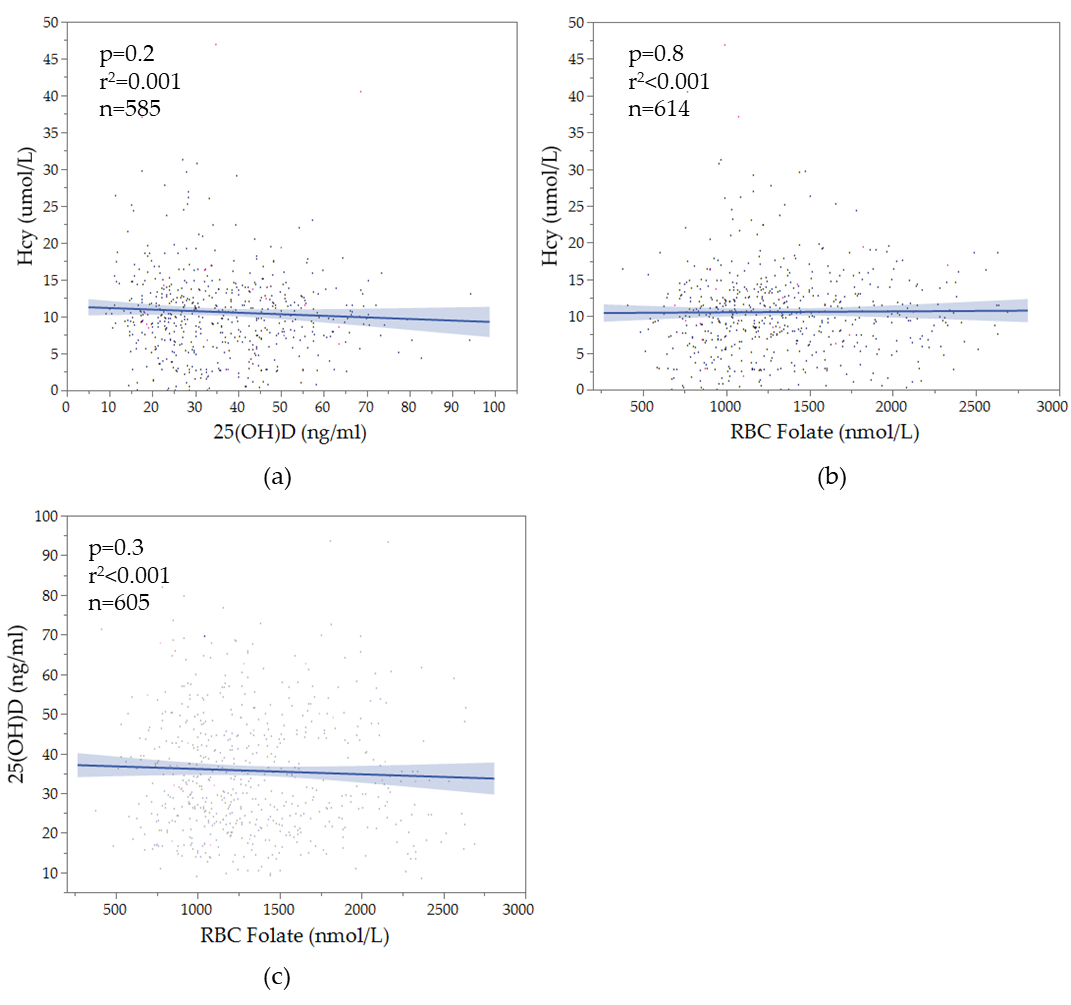
**Supplementary Material**

**Table S1.** Allelic and genotypic frequencies for folate and vitamin D variants and assessment for deviation from Hardy-Weinberg equilibrium

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variant** | **Allele\***  **n (%)** | | **Genotype**  **n (%)** | | **HWE**  **χ2 (p)** |
|  |  |  |  |  |  |
| *MTRR*-rs1801394 | Presence of G allele | 489 (79) | AA | 127 (21) | 1.18 (0.3) |
|  | Absence of G allele | 127 (21) | AG | 320 (52) |  |
|  |  |  | GG | 169 (28) |  |
| *MTR*-rs1805087 | Presence of G allele | 220 (36) | AA | 393 (64) | 0.08 (0.8) |
|  | Absence of G allele | 393 (64) | AG | 197 (32) |  |
|  |  |  | GG | 23 (4) |  |
| *MTHFR*- rs1801131 | Presence of C allele | 322 (52) | AA | 292 (47) | 0.00 (0.9) |
|  | Absence of C allele | 292 (48) | AC | 262 (43) |  |
|  |  |  | CC | 60 (10) |  |
| *MTHFR*- rs1801133 | Presence of T allele | 350 (57) | CC | 266 (43) | 3.9 (0.05) |
|  | Absence of T allele | 266 (43) | CT | 294 (48) |  |
|  |  |  | TT | 56 (0.09) |  |
| *TYMS*-rs11280056 | Presence of deletion | 297 (50) | ins/ins | 295 (50) | 0.9 (0.3) |
|  | Absence of deletion | 295 (50) | ins/del | 239 (40) |  |
|  |  |  | del/del | 58 (10) |  |
| *TYMS*- rs45445694 | Presence of 3-repeat | 478 (79) | 2/2 repeat | 129 (21) | 1.36 (0.2) |
|  | Absence of 3-repeat | 129 (21) | 2/3 repeat | 317 (52) |  |
|  |  |  | 3/3 repeat | 161 (27) |  |
| *SHMT*- rs1979277 | Presence of T allele | 320 (52) | CC | 295 (48) | 3.60 (0.06) |
|  | Absence of T allele | 295 (48) | CT | 247 (40) |  |
|  |  |  | TT | 73 (12) |  |
| *RFC1*- rs1051266 | Presence of A allele | 419 (68) | GG | 195 (32) | 0.04 (0.9) |
|  | Absence of A allele | 195 (32) | GA | 300 (49) |  |
|  |  |  | AA | 119 (19) |  |
| *MTHFD1*- rs2236225 | Presence of A allele | 471 (77) | GG | 144 (23) | 0.06 (0.8) |
|  | Absence of A allele | 144 (23) | GA | 304 (50) |  |
|  |  |  | AA | 167 (27) |  |
| *DHFR*- rs70991108 | Presence of deletion | 415 (67) | ins/ins | 200 (32) | 0.28 (0.6) |
|  | Absence of deletion | 200 (33) | ins/del | 307 (50) |  |
|  |  |  | del/del | 108 (18) |  |
| *DHCR7/NADSYN1*-rs12785878 | Presence of T allele | 525 (95) | GG | 27 (5) | 1.03 (0.3) |
| Absence of T allele | 27 (5) | GT | 209 (39) |  |
|  |  |  | TT | 316 (57) |  |
| *CYP24A1*-rs17216707 | Presence of C allele | 170 (34) | TT | 334 (66) | 1.17 (0.3) |
|  | Absence of C allele | 334 (66) | CT | 157 (31) |  |
|  |  |  | CC | 13 (3) |  |
| *GC*-rs4588 | Presence of A allele | 286 (51) | CC | 267 (49) | ***5.7 (0.02)*** |
|  | Absence of A allele | 267 (49) | CA | 251 (45) |  |
|  |  |  | AA | 35 (6) |  |
| *CYP2R1*-rs10741657 | Presence of A allele | 338 (63) | GG | 200 (37) | 0.05 (0.8) |
|  | Absence of A allele | 200 (37) | GA | 254 (47) |  |
|  |  |  | AA | 84 (16) |  |
| *VDR*-rs4516035 | Presence of G allele | 413 (67) | AA | 200 (33) | 0.24 (0.6) |
|  | Absence of G allele | 200 (33) | AG | 295 (48) |  |
|  |  |  | GG | 118 (19) |  |
| *VDR*-rs1544410^ | Presence of T allele | 387 (63) | CC (bb) | 223 (36) | 0.09 (0.8) |
|  | Absence of T allele | 223 (37) | CT (Bb) | 294 (48) |  |
|  |  |  | TT (BB) | 93 (15) |  |
| *VDR*-rs757343^ | Presence of T allele | 165 (27) | CC (UU) | 450 (73) | 1.28 (0.6) |
|  | Absence of T allele | 450 (73) | CT (Uu) | 148 (24) |  |
|  |  |  | TT (uu) | 17 (3) |  |
| *VDR*-rs2228570^ | Presence of G allele | 507 (85) | AA (ff) | 91 (15) | 0.49 (0.5) |
|  | Absence of G allele | 91 (15) | GA (Ff) | 295 (49) |  |
|  |  |  | GG (FF) | 212 (35) |  |
| *VDR*-rs731236^ | Presence of G allele | 385 (63) | AA (TT) | 229 (37) | 0.71 (0.4) |
|  | Absence of G allele | 229 (37) | AG (Tt) | 300 (49) |  |
|  |  |  | GG (tt) | 85 (14) |  |
| *VDR*-rs11568820 | Presence of G allele | 533 (96) | AA | 24 (4) | 2.74 (0.1) |
|  | Absence of G allele | 24 (4) | AG | 215 (39) |  |
|  |  |  | GG | 318 (57) |  |
| *VDR*-rs7975232^ | Presence of A allele | 485 (79) | CC (aa) | 129 (21) | 0.97 (0.3) |
|  | Absence of A allele | 129 (21) | CA (Aa) | 318 (52) |  |
|  |  |  | AA (AA) | 167 (27) |  |

\*Allelic frequencies reported as presence and absence of polymorphic allele for each variant.

^ VDR genotypes are referenced using base pair notation but are often designated by a lowercase letter and capital letter (e.g. f and F alleles) for the presence and absence of the restriction site respectively in literature – both are given here.



**Figure S1.** Binary associations between biochemical variables of interest (Hcy, 25(OH)D and RBC folate levels).

**Table S2.** Folate variants identified by stepwise regression for inclusion in models for Hcy prediction, with and without adjustments

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Hcy levels** | | |
|  | Unadjusted  (n=609) | Model 1  (n=574) | Model 2  (n=460) |
|  | β (p) | β (p) | β (p) |
| *DHFR*- rs70991108 | -0.07 (0.09) | -0.05 (0.2) | -0.03 (0.5) |
| *MTHFD1*-rs2236225 | -0.05 (0.3) | -0.04 (0.4) | -0.09 (0.05) |

Italics and bold indicate results that are statistically significant. Adjustments; Model 1 – RBC folate and 25(OH)D levels. Model 2 – Model 1 and Hcy determinants; sex, age, creatinine and vitamin B12 levels, reported dietary intake of alcohol, vitamin B6, tea and coffee, smoking status and BMI category. Totals shown are for unadjusted and adjusted models respectively. Total number of participants in each model vary due to missing data.

**Table S3.** Assessment for gene-nutrient interactions in predicting Hcy, with and without adjustments for determinants of Hcy levels.

|  |  |  |
| --- | --- | --- |
|  | **Hcy levels** | |
|  | Unadjusted | Adjusted |
|  | β (p) | β (p) |
| *DHFR*-rs70991108 | -0.08 (0.06) | -0.07 (0.1) |
| RBC folate levels | 0.05 (0.3) | 0.03 (0.5) |
| *DHFR*-rs70991108 *x* RBC folate levels | 0.03 (0.5) | 0.02 (0.7) |
| n=609/489 |  |  |
|  |  |  |
| *MTHFD1*-rs2236225 | -0.06 (0.1) | ***-0.11 (0.02)*** |
| RBC folate levels | 0.05 (0.3) | 0.04 (0.4) |
| *MTHFD1*-rs2236225 *x* RBC folate levels | 0.02 (0.6) | 0.02 (0.6) |
| n=609/488 |  |  |

Italics and bold indicate results that are statistically significant. P values for interactions were compared against a Bonferroni adjusted p threshold of p<0.025 to account for multiple testing.Adjustments; Hcy determinants; sex, age, creatinine and vitamin B12 levels, reported dietary intake of alcohol, vitamin B6, tea and coffee, smoking status and BMI category. Totals shown are for unadjusted and adjusted models respectively. Total number of participants in each model vary due to missing data.