**Table S1: Multivariable linear regression analysis of the determinants of plasma homocysteine, with plasma homocysteine as a continuous dependant variable, examining the effect of MTHFR C677T genotype (n=4006)**

|  |  |  |
| --- | --- | --- |
| **Variable** | **Effect (95% CI)** | **p-value** † |
| **Demographic characteristics** |
| **Age** (rcs, three knots) |  |  | 0.0024 |
| 34 versus 27 years | -0.034 | (-0.095, 0.026) |  |
| **Race** |  |  | <0.0001 |
| Caucasian | Reference |  |  |
| African | -0.087 | (-0.336, 0.163) |  |
| Middle eastern | -0.397 | (-0.594, -0.200) |  |
| Asian | -0.421 | (-0.574, -0.267) |  |
| Other | -0.407 | (-0.707, -0.107) |  |
| **Education** |  |  | 0.5001 |
| College/University completed versus less than completed | 0.036 | (-0.068, 0.140) |  |
| **Health indicators** |
| **BMI** (rcs, three knots) |  |  | 0.0078 |
| 27.3 versus 21.1 kg/m2 | -0.098 | (-0.167, -0.028) |  |
| **Diabetes** |  |  | 0.3769 |
| Yes versus no | -0.142 | (-0.456, 0.173) |  |
| **Chronic hypertension** |  |  | 0.0029 |
| Yes versus no | 0.524 | (0.180, 0.869) |  |
| **Pregnancy characteristics** |
| **Nulliparous** |  |  | 0.0468 |
| Yes versus no | 0.099 | (0.001, 0.196) |  |
| **Smoking**  |  |  | <0.0001 |
| No | Reference |  |
| Second-hand | 0.204 | (-0.190, 0.599) |  |
| Med/light smoker (<10 cigarettes per day) | 0.406 | (0.226, 0.587) |  |
| Heavy smoker (>10 cigarettes per day) | 0.991 | (0.765, 1.216) |  |
| **Hormonal birth control prior to conception** |  |  | 0.1184 |
| No | Reference |  |
| Oral | -0.084 | (-0.172, 0.003) |  |
| Injection or IUD | 0.115 | (-0.199, 0.429) |  |
| **History of PMC** (Preeclampsia, placental abruption, SGA, stillbirth/loss) |  |  | 0.0136 |
| Yes versus no | -0.158 | (-0.283, -0.032) |  |
| **Folic acid supplementation** |  |  | 0.3292 |
| None | Reference |  |
| >0 and <1 mg | -0.043 | (-0.232, 0.146) |  |
| >1 mg  | -0.115 | (-0.323, 0.093) |  |
| **Homocysteine metabolism** |
| **Gestational age at blood work** (rcs, five knots)  |  |  | <0.0001 |
| 13.4 versus 12.4 weeks | 0.018 | (-0.094, 0.130) |  |
| **Serum folate\*MTHFR genotype** ‡ § |  |  | <0.0001 |
| **Serum folate** (rcs, five knots) |  |  | <0.0001 |
| TT: folate 45.1 versus 30.6 nmol/L | 0.304 | (0.011, 0.597) |  |
| CC/CT: folate 45.1 versus 30.6 nmol/L | 0.051 | (-0.055, 0.158) |  |
| **MTHFR genotype** |  |  | <0.0001 |
| CC/ CT (wild type/ heterozygous) | Reference |  |  |
| TT (homozygous) | 0.106 | (-0.125, 0.337) |  |

† Wald test of most meaningful hypotheses, pooled across multiple imputation data sets

‡ Recessive model

§ Interaction effect (Factor + higher order factors)

****

**Figure S1:** For the multivariable model reported in Table S1, associations between homocysteine and continuous variables modeled as a restricted cubic spline: A) gestational age at blood work with five knots at 12.1, 12.4, 12.8, 13.4, and 19; B) maternal age with three knots at 24, 30, and 37; and C) BMI with three knots at 19.6, 23.5, and 32.2. Shaded area represents 95% CI.

**Table S2: Multivariable linear regression analysis of the determinants of plasma homocysteine, with the dependent variable as a continuous z-score calculated from homocysteine mean and SD for gestational week at recruitment (n=7587)**

|  |  |  |
| --- | --- | --- |
| **Variable** | **Effect (95% CI)** | **p-value** † |
| **Demographic characteristics** |
| **Age** (rcs, three knots) |  |  | 0.0014 |
| 34 versus 27 years | -0.014 | (-0.048, 0.021) |  |
| **Race** |  |  | <0.0001 |
| Caucasian | Reference |  |  |
| African | -0.285 | (-0.438, -0.133) |  |
| Middle eastern | -0.348 | (-0.472, -0.225) |  |
| Asian | -0.365 | (-0.460, -0.270) |  |
| Other | -0.309 | (-0.475, -0.144) |  |
| **Education** |  |  | 0.4297 |
| College/University completed versus less than completed | -0.024 | (-0.082, 0.035) |  |
| **Health indicators** |
| **BMI** (rcs, 3 knots) |  |  | 0.0082 |
| 27.3 versus 21.1 kg/m2 | -0.059 | (-0.098, -0.021) |  |
| **Diabetes** |  |  | 0.6017 |
| Yes versus no | -0.047 | (-0.225, 0.130) |  |
| **Chronic hypertension** |  |  | 0.0001 |
| Yes versus no | 0.408 | (0.208, 0.609) |  |
| **Pregnancy characteristics** |
| **Nulliparous** |  |  | <0.0001 |
| Yes versus no | 0.121 | (0.068, 0.173) |  |
| **Smoking**  |  |  | <0.0001 |
| No  | Reference |  |
| Second-hand | 0.265 | (0.105, 0.425) |  |
| Med/light smoker (<10 cigarettes per day) | 0.324 | (0.228, 0.420) |  |
| Heavy smoker (>10 cigarettes per day) | 0.682 | (0.556, 0.808) |  |
| **Hormonal birth control prior to conception** |  |  | 0.2208 |
| No | Reference |  |
| Oral | -0.033 | (-0.082, 0.016) |  |
| Injection or IUD | 0.072 | (-0.070, 0.214) |  |
| **History of PMC** (Preeclampsia, placental abruption, SGA, stillbirth/loss) |  |  | 0.0170 |
| Yes versus no | -0.089 | (-0.161, -0.016) |  |
| **Folic acid supplementation** |  |  | <0.0001 |
| No | Reference |  |
| >0 and < 1 mg | -0.251 | (-0.356, -0.147) |  |
| >1 mg  | -0.268 | (-0.386, -0.151) |  |
| **Homocysteine metabolism** |
| **Gestational age at blood work** (linear)  |  |  | 0.5637 |
| 13.4 versus 12.4 weeks | -0.004 | (-0.017, 0.009) |  |
| **Serum folate** (rcs, five knots) |  |  | <0.0001 |
| 45.1 versus 30.6 nmol/L | 0.106 | (0.048, 0.164) |  |

† Wald test of most meaningful hypotheses, pooled across multiple imputation datasets

**Table S3: Multivariable logistic regression analysis of the determinants of plasma homocysteine, with the binary dependent variable as plasma homocysteine greater than the 90th percentile for gestational week at recruitment (n=7587)**

|  |  |  |
| --- | --- | --- |
| **Variable** | **Effect (95% CI)** | **p-value** † |
| **Demographic characteristics** |
| **Age** (linear) |  |  | 0.4675 |
| 34 versus 27 years | 0.966 | (0.881, 1.060) |  |
| **Race** |  |  | <0.0001 |
| Caucasian | Reference |  |  |
| African | 0.511 | (0.318, 0.822) |  |
| Middle eastern | 0.416 | (0.265, 0.653) |  |
| Asian | 0.491 | (0.357, 0.676) |  |
| Other | 0.565 | (0.335, 0.953) |  |
| **Education** |  |  | 0.4195 |
| College/University completed versus less than completed | 0.939 | (0.806, 1.094) |  |
| **Health indicators** |
| **BMI** (rcs, three knots) |  |  | 0.0356 |
| 27.3 versus 21.1 kg/m2 | 0.880 | (0.794, 0.976) |  |
| **Diabetes** |  |  | 0.1742 |
| Yes versus no | 1.371 | (0.870, 2.162) |  |
| **Chronic hypertension** |  |  | 0.0010 |
| Yes versus no | 2.215 | (1.377, 3.565) |  |
| **Pregnancy characteristics** |
| **Nulliparous** |  |  | 0.0012 |
| Yes versus no | 1.268 | (1.098, 1.465) |  |
| **Smoking**  |  |  | <0.0001 |
| No  | Reference |  |
| Second-hand | 1.409 | (0.953, 2.082) |  |
| Med/light smoker (<10 cigarettes per day) | 1.666 | (1.323, 2.098) |  |
| Heavy smoker (>10 cigarettes per day) | 2.346 | (1.761, 3.125) |  |
| **Hormonal birth control prior to conception** |  |  | 0.0563 |
| No | Reference |  |
| Oral | 0.858 | (0.749, 0.982) |  |
| Injection or IUD | 1.118 | (0.787, 1.590) |  |
| **History of PMC** (Preeclampsia, placental abruption, IUGR, stillbirth, loss) |  |  | 0.1605 |
| Yes versus no | 0.862 | (0.700, 1.061) |  |
| **Folic acid supplementation** |  |  | 0.0003 |
| Yes versus no | 0.626 | (0.486, 0.807) |  |
| **Homocysteine metabolism** |
| **Gestational age at blood work** (rcs, four knots) |  |  | <0.0001 |
| 13.4 versus 12.4 weeks | 0.702 | (0.612, 0.804) |  |
| **Serum folate** (rcs, 5 knots) |  |  | <0.0001 |
|  45.3 versus 30.7 nmol/L | 1.038 | (0.883, 1.220) |  |

† Wald test of most meaningful hypotheses, pooled across multiple imputation datasets