The investigation of the hBCAT proteins in control and diseased human brains: Implications for glutamate toxicity in Alzheimer's disease

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Abstract

Introduction & Aims: The distribution of the BCAT proteins has been extensively mapped in rodent models, and metabolic studies have established that BCAT transamination in the rodent brain is responsible for 30% of *de novo* glutamate synthesis. However, to date the BCAT proteins have not been mapped to the human brain and their role in pathogenic conditions where glutamate toxicity features has not been investigated. To this end, this study aimed to map the hBCAT proteins to several brain regions. Furthermore, the expression of hBCAT in AD relative to matched controls was investigated and correlated with both physiological and pathological features of AD. Finally, metabolic and inflammatory stimuli were examined for their effect on neuronal expression of hBCATc.

Methods: Distribution of the hBCAT proteins were assessed utilising immunohistochemistry and imaged utilising a 12-bit camera mounted on a Leica DM microscope. Western blot analysis and microscopy determined the expressional difference in AD compared to age and gender matched controls in addition to cell types responsible for the increased expression. Further investigation of neuronal hBCATc expression was examined in the immortal cell line IMR32 utilising Western blot analysis, phase contrast microscopy, flow cytometry and ^{14}C radiolabelled activity assay.

Results & Discussion: For the first time this work demonstrates key differences between the animal model of BCAA metabolism and humans. All brain regions contained cell types labelled for hBCATc and hBCATm. However, while this work mirrored animal models in that hBCATc was localised specifically to neurons, hBCATm was absent from astrocytes and instead labelled the vasculature – contrary to animal models. Another novel finding of this work links altered aminotransferase expression to AD pathology. An increase of hBCATm expression of +117% ($p = 2.29 \times 10^{-4}$) and +143% ($p = 7.70 \times 10^{-5}$) in the frontal and temporal cortex of AD subjects relative to matched controls demonstrates the disease association of hBCATm. A non-significant increase of 32% was observed for hBCATc in the frontal region. With hBCATm expression correlating with Braak stage in both the frontal ($p = 1.2 \times 10^{-5}$, ρ +0.468) and temporal ($p = 3.4 \times 10^{-4}$, ρ+0.391) cortex this work posits that altered BCAA metabolism is occurring simultaneously with AD progression and may be a novel therapeutic target for the treatment of dementia. Another novel aspect of this work also demonstrates cell surface expression of hBCATc and relates this to mTOR signalling. Altered cell surface and protein expression was investigated with functional activity. Together this data demonstrated expressional, functional or activity changes in hBCATc due to glutamate, insulin, leucine, TNFα and IL1α.

Posters, presentations and publications

Posters and presentations

The role of hBCAT in glutamate toxicity, PGR presentation, University of Bristol (2011.04.18)

Expressional alteration of the BCAT enzymes in the AD brain, poster presentation, UWE (2012.01.13)

The role of hBCAT in glutamate toxicity, PGR presentation, UWE (2012.08.09)

Pilot study: Expressional changes of hBCATc in the IMR-32 cell line, implications for neurological disease, poster presentation, UWE (2012.12.19)

Co-localisation of hBCATm protein with LC3-II using confocal and electron microscopy: relation to AD pathology, poster presentation, ARUK conference (2013.02.26)

Papers

Distribution of the branched chain aminotransferase proteins in the human brain and their role in glutamate regulation, published paper (2012)

The branched chain aminotransferase proteins: novel redox chaperones for protein disulphide isomerase, published paper (2013)

Upregulation of the BCAT protein in the brains of patients with Alzheimer's disease: implications in glutamate toxicity (in review)

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Abbreviations

αKG – α-Ketoglutarate

AD – Alzheimer's disease

ADP – Adenosine diphosphate

ALS – Amyotrophic lateral sclerosis

APS – Ammonium persulphate

ATP – Adenosine triphosphate

BBB – Blood brain barrier

BCAA – Branched chain amino acids

BCKA – Branched chain α-keto acids

BCKD – Mitochondrial branched chain α-keto acid dehydrogenase enzyme

Bim – Bcl-2 interacting mediator of cell death

BOD – Bcl-2 related ovarian death gene

BSA – Bovine serum albumin

BV – Blood vessels

CHAPS – 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate

CSF – Cerebrospinal fluid

Cys – Cysteine

DAB – 3,3'-Diaminobenzidine

DAPI – 4',6-diamidino-2-phenylindole

DIOC6 – 3,3′-dihexyloxacarbocyanine iodide

DTT – Dithiothreitol

EAAT – Excitatory amino acid transporter

EBM – Eagles basal media

EGM – Endothelial cell growth media

EDTA – Ethylenediaminetetraacetic acid

EGTA – Ethyleneglycoltetraacetic acid

ER – Endoplasmic reticulum

GABA – Gamma-aminobutyric acid

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

GDH – Glutamate dehydrogenase 1

GLUT – glucose transporter

Grx – Glutaredoxin

GSNO – S-nitrosoglutathione

GSH – Glutathione reduced

GSSG – Glutathione oxidized

hBCATc – Human branched chain aminotransferase (cytosolic isoform)

hBCATm – Human branched chain aminotransferase (mitochondrial isoform)

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HRP – Horseradish peroxidase

IMR32 – Human neuroblastoma cell line

IL – Interleukin

IPTG – Isopropyl β-D-1-thiogalactopyranoside

KIC – Ketoisocaproate

KIV – Keto-isovaleric acid

KMV - Keto-β-methylvalerate

L1 – Large neutral amino acid transporter 1

LDS – Lithium dodecyl sulphate

nAChR – Nicotinic acetylcholine receptor

NADH – Nicotinamide adenine dinucleotide

NEAA – Non essential amino acids

NMDA – N-methyl-D-aspartic acid

NOS – Nitric oxide synthetase

MMSE – Mini-mental state examination

MSUD – Maple syrup urine disease

mTOR – Mammalian target of Rapamycin

mTORC1 – Mammalian target of Rapamycin complex 1

mTORC2 – Mammalian target of Rapamycin complex 2

NADPH – Nicotinamide adenine dinucleotide phosphate

NO – Nitric oxide

PDI – Protein Disulphide isomerase

PKC – Protein kinase C

PLP – Pyridoxal phosphate

PMP – Pyridoxine monophosphate

PMSF – Phenylmethyl sulfonyl fluoride

RIPA – Radioimmunoprecipitation assay buffer

RPMI - Roswell park memorial institute medium

SDS – Sodium dodecyl sulphate

TBST – Tris-Buffered Saline/Tween

TCA – Trichloroacetic acid

TEMED – Tetramethylethylenediamine

Trx – Thioredoxin

TNF – Tissue necrosis factor

UO – ubiquionone oxidoreductase

ZIP – zipper interacting protein