Expression studies on cultured primary human hepatocytes with ModeScore reveal metabolic response signatures of statins and agonists of CAR, PXR, and PPARa

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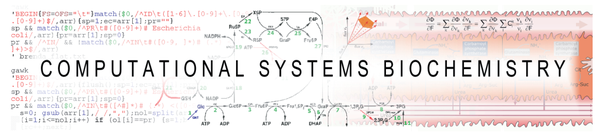


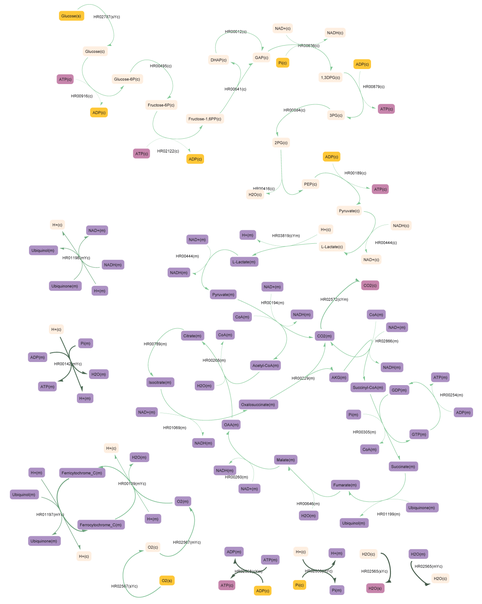
Background. The metabolism of hepatocytes is affected by toxins and pharmaceuticals in a multi-dimensional way. To quantify the effects with targeted metabolomics and proteomics, candidates for relevant metabolic players must be identified first, thus, the bottleneck is often the lack of initial hypotheses. Expression studies show the transcriptional response comprehensively at relatively low cost to form a basis for screening. Using the ModeScore approach it is possible to evaluate them on the level of metabolic functions.

Results: The novel ModeScore approach is applied to two published transcript studies of the response to Atorvastatin, Rosuvastatin, Rifampicin, and specific agonists of CAR and PXR. The method provides an enrichment for the most remarkable functions together with the genes it implements solely based on transcript profiles. Compared with the annotation-based approach, ModeScore relates the transcript changes directly to the enzymes and transporters needed for a metabolic function and not on an artificial definition, thus, present testable hypotheses on the level of cellular function. It is demonstrated how specific response patterns emerge from the ModeScore calculations and how they uncover the cellular strategy of transcriptional regulation.

In conclusion, the novel method provides an enrichment for the most promising functions together with the genes it is based upon for further metabolic analysis solely based on transcript profiles.

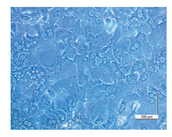
Reference flux distributions (modes)

- Computation with FASIMU [5]



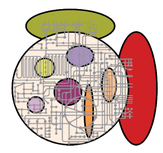
Data.

Full genome RNA transcript profiles (Affymetrix HuGene 2.1) of primary hepatocytes from human donors cultured on collagen monolayer. Treatment with Rifampicin (PXR agonist), Atorvastatin, Rosuvastatin (24h, 48h, SterolTalk experiments [1]), Rifampicin, CITCO (CAR agonist), WY1436 (PPARα agonist) (24h, Stuttgart experiments [2]).



Network.

HepatoNet1b, manually curated network of the human hepatocyte [6], refined to cover more functions, comprises 1500 localized metabolic species, 2702 reactions, 879 annotated genes [3].

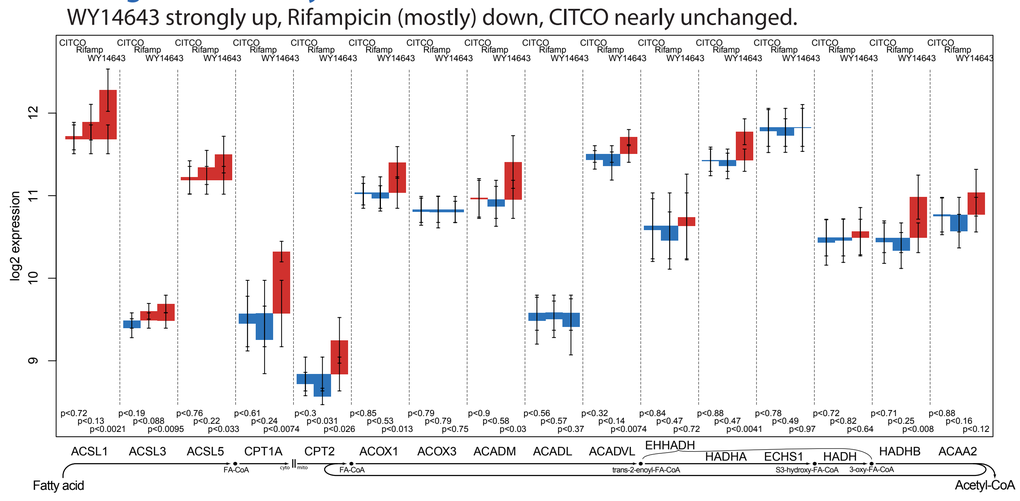


Functions definition [3].

Plethora of metabolic functions (992), three categories:
- Regeneration of important intemediates (72)
- Function of organismic duty (379)
- Synthesis and degradation of cellular constituents (541)

NR agonists — fatty acid activation and beta oxidation

WY14643 strongly up, Rifampicin (mostly) down, CITCO nearly unchanged.

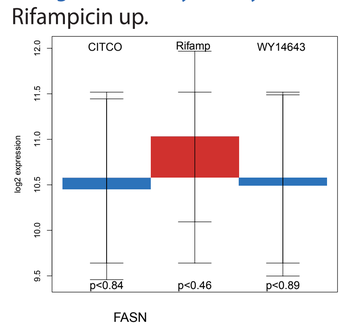


Difference Bar charts

- treated vs. untreated
- blue — down-regulation
- red — up-regulation
- standard deviation
- paired t-test p-value

NR agonists - fatty acid synthesis

Rifampicin up.



Function ranks by ModeScore amplitude

Relative expression scores treated vs. untreated. Only the most up-regulated shown.

	Simulation	Rifamp ampl	Rifamp score	WY14643 ampl	WY14643 score
109	Stearate	0.44	0.54	-0.08	0.56
108	Oleate	0.44	0.5	-0.08	0.49
106	Palmitate	0.42	0.66	-0.08	0.62
46	Palmitolate from Arachidonate	0.41	0.53	-0.08	0.54
43	Palmitolate from Palmitate	0.13	0.42	0.17	0.43
41	Taurine from Cysteine	0.16	0.44	0.1	0.56
121	PI	0.1	0.43	0.13	0.44
55	Bilirubin conjugation	0.25	0.39	-0.05	0.4
125	Cholesterol	0.08	0.47	0.06	0.39
1	Aerobic ATP rephosph (FA)	-0.06	0.59	0.17	0.27
9	GSH red from NADH redox potential	0.1	0.53	-0.01	0.5
8	Aerobic reduction of FAD (FA)	-0.09	0.41	0.17	0.29
73	Oleate degr	-0.09	0.6	0.18	0.59
shortened					

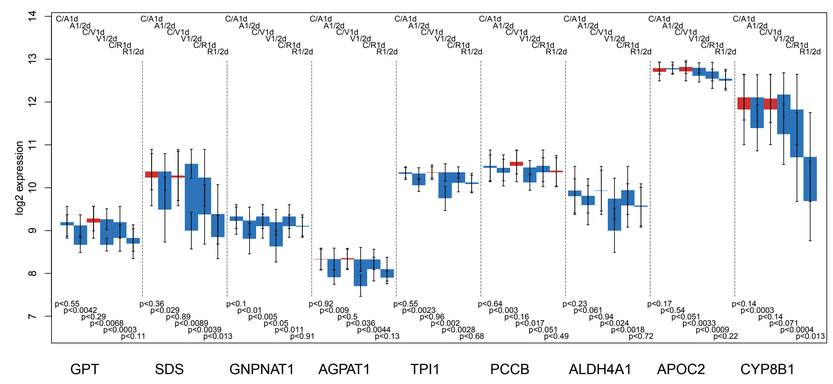
	Simulation	con/Atorv 48h ampl	con/Atorv 48h score	con/Rosuv 48h ampl	con/Rosuv 48h score
27	Isopentenyl-PP	2.7	0.62	2.88	0.57
28	Farnesyl-PP	2.57	0.58	2.71	0.54
148	Cholesterol	1.94	0.6	1.88	0.6
62	Gly-CD-cholate(b)	1.83	0.36	1.84	0.33
61	Glycocholate(b)	1.79	0.35	1.82	0.32
54	Arachidonate from Dihomo-γ-linolenate	1.05	0.48	1.69	0.42
49	γ-Linolenate from Linoleate	1.02	0.46	1.67	0.39
63	Taurocholate(b)	0.23	0.31	1.84	0.26
14	Activated methyl group (THF)	1.08	0.46	0.9	0.49
140	CMP-N-acetylneuraminate	0.71	0.46	0.66	0.47
51	Arachidonate from Linoleate	0.94	0.47	0.2	0.42
124	Glycine	0.84	0.4	0.28	0.33
50	Dihomo-γ-linolenate from Linoleate	0.86	0.44	0.2	0.45
53	Arachidonate from γ-Linolenate	0.86	0.44	0.2	0.45
shortened					

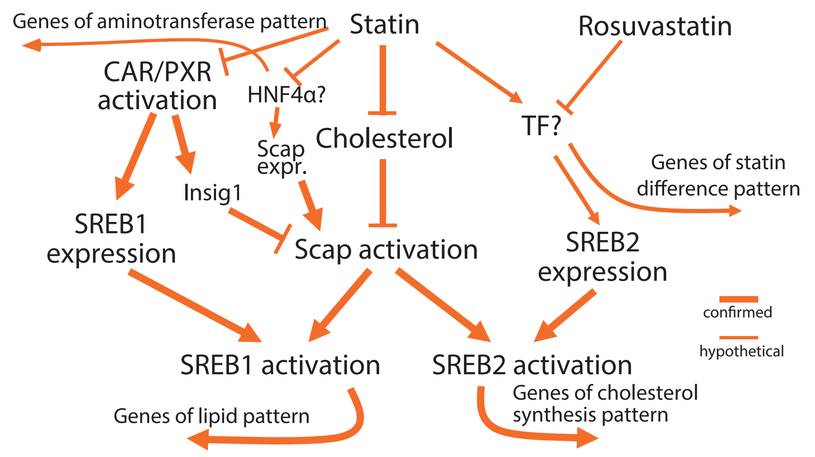
Patterns

Groups of genes show a striking similarity in response to the different treatments. Some of them are obviously functionally related (Cholesterol synthesis), others less so. Presumably, they are commonly regulated. For some of them, the regulation has already been discovered (SREB1/2), for others (Statin difference) it has yet to be confirmed.

Statins - Aminotransferase pattern

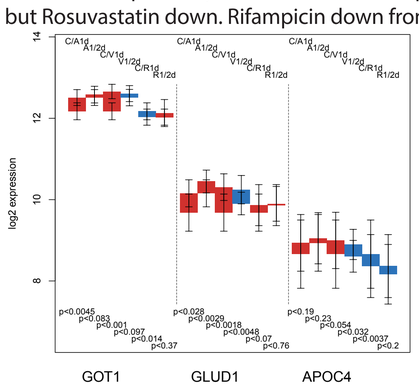
Both statins down at 48h only, Rifampicin down from 24h.





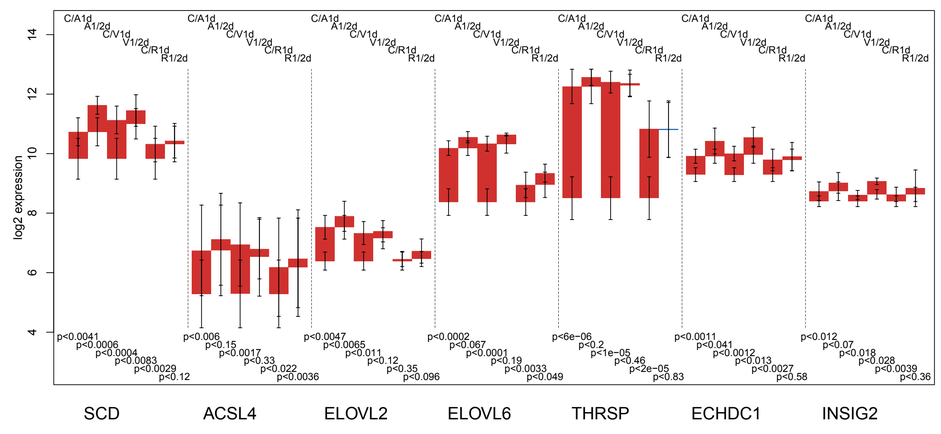
Statins - Statin difference pattern

Both statins up at 24h, Atorvastatin further up at 48h but Rosuvastatin down. Rifampicin down from 24h.



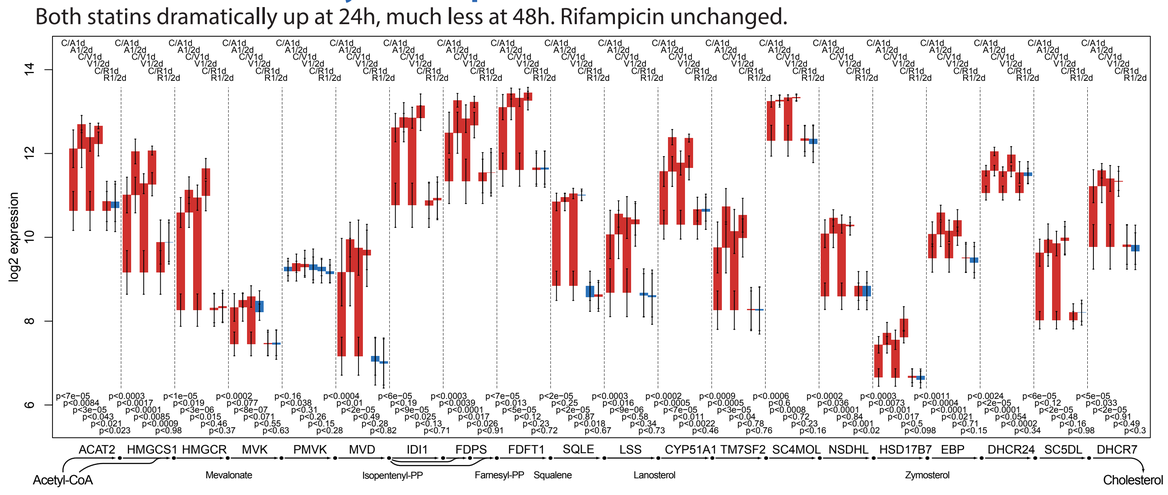
Statins - Lipid pattern

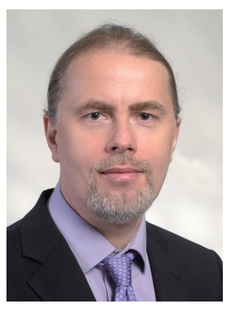
Both statins up in 24h and further at 48h only, Rifampicin similarly up but less amplitude.



Statins - Cholesterol synthesis pattern

Both statins dramatically up at 24h, much less at 48h. Rifampicin unchanged.





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virtual liver network

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