Quantification of Human Blood-Brain Barrier Transporters in Health and in Dementia by Global and Targeted Proteomics

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Introduction

- One of the major challenges of modern day medicine is the understanding and treatment of complex age-related disorders, such as dementia. Attempts to discover effective drugs to treat neurological diseases have proven difficult, partly due to the complexity of the human brain.[1]

- The function of the Blood-Brain Barrier (BBB) is essential in controlling transport between systemic blood and brain tissue and this in turn affects drug efficacy/toxicity.

- ABC and SLC transporters represent a key protective element of the BBB and act as gatekeepers to the CNS, playing a critical role in drug and xenobiotic brain disposition.

- Scaling factors: transporters abundances at the BBB and Brain Microvessels Protein Per Gram Brain (BMvPGB) can inform development of in vitro-in vivo extrapolation (IVIVE) integrated in physiologically-based pharmacokinetic (PBPK) models.

- We developed a sample-preparation protocol and applied targeted and global mass spectrometry for the identification and quantification of proteins at the BBB in 22 brains. We hypothesize that optimised methods for absolute quantification will provide more accurate representations of transporter expression, inter-individual variability between brains and differences between health and disease.

Methods

- **Samples:** Frozen brain frontal cortexes of 22 donors were supplied by the Manchester Brain Bank. The samples were supplied from healthy individuals (n=12), and patients with Alzheimer's Disease (AD) (n=5) or Dementia with Lewy Bodies (DLB) (n=5).

- **Sample preparation:** Several optimization steps were introduced to isolate the microvessel fraction from 22 brains and extract membrane proteins, which were then proteolytically-digested using Filter-Aided Sample Preparation (FASP) and analysed using three LC-MS/MS proteomic methods: Targeted Multi-Reaction Monitoring (MRM), targeted Accurate Mass and Retention Time (AMRT) methodology and label-free global proteomics.

- **QconCATs:** Two transporter QconCAT "TransCAT" standards were used to quantify transporters and cells markers: a liver TransCAT and a brain TransCAT. The "liver TransCAT" has previously been described, [2] the brain TransCAT was designed specifically for this study. [3]

- **Enrichment of microvessel proteins and measurement of protein content of brain microvessels per gram brain (BMvPGB):** Levels of enrichment of microvessel proteins were assessed using a colorimetric method and ATPase activity in isolated microvessels and homogenates
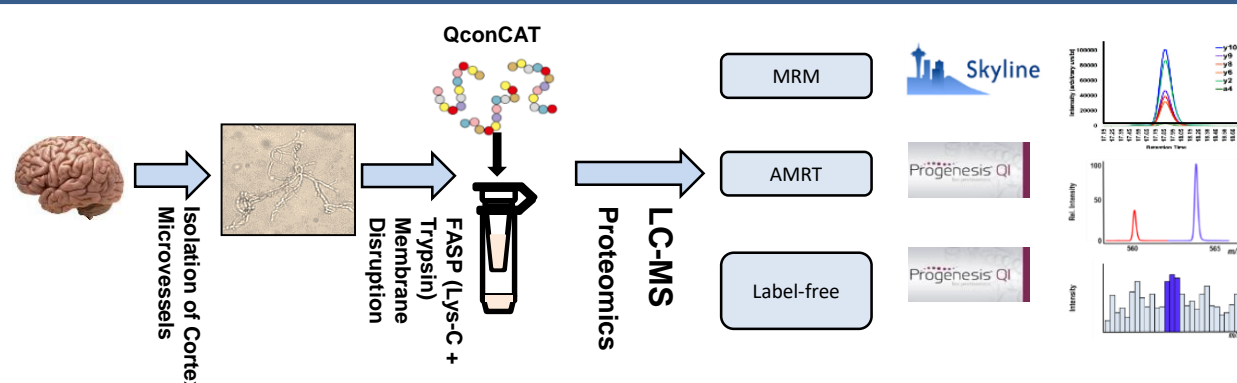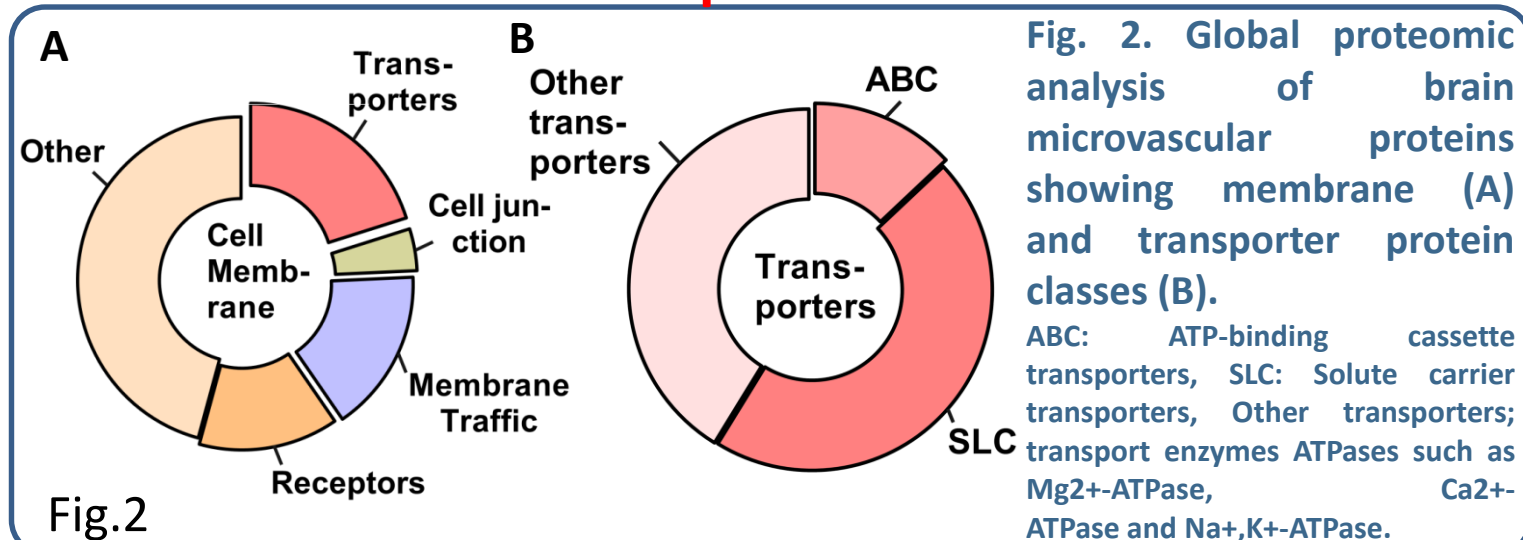

Fig. 1. Experimental workflow and quantitative methods used to analyse the proteins expressed at the BBB.

- **MRM assays and targeted LC–MS/MS analysis:** Peptides were analysed by LC-MRM-MS/MS using an UltiMate® 3000 Rapid Separation LC coupled to a Sciex 6500 QTRAP mass spectrometer.

- **Global LC–MS/MS proteomic analysis:** The BBB global profiles were determined using the a Thermo Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer. Desalted samples were loaded onto an UltiMate® 3000 liquid chromatography (LC) system over 180 min gradient.

- **Bioinformatics:** Skyline software was used for MRM method generation and refinement. For global and AMRT analysis, the LC-MS peptide data were processed and searched using Mascot and Progenesis QI for Proteomics.
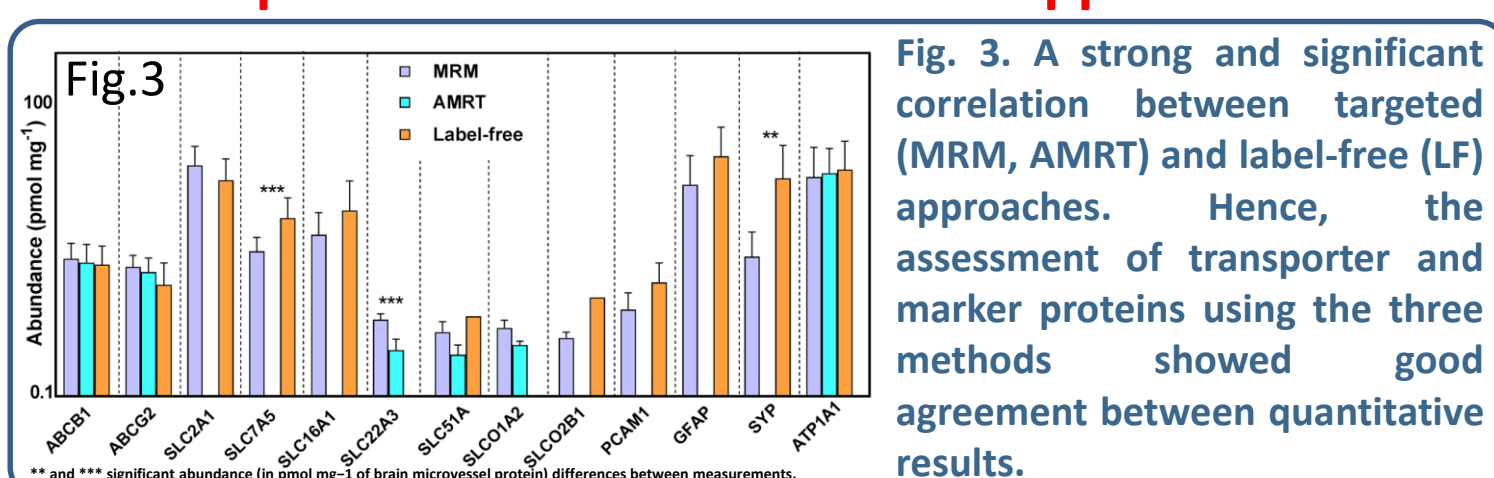
Results

Membrane and Transporter Protein Identification

Fig. 2. Global proteomic analysis of brain microvascular proteins showing membrane (A) and transporter protein classes (B). ABC: ATP-binding cassette transporters, SLC: Solute carrier transporters, Other transporters; transport enzymes ATPases such as Mg2+-ATPase, Ca2+-ATPase and Na+,K+-ATPase.

- 3,390 proteins were identified using global proteomics
- 19% of these proteins were assigned to the plasma membrane
- Importantly, 131 proteins were identified as transporters, out of which ABC and SLC transporters represented 14% and 48%, respectively.

Comparison between Proteomic Approaches

** and *** significant abundance (in pmol mg–1 of brain microvessel protein) differences between measurements.

Fig. 3. A strong and significant correlation between targeted (MRM, AMRT) and label-free (LF) approaches. Hence, the assessment of transporter and marker proteins using the three methods showed good agreement between quantitative results.

Expression of BBB Markers and Transporters

Fig. 4. The purity of the microvascular fraction was assessed by measuring the abundances of cell markers (A); endothelial cells (GLUT1, PECAM1), neurons (SYP), astrocytes (GFAP), and pericytes (NG2); ATP1A1 is used as a plasma membrane marker for quality control. The abundance of ABC and SLC transporters was quantified at the blood-brain barrier (B) and expressed in pmol mg–1 of brain microvessel protein.
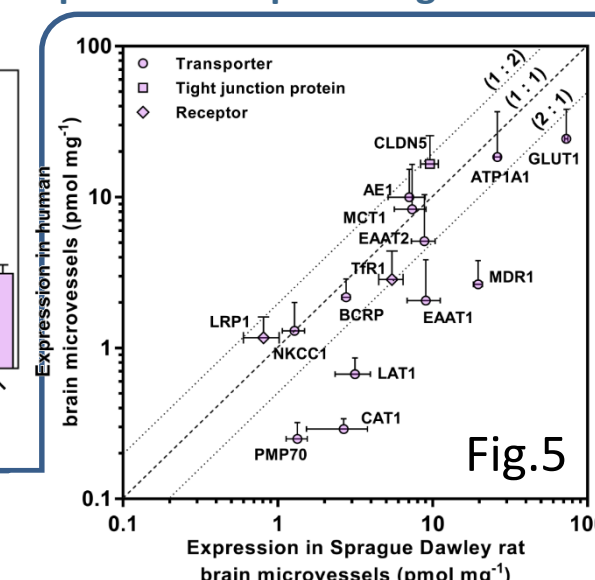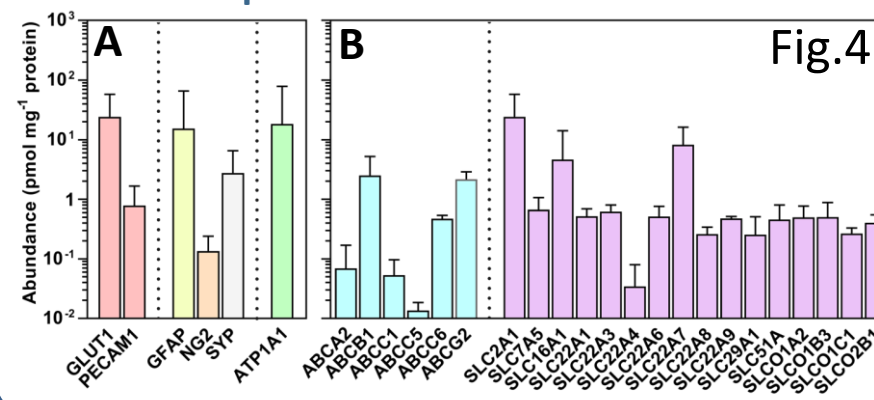

Fig. 5. Comparison of protein expression levels of transporters in brain capillaries between Sprague–Dawley rat and human. Abundance of transporters, tight junction protein, receptors and markers is expressed in pmol mg–1 of brain microvessel protein.
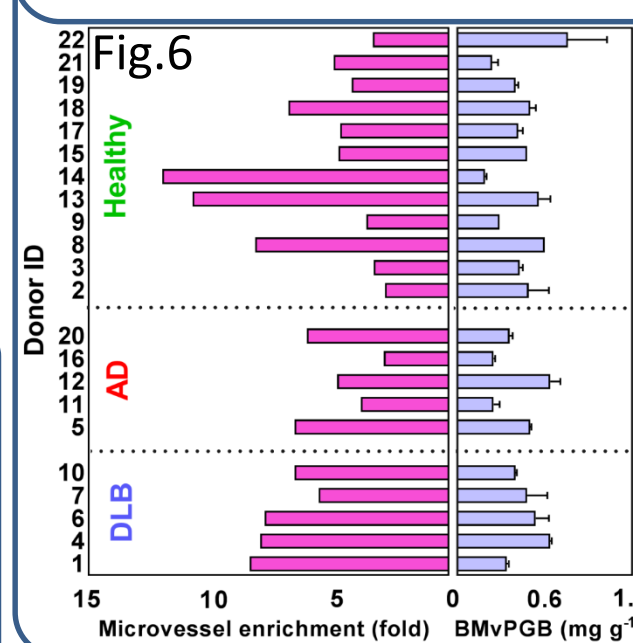

Fig. 6. Brain microvessel protein per gram brain was assessed in the microvascular fraction of each of the 22 brains microvessel samples relative to their homogenates.

Noteworthy, a similar proteomic expression profile between samples from DLB, AD, and healthy donors was observed, both at protein and peptide levels.[3]

Conclusions

This work describes the protein composition of the BBB in health and dementia and quantifies 53 transporters responsible for brain disposition of xenobiotics and endogenous molecules.

Importantly, 19 transporters were measured for the first time, and assessment of the biochemical fingerprint of brain proteins highlighted healthy ageing and development of AD as the main contributors to changes in global expression profiles.

References

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