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**Evidence that the Kennedy and polyamine pathways are dysregulated in human brain in cases of dementia with Lewy bodies**

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## Abstract

Disruptions of brain metabolism are considered integral to the pathogenesis of dementia, but thus far little is known of how dementia with Lewy bodies (DLB) impacts the brain metabolome. DLB is less well known than other neurodegenerative diseases such as Alzheimer's and Parkinson's disease which is perhaps why it is under-investigated. This exploratory study aimed to address current knowledge gaps in DLB research and search for potentially targetable biochemical pathways for therapeutics. It also aimed to better understand metabolic similarities and differences with other dementias. Combined metabolomic analyses of  $^1\text{H}$  NMR and tandem mass spectrometry of neocortical post-mortem brain tissue (Brodmann region 7) from autopsy confirmed cases of DLB (n=15) were compared with age/gender-matched, non-cognitively impaired healthy controls (n=30). Following correction for multiple comparisons, only 2 metabolites from a total of 219 measured compounds significantly differed. Putrescine was suppressed (55.4%) in DLB and O-phosphocholine was elevated (52.5%). We identified a panel of 5 metabolites (PC aa C38:4, O-Phosphocholine, putrescine, 4-Aminobutyrate, and SM C16:0) capable of accurately discriminating between DLB and control subjects. Deep Learning (DL) provided the best predictive model following 10-fold cross validation (AUROC (95% CI) = 0.80 (0.60-1.0)) with sensitivity and specificity equal to 0.92 and 0.88, respectively. Altered brain levels of putrescine and O-phosphocholine indicate that the Kennedy pathway and polyamine metabolism are perturbed in DLB. These are accompanied by a consistent underlying trend of lipid dysregulation. As yet it is unclear whether these are a cause or consequence of DLB onset.

**Keywords:** Dementia with Lewy Bodies; brain; metabolomics;  $^1\text{H}$  NMR; targeted mass spectrometry; metabolic pathways.

## **1. Introduction**

Dementia with Lewy bodies (DLB) is one of the leading causes of progressive and degenerative dementia after Alzheimer's disease (AD) (Walker et al., 2015). It is characterized clinically by fluctuating cognition (McKeith et al., 1992), visual hallucination (Perry et al., 1990) and accompanying spontaneous parkinsonism (McKeith et al., 1994). DLB typically leads to disability and ultimately death (Arnaoutoglou et al., 2019). The median interval between first presentation of cognitive symptoms and death is about 4 years, which is markedly shorter than AD (Price et al., 2017).

The first international consensus diagnostic criteria on DLB was published in 1996 (McKeith et al., 1996), and updated recently in 2017 (McKeith et al., 2017). The revised DLB consensus criteria generate categories of probable and possible DLB according to the presence of essential, core clinical and supportive features as well as indicative and supportive biomarkers. The first three core clinical features include fluctuating cognition, recurrent visual hallucinations, and rapid eye movement sleep disorder (McKeith et al., 2017). In a US population-based study, the incidence rate of DLB was 31.6 per 100,000 people over the age of 65 (Savica et al., 2013). However, this incidence rate is likely an underestimate as the study only included patients with a diagnosis of a parkinsonian disorder (PD) (Walker et al., 2015). In a French study based on screening for symptoms of PD and cognitive impairment, the incidence rate of DLB was 112 per 100,000 people over the age of 65 (Perez et al., 2010). DLB accounted for about 4% all community-diagnosed dementias in a systematic review (Vann Jones and O'Brien, 2014). This frequency can be as high as 20-24% if a detailed neurological examination is undertaken (Aarsland et al., 2008), and as high as 45% following pathological examination of the brain postmortem (PM) (Toledo et al., 2013). Collectively, these findings suggest that DLB is still clinically underdiagnosed because of the difficulty in the objective assessment of

clinical features and the clinical and pathological overlap with other neurodegenerative diseases (Savica et al., 2013; Vann Jones and O'Brien, 2014).

Accumulation of intracytoplasmic misfolded  $\alpha$ -synuclein ( $\alpha$ -syn) inclusions in detergent-insoluble neuronal aggregates known as Lewy bodies are the neuropathologic hallmarks of DLB; however, they are also key neuropathological features of PD (Spillantini et al., 1997). Clinically, DLB may be distinguished from PD dementia by development of the neurocognitive disorder before, concurrently, or within 1 year of onset of motor symptoms (McKeith et al., 1996). However, neuropathologically distinguishing DLB from PD with dementia is complicated because of the common pathologies (Walker et al., 2019). Moreover, 28% of DLB cases had concomitant pathology sufficient to be diagnosed as AD (Walker et al., 2019). Currently, no biomarkers for DLB are available (McKeith et al., 2017).

Over the past few decades, “omics” technologies have emerged as powerful approaches to elucidate biologic mechanisms of pathogenesis and to discover novel biomarkers of neurodegenerative diseases (Alaaeddine et al., 2017; Avramouli and Vlamos, 2017). In terms of dementia including DLB, some efforts have been made to detect biochemical pathway alterations and to differentially diagnose one subtype from the others, using proteomic (Henderson et al., 2017; Remnestal et al., 2016) and lipidomic approaches (Touboul and Gaudin, 2014; Wood et al., 2018). Metabolomics employs high-throughput, analytical chemistry techniques to investigate all metabolites present within biological systems. These metabolites, which include amino acids, sugars, lipids, and other organic molecules, are the closest compounds to the phenotype of living systems (Gonzalez-Riano et al., 2016). Recently, our research groups have identified biochemical profiles and developed novel biomarkers of various neurodegenerative diseases such as AD (Graham et al., 2015; Yilmaz et al., 2017), Huntington’s disease

(Graham et al., 2016; Graham et al., 2018a) and PD (Graham et al., 2018b; Graham et al., 2018c) using targeted and untargeted metabolomic platforms.

Most metabolomic studies have focused on the discovery of biomarkers from biofluids because of limited accessibility to brain tissue (Gonzalez-Riano et al., 2016). However, the metabolomic analysis of brain tissue is still valuable for elucidating the pathological processes and discovering novel CNS biomarkers for neurodegenerative diseases as evidenced by Varma et al. (2018) (Varma et al., 2018). The present exploratory study applied quantitative metabolomics to autopsy confirmed cases of DLB and compared profiles to non-demented control cases. The purpose was to understand the aberrations in metabolism, and to identify potential novel therapeutic targets/pathways. Our principal objective in this exploratory study was to identify and characterize central biomarkers of the disease using an array of machine learning methodologies, while investigating the fundamental biochemical changes associated with the disease.

## **2. Results**

In this exploratory study, we accurately identified and quantified 219 metabolites in PM brain extracts using a combination of  $^1\text{H}$  NMR and direct injection liquid chromatography coupled with mass spectrometry (DI-LC-MS/MS). The results of the univariate analysis are available as Supplementary Table S1. A total of 69 metabolites were significantly different between DLB and control cases ( $p < 0.05$ ). After correction for multiple comparisons only 2 metabolites were significantly different ( $q < 0.05$ ). These were putrescine which was decreased by 55.4% in DLB ( $q = 0.0219$ ), and O-phosphocholine which was increased by 52.5% in DLB ( $q = 0.0219$ ).

Based on logistic regression, a combination of Putrescine, alpha-AAA, sn-Glycero-3-phosphocholine, Asp and PC aa C36:1 markers achieved good DLB diagnosis with an AUC (95%CI) = 0.882 (0.72- 1) with sensitivity = 0.917 (0.917 ~ 1.000) and specificity= 0.708 (0.526 ~ 0.890) after 10-fold cross-validation. The logistic regression model built in this exploratory study is represented as follows:  
$$\text{logit}(P) = \log(P / (1 - P)) = -1.708 - 0.869 \text{ Putrescine} + 0.661 \text{ alpha-AAA} + 0.675 \text{ sn-Glycero-3-phosphocholine} + 1.733 \text{ Asp} - 1.439 \text{ PC aa C36:1}$$

For the development of a biomarker panel discriminating DLB from control cases those metabolites with  $p < 0.05$  were processed using a variety of machine learning techniques. Deep Learning was compared with five other frequently used artificial intelligence methods: RF, SVM, LDA, PAM, and GLM. The performance indices of these include ROC AUC values (including sensitivity and specificity) and 95% CIs which are reported in Table 1. DL performed the best in distinguishing between DLB and control PM brain extracts.

After adding gender, age, and postmortem interval (PMI) to our machine-based learning models, the data shows that demographic variables including PMI, age, and gender have no effect on the metabolomics only results. To understand the diagnostic value of the available demographics variables, we analyzed them alone, and we did not see any improvement (Supplementary statistics). Also, there are no significant correlations between the potential tissue bias or demographic variables and the important metabolites. This justifies the findings in the ML methodology and shows that the demographic variables and the potential tissue bias have no effect on the metabolomics only results. (Supplementary statistics).

## **2.1. Pathway results**

Table 2 displays the results of the pathway analysis. Twelve metabolic pathways were found to be moderately perturbed ( $p < 0.05$ ) in DLB PM brain extracts. The major perturbed biochemical pathways

include: glycerophospholipid metabolism ( $p=2.66 \times 10^{-5}$ ), arginine and proline metabolism ( $p=5.62 \times 10^{-4}$ ), and alanine, aspartate and glutamate metabolism ( $p=5.92 \times 10^{-4}$ ).

Figure 1 summarizes the main biosynthetic and catabolic reactions of the major metabolite classes that are directly linked to the formation of  $\alpha$ -syn, A $\beta$ , Tau, and Lewy bodies and their interactions with the metabolites that we identified to be at significantly different concentrations between DLB and control extracts before obtaining positive false discovery rate (FDR, q values). Figure 2 summarizes the perturbation of polyamine and biogenic amine metabolism based on the varying concentrations of the metabolites measured in our study before applying FDR. Supplementary Figure 1 highlights the changes in energy metabolism intermediates, the amino acids that donate their carbon skeletons to the citric acid cycle intermediates, as well as the ketone body synthesis pathway in DLB brains before multiple comparisons correction tests.

### **3. Discussion**

After AD, DLB is one of the most common types of senile, degenerative dementia. DLB is characterized by the presence of cytoplasmic inclusions of highly conserved amyloidogenic- $\alpha$ -syn proteins that reflects tauopathy in AD (Figure 1). Under normal conditions  $\alpha$ -synuclein exists as an arbitrarily structured and natively unfolded protein, existing as a monomer within the cytoplasm. Misfolding and aggregation of this protein in Lewy body plaques is a pathological characteristic of DLB, little is understood about the effects of  $\alpha$ -syn on the physical properties and composition of the membrane lipid bilayer. A recent review suggested that changes to  $\alpha$ -syn structure are initiated when this protein binds and interacts with lipid surfaces such as phospholipid bilayers of lipid membranes and lipid droplets (Kim et al., 2014).



To better understand the metabolic disturbances underlying DLB pathology we undertook the first <sup>1</sup>H NMR and DI-LC-MS/MS metabolomic analysis of brain tissue from autopsy-confirmed cases of DLB and compared these to tissue from cognitively normal control cases. A panel of 5 metabolites (PC aa C38:4, O-Phosphocholine, putrescine, 4-Aminobutyrate, and SM C16:0) capable of accurately differentiate DLB cases from control was determined by machine learning techniques. After exhaustive testing, we found that Deep Learning performed the best following cross-validation. It had an AUC (95% CI) = 0.8048 (0.6048-1) with sensitivity and specificity equal to 91.7% and 87.8%, respectively.

From more than 200 measured metabolites only two, putrescine and O-phosphocholine, were significantly different after multiple comparisons correction. These differed markedly, brain putrescine levels appeared substantially depressed in DLB pathology, whereas in contrast, O-phosphocholine was substantially elevated. Putrescine is a polyamine, and the maintenance of normal polyamine metabolism is essential for a wide variety of basic cellular functions including cellular proliferation, growth, differentiation, and especially neuronal activities and axonal integrity (Miller-Fleming et al., 2015; Ramani et al., 2014). Several studies highlight that polyamines may modulate cognitive functions, therefore, dysregulation in the polyamine metabolic pathway may impact normal cognitive function (Gupta et al., 2013; Sigrist et al., 2014). Altered polyamine metabolism is associated with certain brain disorders. Excessive polyamine catabolism can lead oxidative stress increasing inflammatory responses, possibly occurring in cases of stroke and several neurological diseases (Hussain et al., 2017). Polyamine levels are thought to be affected by aging, but the change may vary considerably among tissues and age groups. Plasma levels of putrescine (along with spermidine and spermine) significantly decreased with advancing age, yet levels of their ornithine precursor are unchanged (Pan et al., 2018). Polyamine content is very tightly controlled with the key enzymes in biosynthesis and interconversion having multiple levels of regulation in response to hormonal stimulation and polyamine content. The likely

molecular cause for suppressed putrescine levels in DLB, would be the inhibition or interference of spermidine/spermine N1-acetyltransferase (SSAT). For instance, it has been shown that that SSAT null mice (SSAT<sup>-/-</sup>) have significantly lower brain levels of putrescine than the wild-type (Sandusky-Beltran et al., 2019). Therefore, it is possible that DLB pathology, perhaps via  $\alpha$ -syn, affects SSAT activity. This is merely speculation, but it is known that tau pathology elevates brain putrescine rather than suppressing it (Sandusky-Beltran et al., 2019).

O-phosphocholine is an intermediate molecule in the synthesis of phosphatidylcholine in tissues. The increased brain levels of O-phosphocholine in DLB cases were associated with a general trend towards lower levels in many different species of phosphatidylcholine. This suggests that phosphatidylcholine synthesis is impaired during or after DLB pathogenesis. There is some evidence for this. Phosphatidylcholines (PCs) with 20:5 substitutions are decreased in several types of dementia including DLB with intermediate Alzheimer's pathology, DLB with Alzheimer's pathology, and Parkinson's disease subjects (Wood et al., 2018). Total PC and specific PC species levels have been proposed as valid biomarkers, with diminished PC levels in the CSF of patients with AD accompanied by lowered LPC and increased PC hydrolytic products such as glycerophosphocholine and phosphocholine, suggesting that PC breakdown might be enhanced in dementia pathogenesis (Walter et al., 2004).

Disturbances in lipid metabolic pathways have emerged as a frequent biochemical feature of DLB and may play a part in the severe neurodegeneration responsible for cognitive decline. The majority of published articles affirm lipids as the most consistently altered class of molecules in dementia (Koal et al., 2015; Touboul and Gaudin, 2014; Varma et al., 2018; Wood et al., 2018). Among these, changes in sphingomyelin have been reported and are considered to be important components of lipid rafts associated with early, preclinical AD (Koal et al., 2015). Although not statistically significant, we report

concentrations of sn-glycero-3-phosphocholine to be higher in DLB brain extracts while we found several sphingomyelins to be downregulated here. This is interesting due to their biochemical connection with ceramide synthesis, an essential pathway affecting A $\beta$ , tau, and Lewy body synthesis (Figure 1). Typically, unesterified free fatty acids entering brain cells are incorporated into glycerolipids or ceramides in the endoplasmic reticulum. Then glycerolipids and ether lipids are converted to diacyl or acyl-alkyl phosphatidylcholine species through the Kennedy pathway. Phospholipase and sphingomyelinase enzymes metabolize phosphatidylcholines to recycle phosphatidic acid and diacylglycerol or to generate sphingomyelin. It is well known that these lipids are essential for the formation of lipid rafts, which are crucial for neuronal function and structural domains. High ceramide/sphingomyelin ratios are due to the remodeling of lipid rafts, considered one of the essential elements in A $\beta$  production in AD and DLB. Calmodulin kinase activity can be augmented by dysregulation of calcium signaling (data not presented), which in turn results in tau hyperphosphorylation in coordination with the ceramide-PPA2-GSK3 $\beta$  pathway. A recent systematic review provided evidence that several metabolites, including lipids (mainly phosphatidyl choline (PC), lyso PC, sphingomyelin (SM), and high density lipoproteins (HDL) subfractions), and steroids are associated with changes in cognitive performance and increasing risk of dementia (Jiang et al., 2019).

Higher levels of  $\alpha$ -syn in DLB brain extracts may be linked to the changes in the composition of endogenous brain fatty acid species. A recent study by our group provided an insight into the changes of fatty acid metabolism in the development of both AD and DLB (Nasaruddin et al., 2018). Regulation of chemical signaling in neuronal cells is achieved by the fusion of the active zone of the nerve terminal plasma membrane with the synaptic vesicles (Sudhof, 2004).  $\alpha$ -syn has been known to have specific interactions with the presynaptic membranes, including presynaptic active zones and small synaptic vesicles (Fortin et al., 2004). It has also been suggested that when isolated  $\alpha$ -syn monomers from human

neurons are exposed to synthetic lipid membranes, they readily bind to the membrane surface and form subsequent dimers and oligomers (Davidson et al., 1998). Such an interaction is thought to induce a dramatic change in  $\alpha$ -syn structure from its unfolded form to a folded  $\alpha$ -helical secondary structure. How such membrane-dependent mechanisms are related to lipid content, neurodegeneration, and the aggregation propensity of  $\alpha$ -syn remain largely unknown and further work is required to uncover the direct relation between them.

There are a number of strengths to the present study. These include the standardized brain collections, the use of age- and gender-matched controls, and a combined quantitative metabolomic approach - providing the most comprehensive metabolite coverage of DLB brain thus far. The relatively small sample size is one limitation, but there is inherent difficulty in obtaining such specialized and well characterized tissues. The examination of well characterized samples, even small sizes can develop models of high diagnostic accuracy. It would be worthwhile to expand the analysis carried out here to other brain regions to assess the wider metabolic disturbances in the DLB brain. Brain tissue is far from an ideal matrix for discovering dementia biomarkers, and it will be necessary to validate our findings in more accessible, non-invasive biomatrices such as blood serum/plasma as evidenced by Varma et al. for AD (Varma et al., 2018). Another major limitation which may have provided some additional, useful insight to our exploratory study is the lack of medication information on which each of our donors were taking prior to death. This is particularly important when one considers the effects of some drugs on specific metabolites such as phosphocholine and acetylcholine.

#### **4. Conclusions**

Herein we report for the first time the use of quantitative metabolomics to biochemically profile PM human brain from people who died from DLB and compared them with healthy, non-cognitively impaired controls. We identified a potential biomarker panel which is capable of accurately

discriminating DLB from control brain with a high degree of accuracy. Although brain tissue is not an ideal matrix for developing biomarker tests, there is value in identifying central biomarkers for subsequently measuring in more accessible biomatrices, potentially for diagnosing DLB. Improved diagnostic tests would allow for better patient stratification potentially providing more effective treatment modalities when these become available. To this end, we have identified biochemical pathways perturbed in the presence of the disease. We have highlighted the Kennedy pathway and polyamine metabolism in particular, which have been previously linked with other neurodegenerative diseases. Furthermore, we have highlighted lipid and energy metabolism as linked to the etiopathophysiology of DLB. Our findings provide new insights into the biochemical perturbations that accompany neurodegeneration in DLB. Further work is required to determine the translational potential of our results and how these could benefit DLB patients in the future.

## **5. Experimental Procedure**

### **5.1. Samples**

Human brain samples were collected from pathologically confirmed DLB patients and control subjects with no concomitant neurological disorders. Tissue samples were harvested from the neocortex (Brodmann region 7) of DLB patients (n=15) and age-, and gender-matched control subjects (n=30). Tissues were provided by the Brains for Dementia Research Group, Institute of Clinical Neurosciences, School of Clinical Sciences, University of Bristol, Bristol, UK. This exploratory study was approved by the Beaumont Health System's Human Investigation Committee (HIC No.: 2018-387). The methods were carried out in accordance with the approved guidelines. Details such as age, gender, race, and PM delay can be found in Table S2 in the Supplementary Materials. Limited information on brain pH and cause of death are also provided.

## 5.2. NMR Analysis

Samples were analyzed as previously described by our group (Graham et al., 2016; Graham et al., 2017a; Graham et al., 2017b; Graham et al., 2018c). In brief, samples previously stored at  $-80^{\circ}\text{C}$  were lyophilized and milled to a fine powder under liquid nitrogen minimizing heat production. For  $^1\text{H}$  NMR, 50 mg samples were extracted in 50% methanol/water (1 g/mL) in a sterile 2 mL Eppendorf tube. Samples were mixed for 20 min and sonicated for 20 min at  $4^{\circ}\text{C}$ . Macromolecules and larger proteins were removed via centrifugation at  $13,000\times g$  at  $4^{\circ}\text{C}$  for 30 min. Supernatants were collected, dried under vacuum using a Savant DNA SpeedVac (Thermo Scientific, Waltham, MA USA), and reconstituted in 285  $\mu\text{L}$  of 50 mM potassium phosphate buffer (pH 7.0), 30  $\mu\text{L}$  of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and 35  $\mu\text{L}$  of  $\text{D}_2\text{O}$  (Ravanbakhsh et al., 2015). 200  $\mu\text{L}$  of the reconstituted sample was transferred to a 3 mm Bruker NMR tube for analysis. All samples were housed at  $4^{\circ}\text{C}$  in a thermostatically controlled SampleJet autosampler (Bruker-Biospin, USA) and heated to room temperature over 3 min prior to analysis by NMR.

All 1D  $^1\text{H}$  NMR data were recorded at 300 ( $\pm 0.5$ ) K on a Bruker Ascend HD 600 MHz spectrometer (Bruker-Biospin, Billerica, MA, USA) coupled with a 5 mm TCI cryoprobe in a randomized fashion. For each sample, 256 transients were collected as 64k data points with a spectral width of 11.964 Hz (20ppm), using a pulse sequence called CPP WaterSupp (Bruker pulse program: puseoesypr1d) developed by Mercier et al. (Mercier et al., 2011) and an inter-pulse delay of 9.65s. The data collection protocol included a 3 min. equilibration period, fast 3D shimming using the z-axis profile of the  $^2\text{H}$  NMR solvent signal, receiver gain adjustment, and acquisition. The free induction decay signal was zero filled to 128k points prior to Fourier transformation, and 0.1 Hz of line broadening was applied. The singlet produced by the DSS methyl groups was used as an internal standard for chemical shift referencing (set to 0 ppm, concentration 500  $\mu\text{M}$ ) and for quantification. All  $^1\text{H}$ -NMR spectra were

processed and analyzed using the Chenomx NMR Suite Professional Software package version 8.1 (Chenomx Inc, Edmonton, AB).

### **5.3. Targeted Mass Spectrometry Analysis**

Metabolite quantification in PM brain tissue was completed as previously described by our group using the commercially available AbsoluteIDQ p180 Kit (Biocrates Life Sciences AG) (Graham et al., 2017a; Graham et al., 2017b; Graham et al., 2018a; Graham et al., 2018c). In brief, 10 mg of lyophilized and powdered brain tissue was extracted using 300  $\mu$ l of extraction solvent (85% ethanol and 15% phosphate-buffered saline solution). The samples were ultrasonicated for 10 min on ice and vortexed for 1 min. Proteins and other impurities were removed by centrifugation at 13000 $\times$ g for 15 min at 4°C. The supernatant was collected and 10  $\mu$ L was used for analysis. Data were acquired using an Acquity I-class (Waters, USA) coupled with a Xevo TQ-S mass spectrometer (Waters, USA). Metabolite concentrations were calculated using the MetIDQ software (Biocrates Life Sciences AG).

### **5.4. Statistical Analysis**

#### **5.4.1. Data Preparation**

Missing values were detected and replaced with a small value (half of the minimum positive values in the original data) assumed to be the detection limit. The assumption of this approach is that most missing values are due to low abundance metabolites (i.e. below the detection limit). This method of sample normalization allows for general-purpose adjustment for differences among the samples; data transformation and scaling are two different approaches to make individual features more comparable. In this data set, the log value of each metabolite was centered by its mean and auto scaled by its standard deviation. Data were subsequently quantile normalized to reduce sample-to-sample variation. We used logistic regression to define relative contributions of different features on the results.

### **5.4.2. Software Tools**

We used the H2O R package (Candel et al., 2018) (Author The H2O.ai team Maintainer Tom Kraljevic <tomk@0xdata.com>) to tune the parameters of the Deep Learning (DL) model and the caret R package (<https://cran.r-project.org/web/packages/caret/caret.pdf>, Maintainer Max Kuhn <mxkuhn@gmail.com>, December 10, 2017 ) to tune the parameters in the other artificial intelligence models which include DL, random forest (RF), support vector machine (SVM), linear discriminant analysis (LDA), prediction analysis for microarrays (PAM), and generalized linear model (GLM) (Kuhn, 2008). The variable importance functions varImp in H2O and varImp in caret R packages were utilized to rank the model features in each of the predictive algorithms. Further, we employed the pROC R package to compute area under the curve (AUC) of a receiver-operating characteristic (ROC) curve, plus specificity and sensitivity values to assess the overall performance of the models (Robin et al., 2011).

### **5.4.3. Modeling & Evaluation**

The data were split into training (80%) and test sets (20%). 10-fold cross validation on the training data was performed during the model construction process. In addition, the process was repeated ten times and the average AUC, sensitivity, specificity and 95% CIs for the test set were calculated. The contribution of a feature to the model performance is considered using a model-based approach. We ranked the importance of the features in each of the predictive algorithms by using the variable importance functions varImp in H2O and varImp in caret R packages (See a detailed description in the supplementary information).

### **5.4.4. Univariate analysis**



All data were analyzed using MetaboAnalyst (v4.0) (Chong et al., 2018) and the univariate analysis consisted of a Student's t-test for metabolites exhibiting a normal distribution and the Wilcoxon Mann-Whitney test for metabolites exhibiting non-normal distributions.

#### **5.4.5. Metabolomics pathway topology analysis**

Metabolites that were found to be significantly different (p-value < 0.05) between DLB and controls were analyzed using the pathway topology search tool in MetaboAnalyst (v4.0) (Chong et al., 2018). The pathway library chosen was for *Homo sapiens* (human) and all compounds in the selected pathways were used when referencing the specific metabolome. Fisher's exact test was applied to perform over-representation analysis and “relative betweenness centrality” was chosen for the pathway topology testing. Pathways that had a p-value < 0.05 were considered to be perturbed due to DLB. Bonferroni's correction for multiple comparisons was used to determine significant differences between mean values for categories among normally-distributed continuous variables.

## **Abbreviations**

AD, Alzheimer's disease; A $\beta$ , Amyloid  $\beta$ ;  $\alpha$ -syn,  $\alpha$ -synuclein; AUC, Area under the curve; CDP, Cytidine diphosphate; DI-LC-MS/MS, Direct injection liquid chromatography coupled with mass spectrometry; DL, Deep Learning; DLB, Dementia with Lewy bodies; GLM, Generalized linear model; GSK-3 $\beta$ , Glycogen synthase kinase 3 $\beta$ ; HDL, High density lipoproteins; HIC, Human Investigation Committee; LDA, Linear discriminant analysis; PAM, Prediction analysis for microarrays; PC, Phosphatidyl choline; PD, Parkinsonian disorder; PP2A, Protein phosphatase 2a; RF, Random forest; ROC, Receiver-operating characteristic; SM, Sphingomyelin; SVM, Support vector machine; TAU, A microtubule-associated protein in neurons; UPS, Ubiquitin-proteasome system.

## **Declarations**

### **Ethics approval and consent to participate**

This study was approved by the Beaumont Health System's Human Investigation Committee (HIC No.: 2018-387).

### **Consent for publication**

Not applicable.

### **Availability of supporting data**

The data used in this exploratory study are not publicly available but may be provided upon reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' contributions**

This exploratory study was designed by SFG, BDG, PP, PGK, BMG. AY and ZU acquired all the metabolomics data and BA performed all the statistical analyses. SA, KJO, MM and SFG composed the

first draft of the manuscript which was approved by all. SA, BDG and SFG edited and approved the final submission.

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## Figure legends

**Figure 1.** The processing of  $\alpha$ -Synuclein ( $\alpha$ -syn), A $\beta$  and TAU, and Lewy bodies and the interactions with lipid homeostasis in DLB. Under pathological conditions,  $\alpha$ -syn undergoes a series of membrane-dependent conformational/structural transitions that may be implicated in neurodegeneration. Ultimately,  $\alpha$ -syn aggregates with each other into insoluble fibrillar plaques known as Lewy bodies that accumulate in neurons in DLB brain. Metabolites represented in red and green indicate higher and lower levels, respectively. Bigger font sizes in metabolites represent statistically significant changes in DLB brain extracts compared to those of controls. A $\beta$ , Amyloid  $\beta$ ; CDP, cytidine diphosphate; GSK-3 $\beta$ , Glycogen synthase kinase 3 $\beta$ ; PP2A, protein phosphatase 2a; TAU, A microtubule-associated protein in neurons; UPS, ubiquitin-proteasome system.

**Figure 2.** The changes in polyamines and biogenic amines in brain extracts from patients with DLB. Metabolites represented in red and green indicate higher and lower levels, respectively. Bigger font sizes in metabolites represent statistically significant changes in DLB brain extracts compared to those of controls.

**Supplementary Figure 1.** The changes of energy metabolism intermediates, ketone bodies, and amino acid carbon skeletons that participate in energy metabolism pathways in the brains of patients with DLB. The carbon skeletons of the diverse set of 20 fundamental amino acids are funneled into only several molecules such as pyruvate, acetyl-CoA, etc. Metabolites represented in red and green indicate higher and lower levels, respectively. Bigger font sizes in metabolites represent statistically significant changes in DLB brain extracts compared to those of controls.

