Abundance of Hepatic Transporters in Caucasians: A Meta-Analysis

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Background

- Physiologically-based pharmaco-kinetic (PBPK) models often use *in vitro-in vivo* extrapolation (IVIVE) factors that account for differences in enzyme and drug transporter expression based on quantitative protein abundance as a surrogate for activity.
- New proteomics techniques based on LC-MS/MS and quantitative immunoblotting provide a quantitative means for measuring transporter abundances without relying on pure standards of whole membrane proteins.
- Abundances have been quantified in liver samples from diverse backgrounds.

Objectives

To characterise the abundance of key hepatic transporters in a Caucasian healthy population.

Methods

- A search for abundance data on 19 hepatic transporter proteins was performed through a keyword search using the electronic database, PubMed.
- Where individual data were not reported, data were extracted via GetData Graph Digitizer or individual donor data were requested from authors.
- Data were scaled to pmol per million hepatocytes.
- Weighted mean, geometric mean, and coefficient of variation (CV) were calculated. Data was tested for homogeneity.
- Inclusion criteria for the final dataset:
  - Abundance quantified using LC-MS/MS or quantitative Western blot in crude membrane fractions
  - Adult healthy Caucasian liver
  - Wild-type (Extensive Transporter) phenotype
  - Samples not used in more than one study *i.e.* not double counted

Results

- Suitable data were not found for: ENT1, ENT2, MCT1, OAT2, OAT7, OSTα/β, MRP4 and MRP6.
- A total of 1622 measurements were collated for 19 hepatic transporters, of which 282 measurements matched our inclusion criteria **(Figure 1)**.
- More than 1/4 of the data was excluded due to use of disease tissue (*i.e.* hepatocellular carcinoma and acute liver injury).
- Variability was reduced significantly when using the inclusion criteria and accounting for co-factors, such as disease and phenotypic background. The CV of OATP1B1 abundance was reduced from 95% (n=262)[1,2,3,4,5,6,7,8] down to 56% (n=66)[1-4].

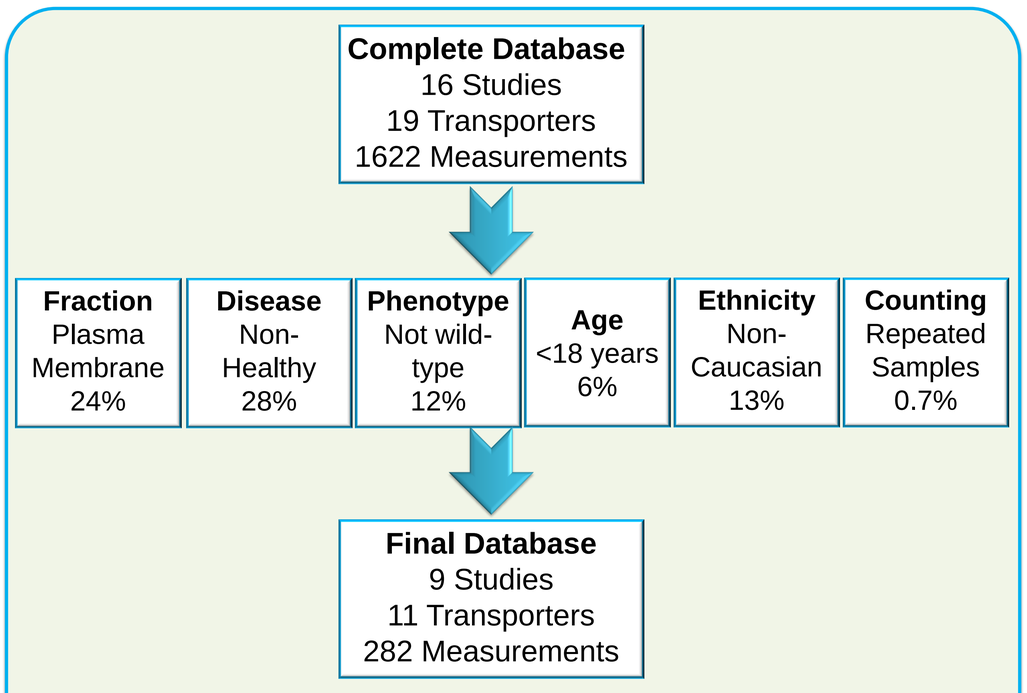


Figure 1 – *Breakdown of the exclusion criteria used to ensure a homogeneous baseline population. Percentages indicate the fraction of data points that were excluded based on the complete database.*

- Highest variability was observed for OATP1B3 and OATP1B1, followed by MRP2 and P-gp **(Table 1)**.
- OATP1B1 and OATP1B3 also showed the highest population abundance in Caucasians, followed by OCT1 and OATP2B1 **(Table 1)**.

Table 1 – Abundance of hepatic transporters (pmol/million hepatocytes) in healthy adult Caucasians obtained through meta-analysis. The data is presented as weighted mean and CV. Heterogeneity was assigned as low ($p>0.05$), moderate ($p<0.05$) or high ($p<0.001$).

Transporter	Mean Abundance	CV (%)	Range	N	Heterogeneity
OATP1B1[1,2,3,4]	4.28	56	1.06 - 23.4	66	High
OATP1B3[1,2,3,4]	4.30	79	0.73 – 38.1	66	High
OATP2B1[1,2,3,4]	1.38	41	1.22 – 7.6	66	Low
OCT1[8]	1.63	42	n/a	55	--
NTCP[8]	0.81	41	n/a	55	--
MATE1[8]	0.18	31	n/a	55	--
P-gp[3,4,9]	0.21	48	0.13 – 1.3	78	Low
BSEP[8,10]	0.89	14	n/a	70	Low
MRP2[9,11,12]	0.37	54	0.17 – 2.9	37	Low
MRP3[8]	0.19	29	n/a	55	--
BCRP[9,10,13]	0.05	37	0.05 – 0.23	40	Moderate

- A simulation run using the Simcyp Simulator in 2000 North European Caucasian virtual individuals was able to replicate the reported abundance values and the reported population variability for the 11 hepatic transporters **(Figure 2)**.

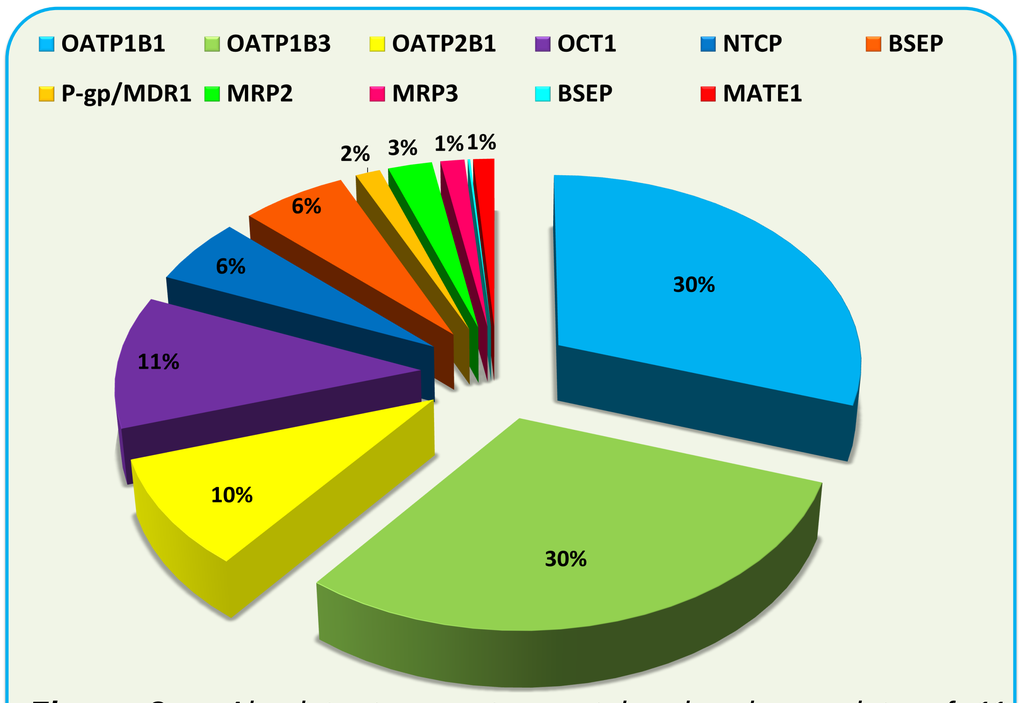
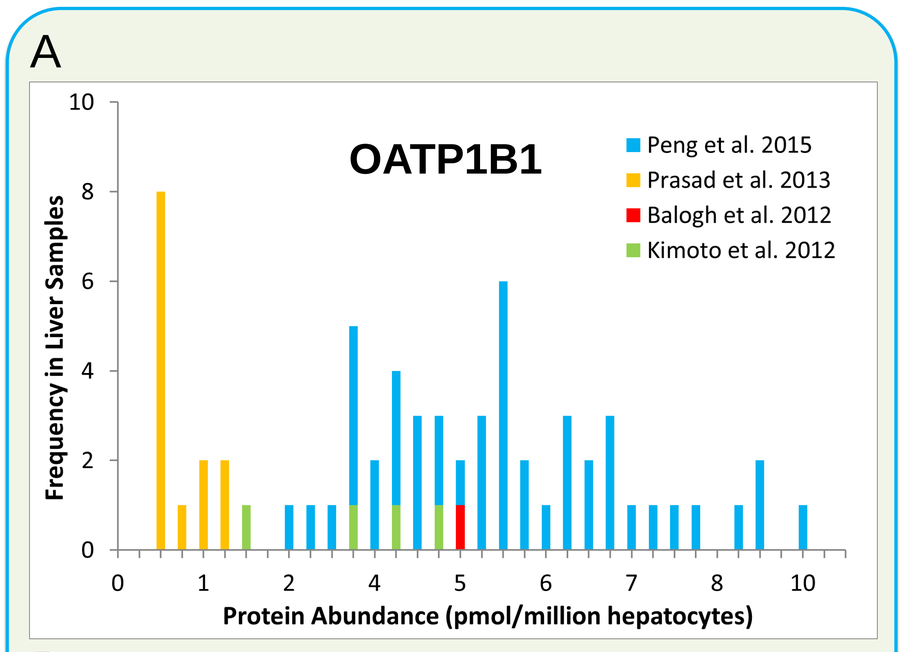


Figure 2 – *Absolute transporter protein abundance data of 11 hepatic transporters as a percentage of the total abundance following a simulation of 2000 virtual individuals using the Simcyp Simulator (V15).*

- Analysis of absolute abundance data for OATP1B1 and OATP1B3 indicated a biphasic distribution, suggesting heterogeneity of the available data **(Figures 3A and B)**. The heterogeneity was observed even after accounting for differences in age, disease, genotype and ethnicity[1-4].



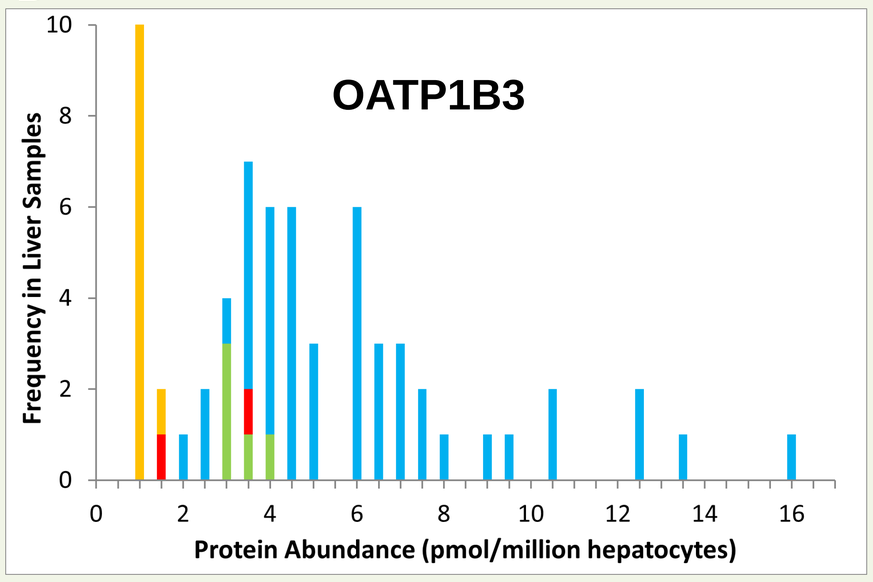


Figure 3 – *Distribution of the individual absolute protein abundance values obtained for OATP1B1 and OATP1B3 by four independent studies[1-4].*

Discussion and Conclusion

- The final database contained 11 hepatic transporters with only 20% of the total data were suitable for characterising abundance in healthy Caucasian adults.
- This study demonstrates the importance of characterizing tissue(s) background prior to study commencement of proteomic transporter expression data or prospectively and clearly reporting them in publications and emphasizes the need for cross-lab comparisons[16].
- The heterogeneity observed for OATPs may be attributed to:
  - Tissue quality and preparation method
  - Significant differences in the yield of the crude membrane fraction
  - Genotypes that are not yet evaluated
- When using these data for PBPK modelling, it is important to account for activity differences for transporters where a clear link between activity and expression has been shown, *e.g.* OATP1B1 and OATP1B3 polymorphisms have been shown to result in different transporter activities in Caucasians[14,15].
- Similar studies will be warranted in other populations, including Chinese and Japanese. 13% of data that was not from the Caucasian population were from Asian, non-Hispanic black and African Americans[2-4, 12,13].

This is the first in-depth analysis of current quantitative abundance data for a wide array of hepatic transporters with the aim of using these data for IVIVE.

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