevated in the serum of MCI and LOAD patients as we [10–11] and others [14] have previously reported. DAG 34:2 and DAG 36:2 were robust biomarkers being elevated in both MCI and LOAD patients with an MMSE score of 10 to 18 while DAG 36:2 also was elevated in MCI and LOAD patients with an MMSE score of 19 to 24 (Table 2). Our previous studies have shown that DAG 34:2 is comprised mainly of DAG 16:0/18:2 while DAG 36:2 is 66% DAG 18:1/18:1 and 33% DAG 18:0/18:2, with no differences in fatty acid composition of these DAGs between aged-matched control, MCI and LOAD subjects [14]. Elevations in these lipid levels, which are normally tightly regulated [26–27], is of significant clinical interest considering the diverse roles of DAGs as precursors of structural glycerophospholipids and neutral lipids, as structural components of nuclear and endoplasmic reticular membranes, as mediators of signal transduction, as mediators of nuclear signal transduction, and in Golgi transport carrier biogenesis [26–27]. Alterations in any or all of these DAG-dependent functions could contribute to the development of neuronal dysfunction and ultimately to cognitive decline in MCI and LOAD patients.

Table 1
Patient demographics for the clinical cohorts.

Parameter	Controls	MCI-1	MCI-2	LOAD-1	LOAD-2
MMSE	25–30	19–24	10–18	19–24	10–18
N	51	64	13	57	33
Age	76 ± 0.8	78.2 ± 0.062	77 ± 1.3	78 ± 0.7	78.2 ± 0.7
Gender (% Male)	35.3	23.4	38.5	22.8	30.3
Glucose (mg/dL)	95.3 ± 2.1	95.8 ± 1.5	93.4 ± 2.5	96.5 ± 2.5	95.3 ± 3.3
Total Cholesterol (mg/dL)	207.4 ± 4.1	212.8 ± 4.4	210.8 ± 15.6	213.8 ± 4.7	218.3 ± 6.6
HDL/LDL Ratio	0.55 ± 4.1	0.52 ± 0.022	0.44 ± 0.057	0.53 ± 0.023	0.51 ± 0.031
Triglycerides (mg/dL)	109.9 ± 7.7	115.7 ± 6.4	150.4 ± 29.1	110.7 ± 6.0	110.5 ± 7.8

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