**S2 File**

Supporting information

**Hepatic DNA methylation and expression profiles under prenatal restricted diet in three generations of female rat fetuses**

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**Table A.** Location of the studied cytosines in the CpG islands, primers used in pyrosequencing, and amplicon lengths.

|  |  |  |  |
| --- | --- | --- | --- |
| Cytosines genomic location (rn6/RGSC6.0) | Location in a gene context | Primers sequences | amplicon length (bp) |
| 12:48316548 | 174 bp downstream of the *Usp30* | F: 5’ GGGTGGGAAAGTTTTGTAGTAAGR: 5’ TTCTAAACCCACTCCAACCCCCTCAATCTAseq: 5’ GGAGGTTATTTTGGTTATT | 178 |
| 14:92117731 | 192 bp downstream of the *Grb10* | F: 5’ AGTATAGGTGTTGGGAAGTTTR: 5’ AAACTACAAACCCTATTCCTATTCTTACCCseq: 5’ GGGAATTGATTGGGTAT | 327 |
| 2:113652068 | 280 bp upstream of the *Pld1* | F: 5’ AGGGATTTGTAAGAAGGTTATAGTR: 5’ ACCTTCCTCCATACCTCAATTCTAAseq: 5’ AGTTGGTTTTTTGTTGT | 293 |
| 2:258052775 | 60 bp downstream of the *St6galnac5* | F: 5’ TTTTTGGATGTTGGAATGTGR: 5’ CCCCTACCTTACAACCCTTAAAseq: 5’ GTGGGGGTTTTGTTGA | 206 |
| 5:140802879 | 759 bp upstream of the *Oxct2b* | F: 5’ AGTTTGTTTGGGGTTGGATTAR: 5’ ATCACCAAAAACTACCAATACTAAACseq: 5’ GTTTTAGAGGAAAGTTAATAGTT | 200 |
| 6:26821801 | 842 bp upstream of the *Khk* | F: 5’ GTGGTAGGATGAGGTTGAAGATTTTTAGAR: 5’ AACCACCCTCTCCCTTCCTAACTATCATseq: 5’ AGTTTTTAGAGTTTATG | 256 |
| X:113660107 | 163 bp upstream of the *Acsl4* | F: 5’ GGGGGAGTATAGTGGTGTGR: 5’ ACCCCCCAAAAAACTTTCATCCTAAATseq: 5’ GTTAAGGAAGTTATTATTAAAGAGG | 330 |

**Table B.** Sequences of primers used for real-time PCR studies.

|  |  |  |
| --- | --- | --- |
| Gene | Primers | Amplicon length |
| *Usp30* | F: 5’ TGGGGTCCCATTACAGAGAGR: 5’ TCAGCAGTGTCAAGGACAGG | 208 |
| *Grb10* | F: 5’ GATGGGACAAGCAAAGTGGTR: 5’ ACGATCTCATGGTCCTCCAG | 164 |
| *Pld1* | F: 5’ ATCTACCTCTCTGGCTGTCCTGR: 5’ GTATGTCTTTTGGTGGGAATGG | 220 |
| *St6galnac5* | F: 5’ CAACACTTGGCTGAGCACTGR: 5’ TTGTACATTCATCGGGTCCA | 164 |
| *Oxct2b* | F: 5’ GAGCACTGCACCAAGACAAAR: 5’ CCTTAACCTCGTCCAACGAC | 175 |
| *Khk* | F: 5’ TTCGGCTATGGAGAGGTGGTR: 5’ TGAGCGTAGCCCCTTTCTTC | 118 |
| *Acsl4* | F: 5’ AGCCGCACTGAAGAATTGTCR: 5’ TCTACCCCCTTCTGTTGTGC | 233 |

**Table C.** Clustering analysis into functional pathways of the 374 genes with significantly differed cytosines (accorging to KEGG database).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pathway** | **No of genes** | **P value** | **P value (after Bonferroni correction)** | **Gene’s ID** |
| rno01100: Metabolic pathways | 144 | 1.32306E-44 | 3.09595137598173E-42 | ENSRNOG00000000466, ENSRNOG00000005512, ENSRNOG00000002781, ENSRNOG00000010626, ENSRNOG00000024629, ENSRNOG00000019180, ENSRNOG00000001344, ENSRNOG00000019372, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000014365, ENSRNOG00000001992, ENSRNOG00000014856, ENSRNOG00000001796, ENSRNOG00000009345, ENSRNOG00000049912, ENSRNOG00000017087, ENSRNOG00000032908, ENSRNOG00000017571, ENSRNOG00000008099, ENSRNOG00000012055, ENSRNOG00000046345, ENSRNOG00000021177, ENSRNOG00000010633, ENSRNOG00000013816, ENSRNOG00000005668, ENSRNOG00000008209, ENSRNOG00000009157, ENSRNOG00000003223, ENSRNOG00000009889, ENSRNOG00000019804, ENSRNOG00000016776, ENSRNOG00000003070, ENSRNOG00000006388, ENSRNOG00000001701, ENSRNOG00000007967, ENSRNOG00000012210, ENSRNOG00000010993, ENSRNOG00000014508, ENSRNOG00000012616, ENSRNOG00000017291, ENSRNOG00000028292, ENSRNOG00000020479, ENSRNOG00000028156, ENSRNOG00000021265, ENSRNOG00000059453, ENSRNOG00000018077, ENSRNOG00000005993, ENSRNOG00000014511, ENSRNOG00000011366, ENSRNOG00000017817, ENSRNOG00000032134, ENSRNOG00000012698, ENSRNOG00000012559, ENSRNOG00000010881, ENSRNOG00000011419, ENSRNOG00000003114, ENSRNOG00000007951, ENSRNOG00000029280, ENSRNOG00000016214, ENSRNOG00000038951, ENSRNOG00000046864, ENSRNOG00000004027, ENSRNOG00000024112, ENSRNOG00000026646, ENSRNOG00000018281, ENSRNOG00000042359, ENSRNOG00000000257, ENSRNOG00000029528, ENSRNOG00000005358, ENSRNOG00000018816, ENSRNOG00000003554, ENSRNOG00000006369, ENSRNOG00000008945, ENSRNOG00000008047, ENSRNOG00000000168, ENSRNOG00000026705, ENSRNOG00000015859, ENSRNOG00000006947, ENSRNOG00000008944, ENSRNOG00000000437, ENSRNOG00000016791, ENSRNOG00000016989, ENSRNOG00000006116, ENSRNOG00000013331, ENSRNOG00000002636, ENSRNOG00000018764, ENSRNOG00000059622, ENSRNOG00000018507, ENSRNOG00000007446, ENSRNOG00000032297, ENSRNOG00000060045, ENSRNOG00000046515, ENSRNOG00000004234, ENSRNOG00000003653, ENSRNOG00000005424, ENSRNOG00000000177, ENSRNOG00000002755, ENSRNOG00000019843, ENSRNOG00000028368, ENSRNOG00000009630, ENSRNOG00000037229, ENSRNOG00000020902, ENSRNOG00000018367, ENSRNOG00000037225, ENSRNOG00000052704, ENSRNOG00000016470, ENSRNOG00000009980, ENSRNOG00000016643, ENSRNOG00000018516, ENSRNOG00000011922, ENSRNOG00000007735, ENSRNOG00000008163, ENSRNOG00000000800, ENSRNOG00000052070, ENSRNOG00000008569, ENSRNOG00000011528, ENSRNOG00000017777, ENSRNOG00000047708, ENSRNOG00000043377, ENSRNOG00000028717, ENSRNOG00000011329, ENSRNOG00000018319, ENSRNOG00000042503, ENSRNOG00000009994, ENSRNOG00000028711, ENSRNOG00000007775, ENSRNOG00000019979, ENSRNOG00000018129, ENSRNOG00000001440, ENSRNOG00000021886, ENSRNOG00000017593, ENSRNOG00000039859, ENSRNOG00000028047, ENSRNOG00000001736, ENSRNOG00000011053, ENSRNOG00000016122, ENSRNOG00000056954, ENSRNOG00000038085, ENSRNOG00000008475, ENSRNOG00000002642, ENSRNOG00000049110, ENSRNOG00000007583, ENSRNOG00000021150 |
| rno04910: Insulin signaling pathway | 28 | 1.98241E-13 | 4.63989E-11 | ENSRNOG00000021147, ENSRNOG00000006388, ENSRNOG00000052343, ENSRNOG00000021689, ENSRNOG00000011377, ENSRNOG00000015480, ENSRNOG00000010957, ENSRNOG00000018494, ENSRNOG00000014366, ENSRNOG00000050578, ENSRNOG00000014904, ENSRNOG00000006116, ENSRNOG00000002833, ENSRNOG00000029986, ENSRNOG00000009079, ENSRNOG00000053869, ENSRNOG00000004060, ENSRNOG00000019228, ENSRNOG00000003463, ENSRNOG00000002946, ENSRNOG00000018903, ENSRNOG00000020902, ENSRNOG00000001868, ENSRNOG00000004978, ENSRNOG00000020657, ENSRNOG00000003919, ENSRNOG00000007583, ENSRNOG00000023428 |
| rno04932: Non-alcoholic fatty liver disease (NAFLD) | 27 | 4.56661E-11 | 1.06859E-08 | ENSRNOG00000005512, ENSRNOG00000018816, ENSRNOG00000001068, ENSRNOG00000007967, ENSRNOG00000008569, ENSRNOG00000020417, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000028717, ENSRNOG00000013331, ENSRNOG00000002833, ENSRNOG00000042503, ENSRNOG00000029986, ENSRNOG00000018129, ENSRNOG00000017571, ENSRNOG00000019228, ENSRNOG00000003463, ENSRNOG00000021177, ENSRNOG00000017817, ENSRNOG00000032134, ENSRNOG00000002946, ENSRNOG00000018903, ENSRNOG00000005668, ENSRNOG00000026646, ENSRNOG00000016470, ENSRNOG00000018281, ENSRNOG00000023428 |
| rno00190: Oxidative phosphorylation | 24 | 7.0201E-10 | 1.6427E-07 | ENSRNOG00000005512, ENSRNOG00000017571, ENSRNOG00000008163, ENSRNOG00000018816, ENSRNOG00000008569, ENSRNOG00000007967, ENSRNOG00000021177, ENSRNOG00000017817, ENSRNOG00000000557, ENSRNOG00000032134, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000005668, ENSRNOG00000028717, ENSRNOG00000038951, ENSRNOG00000001992, ENSRNOG00000052704, ENSRNOG00000013331, ENSRNOG00000016470, ENSRNOG00000026646, ENSRNOG00000042503, ENSRNOG00000018281, ENSRNOG00000049912, ENSRNOG00000018129 |
| rno04931: Insulin resistance | 20 | 5.92681E-09 | 1.38687E-06 | ENSRNOG00000053869, ENSRNOG00000042411, ENSRNOG00000006388, ENSRNOG00000019228, ENSRNOG00000021689, ENSRNOG00000003463, ENSRNOG00000015480, ENSRNOG00000018494, ENSRNOG00000002946, ENSRNOG00000018903, ENSRNOG00000020902, ENSRNOG00000014904, ENSRNOG00000019742, ENSRNOG00000002833, ENSRNOG00000003919, ENSRNOG00000029986, ENSRNOG00000018507, ENSRNOG00000007583, ENSRNOG00000023428, ENSRNOG00000019057 |
| rno00510: N-Glycan biosynthesis | 14 | 9.36456E-09 | 2.19131E-06 | ENSRNOG00000017087, ENSRNOG00000004234, ENSRNOG00000046515, ENSRNOG00000000800, ENSRNOG00000014511, ENSRNOG00000006369, ENSRNOG00000012055, ENSRNOG00000011528, ENSRNOG00000046345, ENSRNOG00000010993, ENSRNOG00000012559, ENSRNOG00000016989, ENSRNOG00000049110, ENSRNOG00000011922 |
| rno00310: Lysine degradation | 14 | 1.57418E-08 | 3.68358E-06 | ENSRNOG00000016680, ENSRNOG00000001417, ENSRNOG00000024629, ENSRNOG00000001344, ENSRNOG00000061499, ENSRNOG00000015621, ENSRNOG00000028207, ENSRNOG00000016790, ENSRNOG00000004027, ENSRNOG00000013045, ENSRNOG00000020386, ENSRNOG00000017508, ENSRNOG00000021614, ENSRNOG00000023317 |
| rno04150: mTOR signaling pathway | 15 | 1.80238E-08 | 4.21756E-06 | ENSRNOG00000042411, ENSRNOG00000052343, ENSRNOG00000019228, ENSRNOG00000021689, ENSRNOG00000037505, ENSRNOG00000002763, ENSRNOG00000010957, ENSRNOG00000050578, ENSRNOG00000018903, ENSRNOG00000012061, ENSRNOG00000057078, ENSRNOG00000017426, ENSRNOG00000017297, ENSRNOG00000003919, ENSRNOG00000023428 |
| rno00564: Glycerophospholipid metabolism | 17 | 1.40689E-07 | 3.29207E-05 | ENSRNOG00000018077, ENSRNOG00000008320, ENSRNOG00000000177, ENSRNOG00000025012, ENSRNOG00000026705, ENSRNOG00000000437, ENSRNOG00000028368, ENSRNOG00000016791, ENSRNOG00000009980, ENSRNOG00000014856, ENSRNOG00000024112, ENSRNOG00000028156, ENSRNOG00000018319, ENSRNOG00000016643, ENSRNOG00000012269, ENSRNOG00000021265, ENSRNOG00000001796 |
| rno05012: Parkinson's disease | 21 | 2.65611E-07 | 6.2151E-05 | ENSRNOG00000005512, ENSRNOG00000017571, ENSRNOG00000018816, ENSRNOG00000008569, ENSRNOG00000007967, ENSRNOG00000021177, ENSRNOG00000017817, ENSRNOG00000019465, ENSRNOG00000032134, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000005668, ENSRNOG00000028717, ENSRNOG00000004978, ENSRNOG00000013331, ENSRNOG00000016470, ENSRNOG00000026646, ENSRNOG00000042503, ENSRNOG00000018281, ENSRNOG00000049912, ENSRNOG00000018129 |
| rno05010: Alzheimer's disease | 23 | 3.0069E-07 | 7.03589E-05 | ENSRNOG00000005512, ENSRNOG00000021147, ENSRNOG00000017571, ENSRNOG00000004060, ENSRNOG00000018816, ENSRNOG00000008569, ENSRNOG00000007967, ENSRNOG00000021177, ENSRNOG00000017817, ENSRNOG00000032134, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000005668, ENSRNOG00000028717, ENSRNOG00000013331, ENSRNOG00000002833, ENSRNOG00000026646, ENSRNOG00000016470, ENSRNOG00000042503, ENSRNOG00000018281, ENSRNOG00000049912, ENSRNOG00000018129, ENSRNOG00000021150 |
| rno05231: Choline metabolism in cancer | 16 | 1.85099E-06 | 0.000433038 | ENSRNOG00000008320, ENSRNOG00000019228, ENSRNOG00000001068, ENSRNOG00000021689, ENSRNOG00000000177, ENSRNOG00000050578, ENSRNOG00000026705, ENSRNOG00000018903, ENSRNOG00000016791, ENSRNOG00000012061, ENSRNOG00000009980, ENSRNOG00000024112, ENSRNOG00000028156, ENSRNOG00000003919, ENSRNOG00000001796, ENSRNOG00000023428 |
| rno00600: Sphingolipid metabolism | 11 | 5.51419E-06 | 0.001289491 | ENSRNOG00000002781, ENSRNOG00000009980, ENSRNOG00000032942, ENSRNOG00000000177, ENSRNOG00000012210, ENSRNOG00000003223, ENSRNOG00000010626, ENSRNOG00000036866, ENSRNOG00000012953, ENSRNOG00000009345, ENSRNOG00000000257 |
| rno00514: Other types of O-glycan biosynthesis | 8 | 6.277E-06 | 0.001467745 | ENSRNOG00000019843, ENSRNOG00000046515, ENSRNOG00000050298, ENSRNOG00000012146, ENSRNOG00000028207, ENSRNOG00000010104, ENSRNOG00000001417, ENSRNOG00000023317 |
| rno05016: Huntington's disease | 22 | 7.18234E-06 | 0.001679261 | ENSRNOG00000005512, ENSRNOG00000017571, ENSRNOG00000018816, ENSRNOG00000008569, ENSRNOG00000000604, ENSRNOG00000007967, ENSRNOG00000019298, ENSRNOG00000021177, ENSRNOG00000017817, ENSRNOG00000032134, ENSRNOG00000048320, ENSRNOG00000011949, ENSRNOG00000005668, ENSRNOG00000028717, ENSRNOG00000013331, ENSRNOG00000016470, ENSRNOG00000026646, ENSRNOG00000042503, ENSRNOG00000018281, ENSRNOG00000049912, ENSRNOG00000018129, ENSRNOG00000021150 |
| rno04012: ErbB signaling pathway | 14 | 1.40695E-05 | 0.003286883 | ENSRNOG00000021147, ENSRNOG00000019228, ENSRNOG00000021689, ENSRNOG00000010957, ENSRNOG00000006450, ENSRNOG00000014366, ENSRNOG00000018903, ENSRNOG00000017879, ENSRNOG00000012061, ENSRNOG00000001868, ENSRNOG00000002833, ENSRNOG00000020657, ENSRNOG00000003919, ENSRNOG00000023428 |
| rno00534: Glycosaminoglycan biosynthesis - heparan sulfate / heparin | 8 | 1.61706E-05 | 0.003776809 | ENSRNOG00000016776, ENSRNOG00000017659, ENSRNOG00000024591, ENSRNOG00000010598, ENSRNOG00000021886, ENSRNOG00000019979, ENSRNOG00000019804, ENSRNOG00000008944 |
| rno04070: Phosphatidylinositol signaling system | 14 | 2.53482E-05 | 0.005913996 | ENSRNOG00000056954, ENSRNOG00000012061, ENSRNOG00000004060, ENSRNOG00000019228, ENSRNOG00000020479, ENSRNOG00000024112, ENSRNOG00000021265, ENSRNOG00000001796, ENSRNOG00000018516, ENSRNOG00000023428, ENSRNOG00000026705, ENSRNOG00000060045, ENSRNOG00000021150, ENSRNOG00000018903 |
| rno00563: Glycosylphosphatidylinositol(GPI)-anchor biosynthesis | 8 | 2.81363E-05 | 0.006562353 | ENSRNOG00000029280, ENSRNOG00000003070, ENSRNOG00000003554, ENSRNOG00000011366, ENSRNOG00000059622, ENSRNOG00000049110, ENSRNOG00000042359, ENSRNOG00000007735 |
| rno00561: Glycerolipid metabolism | 11 | 3.0989E-05 | 0.007225319 | ENSRNOG00000000437, ENSRNOG00000018077, ENSRNOG00000004027, ENSRNOG00000009980, ENSRNOG00000000177, ENSRNOG00000024112, ENSRNOG00000001796, ENSRNOG00000001344, ENSRNOG00000028711, ENSRNOG00000014508, ENSRNOG00000026705 |
| rno01130: Biosynthesis of antibiotics | 21 | 7.29922E-05 | 0.016935737 | ENSRNOG00000003653, ENSRNOG00000032908, ENSRNOG00000001440, ENSRNOG00000005424, ENSRNOG00000007967, ENSRNOG00000024629, ENSRNOG00000001344, ENSRNOG00000043377, ENSRNOG00000011329, ENSRNOG00000016122, ENSRNOG00000018367, ENSRNOG00000038085, ENSRNOG00000006116, ENSRNOG00000013331, ENSRNOG00000028292, ENSRNOG00000004027, ENSRNOG00000009994, ENSRNOG00000018507, ENSRNOG00000032297, ENSRNOG00000009889, ENSRNOG00000029528 |
| rno04152: AMPK signaling pathway | 15 | 0.000137029 | 0.031558398 | ENSRNOG00000019228, ENSRNOG00000021689, ENSRNOG00000003463, ENSRNOG00000010728, ENSRNOG00000050578, ENSRNOG00000018911, ENSRNOG00000018903, ENSRNOG00000016122, ENSRNOG00000013552, ENSRNOG00000020902, ENSRNOG00000005389, ENSRNOG00000017297, ENSRNOG00000003919, ENSRNOG00000029986, ENSRNOG00000023428 |
| rno04666: Fc gamma R-mediated phagocytosis | 12 | 0.00016187 | 0.037172188 | ENSRNOG00000012061, ENSRNOG00000001868, ENSRNOG00000019228, ENSRNOG00000001068, ENSRNOG00000021689, ENSRNOG00000009980, ENSRNOG00000028156, ENSRNOG00000000177, ENSRNOG00000003919, ENSRNOG00000010626, ENSRNOG00000023428, ENSRNOG00000018903 |

**Table D.** Genes involved in oxidative phosphorylation with altered cytosine methylation in R group when comparing to C group.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mitochondrial membrane complex** | **Genes with altered cytosine methylation** | **Chromosome and cytosine position (rn6/RGSC6.0)** | **% of difference in methylation in R group comparing to C group** |
| I - NADH-coenzyme Q oxidoreductase | *Ndufa10* | 9:99651701 | 2.6415 |
| *Ndufa11* | 9:10338253 | 1.7341 |
| *Ndufa2* | 18:29587615 | -1.7483 |
| *Ndufa2* | 18:29587928 | 1.4493 |
| *Ndufa4* | 4:38241166 | -1.9520 |
| *Ndufa6* | 7:123586929 | 1.7263 |
| *Ndufa6* | 7:123586974 | -1.9820 |
| *Ndufa8* | 3:15379153 | -3.3846 |
| *Ndufab1* | 1:192057610 | 1.9727 |
| *Ndufb5* | 2:119139606 | -1.3447 |
| *Ndufb7* | 19:24701019 | 2.1622 |
| *Ndufs5* | 5:141405271 | 2.4540 |
| *Ndufv2* | 9:113900145 | -2.1638 |
| *Ndufv2* | 9:113900307 | -2.4433 |
| II - Succinate-Q oxidoreductase | *Sdha* | 1:31545326 | 1.2642 |
| *Sdhb* | 5:159484430 | 1.6000 |
| III - Q-cytochrome c oxidoreductase | *Uqcrc1* | 8:117679138 | 1.7568 |
| *Uqcrfs1* | 17:35678011 | 2.4272 |
| IV - Cytochrome c oxidase | *Cox17* | 11:64968378 | -3.2258 |
| *Cox4i1* | 19:54245885 | 2.1033 |
| *Cox5a* | 8:62298290 | -2.2312 |
| *Cox5a* | 8:62298415 | -2.7837 |
| *Cox8a* | 1:222468938 | 1.4742 |
| V - ATP synthase | *Ppa1* | 20:31265624 | 2.2409 |
| *Atp6v1a* | 11:61530667 | 3.0641 |
| *Atp6v1g1* | 5:79367706 | -2.6634 |

+ or – means that the methylation in R group was higher or lower than in C group, respectively

**Table E.** Mean coverage after tNGS (± SD) for the analysed cytosines in the seven selected genes

|  |  |  |
| --- | --- | --- |
| **Gene** | **C-group** | **R-group** |
| **Mean** | **± SD** | **Mean** | **± SD** |
| *Usp30* | 54.67 | 7.69 | 46.50 | 18.36 |
| *Grb10* | 40.83 | 4.75 | 40.00 | 9.94 |
| *Pld1* | 35.50 | 13.44 | 33.50 | 12.29 |
| *St6galnac5* | 54.00 | 8.07 | 51.17 | 14.86 |
| *Oxct2b* | 67.33 | 20.87 | 72.33 | 30.19 |
| *Khk* | 41.67 | 5.01 | 39.00 | 11.40 |
| *Acsl4* | 41.67 | 14.36 | 37.33 | 13.03 |

**Table F.** The assignment of the selected seven genes to particular KEGG pathways.

|  |  |
| --- | --- |
| **Gene** | **Pathways (rat database)** |
| ***Grb10*** *(growth factor receptor bound protein 10)* | rno04150: mTOR signalling pathway |
| ***Oxct2b*** *(3-oxoacid CoA transferase 2B)* | no pathway for rat |
| ***Usp30*** *(ubiquitin specific peptidase 30)* | rno04137: Mitophagy - animal |
| ***Pld1*** *(phospholipase D1)* | rno00564: Glycerophospholipid metabolismrno00565: Ether lipid metabolismrno01100: Metabolic pathwaysrno04014: Ras signalling pathwayrno04024: cAMP signalling pathwayrno04071: Sphingolipid signalling pathwayrno04072: Phospholipase D signalling pathwayrno04144: Endocytosisrno04666: Fc gamma R-mediated phagocytosisrno04724: Glutamatergic synapserno04912: GnRH signalling pathwayrno04928: Parathyroid hormone synthesis, secretion and actionrno05212: Pancreatic cancerrno05231: Choline metabolism in cancer |
| ***Khk*** *(ketohexokinase)* | rno00051: Fructose and mannose metabolismrno01100: Metabolic pathways |
| ***St6galnac5*** *(ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5)* | rno00604: Glycosphingolipid biosynthesis - ganglio seriesrno01100: Metabolic pathways |
| ***Acsl4*** *(acyl-CoA synthetase long-chain family member 4)* | rno00061: Fatty acid biosynthesisrno00071: Fatty acid degradationrno01100: Metabolic pathwaysrno01212: Fatty acid metabolismrno03320: PPAR signalling pathwayrno04146: Peroxisomerno04216: Ferroptosisrno04714: Thermogenesisrno04920: Adipocytokine signalling pathway |

**Table G.** Methylation level analysis of particular cytosines across generations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **CpG** | **Group** | **P value** | **P value (after Bonferroni correction)** | **Tukey Honest Significant Test****(mean difference and S: significant or NS: not significant)** |
| **F1 versus F2** | **F1 versus F3** | **F2 versus F3** |
| *Grb10* | CpG1 | C | 0.2339 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.2888 | 1.0000 | NA | NA | NA |
| CpG2 | C | 0.3149 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.5150 | 1.0000 | NA | NA | NA |
| CpG3\*\* | C | 0.0000 | 0.0005 | 13.833 (S) | 12.666 (S) | -1.166 (NS) |
| CpG3\* | R | 0.0001 | 0.0036 | 12.500 (S) | 10.833 (S) | -1.666 (NS) |
| CpG4\* | C | 0.0000 | 0.0019 | 14.500 (S) | 15.000 (S) | 0.500 (NS) |
| CpG4\*\* | R | 0.0000 | 0.0000 | 16.333 (S) | 16.500 (S) | 0.166 (NS) |
| CpG5\*\* | C | 0.0000 | 0.0003 | 15.333 (S) | 15.000 (S) | -0.333 (NS) |
| CpG5\*\* | R | 0.0000 | 0.0000 | 16.333 (S) | 16.166 (S) | -0.166 (NS) |
| *Oxct2b* | CpG1 | C | 0.7613 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.4800 | 1.0000 | NA | NA | NA |
| CpG2 | C | 0.8426 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.0889 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.7217 | 1.0000 | NA | NA | NA |
| CpG3 | R | 0.3066 | 1.0000 | NA | NA | NA |
| CpG4 | C | 0.3398 | 1.0000 | NA | NA | NA |
| CpG4 | R | 0.0779 | 1.0000 | NA | NA | NA |
| *Usp30* | CpG1 | C | 0.8263 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.3512 | 1.0000 | NA | NA | NA |
| CpG2 | C | 0.7512 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.4797 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.7257 | 1.0000 | NA | NA | NA |
| CpG3 | R | 0.5072 | 1.0000 | NA | NA | NA |
| CpG4 | C | 0.0462 | 1.0000 | NA | NA | NA |
| CpG4 | R | 0.3911 | 1.0000 | NA | NA | NA |
| *Pld1* | CpG1 | C | 0.8036 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.0917 | 1.0000 | NA | NA | NA |
| CpG2 | C | 0.6844 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.9256 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.1609 | 1.0000 | NA | NA | NA |
| CpG3 | R | 0.2423 | 1.0000 | NA | NA | NA |
| CpG4 | C | 0.5425 | 1.0000 | NA | NA | NA |
| CpG4 | R | 0.0600 | 1.0000 | NA | NA | NA |
| *St6galnac5* | CpG1 | C | 0.1230 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.0041 | 0.2453 | NA | NA | NA |
| CpG2 | C | 0.6291 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.4814 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.0041 | 0.2478 | NA | NA | NA |
| CpG3\* | R | 0.0001 | 0.0046 | 8.166 (S) | 7.000 (S) | -1.166 (NS) |
| *Khk* | CpG1 | C | 0.0575 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.2216 | 1.0000 | NA | NA | NA |
| CpG2 | C | 0.1402 | 1.0000 | NA | NA | NA |
| CpG2 | R | 0.0269 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.3828 | 1.0000 | NA | NA | NA |
| CpG3 | R | 0.1012 | 1.0000 | NA | NA | NA |
| CpG4 | C | 0.0431 | 1.0000 | NA | NA | NA |
| CpG4 | R | 0.1130 | 1.0000 | NA | NA | NA |
| CpG5 | C | 0.1042 | 1.0000 | NA | NA | NA |
| CpG5 | R | 0.5638 | 1.0000 | NA | NA | NA |
| *Acsl4* | CpG1 | C | 0.2444 | 1.0000 | NA | NA | NA |
| CpG1 | R | 0.0009 | 0.0540 | NA | NA | NA |
| CpG2 | C | 0.0143 | 0.8568 | NA | NA | NA |
| CpG2 | R | 0.0187 | 1.0000 | NA | NA | NA |
| CpG3 | C | 0.1625 | 1.0000 | NA | NA | NA |
| CpG3 | R | 0.0066 | 0.3974 | NA | NA | NA |
| CpG4 | C | 0.0785 | 1.0000 | NA | NA | NA |
| CpG4 | R | 0.7792 | 1.0000 | NA | NA | NA |
| CpG5 | C | 0.0518 | 1.0000 | NA | NA | NA |
| CpG5 | R | 0.0380 | 1.0000 | NA | NA | NA |

\* p value < 0.05; \*\* p value < 0.001 after Bonferroni correction; NA-not analysed

**Figure A.** The histogram of absolute differences in methylation levels of cytosines, between studied groups from F1 fetuses. Y-axis indicates frequency of cytosines with detected absolute difference in methylation level on X-axis. Methylation levels differences are expressed as percentages. Only cytosines with absolute difference higher than 10% were selected for further analyses.



**Figure B.** Transgenerational study of cytosine (CpG) methylation levels by pyrosequencing. Values are means ± SDs. A: *Khk*; B: *Grb10*; C: *Pld1*; D: *Acsl4*. The underlined cytosines are those selected from NGS.



**Figure C.** The transgenerational analysis of methylation level across generation. The boxes indicate the lower and upper quartiles, with the median marked by the horizontal line. Whiskers indicate 1.5 of the interquartile range. Outlying observations are marked with diamonds. Ona-way ANOVA was applied to compare group means. A star and double star, indicate a significant difference of p < 0.05 and p < 0.001, respectively.



**Figure D.** The hepatic relative transcript level in three generations of rats. The results show the deltaCt value. The boxes indicate the lower and upper quartiles, with the median marked by the horizontal line. Whiskers indicate 1.5 of the interquartile range. Outlying observations are marked with diamonds. Ona-way ANOVA was applied to compare group means. A star indicates a significant difference (p = 0.0295 for *Pld1* and p = 0.0253 for *Oxct2b*, respectively).

