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Organic Anion Transporter 7 (OAT7) – A Novel Pravastatin Uptake Transporter in Human Liver, Regulated by HNF4α

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BACKGROUND

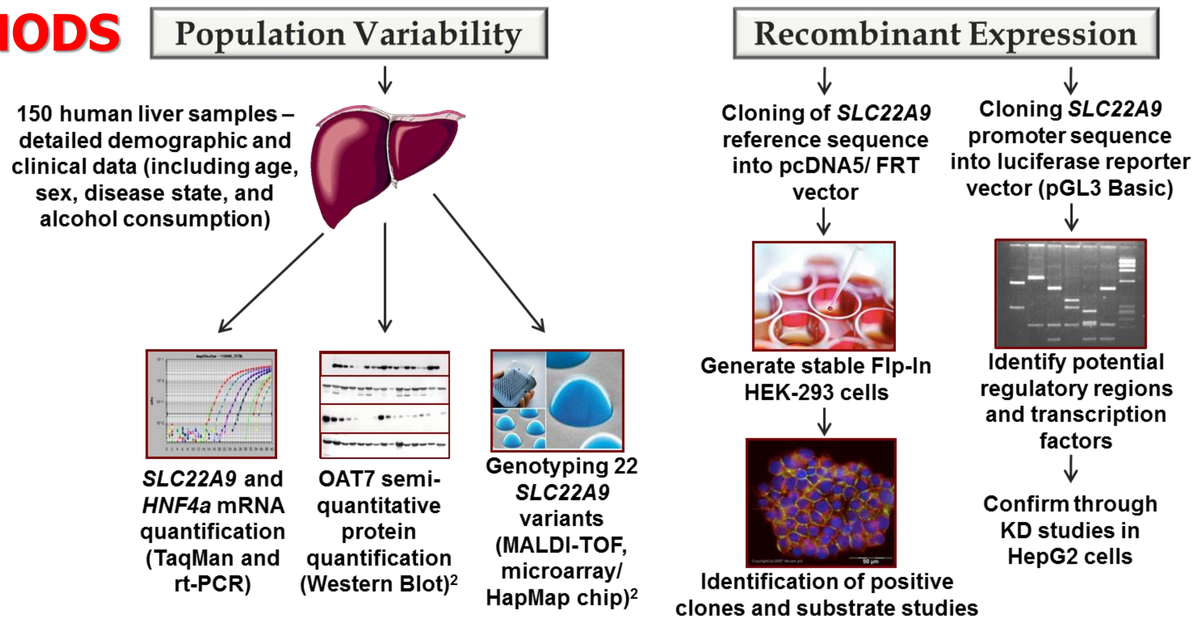
Organic anion transporter 7 (OAT7, *SLC22A9*) was identified in 2007 as a novel member of the SLC22 transporter family and is the first liver-specific functional OAT member in humans to date[1].

Hepatic uptake transporters have been shown to play a significant role in the absorption, distribution, toxicity and excretion of various xenobiotics, including HMG-CoA reductase inhibitors (statins).

Though several transporters have been implicated in the hepatic uptake of statins, they seem to have only a partial contribution to the disposition of statins.

OBJECTIVE AND METHODS

1. **To investigate the potential contribution of OAT7 to the hepatic uptake of statins.**
2. **To identify factors that may contribute to variability in *SLC22A9*/OAT7 expression and function.**



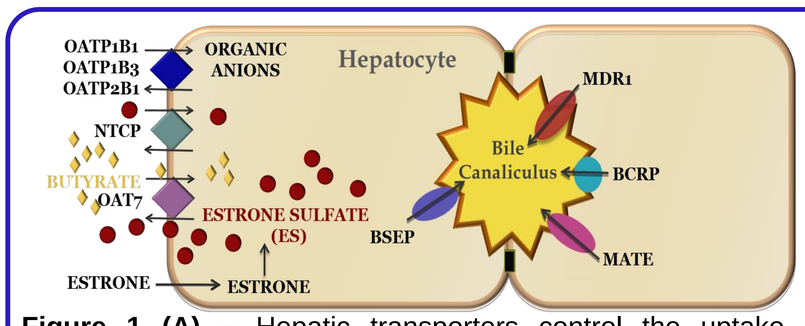
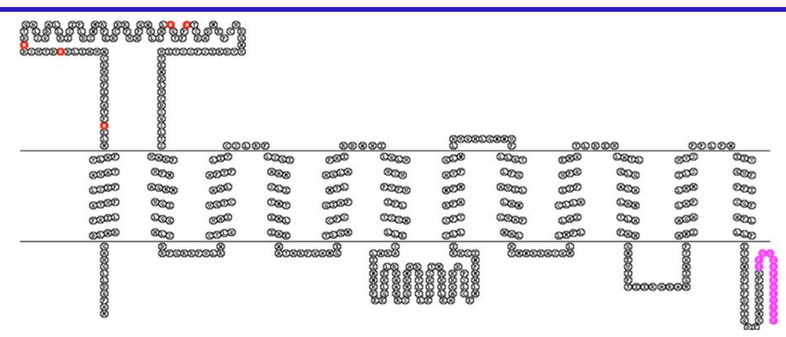


Figure 1 (A) – Hepatic transporters control the uptake and excretion of endogenous compounds and xenobiotics. OAT7 mediates the sodium-independent uptake of butyrate into hepatocytes in exchange for estrone sulfate.



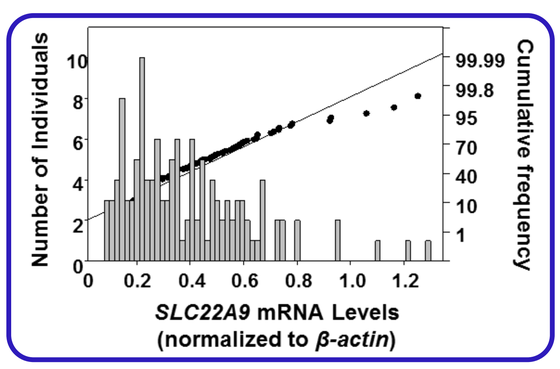
(B) – The expected location of the anti-OAT7 antiserum binding (marked in magenta) on the 12 transmembrane domain structure of human OAT7 protein (Image created using TOPO2).

RESULTS

SLC22A9 mRNA Expression

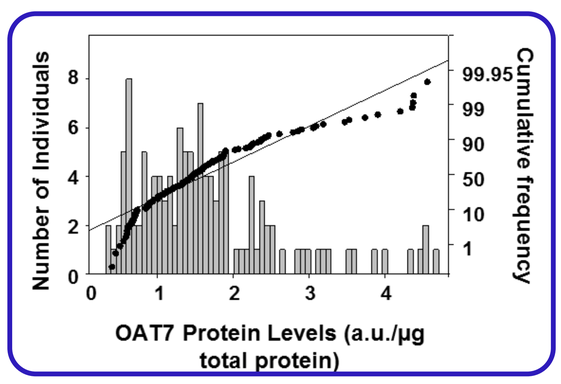
Investigation of normalized cDNAs from 20 normal and tumor human tissues showed predominant expression of *SLC22A9* in the liver.

Other tissues, including kidney and pancreas, expressed approximately 60-fold lower *SLC22A9* mRNA levels.

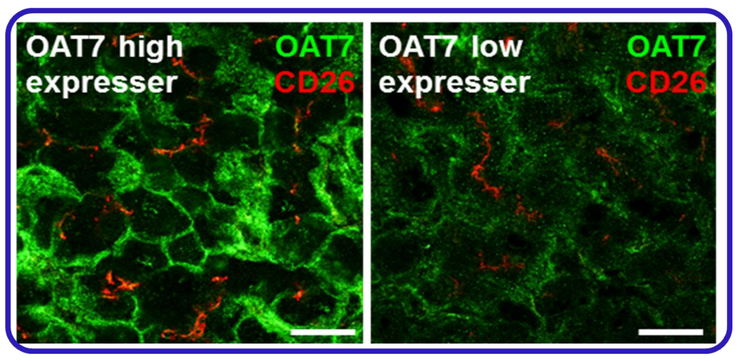


Across the 126 liver samples, *SLC22A9* mRNA expression was not normally distributed and showed 16-fold variability.

OAT7 Protein Expression



OAT7 protein expression measured across the 126 liver samples showed a 25-fold variability and was not normally distributed.

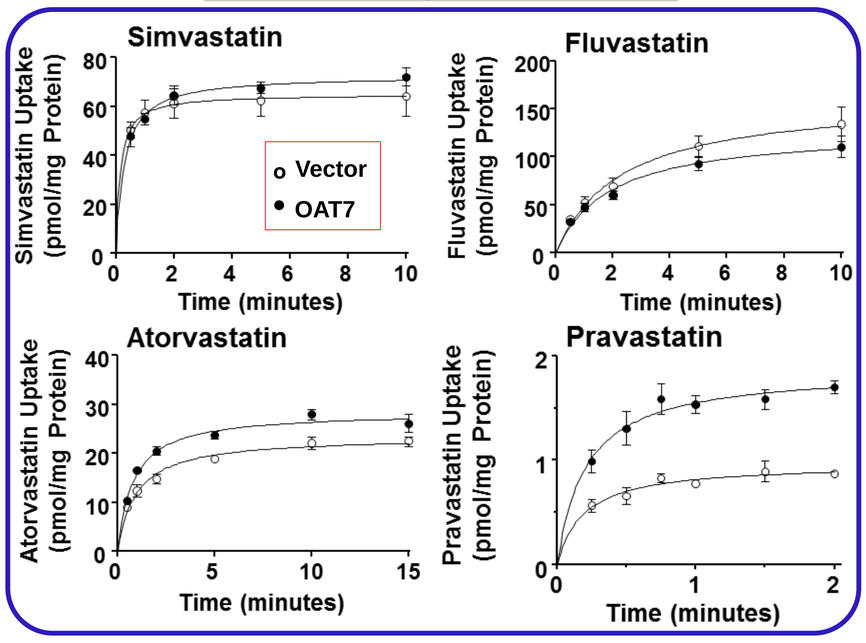


OAT7 expression was investigated in cryosections of human liver in high- and low-expresser individuals (B). Staining of sinusoidal hepatocyte membrane (green) was observed, whereas, canalicular membrane staining (red) did not show co-staining with the OAT7 signal.

No correlation was observed between *SLC22A9* mRNA and OAT7 protein expression in the human liver samples, likely due to post-transcriptional/translational regulation.

RESULTS (cont'd)

OAT7 Transport Function

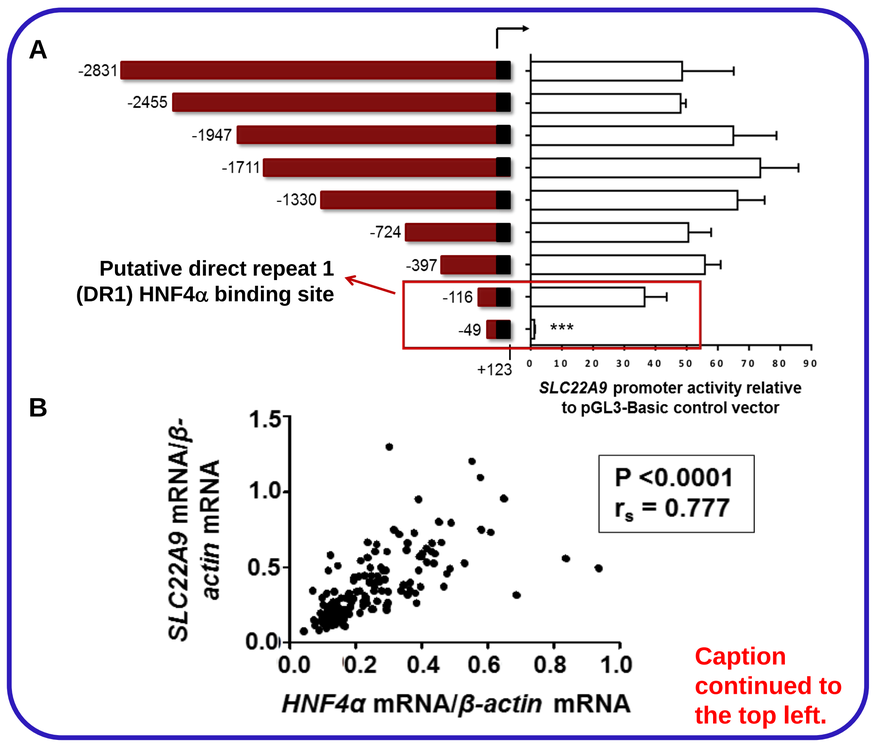


The uptake of simvastatin, fluvastatin, atorvastatin and pravastatin was tested in vector-transfected (o) and OAT7-transfected (•) cells – of these, only pravastatin showed significantly higher accumulation (~2x) in OAT7- compared to vector-transfected cells.

Determination of kinetic properties of pravastatin uptake showed that OAT7 is a high-capacity, low-affinity transporter of pravastatin with:

V_{max} of 2.3 ± 0.3 nmol/mg.min and K_m = 1.0 ± 0.3 mM

OAT7/*SLC22A9* Regulation



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RESULTS (cont'd)

OAT7/*SLC22A9* Regulation

SLC22A9 promoter activity dropped significantly upon deletion beyond position -116, which contains the putative direct repeat element of hepatic nuclear factor 4α (A). HNF4α mRNA levels further correlated significantly with *SLC22A9* mRNA expression (B).

Inter-individual Variability

Multivariate analyses of the influence of non-genetic factors on *SLC22A9*/OAT7 expression indicated a significant association between *SLC22A9* mRNA expression and regular alcohol consumption and OAT7 protein expression and primary liver disease.

Among the 22 variants identified, only rs61742518 resulted in a non-synonymous mutation, i.e. T433M (rs61742518). Overall, genetic variants were found to have only a minor effect on *SLC22A9*/OAT7 expression.

Inter-individual variability in *SLC22A9* mRNA expression could be mainly explained by HNF4α regulation (46%), whereas, variability in OAT7 protein expression is most likely influenced by additional factors, such as epigenetics.

CONCLUSION

The OATP1B1 contribution to the hepatic uptake clearance of pravastatin has been recently calculated to amount to 66%[4]. Furthermore, inhibition of OATP1B3, 2B1 and NTCP, only partially account for reduced pravastatin uptake, suggesting the contribution of additional uptake mechanisms[5,6].

We show for the first time that human OAT7 is a high-capacity, low-affinity transporter for pravastatin.

Contrary to previous publications, *SLC22A9* variability is predominantly influenced by HNF4α regulation and not genetic factors[7].

REFERENCES

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