

Maternal serum vitamin B12 during pregnancy and offspring Autism Spectrum Disorder

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Supplement

Table S1: Review of previous studies on maternal vitamin B12 and multivitamin and autism spectrum disorders, by primary method of exposure assessment

Author/ Country/	Diagnosis	Study design	Diagnostic criteria	Measures of exposure	Dosage	Sample size/Age range	Covariates	Results, Effect size estimate and 95% CI
Biomarker studies								
Raghavan et al., 2018 USA	ASD	Prospective cohort, Clinical Boston Medical Center Birth	ICD-9	Maternal blood samples collected 24-72 hours post-delivery Self-reported maternal multivitamin supplement use	NA	ASD, n=86 TD, n=1171	Child: gestational age, sex and year of birth Mother: age, education, parity, BMI, smoking status, diabetes status, race and MTHFR genotype	Plasma B ₁₂ (>600 pmol/L), HR 3.0 (1.6-5.7); ref (≥200-≤600 pmol/L) Plasma B ₁₂ (≥90th percentile), HR 2.5 (1.4-4.5), ref ((≥10th to <90th percentile) Plasma folate at birth (≥60.3 nmol/L), HR 2.5 (1.3-4.6), ref (>14.7 to <60.3) Multivitamin use: First trimester ≤ 2/week HR 3.4 (1.6, 7.2), >5/week HR 2.3 (1.2, 3.9) Second trimester ≤ 2/week HR 3.8 (1.8, 8.0), >5/week HR 2.1 1.2, 3.6 Third trimester ≤ 2/week HR 3.5 (1.7, 7.4), >5/week HR 2.1 1.2, 3.6 ref: multivitamins 2–5 times/week
Egorova et al., 2020 Sweden	ASD	Case-control	ICD-10	Maternal serum folate concentrations at 14 weeks of pregnancy	NA	ASD, n=100 (74-infantile autism, 26-Asperger syndrome) TD, n=100	Child: sex Maternal: age at sampling, serum cotinine and 5-methyl tetrahydrofolate levels Other: year of sampling	Folate, nmol/L OR 1.70 (1.22-2.37)

Steenweg-de Graaff et al., 2014 Netherlands	Autistic traits	Population-based cohort, Generation R	SRS, CBCL-Parent reported	Maternal plasma folate concentration: median 13.2 weeks of gestation (10.5-17.2 weeks) FA use from preconception to 10 weeks of pregnancy	NA	Autistic traits, n=3893	Child: age, sex, gestational age at venipuncture	FA before conception OR -0.04 (-0.07 to -0.02), FA first 10 weeks of pregnancy, OR -0.04 (-0.07 to -0.02) FA after first 10 weeks of pregnancy, OR -0.06 (-0.09 to -0.03) ref no use Folate concentrations, nmol/L B -0.004 (-0.013 to 0.004)
Braun et al., 2014 USA	Autistic behaviors	Population-based cohort, HOME	SRS-Mother report	Whole blood folate: 11-21 weeks gestation Prenatal vitamin use: 14-39 weeks gestation	NA	Autistic traits, n=209	Maternal: age, race, education, prenatal vitamin use, marital status, employment insurance status, depressive symptoms, serum cotinine concentrations food security, fresh fruit/vegetable intake Household: income	Prenatal vitamin weekly/daily OR 0.26 (0.08-0.89), ref no use Folate concentration, nmol/L OR 1.42 (0.81-2.49)
Record based								
Moser et al., 2019 Israel	ASD	Nested case-control	DSM	Dispensing record for FA with or without multivitamin supplements one year prior to child's birth	Cumulative FA dose ^a	ASD, n=2009 TD, n=19,886	Child: sex, birth year, birth order Mother: age, region of residence, poverty index, subfertility, number of family physician and obstetric obgyn visits, diabetes mellitus, hypertension, cardiovascular disease, cancer, epilepsy, antifolate medications ^b	FA supplement, ref ≤0.2 mg/day 0.2-<0.4mg/day OR 1.27 (0.98-1.65) 0.4-<1mg/day OR 1.10 (0.98-1.24) 1-<3mg/day OR 1.14 (0.98-1.34) ≥3mg/day OR 1.01 (0.60-1.70)

Levine et al 2018 Israel	ASD	Case-control cohort	ICD-9	Pharmacy records, multivitamins and /or FA supplements before and during pregnancy	NA	ASD, n=572 Controls, n=45300	Child: sex, birth year Maternal: parity, SES (high vs low), psychiatric diagnosis at childbirth, age Paternal psychiatric diagnosis and age	FA and/or multivitamin supplements, ref no exposure Before pregnancy (540-271 days before childbirth), RR, 0.39 (0.30-0.50) During pregnancy (270 days before childbirth up to the date of childbirth), RR, 0.27 (0.22-0.33), FA before pregnancy, RR, 0.56 (0.42-0.74) FA during pregnancy, RR, 0.32 (0.26-0.41) Multivitamin before pregnancy, RR 0.36 (0.24-0.52) Multivitamin during pregnancy, RR 0.35 (0.28-0.44)
Self-report								
Brieger et al., 2021	ASD	Prospective cohort of high risk families, EARLI	DSM	Multivitamin, prenatal and folic acid intake during pregnancy	FA (<400mcg, 400-1000mcg, >1000mcg) in one month	ASD, n=38 Non-ASD, n=153	Child: sex Maternal: education	Prenatal vitamin use, ref no use OR 0.70 (0.32-1.53) Folic acid, ref 400-1000 mcg <400 mcg OR 0.75 (0.32-1.69) >1000 mcg OR 1.64 (0.44-5.47)
Tan et al., 2020 China	ASD	Case-control	DSM	FA supplementation 12 weeks before and 12 weeks after LMP Micronutrient supplementation from LMP to birth	FA: 400µg daily Micronutrient: NA	ASD, n=416 TD, n=201	Child; age, sex, gestational age, birth weight Maternal; residence (rural/urban), labor mode Paternal; age Household: income	No FA supplementation, OR 1.91 (1.24-2.93), No micronutrient supplements OR 1.721 (1.19-2.47) ref any supplement use
DeVilbiss et al 2017 Sweden	ASD	Prospective cohort study (Stockholm Youth cohort)	ICD 10 DSM-IV-TR	Self-reported multivitamin use during pregnancy	NA	ASD without ID sibling cohort, n=173209	Child: sex, birth year, years reside in Stockholm County, socio-economic indicators (education,	Multivitamin use ASD, OR 0.89 (0.82-0.97) ASD with ID, OR 0.69 (0.57-0.84) ASD without ID, OR 0.94 (0.85-1.03)

						ASD sibling cohort, n=174428 ASD with ID, n=169760	family income, maternal birth country) Maternal:(age, BMI, parity, smoking status, medications (antidepressants or antiepileptics), neuropsychiatric conditions ^c	Folic acid supplement ASD, OR 1.27 (1.01-1.60) ASD without ID, OR 1.29 (0.99-1.67) ASD with ID, OR 1.20 (0.71-2.01)
Surén et al, 2013 Norway	ASD	Prospective cohort MoBa	ADI-R ADOS DSM-IV ICD-10	Self-reported multivitamin, FA supplement 4 weeks before LMP and 8 weeks after LMP	NA	ASD, n=114 85,176	Child: birth year Maternal: planned pregnancy, smoking, BMI, parity, weight gain at 18 and 30 weeks Parental: education, age	FA, OR 0.61 (0.41–0.90), ref no supplement use
Nilsen et al., 2013 Norway	ASD	Population based cohort, MoBA	ICD-10	Self-reported FA intake Before and/or during pregnancy	NA	ASD, n=234	Child; birth year Maternal; age, marital status, parity, hospital size Paternal; age	FA OR 0.86 (0.78, 0.95), ref no supplement use
Interview								
Schmidt et al. 2019 USA	ASD	Prospective cohort of high risk families MARBLES	MSEL ADOS at 3 years of age	Vitamin and supplement use ^d 6 months preconception and each month during pregnancy	NA	Siblings of children with ASD- total, n=241 ASD, n=55 TD, n=126 Non-TD, n=60	Child: birthplace, sex, year of birth Maternal: education, age, pre-pregnancy BMI, planned pregnancy, race/ethnicity, home ownership, insurance delivery type	Prenatal vitamin RR 0.50 (0.30-0.81), ref, no supplement use Folic acid supplement ≥600 µg/day RR 0.51 (0.31-0.82), ref < 600 µg/day
Li et al 2018 China	ASD	Retrospective case-control	SRS DSM-IV	FA supplement use and food preference in preconception and during pregnancy assessed 3–6 years after delivery	NA	ASD, n=374 TD, n=354	Child: age, premature birth, sex Maternal: pre-pregnancy BMI and delivery, premature	FA before pregnancy OR 0.95 (0.61-1.50) FA during pregnancy OR 0.64 (0.41-1.00) ref, no supplement use

							delivery, intake of other supplements Parental age and education	
Strom et al. 2018 Denmark	ASD	Prospective cohort DNBC	ICD-10	FA use and multivitamin:4-8 weeks of gestation and mid gestation, 25 weeks gestation	FA use 0, <400, ≥400 µg/day	ASD, n=1234 Childhood autism, n=312	Child: sex Maternal: age, parity, smoking, education, SES, planned pregnancy, pre-pregnancy BMI Paternal: age	Any FA, ref no supplement use At GW 4- 8, HR 1.06, (0 .94- 1.19), Mid pregnancy, <400 µg/day, HR 1.01 (0.76-1.34) 400 µg/day, HR 0.98 (CI 0.75-1.29) Folate from food Q2: HR 0.82 (0.67, 1.01), Q3: HR 0.96 (0.78, 1.17), Q4: HR 0.85 (0.69, 1.04) Q5: HR 0.94 (0.77, 1.16) (ref: Q1 quintile)
Virk et al 2016 Denmark	ASD	Prospective cohort DNBC	ICD-10	Self-reported FA or multivitamin 4 weeks prior LMP and 8 weeks after LMP, 4-8 weeks	NA	ASD, n=552 Autistic, n=198 Asperger's syndrome, n=109 PDD-NOS, n=245	Child: sex, birth weight, age Maternal: age, smoking, alcohol consumption, mental, pre-pregnancy BMI, parity, mental illness Paternal: age Household: SES	Folate, ref no use ASD: RR 1.06 (0.82-1.36) Autism: RR 1.18 (0.76-1.84) Asperger's syndrome: RR 0.85 (0.46-1.53) PDD-NOS: RR 1.07 (0.75-1.54) Multivitamin users, ref no use ASD: RR 1.00 (0.82-1.22) Autism: RR 1.22 (0.87-1.69) Asperger's syndrome: RR 0.95 (0.62-1.46) PDD-NOS: RR 0.87 (0.65-1.17)
Schmidt et al. 2012 USA	ASD	Case control CHARGE	MSEL ADI-R ADOS	Self-reported FA and multivitamin intake 3 months before and during pregnancy	FA intake calculated based on brand, dose, frequency of consumption product	ASD, n=429 DD, n=130 TD, n=278	Child: birth year, sex, race Maternal: race, age, educational, pre-pregnancy BMI, birthplace, residing with a smoker, smoking status, alcohol consumption, other nutrient intakes (vitamin A, B ₆ , C, D, E)	FA, ≥600 µg during first month of pregnancy, OR 0.62 (0.42-0.92) ref <600 µg) Among mother and children with MTHFR 677 C>T variant genotypes, OR 0.3 (0.10-0.90)

ABC, Autism Behavior Checklist; ADI-R, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule-Generic; ASD, Autism Spectrum Disorder; BMI, Body Mass Index; CARS, Childhood Autism Rating Scale; CBCL, Child Behavior Checklist; DD, Developmental delay; CHARGE, Childhood Autism Risks from Genetics and Environment; DNBC, Danish National Birth Cohort; DSM, Diagnostic and Statistical Manual of Mental Disorders; EARLI, Early Autism Risk Longitudinal Investigation; FA, Folic Acid; HR, Hazard ratio; HOME, Health Outcomes and Measures of the Environment; ICD, International Classification of Diseases; LMP, Last menstrual period; MARBLES, Markers of Autism Risk in Babies: Learning Early Signs; MoBa, Norwegian Mother and Child Cohort Study; MSEL, Mullen Scales of Early Learning; NA, not available; OR Odds ratio; SES, socioeconomic status; SRS, Social Responsiveness Scale; TD, typical development.

^aUn-supplemented or very low (median daily dispensed dose<0.2mg, typical of dose levels in women not receiving FA supplementation); low-supplemented (0.2 to <0.4 mg/day); typically-supplemented (0.4 to <1 mg/day, reflecting typical dose levels of women supplementing with commonly available prenatal vitamins); high-supplemented (1 to <3 mg/day) and very high supplemented (>3 mg/day, reflecting clinical recommendations for FA supplementation for some high-risk pregnancies).

^bAntifolate medication (proguanil, methotrexate, sulfasalazine, sulphamethoxazole and trimethoprim, pyrimethamine, valproate, carbamazepine, phenytoin and phenobarbital)

^cAnxiety disorder, autism, bipolar disorder, depression, epilepsy, intellectual disability, non-affective psychotic disorders, and stress disorders)

¹includes prenatal, multivitamin, Vit -A, B6, B12, C, D, E, folic acid, iron, calcium, zinc, cereals other supplements: slim fast, instant breakfast, protein powder, brewer's yeast, st.john's worts, ginseng, omega-3 fatty acids, fish oil.

Table S2. Odds ratios and 95% CIs for the association between log-transformed maternal serum Vitamin B12 (continuous) and offspring ASD and subtypes adjusting for additional covariates

	Model I		Model II	
Log-transformed maternal vitamin B12 levels (pmol/L)	Adjusted^a Odds Ratio (95% CI)	P- value	Adjusted^b Odds Ratio (95% CI)	P- value
ASD	0.93 (0.79-1.09)	0.406	0.93 (0.79-1.11)	0.423
Childhood autism	0.96 (0.72-1.28)	0.764	1.00 (0.75-1.35)	0.989
Asperger's	1.04 (0.77-1.40)	0.794	1.00 (0.74-1.36)	0.979
PDD/PDD-NOS	0.87 (0.66-1.15)	0.339	0.86 (0.64-1.15)	0.300

ASD, autism spectrum disorder; CI, confidence interval; PDD, Pervasive Developmental Disorder; PDD-NOS, PDD-not otherwise specified.

^aAdjusted for maternal socioeconomic status, gestational week of blood draw and offspring gestational age.

^bAdjusted for maternal: history of psychopathology, substance abuse, socioeconomic status, gestational week of blood draw and offspring gestational age.

Table S3: Odds ratios and 95% CIs for the association between maternal serum vitamin B12 levels (in quintiles) and offspring ASD and subtypes adjusting for additional covariates

Maternal serum vitamin B12 in quintiles (pmol/L)	Model I		Model II	
	Adjusted ^a Odds Ratio (95% CI)	P- value	Adjusted ^b Odds Ratio (95% CI)	P- value
1. ASD				
0-20 (0-80.8)	1.06 (0.84-1.33)	0.631	1.05 (0.83-1.33)	0.661
21-40 (80.9-105.4)	1.08 (0.86-1.36)	0.492	1.11 (0.88-1.39)	0.388
41-60 (105.5-128.9)	Reference		Reference	
61-80 (128.8-164.9)	1.02 (0.81-1.29)	0.839	1.00 (0.79-1.27)	0.981
≥81 (≥165)	1.01 (0.80-1.27)	0.943	1.03 (0.81-1.30)	0.812
2. Childhood autism				
0-20 (0-80.8)	1.49 (0.98-2.27)	0.061	1.53 (0.99-2.35)	0.054
21-40 (80.9-105.4)	1.29 (0.88-1.89)	0.197	1.35 (0.91-1.99)	0.141
41-60 (105.5-128.9)	Reference		Reference	
61-80 (128.8-164.9)	0.96 (0.64-1.45)	0.857	1.00 (0.66-1.52)	0.996
≥81 (≥165)	1.59 (1.06-2.42)	0.027	1.75 (1.14-2.67)	0.010
3. Asperger's				
0-20 (0-80.8)	0.89 (0.61-1.29)	0.538	0.87 (0.59-1.27)	0.468
21-40 (80.9-105.4)	1.03 (0.69-1.53)	0.877	1.02 (0.68-1.53)	0.918
41-60 (105.5-128.9)	Reference		Reference	
61-80 (128.8-164.9)	1.27 (0.84-1.89)	0.255	1.19 (0.79-1.82)	0.400
≥81 (≥165)	0.99 (0.67-1.47)	0.970	0.95 (0.63-1.42)	0.802
3. PDD/PDD- NOS				
0-20 (0-80.8)	0.87 (0.56-1.33)	0.511	0.86 (0.55-1.34)	0.507
21-40 (80.9-105.4)	0.93 (0.61-1.40)	0.725	0.96 (0.62-1.48)	0.847
41-60 (105.5-128.9)	Reference		Reference	
61-80 (128.8-164.9)	0.88 (0.58-1.35)	0.562	0.83 (0.54-1.29)	0.414
≥81 (≥165)	0.67 (0.44-1.03)	0.067	0.68 (0.44-1.06)	0.086

ASD, autism spectrum disorder; CI, confidence interval; PDD, Pervasive Developmental Disorder; PDD-NOS, PDD-not otherwise specified.

^aAdjusted for maternal socioeconomic status, gestational week of blood draw and offspring gestational age.

^bAdjusted for maternal: history of psychopathology, substance abuse, socioeconomic status, gestational week of blood draw and offspring gestational age.

Table S4. Odds ratios and 95% CI of the association between log-transformed maternal serum Vitamin B12 (continuous, quintiles) and offspring autism with and without Intellectual Disability (ID)

Maternal serum vitamin B12 (pmol/L)	Association with offspring autism with ID					
Continuous	Cases (N=221) Median	Controls (N=221) Median	Odds Ratio Unadjusted (95% CI)	P- value	Odds Ratio Adjusted^a (95% CI)	P- value
Log-transformed analysis	4.75	4.74	1.05 (0.68-1.61)	0.825	1.06 (0.69-1.64)	0.796
Quintiles	n (%)	n (%)	Odds Ratio Unadjusted (95% CI)	P- value	Odds Ratio Adjusted^a (95% CI)	P- value
0-20 (0-80.8)	51 (23.08)	42 (19.00)	1.55 (0.70-3.41)	0.279	1.74 (0.77-3.93)	0.187
21-40 (80.9-105.4)	36 (16.29)	51 (23.08)	0.67 (0.32-1.39)	0.281	0.71 (0.33-1.52)	0.377
41-60 (105.5-128.9)	46 (20.81)	40 (18.10)	Reference		Reference	
61-80 (128.8-164.9)	40 (18.10)	56 (25.34)	0.66 (0.30-1.42)	0.285	0.66 (0.30-1.46)	0.308
≥81 (≥165)	48 (21.72)	32 (14.48)	1.63 (0.73-3.64)	0.232	1.87 (0.80-4.38)	0.148
Maternal serum vitamin B12 (pmol/L)	Association with offspring autism without ID					
Continuous	Cases (N=1337) Median	Controls (N=1337) Median	Odds Ratio Unadjusted (95% CI)	P- value	Odds Ratio Adjusted^a (95% CI)	P- value
Log-transformed analysis	4.73	4.76	0.88 (0.74-1.05)	0.149	0.92 (0.77-1.10)	0.369
Quintiles	n (%)	n (%)	Odds Ratio Unadjusted (95% CI)	P- value	Odds Ratio Adjusted^a (95% CI)	P- value
0-20 (0-80.8)	280 (20.94)	270 (20.19)	1.54 (0.94-2.52)	0.086	1.42 (0.86-2.34)	0.172
21-40 (80.9-105.4)	292 (21.84)	262 (19.60)	1.64 (1.04-2.57)	0.033	1.58 (0.99-2.49)	0.052
41-60 (105.5-128.9)	244 (18.25)	270 (20.19)	Reference		Reference	
61-80 (128.8-164.9)	270 (20.19)	256 (19.15)	1.23 (0.76-1.99)	0.391	1.09 (0.67-1.78)	0.729
≥81 (≥165)	251 (18.77)	279 (20.87)	1.47 (0.91-2.36)	0.112	1.49 (0.92-2.43)	0.103

CI, confidence interval; Intellectual Disability, ID.

^aAdjusted for gestational week of blood draw and maternal socioeconomic status.