**SUPPLEMENTARY MATERIALS**

**Tab 1S.** Primers’ sequences used for TL analysis and DNA methylation analyses for LINE-1 and 6CpG-EA calculation.

|  |  |  |  |
| --- | --- | --- | --- |
| **A) Primers for TL relative quantification** | |  |  |
|  | Fw (5'-3') | Rv (5'-3') |  |
| TEL | CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT | GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT |  |
| IFNB1 | GGT TAC CTC CGA AAC TGA AGA | CCT TTC ATA TGC AGT ACA TTA GCC |  |
|  |  |  |  |
| **B) Primers for amplification and DNA methylation assessment** | | | |
|  | Fw (5'-3') | Rv (5'-3') | seq (5'-3') |
| LINE-1 | TTT TGA GTT AGG TGT GGG ATA TA | Bio-AAA ATC AAA AAA TTC CCT TTC | AGT TAG GTG TGG ATA TAG T |
| FHL2 | GTG TTT TTA GGG TTT TGG GAG TAT AGT AGT | Bio-CAC CTC CTA AAA CTT CTC CAA TCT CC | GGT TTT GGG AGT ATA GTA GTT |
| IGSF11 | GTT GGA TAG TTT GTG GGT AGA AAA TTT A | Bio-ATT ATT CAT TCA TTA TTC TCC TTA AAA AAA TCT TAT T | AGA AGT TAA GAA GGT ATA GAT A |
| CCDC120 | TGT TGA GGG AGG GGA ATG TTT GTA TTT AT | Bio-CCA ATA ATA TCT ATA TCA TCA ACA TTT CTA CAA CTT | GGA GGG GAA TGT TTG |
| MEIS1 | TTG AAT AAT TAG TAA GAT TTT TGT TTG AAG GTT T | Bio-TTA CCT TTA AAA CAA CAA AAT AAA TCA CAC TAA CC | TTA GTA AGA TTT TTG TTT G |
| ELOVL | Bio-GGG AGG GGA GTA GGG TAA GTG A | CCA TCT AAA CAA CCA ATA AAT ATT CCT AAA AC | AAT AAA TAT TCC TAA AAC TC |
| COL1A2 | TTG AAG GGA AGA GGT AAG GAA GAT TTT A | Bio-TAA CCC ATC TTT TTC CTT CTT CTC A | AAT TTG TAT AGA GAG TGT TTA TTG |

**Table 2S.** CpG ID and associated genes for the selected 6 CpGs used for the EA calculation according to Han et al. are displayed. The equation proposed by Han et al. for the EA calculation according to the 6CpGs is shown at the bottom of the table.

|  |  |  |
| --- | --- | --- |
| **Gene Name** | **CpG ID** | **Methylation level code in the algorithm** |
| FHL2 | cg22454769 | α |
| IGSF11 | cg00329615 | β |
| CCDC102B | cg19283806 | γ |
| MEIS1-AS3 | cg11807280 | ε |
| ELOVL2 | cg16867657 | δ |
| COL1A1 | cg18618815 | ϕ |
| EA = 32.07 + 0.99\*α + (-0.12)\*β + (-0.99)\*γ + (-0.09)\*ε + 0.05\*δ + 0.11\*ϕ | | |

**Table 3S.** Diet quality and nutrient intakes in the healthy and western diet groups. Circulating levels of homocysteine in the two groups are also shown.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy diet group** | | | | **Western diet group** | | | | |
|  | min | max | median | IQR | min | max | median | IQR | p |
| B1 intake (mg/dl) | 0.59 | 14.41 | 1.46 | 0.84 | 0.54 | 16.09 | 1.38 | 0.83 | 0.155 |
| B2 intake (mg/dl) | 0.78 | 15.37 | 2.00 | 1.00 | 0.72 | 21.43 | 1.73 | 0.70 | **0.006** |
| B6 intake (mg/dl) | 0.89 | 51.23 | 2.41 | 1.15 | 0.6 | 37.68 | 1.85 | 1.11 | **1.7\*10-4** |
| B9 intake (µg/dl) | 170.25 | 1532.85 | 408.17 | 209.86 | 126.05 | 1032.33 | 312.07 | 121.94 | **1.84\*10-7** |
| B12 intake (µg/dl) | 0.70 | 500.84 | 4.58 | 3.53 | 0.73 | 37.46 | 3.51 | 2.56 | 0.091 |
| Choline intake (mg/dl) | 81.92 | 1687.81 | 410.24 | 273.71 | 92.03 | 1363.75 | 368.85 | 221.34 | 0.325 |
| Betaine intake (mg/dl) | 8.40 | 482.46 | 62.08 | 79.98 | 6.70 | 422.38 | 111.05 | 118.76 | **5.9\*10-6** |
| Plasma Homocysteine (µM) | 4.82 | 19.43 | 10.09 | 3.63 | 7.17 | 21.32 | 10.97 | 4.16 | *0.034* |
| Bonf P=0.006 | |  |  |  |  |  |  |  |  |

**Table 4S.** Intakes of nutrients involved in 1CC in the two genders.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **M** | | | | **F** | | | |  |
|  | Min | Max | Mean | SD | Min | Max | Mean | SD | p |
| B1 intake (mg/dl) | 0.54 | 16.09 | 2.0203 | 2.04466 | 0.59 | 11.21 | 1.6034 | 1.6844 | **2.3\*10-7** |
| B2 intake (mg/dl) | 0.85 | 17.47 | 2.4732 | 2.22171 | 0.72 | 21.43 | 2.1675 | 2.45831 | **4.2\*10-5** |
| B6 intake (mg/dl) | 0.64 | 37.68 | 3.2504 | 4.24812 | 0.6 | 51.23 | 3.0203 | 5.55344 | **2.3\*10-4** |
| B9 intake (µg/dl) | 139.56 | 1532.85 | 427.1592 | 201.6235 | 126.05 | 1140.86 | 354.4114 | 175.2046 | **4.5\*10-4** |
| B12 intake (µg/dl) | 0.7 | 500.84 | 11.1915 | 49.84499 | 0.73 | 12.27 | 3.7916 | 2.3545 | **7.2\*10-6** |
| Choline intake (mg/dl) | 81.92 | 1470.13 | 501.5905 | 236.017 | 83.83 | 1687.81 | 355.4558 | 230.6262 | **3.04\*10-8** |
| Betaine intake (mg/dl) | 9.39 | 422.38 | 131.0665 | 102.9698 | 6.7 | 482.46 | 87.207 | 69.94304 | **0.005** |

**Table 5S.** Description of PCs identified in the PCA. A) Principal Component Analysis (PCA). Eigenvalues for each PC and % of variance explained are shown. 2 PC have been selected, cumulatively explaining 57.5% of the variance. B) PCA Loadings defining which is the contribution of the original variables to the principal components. Varimax rotation with Kaiser normalization has been applied.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **A** | |  |  | |  |  | |
| PC | |  | Eigenvalues | |  | Weight of rotated factors | |
|  |  | Total | % variance | % cumulative | total | % variance | % cumulative |
|  | 1 | 2.886 | 41.223 | 41.223 | 2.873 | 41.046 | 41.046 |
|  | 2 | 1.14 | 16.289 | **57.512** | 1.153 | 16.466 | 57.512 |
|  | 3 | 0.98 | 13.997 | 71.508 |  |  |  |
|  | 4 | 0.88 | 12.571 | 84.079 |  |  |  |
|  | 5 | 0.596 | 8.514 | 92.593 |  |  |  |
|  | 6 | 0.442 | 6.308 | 98.902 |  |  |  |
|  | 7 | 0.077 | 1.098 | 100 |  |  |  |

|  |  |  |
| --- | --- | --- |
| **B** | Rotated matrix | |
|  | Component | |
|  | PC1 | PC2 |
| B1 intake | **0.934** | 0.027 |
| B2 intake | **0.923** | 0.011 |
| B6 intake | **0.723** | -0.2 |
| B12 intake | 0.086 | **-0.468** |
| Choline intake | 0.41 | **0.564** |
| Betaine intake | -0.029 | **0.747** |
| B9 intake | **0.671** | 0.129 |

**Supplementary figure 1.** Box plots showing mean difference of chronological age (A) and 6CpG-EA (B) between males and females in this cohort.

