Establishing the Relationship between mRNA Expression and Protein Abundance for Seven Transporters in Human Small Intestine: A Pilot Study

Matthew D Harwood[a,b], Stephanie L Gibson[a], Brahim Achour[c], Pamela J Davies[a], Gordon L Carlson[a], Amin Rostami-Hodjegan[b,c] and Geoffrey Warhurst[a].

[a] Gut Barrier Group, Salford Royal Hospital Trust, University of Manchester, UK

[b] Simcyp Ltd (a Certara Company), Sheffield, UK

[c] Centre for Applied Pharmacokinetic Research , Manchester Pharmacy School, University of Manchester, UK

Introduction, Aims & Objectives

Previous literature meta-analyses have described the regional-specific intestinal expression of transporters in humans based on a combination of mRNA and protein expression data from relative quantification methods for incorporation into mechanistic Physiologically-based Pharmacokinetic (PBPK) models[1]. Within PBPK models, mRNA expression has been used as a surrogate for transporter function[2], whereas in other studies, PBPK models use protein expression data as a proxy for activity[3]. Quantitative proteomic techniques facilitate the relationship between mRNA and protein abundance to be defined, and while it is suggested that these factors can be poorly correlated[4], specific data from human intestine is limited[5].

This aim of this study is to assess the relationship between relative mRNA expression and protein abundances for seven transporters expressed in human jejunum by a targeted proteomic technique.

Methods

Human jejunum (n=4) was harvested under informed consent (REC 06/1410/126) from donors undergoing elective surgery at Salford Royal Hospital. Fresh mucosal tissue obtained after blunt dissection was snap frozen in liquid nitrogen.

Crude membranes were ground into a powder by pestle and mortar and the extract was suspended in buffer (150mM NaCl, 1.0%, Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50mM Tris Base, pH8.0) with protease inhibitors and spun at 10,000 rpm for 5 min at 4°C.

The mRNA (gene) expression of seven transporters; P-gp, BCRP, MRP2, OATP2B1, HPT1, OST-α, OST-β was quantified by the $2^{-\Delta ct}$ RT-PCR method with normalisation against two reference proteins (GAPDH and Villin) after Trizol® and chloroform-methanol extraction.

The protein abundance of the seven transporters in crude membranes was determined by the Quantification conCATemer (QconCAT) LC-MS/MS targeted proteomics technique after correction for peptide losses during the digestion procedure[6]. Protein abundances are expressed as fmol/µg crude membrane protein.

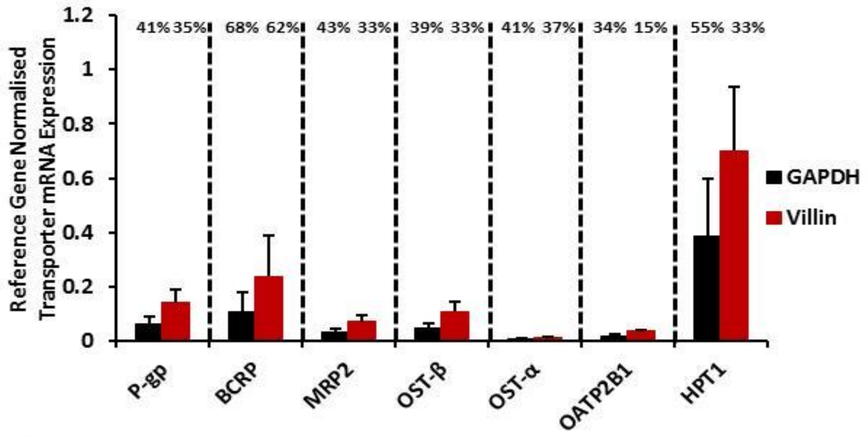

Figure 1. Relative mRNA expression of 7 transporters in human jejunum (n=4) normalised the reference genes GAPDH (black bars) and Villin (red bars). The samples were run in duplicate. The values (%) above the bars relate to the precision (CV%) between the 4 samples. The bars represent mean ± SD.

mRNA and protein expression levels for seven transporters in human jejunum were quantified (**Figures 1 & 2**). Relative mRNA expression was reference gene-dependent; however the between sample precision of expression was systematically higher using villin (**Figure 1**), which may be due to its' epithelial-specificity.

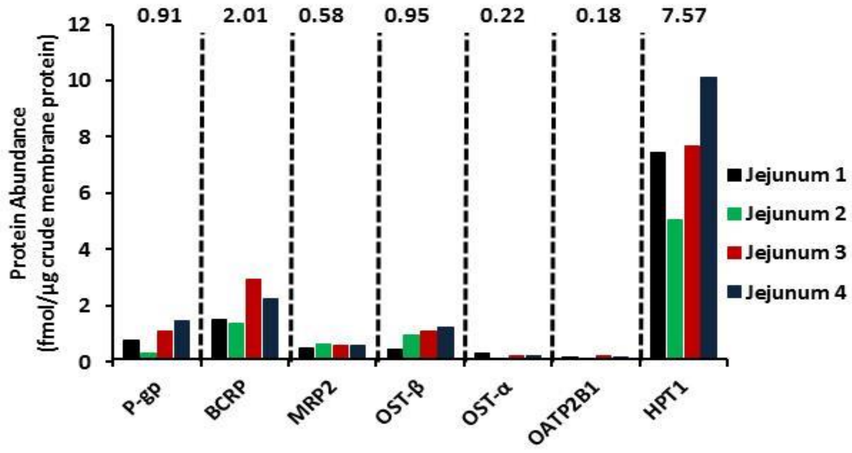

Figure 2. Protein abundance determination for seven transporters in human jejunum (n=4) crude membranes. The bars represent the mean protein abundance for each jejunum over triplicate LC-MS/MS runs, with the mean abundance values provided as text above the bars.

The rank order of protein expression was in-line with a previous study[6] which utilised a different set human small intestinal samples, where expression for HPT1 > BCRP > P-gp > MRP2 > OST-α. The rank order of mRNA expression also broadly follows that of protein with HPT1, BCRP, P-gp and OST-β consistently demonstrating the highest expression.

Results

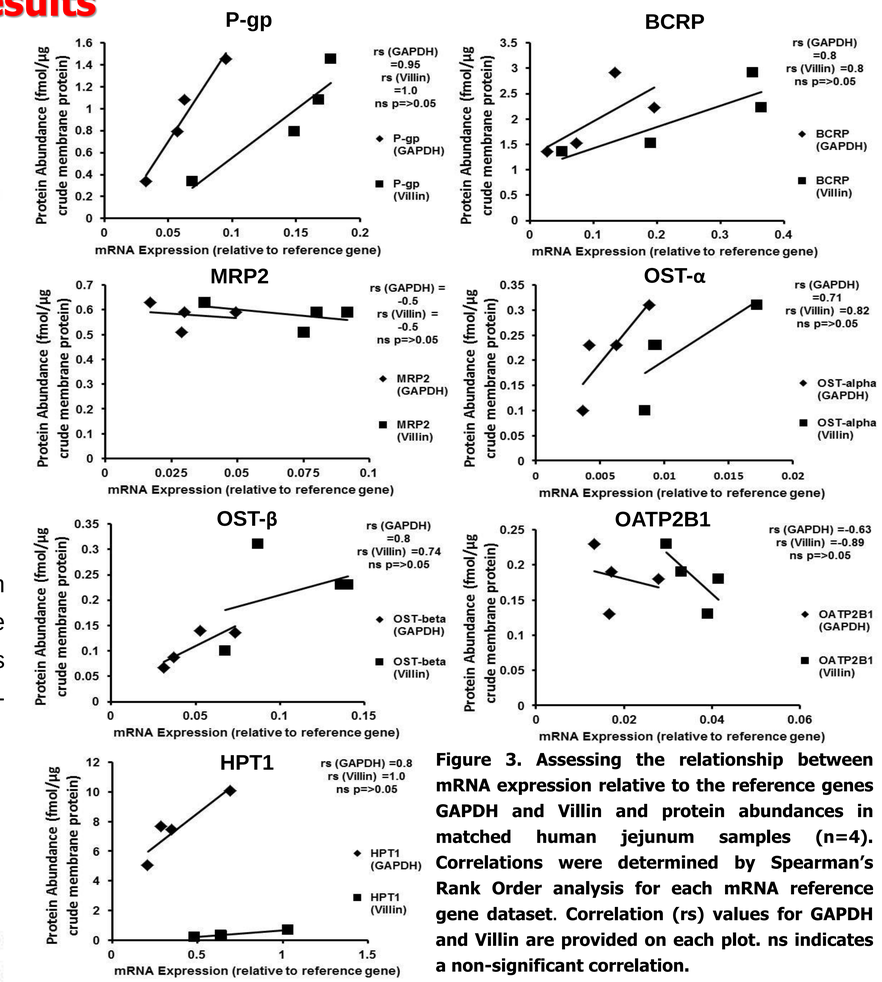

Figure 3. Assessing the relationship between mRNA expression relative to the reference genes GAPDH and Villin and protein abundances in matched human jejunum samples (n=4). Correlations were determined by Spearman's Rank Order analysis for each mRNA reference gene dataset. Correlation (rs) values for GAPDH and Villin are provided on each plot. ns indicates a non-significant correlation.

HPT1, P-gp, BCRP, OST-α and OST-β mRNA-protein expression were highly correlated (rs = > 0.7), whereas no correlation existed (rs = < 0.4) for MRP2 and OATP2B1 (**Figures 3**). None of the correlations reached significance (α = 0.05), likely owing to the limited dataset (n=4).

Discussion & Conclusion:

- The findings reported here suggest that mRNA-protein expression is transporter-dependent. Yet, clearly more data are required; however given the discrepancies in mRNA-protein expression, caution is advised if using jejunal mRNA transporter expression in PBPK models.
- A further 28 intestines require analysis to substantiate these relationships.

References:

1. Harwood *et al.*, 2013., BDD, 34, 2; **2.** Meyer *et al.*, 2012, DMD, 40, 892
3. Neuhoff *et al.,* 2013, JPS, 42, 1541; **4.** Vogel *et al.*, 2012, Nat Rev Genet, 13, 232
5. Drozdzik *et al.*, 2014, Mol Pharm, 11, 3457; **6.** Harwood *et al.*, 2015, JPBA, 110, 27