

**A targeted analysis reveals relevant shifts in the methylation and transcription of genes responsible for bile acid homeostasis and drug metabolism in non-alcoholic fatty liver disease**

Supplement

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**Additional file 3: Table S3.** Methylation changes in genes involved in bile acid homeostasis (gene cluster 1) – percent methylation of significantly changed CpG sites in healthy individuals and in patients with different stages of NAFLD

**Table S3** Methylation changes in genes involved in bile acid homeostasis – percent methylation of significantly changed CpG sites in healthy individuals and in patients with different stages of NAFLD

Gene	CpG-sites			ID	Discovery Group (n=74)**				Validation Cohort (n=54)***			
	Total	Sign.	Sign. (%)*		Healthy		NAFLD&NASH		Mild fibrosis stage		Advanced fibrosis stage	
					Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>ABCG8</b>	1	1	100%	cg00760272	0.40	0.049	0.45	0.075	0.39	0.051	0.46	0.097
<b>UGT2B11</b>	1	1	100%	cg27372994	0.26	0.031	0.28	0.044	0.26	0.037	0.30	0.056
<b>ABCG5</b>	12	11	92%	cg01186613	0.40	0.055	0.45	0.074	0.38	0.046	0.45	0.081
				cg00009421	0.37	0.033	0.40	0.043	0.35	0.033	0.39	0.067
				cg07681696	0.43	0.039	0.47	0.055	0.42	0.044	0.47	0.075
				cg16451365	0.39	0.036	0.43	0.048	0.39	0.036	0.43	0.069
				cg11467440	0.48	0.029	0.51	0.040	0.45	0.034	0.48	0.048
				cg18281102	0.38	0.038	0.42	0.049	0.37	0.037	0.41	0.075
				cg25781162	0.42	0.031	0.46	0.043	0.42	0.041	0.46	0.073
				cg00705576	0.40	0.027	0.43	0.041	0.39	0.036	0.42	0.060
				cg11113753	0.37	0.029	0.40	0.042	0.35	0.028	0.38	0.057
				cg08453096	0.48	0.045	0.51	0.050	0.49	0.042	0.52	0.081
<b>HNF1A</b>	8	7	88%	cg13341470	0.38	0.032	0.41	0.040	0.37	0.031	0.40	0.053
				cg01341572	0.47	0.031	0.50	0.048	0.39	0.042	0.44	0.072
				cg05934698	0.39	0.031	0.42	0.042	0.38	0.036	0.43	0.075
				cg25477769	0.51	0.024	0.54	0.032	0.48	0.033	0.52	0.047
				cg23661013	0.41	0.038	0.45	0.053	0.39	0.042	0.43	0.064
				cg02153339	0.38	0.040	0.42	0.050	0.35	0.041	0.39	0.073
				cg14996810	0.49	0.039	0.53	0.052	0.48	0.051	0.52	0.083
<b>OSTalpha</b>	7	6	86%	cg06444781	0.46	0.026	0.49	0.039	0.45	0.046	0.49	0.070
				cg17526770	0.40	0.034	0.44	0.046	0.39	0.030	0.43	0.072
				cg27558485	0.37	0.031	0.40	0.042	0.36	0.037	0.40	0.072
				cg08877188	0.39	0.027	0.41	0.038	0.31	0.028	0.34	0.047
				cg21748136	0.39	0.034	0.42	0.049	0.37	0.038	0.42	0.076
				cg05473677	0.35	0.029	0.37	0.041	0.34	0.025	0.37	0.060
<b>CYP8B1</b>	6	5	83%	cg04478991	0.31	0.031	0.33	0.037	0.30	0.035	0.33	0.058
				cg21979032	0.35	0.035	0.38	0.049	0.33	0.035	0.38	0.062
				cg20365867	0.46	0.042	0.50	0.063	0.45	0.046	0.50	0.076
				cg26124860	0.39	0.046	0.44	0.063	0.39	0.041	0.44	0.090
				cg07147573	0.38	0.041	0.41	0.053	0.36	0.039	0.40	0.079
<b>NR2A1</b>	18	15	83%	cg14404298	0.36	0.035	0.39	0.045	0.34	0.043	0.37	0.063
				cg27420224	0.48	0.050	0.52	0.056	0.43	0.040	0.49	0.070
				cg23792485	0.52	0.045	0.56	0.054	0.48	0.050	0.53	0.072
				cg06640637	0.52	0.040	0.56	0.053	0.46	0.048	0.52	0.070
				cg08314996	0.35	0.042	0.39	0.053	0.32	0.046	0.36	0.068
				cg21081369	0.37	0.029	0.40	0.036	0.34	0.031	0.38	0.056
				cg19717150	0.30	0.034	0.33	0.046	0.25	0.034	0.29	0.049
				cg07006673	0.35	0.039	0.39	0.061	0.34	0.046	0.38	0.074
cg23834593	0.34	0.036	0.37	0.046	0.32	0.036	0.35	0.058				

				cg03862380	0.31	0.032	0.34	0.044	0.29	0.037	0.32	0.065
				cg16221969	0.44	0.029	0.46	0.038	0.42	0.040	0.45	0.056
				cg21559386	0.33	0.038	0.36	0.048	0.31	0.034	0.33	0.061
				cg06126829	0.43	0.032	0.47	0.048	0.42	0.042	0.45	0.065
				cg20265805	0.34	0.030	0.36	0.036	0.32	0.033	0.34	0.048
				cg24084358	0.43	0.034	0.46	0.046	0.41	0.041	0.44	0.072
<b>NR1I3</b>	4	3	75%	cg06277277	0.30	0.035	0.34	0.054	0.29	0.035	0.33	0.065
				cg25214346	0.31	0.029	0.34	0.042	0.31	0.028	0.34	0.064
				cg17028814	0.35	0.036	0.39	0.051	0.35	0.037	0.38	0.066
<b>NR1H4</b>	4	3	75%	cg01202639	0.31	0.041	0.36	0.054	0.32	0.047	0.36	0.067
				cg08098450	0.46	0.052	0.51	0.074	0.49	0.054	0.56	0.082
				cg03771004	0.30	0.043	0.35	0.058	0.32	0.041	0.36	0.088
<b>EPHX1</b>	13	9	69%	cg26187962	0.36	0.038	0.40	0.058	0.36	0.056	0.39	0.075
				cg03337430	0.52	0.043	0.57	0.072	0.54	0.053	0.59	0.079
				cg24928687	0.28	0.042	0.33	0.070	0.26	0.037	0.33	0.083
				cg17468616	0.41	0.042	0.45	0.058	0.40	0.035	0.45	0.071
				cg23096144	0.25	0.028	0.28	0.048	0.23	0.024	0.27	0.052
				cg03459809	0.17	0.027	0.20	0.053	0.16	0.029	0.18	0.048
				cg05385434	0.20	0.043	0.22	0.063	0.20	0.040	0.26	0.098
				cg25152404	0.28	0.031	0.31	0.044	0.26	0.030	0.30	0.073
				cg24868305	0.17	0.032	0.18	0.048	0.16	0.024	0.20	0.073
<b>ABCB11</b>	3	2	67%	cg20118424	0.43	0.047	0.48	0.069	0.44	0.054	0.50	0.093
				cg00295325	0.30	0.036	0.33	0.055	0.32	0.036	0.36	0.071
<b>CYP3A4</b>	3	2	67%	cg23326197	0.77	0.025	0.74	0.033	0.82	0.023	0.79	0.035
				cg22821554	0.85	0.017	0.84	0.024	0.89	0.021	0.87	0.029
<b>SLCO2B1</b>	9	6	67%	cg12537437	0.39	0.040	0.44	0.076	0.37	0.047	0.45	0.082
				cg25367084	0.44	0.041	0.49	0.071	0.38	0.035	0.45	0.079
				cg23577865	0.48	0.029	0.51	0.048	0.44	0.025	0.48	0.056
				cg20358275	0.44	0.032	0.46	0.050	0.39	0.038	0.43	0.055
				cg15751948	0.46	0.045	0.50	0.054	0.44	0.044	0.48	0.074
				cg18589858	0.77	0.022	0.75	0.020	0.81	0.029	0.78	0.032
<b>ABCC2</b>	5	3	60%	cg17044311	0.38	0.050	0.42	0.070	0.37	0.050	0.44	0.093
				cg14947634	0.41	0.052	0.46	0.071	0.41	0.052	0.47	0.091
				cg09448875	0.40	0.049	0.45	0.067	0.40	0.045	0.46	0.093
<b>NROB2</b>	5	3	60%	cg16177693	0.29	0.033	0.32	0.049	0.28	0.030	0.32	0.065
				cg06650260	0.30	0.033	0.33	0.045	0.29	0.036	0.33	0.074
				cg16762386	0.36	0.034	0.40	0.052	0.37	0.040	0.41	0.069
<b>NR1I2</b>	5	3	60%	cg22276685	0.37	0.038	0.40	0.052	0.35	0.037	0.40	0.077
				cg00836482	0.60	0.024	0.57	0.034	0.62	0.036	0.59	0.033
				cg25562664	0.47	0.024	0.45	0.029	0.50	0.041	0.45	0.042
<b>BAAT</b>	2	1	50%	cg05770171	0.48	0.051	0.53	0.070	0.51	0.059	0.57	0.079
<b>SLC10A1</b>	6	3	50%	cg05633152	0.41	0.039	0.45	0.058	0.41	0.036	0.46	0.072
				cg21088438	0.30	0.044	0.33	0.050	0.29	0.045	0.34	0.075
				cg01448863	0.25	0.035	0.27	0.045	0.25	0.033	0.28	0.069
<b>SLCO1B1</b>	2	1	50%	cg00995065	0.36	0.050	0.41	0.073	0.38	0.052	0.46	0.074
<b>SLC27A5</b>	13	5	38%	cg19469742	0.40	0.033	0.43	0.056	0.40	0.049	0.46	0.090

				cg18495710	0.40	0.041	0.45	0.055	0.40	0.041	0.44	0.073
				cg16107172	0.44	0.032	0.47	0.050	0.43	0.037	0.47	0.069
				cg07726085	0.40	0.033	0.43	0.053	0.37	0.039	0.40	0.068
				cg16278661	0.31	0.028	0.34	0.045	0.30	0.030	0.33	0.063
<b>SULT2A1</b>	3	1	33%	cg19139729	0.32	0.032	0.36	0.046	0.31	0.040	0.34	0.076
<b>CYP7B1</b>	12	4	33%	cg01510388	0.30	0.054	0.32	0.066	0.25	0.052	0.32	0.074
				cg00054210	0.26	0.054	0.29	0.067	0.20	0.049	0.25	0.074
				cg04418492	0.04	0.013	0.05	0.014	0.03	0.010	0.05	0.048
				cg24990212	0.10	0.023	0.11	0.033	0.10	0.021	0.13	0.069
<b>HSD3B7</b>	10	3	30%	cg07359991	0.50	0.037	0.53	0.044	0.53	0.046	0.58	0.067
				cg10917602	0.35	0.030	0.38	0.046	0.34	0.036	0.37	0.075
				cg09162137	0.61	0.031	0.59	0.039	0.69	0.040	0.65	0.036
<b>SCP2</b>	7	2	29%	cg05232215	0.14	0.037	0.16	0.055	0.09	0.021	0.14	0.074
				cg06621784	0.19	0.045	0.15	0.053	0.22	0.065	0.13	0.047
<b>CYP27A1</b>	5	1	20%	cg03460682	0.09	0.011	0.10	0.019	0.09	0.016	0.12	0.054
<b>FGF19</b>	17	1	6%	cg24454109	0.67	0.034	0.64	0.046	0.72	0.040	0.69	0.050
<b>NR2B1</b>	9	1	11%	cg14651936	0.77	0.027	0.76	0.026	0.84	0.022	0.81	0.029
<b>ABCB4</b>	4	1	25%	cg04996195	0.23	0.049	0.26	0.059	0.18	0.035	0.24	0.072
<b>SLCO1A2</b>	4	1	25%	cg16923485	0.66	0.036	0.63	0.050	0.72	0.050	0.68	0.058
<b>NR5A2</b>	21	5	24%	cg08158862	0.19	0.026	0.22	0.040	0.20	0.044	0.23	0.058
				cg16993043	0.05	0.025	0.06	0.028	0.04	0.019	0.07	0.070
				cg17804356	0.11	0.015	0.12	0.020	0.11	0.021	0.13	0.053
				cg21851672	0.55	0.028	0.54	0.025	0.58	0.031	0.55	0.029
				cg04308769	0.65	0.044	0.61	0.059	0.71	0.043	0.67	0.049
<b>ATP8B1</b>	9	2	22%	cg12517027	0.60	0.063	0.54	0.093	0.66	0.075	0.53	0.133
				cg21357291	0.12	0.036	0.14	0.046	0.11	0.024	0.15	0.080
<b>FGFR4</b>	12	2	17%	cg00618323	0.21	0.024	0.23	0.036	0.19	0.017	0.21	0.039
				cg04849878	0.25	0.038	0.29	0.061	0.25	0.038	0.29	0.076
<b>ABCC3</b>	8	1	13%	cg27222669	0.75	0.033	0.72	0.043	0.79	0.038	0.75	0.049
<b>ABCC4</b>	8	1	13%	cg24476569	0.55	0.056	0.51	0.064	0.60	0.066	0.54	0.104

Shown are all targets that displayed significantly differentially methylated CpG sites. Listed are the CpG sites that are NAFLD dependently significantly differentially methylated and validated. Targets are ordered according to the percentage of affected CpG sites within the TSS1500 interval.

\* Percentage of differentially methylated CpG sites in NAFLD within the TSS1500 interval as confirmed in two cohorts.

\*\* The discovery cohort is composed of liver healthy and NAFLD patients.

\*\*\* The validation cohort is composed of NAFLD patients with different stages of steatosis and fibrosis.

Mean, percentage methylation of the CpG site; SD, standard deviation; Total, total number of investigated CpG sites of a gene; n, number; coef, coefficient; FDR, false discovery rate