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Metabolomic Profiling of Post-Mortem Brain Reveals Changes in Amino Acid and Glucose Metabolism in Mental Illness Compared with Controls

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ABSTRACT

Metabolomic profiling was carried out on 53 post-mortem brain samples from subjects diagnosed with schizophrenia, depression, bipolar disorder (SDB), diabetes, and controls. Chromatography on a ZICpHILIC column was used with detection by Orbitrap mass spectrometry. Data extraction was carried out with m/z Mine 2.14 with metabolite searching against an in-house database. There was no clear discrimination between the controls and the SDB samples on the basis of a principal components analysis (PCA) model of 755 identified or putatively identified metabolites. Orthogonal partial least square discriminant analysis (OPLSDA) produced clear separation between 17 of the controls and 19 of the SDB samples (R2CUM 0.976, Q2 0.671, p-value of the cross-validated ANOVA score 0.0024). The most important metabolites producing discrimination were the lipophilic amino acids leucine/isoleucine, proline, methionine, phenylalanine, and tyrosine; the neurotransmitters GABA and NAAG and sugar metabolites sorbitol, gluconic acid, xylitol, ribitol, arabinotol, and erythritol. Eight samples from diabetic brains were analysed, six of which grouped with the SDB samples without compromising the model (R2 CUM 0.850, Q2 CUM 0.534, p-value for cross-validated ANOVA score 0.00087). There appears on the basis of this small sample set to be some commonality between metabolic perturbations resulting from diabetes and from SDB.

1. Introduction

Mental illness, most commonly schizophrenia, depression, and bipolar disorder ('SDB') is common: schizophrenia has a European prevalence of 0.2–2.6%, depression 3.1–10.1%, and bipolar disorder 0.2–1.1% [1]. These conditions are a major burden on the health-care systems and on the relatives of affected people. Clinically, such illnesses are heterogeneous and present with psychosis or mood state features that vary over time and across individuals. Thus, it would be of great value to have an objective means to assist in diagnosis and categorisation of such illnesses and also give an insight into the best way to manage them [2]. Much diagnosis of mental illness remains subjective due to complex and poorly defined mechanisms underlying these diseases; there are no biomarkers and mental illness might be better viewed as a continuum rather than using absolute labelling [3].The significance of low-molecular-weight metabolites in driving or reflecting the aetiology of psychiatric disease has been researched for many years using serum samples that are a pragmatic choice for diagnostic testing and, additionally, brain tissue to investigate the central pathologies [4,5]. In the past 10 years, mass spectrometry-based metabolomics has evolved as a method for profiling a wide range of low-molecular-weight metabolites [6,7]. Metabolomics is a natural fit with metabolite profiling in mental illness where, for many years, targeted analysis was carried out in order to profile, for instance, biogenic amines in order to determine whether or not abnormalities in their levels might be causative [8,9]. There have been several studies which have carried out metabolomic profiling in mental illness [10–17], but these have not been as extensive as those into other diseases such as cancer.

There have been no untargeted metabolomics studies of human post-mortem brain samples although there was a study which examined disturbed glucose metabolism in post-mortem brains from psychotic patients [18]. In the current study, the availability of a unique library of post-mortem brain samples with extensive associated medical information allowed us to investigate whether or not these samples might reveal any underlying pathology which could be related to metabolic differences. Thus, we applied our established LC-MS-based metabolomic profiling methods [19,20] to determine if it was possible

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to individually classify healthy control, depressive, schizophrenic, and bipolar brains. The observation of 'metabolic syndrome'-like features in those diagnosed with mental illness [21,22] prompted us to determine whether or not there was an overlap between metabolic perturbations in mental illness and diabetes. If such a link between mental illness and diabetes could be established then this might give some rationale for the evaluation of medicines used in the treatment of diabetes in the treatment of mental illness.

2. Materials and Methods

2.1. Chemicals

HPLC-grade acetonitrile was obtained from Fisher Scientific, UK. Ammonium carbonate, ammonium hydroxide solution (28–30%), acetic anhydride, pyridine, and methanol were purchased from Sigma–Aldrich, UK. HPLC grade water was produced by a Direct-Q 3 Ultrapure Water System from Millipore, UK. The mixtures of metabolite authentic standards were prepared as previously described [19,23] from standards obtained from Sigma–Aldrich, UK.

2.2. Post-Mortem Brain Samples

Post-mortem brain samples were obtained from the Sudden Death Bank collection held in the MRC Edinburgh Brain and Tissue Banks. Psychiatric diagnosis annotations for each sample were made by detailed study of donor case notes by qualified psychiatrists. Full ethics permission has been granted to the Banks for collection of samples and distribution to approved researchers (LREC 2003/8/37). The University of Strathclyde Ethics Committee also approved the local study of this material (UEC101123). Details of the brain samples are given in Table A1 in the Appendix. The information regarding the brain samples is summarised in Table 1.

2.3. Sample Extraction

The brain samples were thawed and then a sample of brain tissue (50 mg) was homogenised in ice cold methanol/water (1:1, 1.5 ml) using a handheld Lab Gen 7B homogeniser. The samples were then centrifuged at 16,000*g*, 15 min 4 °C and the supernatant was removed and the pellet reserved for further extraction to remove lipids. Lipids were extracted from the pellet with chloroform/methanol (3:1, 1.6 ml). The methanol/water extract was dried under a stream of nitrogen at 37 °C and redissolved in acetonitrile/water (80:20, 200 μl), the sample was centrifuged 16,000*g*, 15 min 4 °C to remove any insoluble material and then analysed by ZICHILIC and ZICpHILIC chromatography. The chloroform/methanol extract was dried under a stream of nitrogen at 37 °C and re-dissolved in either methanol/water (1:1, 200 μl) or methanol/chloroform (1:1, 200 μl) prior to chromatography on either C18 column or silica gel, respectively.

2.4. HILIC–HRMS Analysis

Sample analysis was carried out on an Accela 600 HPLC system combined with an Exactive (Orbitrap) mass spectrometer (Thermo Fisher Scientific, UK). An aliquot of each sample solution (10 μl) was injected onto a ZIC-pHILIC column (150 × 4.6 mm, 5 μm; HiChrom, Reading UK) with mobile phase A: 20 mM ammonium carbonate in HPLC grade water (pH 9.2), and B: HPLC grade acetonitrile. The LC and the MS conditions were as described previously [19,20]. Samples were submitted in random order for LC-MS analysis, and pooled quality control samples were injected at the beginning, middle, and end of the experiment to monitor the stability of the instrumentation. Standard mixtures containing authentic standards for 220 compounds were run in order to calibrate the column. Further analysis of the polar extract and of the lipophilic extracts were carried out on a ZICHILIC column (150 × 4.6 mm, 5 μm), ACE C18 column (150 × 3 mm, 3 μm), and an ACE silica gel column according to our previously described methods [23,24].

2.5. Analysis of Sugar Acids and Polyols by GC-MS

The individual standards for the polyols (100 μg) were treated with acetic anhydride/pyridine (1:1, 100 μl) for 30 min at 70 °C. The reagent was removed under a stream of nitrogen and the sample was re-dissolved in ethyl acetate 1 ml. The individual standards for the polyol acids were treated with methanol containing 1% HCl for 30 min at 70 °C, the reagent was removed under a stream of nitrogen and the sample was then treated as for the polyols. Brain tissue (200 mg) was extracted with acetonitrile/water (1:1, 1 ml) containing 2 μg/ml of pinitol internal standard, centrifuged and the supernatant was removed and evaporated to dryness with a stream of nitrogen at 70 °C and treated as for the polyol acids except that the residue was re-dissolved in 0.2 ml of ethyl acetate. GC-MS analysis was carried out on a DSQ GC-MS system (Thermo Fisher Scientific, UK) fitted with a GL Sciences Inert Cap 1 MS column from Hichrom, Reading UK(30 m × 0.25 mm × 0.25 μm film). The oven was programmed from 100 °C to 320 °C at 5 °C/min. The MS was operated in EI mode at 70 eV. For quantification of the sugars in brains selected, ion monitoring was carried out for ions at m/z 217, 200, 187, 145, 142, and 140, which are typical fragments of alditol acetates [25].

2.6. Data Extraction and Metabolite Identification

MZMine 2.14 [26] was used for peak extraction and alignment, as previously described [19,20]. Putative identification of metabolites was also conducted in MZMine by searching the accurate mass against our in-house database [18,19,23]. Background peaks present in the blank were removed in MZmine before transferring the data to an Excel file. Manual editing of the data was carried out in order to remove idiosyncratic peaks such as metabolites identified as drugs which were presumably from patient treatments and also nicotine metabolites which were particularly abundant in the brains of schizophrenic patients because of their well-established tendency to smoke much more than the general population [27] and ethyl sulphate which is from alcohol metabolism. The GC-MS data were extracted by using Sieve 1.3 (ThermoFisher Scientific UK), and the ions corresponding to the retention time of the sugar standards were extracted in order to build the OPLS-DA model.

Table 1
Summary information for the different groups of brain samples.

Group	Number	Male	Age range	Mean age ± RSD	Female	Age range	Mean age ± RSD
Control	21	18	26–74	47.4 ± 29.5	3	42–60	50.7 ± 17.8
Schizophrenic	11	10	25–69	44.4 ± 34.7	1	40	–
Bipolar	6	1	48	–	5	39–57	45.6 ± 16.2
Depressive	7	4	24–74	47.5 ± 49.1	3	20–57	60.3 ± 33.7
Diabetic	8	8	20–69	44.9 ± 35.2	0	–	–