**Supplementary Data**

**Title:** Maternal plasma vitamin B12 concentrations during pregnancy and infant cognitive outcomes at 2 years of age

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| **Supplementary Table 1:** Characteristics of included versus excluded mother-offspring pairs for analysis of maternal plasma vitamin B12 concentrations and infant cognitive development at 2 years in the Growing Up in Singapore Towards healthy Outcomes study. | | | | | | | |
|  | Total  n=998 | | Included  n=443 | | Excluded  n=555 | |  |
|  | *n*, mean, median | %, SD,  IQR | *n*, mean, median | %, SD,  IQR | *n*, mean, median | %, SD,  IQR | *P* |
| **Maternal Characteristics** |  |  |  |  |  |  |  |
| Age, year | 30.6 | 5.1 | 30.9 | 5.1 | 30.3 | 5.1 | 0.07 |
| Ethnicity |  |  |  |  |  |  | 0.42 |
| Chinese | 537 | 55.5 | 246 | 55.5 | 291 | 53.3 |  |
| Malay | 262 | 26.5 | 120 | 27.1 | 142 | 26.0 |  |
| Indian | 190 | 27.1 | 77 | 17.4 | 113 | 20.7 |  |
| Education |  |  |  |  |  |  | 0.09 |
| Secondary or lower | 300 | 30.7 | 120 | 27.2 | 180 | 33.6 |  |
| Post-secondary | 344 | 35.3 | 162 | 36.7 | 182 | 34.0 |  |
| University or higher | 332 | 34.0 | 159 | 36.1 | 173 | 32.3 |  |
| Plasma vitamin B12 concentrations, pmol/L | 219.6 | 78.5 | 220.6 | 80.5 | 218.8 | 77.0 | 0.72 |
| Plasma vitamin B6 concentrations, nmol/L | 61.8 | 25.9, 113.0 | 59.9 | 24.8, 108.3 | 62.0 | 27.6, 115.9 | 0.53 |
| Plasma folate concentrations, nmol/L | 34.4 | 24.5, 44.4 | 34.0 | 24.5, 46.0 | 34.7 | 24.0, 44.0 | 0.68 |
| Plasma homocysteine concentrations, µmol/L | 5.0 | 1.1 | 5.0 | 1.1 | 5.1 | 1.2 | 0.61 |
| Pre-pregnancy BMI, kg/m2 | 22.7 | 4.3 | 22.9 | 4.6 | 22.5 | 4.1 | 0.14 |
| EPDS score | 7 | 4, 10 | 8 | 5, 10 | 7 | 4, 10 | 0.06 |
| STAI-state score | 34.0 | 10.0 | 33.8 | 10.1 | 34.2 | 10.0 | 0.50 |
| Gestational Diabetes |  |  |  |  |  |  | 0.65 |
| Yes | 170 | 17.8 | 79 | 18.4 | 91 | 17.3 |  |
| No | 785 | 82.2 | 350 | 81.6 | 435 | 82.7 |  |
| Parity |  |  |  |  |  |  | 0.65 |
| Nulliparous | 420 | 42.5 | 192 | 43.3 | 228 | 41.9 |  |
| Primi/Multiparous | 567 | 57.5 | 251 | 56.7 | 316 | 58.1 |  |
| Maternal Diet |  |  |  |  |  |  |  |
| Diet quality (HEI-SGP) | 52.4 | 13.7 | 52.4 | 13.5 | 52.4 | 13.8 | 0.95 |
| Animal-based protein foods, g | 147 | 75, 233 | 158 | 86, 236 | 141 | 67, 228 | 0.06 |
| Dairy products, g | 250 | 0, 350 | 250 | 0, 323 | 250 | 0, 350 | 0.43 |
|  |  |  |  |  |  |  |  |

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| **Supplementary Table 1** *(cont’)* |  |  |  |  |  |  |  |
|  | Total  n=998 | | Included  n=443 | | Excluded  n=555 | |  |
|  | *n*, mean, median | %, SD,  IQR | *n*, mean, median | %, SD,  IQR | *n*, mean, median | %, SD,  IQR | *P* |
| **Infant Characteristics** |  |  |  |  |  |  |  |
| Sex |  |  |  |  |  |  | 0.51 |
| Male | 513 | 51.9 | 278 | 50.9 | 235 | 53.1 |  |
| Female | 476 | 48.1 | 268 | 49.1 | 208 | 46.9 |  |

BMI, body mass index; EPDS, Edinburgh Postnatal Depression Scale; STAI, State-Trait Anxiety Inventory; HEI-SGP, Healthy Eating Index for Singapore Pregnant women †*P*-values (\**P*<0.05) were obtained from chi-square test; independent t-test or Wilcoxon rank-sum tests.

**Supplementary Table 2:** Associations of maternal plasma vitamin B12 concentrations with infant cognitive development (Bayley Scale of Infant and Toddler Development –III) at 24 months of age in the Growing Up in Singapore Towards healthy Outcomes study (n=443).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cognitive | | | Receptive language | | | Expressive language | | | Fine motor | | | Gross motor | | |
|  | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P |
| Maternal B12 concentrations† | | |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Model 1‡ | 0.11 | 0.02, 0.20 | 0.018\* | 0.05 | -0.04, 0.15 | 0.26 | 0.04 | -0.05, 0.13 | 0.40 | 0.04 | -0.06, 0.13 | 0.46 | 0.05 | -0.04, 0.14 | 0.29 |
| Model 2 | 0.10 | 0.01, 0.19 | 0.034\* | 0.05 | -0.04, 0.14 | 0.28 | 0.04 | -0.05, 0.13 | 0.42 | 0.03 | -0.06, 0.13 | 0.50 | 0.04 | -0.05, 0.14 | 0.40 |
| Model 3 | 0.10 | 0.004, 0.19 | 0.041\* | 0.04 | -0.05, 0.14 | 0.38 | 0.04 | -0.06, 0.13 | 0.45 | 0.02 | -0.07, 0.12 | 0.67 | 0.03 | -0.07, 0.12 | 0.60 |

† Effect estimates are per SD increment in maternal plasma vitamin B12 concentrations, and per SD BSID-III score

‡ Model 1 – adjusted for infant’s age at cognitive testing

§ Model 2 – adjusted as for Model 1 and maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes status, antenatal depression and anxiety levels.

|| Model 3 – adjusted as for Model 2 and maternal plasma folate and vitamin B6 concentrations.

**Supplementary Table 3:** Associations of maternal folate and vitamin B6 with infant cognitive development (Bayley Scale of Infant and Toddler Development –III) at 24 months of age in the Growing Up in Singapore Towards healthy Outcomes study (n=443).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Cognitive | | | Receptive language | | | Expressive language | | | Fine motor | | | Gross motor | | |
|  | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P | β | 95% CI | P |
| Maternal Folate concentrations†§ | -0.02 | -0.11, 0.08 | 0.72 | -0.04 | -0.13, 0.06 | 0.46 | -0.001 | -0.10, 0.09 | 0.99 | -0.002 | -0.10, 0.09 | 0.97 | 0.02 | -0.08, 0.11 | 0.76 |
| Maternal Folate status‡§ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Insufficient | -0.18 | -0.50, 0.15 | 0.29 | 0.14 | -0.18, 0.47 | 0.39 | -0.16 | -0.49, 0.17 | 0.34 | -0.13 | -0.46, 0.20 | 0.44 | -0.15 | -0.49, 0.19 | 0.39 |
| Sufficient | **Reference** | | | | | | | | | | | | | | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Maternal B6 concentrations†|| | 0.01 | -0.08, 0.11 | 0.76 | 0.05 | -0.04, 0.14 | 0.27 | 0.01 | -0.08, 0.11 | 0.79 | 0.06 | -0.03, 0.16 | 0.18 | 0.08 | -0.02, 0.17 | 0.12 |
| Maternal B6 status‡|| |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Insufficient | -0.16 | -0.41, 0.10 | 0.23 | -0.07 | -0.32, 0.19 | 0.60 | -0.05 | -0.31, 0.21 | 0.70 | -0.003 | -0.26, 0.26 | 0.98 | -0.08 | -0.35, 0.18 | 0.54 |
| Sufficient | **Reference** | | | | | | | | | | | | | | |

† Effect estimates are per SD increment in maternal plasma folate and vitamin B6 concentrations, and per SD BSID-III score

‡ Folate status: n=46 insufficient (<13.6nmol/L), n=397 sufficient (≥13.6nmol/L); vitamin B6 status: n=66 insufficient (<20 nmol/L), n=377 sufficient (>20 nmol/L)

Models were adjusted for infant’s age at cognitive testing; maternal age, ethnicity, education, pre-pregnancy BMI, parity, gestational diabetes status, antenatal depression and anxiety levels; maternal plasma vitamin B12, and §vitamin B6 or ||folate concentrations.