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Metabolic signatures of Huntington's disease (HD): ^{1}H NMR analysis of the polar metabolome in post-mortem human brain

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ABSTRACT

Huntington's disease (HD) is an autosomal neurodegenerative disorder affecting approximately 5–10 persons per 100,000 worldwide. The pathophysiology of HD is not fully understood but the age of onset is known to be highly dependent on the number of CAG triplet repeats in the huntingtin gene. Using ^{1}H NMR spectroscopy this study biochemically profiled 39 brain metabolites in post-mortem striatum ($n = 14$) and frontal lobe ($n = 14$) from HD sufferers and controls ($n = 28$). Striatum metabolites were more perturbed with 15 significantly affected in HD cases, compared with only 4 in frontal lobe ($p < 0.05$; $q < 0.3$). The metabolite which changed most overall was urea which decreased 3.25-fold in striatum ($p < 0.01$). Four metabolites were consistently affected in both brain regions. These included the neurotransmitter precursors tyrosine and L-phenylalanine which were significantly depleted by 1.55–1.58-fold and 1.48–1.54-fold in striatum and frontal lobe, respectively ($p = 0.02$–0.03). They also included L-leucine which was reduced 1.54–1.69-fold ($p = 0.04$–0.09) and myo-inositol which was increased 1.26–1.37-fold ($p < 0.01$). Logistic regression analyses performed with MetaboAnalyst demonstrated that data obtained from striatum produced models which were profoundly more sensitive and specific than those produced from frontal lobe. The brain metabolite changes uncovered in this first ^{1}H NMR investigation of human HD offer new insights into the disease pathophysiology. Further investigations of striatal metabolite disturbances are clearly warranted.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the extension of a CAG repeat at exon 1 of chromosome 4 (4p63) and is clinically characterized by chorea and dystonia, cognitive decline and behavioural changes [1–6]. It affects 30,000 US citizens (1 in every 10,000) and it is estimated that an additional 150,000–200,000 are at greater risk because they have at least one parent with HD [7]. The appearance of symptoms is inversely correlated to the number of CAG repeats, which is also an influential factor in determining the age of HD onset (it is responsible for ~50–70% of the variance) [8]. The unaffected range is $(CAG)_{6–35}$ repeats, alleles of $(CAG)_{>40}$ are considered fully penetrant and these individuals carry a 100% lifetime risk of developing HD and CAG repeat size with alleles of $(CAG)_{>60}$ causes juvenile onset. Although HD can present itself at any age, the age of onset is typically 40–45 years with death typically occurring 15–20 years after the initial manifestation [3,9–11]. Currently there is no neuroprotective therapy [3] or ultimate "cure" for this debilitating neurodegenerative disease [5,8].

There are major knowledge gaps regarding the underlying biomolecular mechanisms of HD [2,11]. However, there is some evidence that mechanisms contributing to HD pathogenesis include: polyglutamine aggregation and misfolding [12], oxidative stress and mitochondrial dysfunction [9], misregulation of energy expenditure [10], transcriptional deregulation [13,14], excitotoxicity [15,16] and dopamine toxicity [17,18]. Despite research advances in the last two decades there

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; AD, Alzheimer's disease; AUC, area under the curve; AUROC, area under the receiver operating characteristic curve; BBB, blood brain barrier; BCAA, branched-chain amino acid; CNS, central nervous system; CSF, cerebral spinal fluid; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; GC-Tof-MS, gas chromatography time of flight mass spectrometry; HD, Huntington's disease; IGF-1, insulin like growth factor; MRI, magnetic resonance imaging; MAS-NMR, magic angle spinning NMR; MRS, magnetic resonance spectroscopy; ROC, receiver operating characteristic.

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has been no meaningful progress in medical treatments for HD. Few drugs are available for HD treatment and these offer only symptomatic relief (of chorea only) [2]. The most promising research to date has been with co-enzyme Q10 (currently in Phase 3 clinical trial (n = 608 participants); 2Care, The Huntington Study Group) which acts in part to enhance mitochondrial anti-oxidative and free radical scavenging mechanisms [8]. The aim would be to target new treatments to pre-manifest patients as the discovery that changes due to HD happen many years prior to diagnosable onset. Significant technological advances now make it possible to measure, screen and identify thousands of potential biomarkers in biosamples. There is an unprecedented opportunity to identify reliable "state" biomarkers of pre-manifest HD progression that can be used as outcome measures in preventative clinical trials.

Most biomarker research in HD has concentrated on identifying clinical and neuroimaging biomarkers of disease. Clinical biomarkers are standardised clinical tests and rating scales that measure the progression of various characteristics of the HD phenotype, such as cognition and motor deterioration [3]. Data from the full PREDICT-HD study reported that a standardised cognitive tasks (n = 51) demonstrate psychomotor processing, emotion recognition and working memory to be very sensitive when differentiating individuals according to time to predicted HD onset [3,19]. However clinical biomarkers are limited when differentiating between symptomatic improvement and progression of the disease [20]. Additionally they seldom provide any information pertaining to the fundamental disease mechanisms or disease pathogenesis, emphasizing the need for additional non-clinical biomarkers [3].

Very few studies have investigated the potential of metabolomics methodologies to discover novel biochemical biomarkers for HD. A range of studies have demonstrated the utility of metabolomic profiling techniques in accurately distinguishing neurodegenerative diseases from healthy controls [9,10,21–30]. Indeed, it has been successful in identifying plasma biomarker panels for the clinical diagnosis of Alzheimer's disease (AD) in individuals with amnestic mild cognitive impairment [31]. However there is a significant paucity of reliable "state" biomarkers which accurately discriminates pre-manifest HD from manifest HD. The majority of HD metabolomics experiments have been conducted with rodent models which mimic some of the

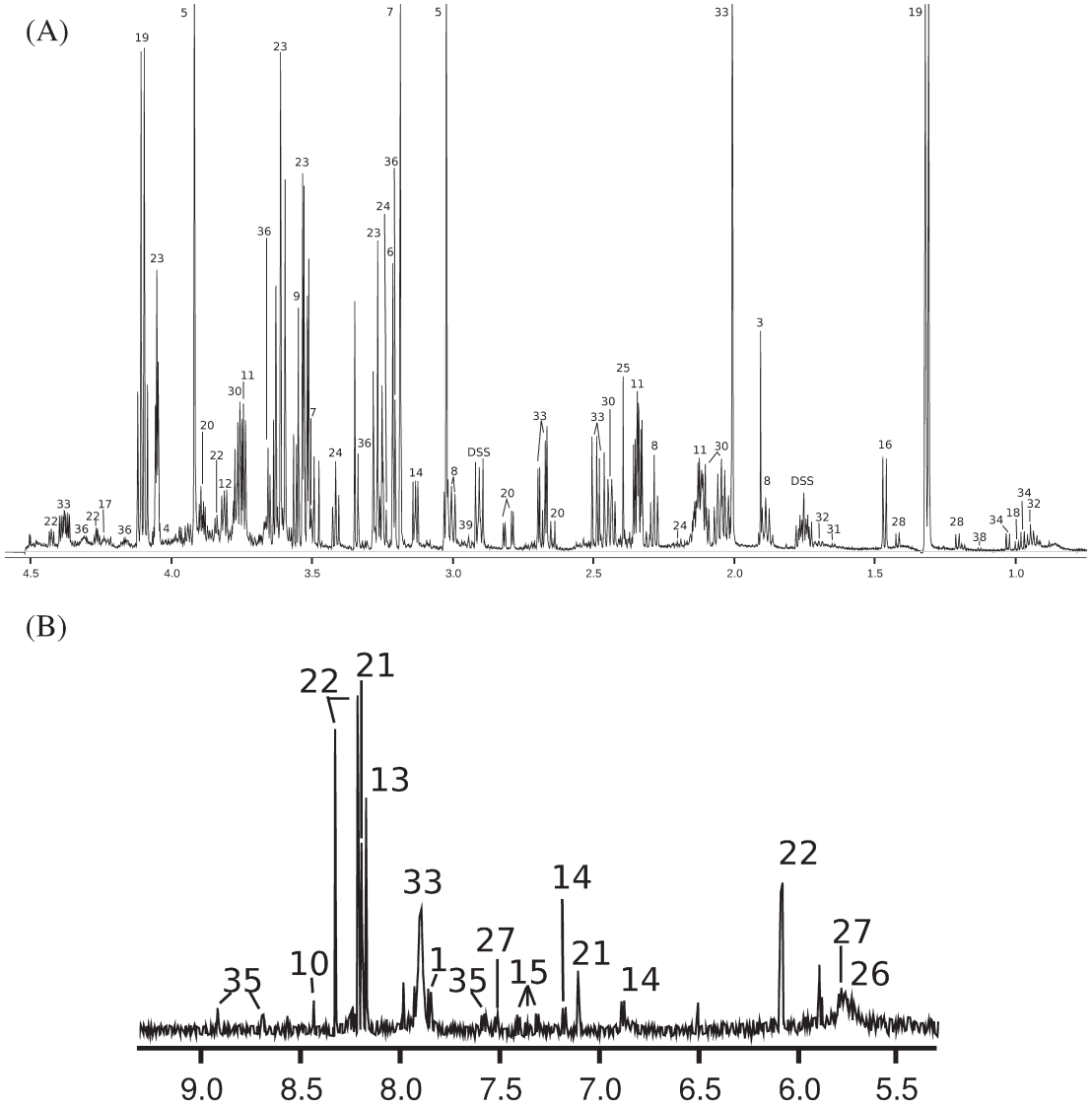

Fig. 1. NMR spectroscopy of Huntington's disease (HD) brain extract (frontal lobe). Typical 1D ^{1}H NMR spectrum of a polar extract taken from HD striatum with identified metabolites labelled in the aliphatic (A) and aromatic regions of the spectrum (B). 1,1-Methylhistidine; 2, adenine; 3, acetic acid; 4, ascorbic acid; 5, creatine; 6, glycerophosphocholine; 7, choline; 8, 4-aminobutyrate; 9, glycine; 10, formate; 11, L-glutamic acid; 12, ethanolamine; 13, hypoxanthine; 14, tyrosine; 15, L-phenylalanine; 16, L-alanine; 17, L-threonine; 18, isoleucine; 19, L-lactic acid; 20, aspartate; 21, anserine; 22, inosine; 23, myo-inositol; 24, taurine; 25, succinate; 26, urea; 27, uracil; 28, 3-hydroxybutyric acid; 29, adenosine triphosphate; 30, L-glutamine; 31, homocitrulline; 32, L-leucine; 33, *N*-acetylaspartic acid; 34, valine; 35, niacinamide; 36, phosphorylcholine; 37, isobutyric acid; 38, propylene glycol; 39, glutathione-oxidized.