WEB MATERIAL

Maternal Intake of Supplemental Iron and Risk of Autism Spectrum Disorders

Rebecca J. Schmidt*, Daniel J. Tancredi, Paula Krakowiak, Robin L. Hansen, and Sally Ozonoff

*Correspondence to Dr. Rebecca J. Schmidt, Department of Public Health Sciences, School of Medicine, University of California, Davis, 123 MS1C, 1 Shields Avenue, Davis, CA 95616-8638 (e-mail: rjschmidt@ucdavis.edu).

Abbreviations: ASD, autism spectrum disorder; CHARGE, Childhood Autism Risks from Genetics and Environment; CI, confidence interval; OR, odds ratio; TD, typical development.

Web Table 1. Adjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) for Associations Between Taking an Iron-Specific Supplement and Child's Risk of ASD in the CHARGE Study, California, United States, 2003–2009

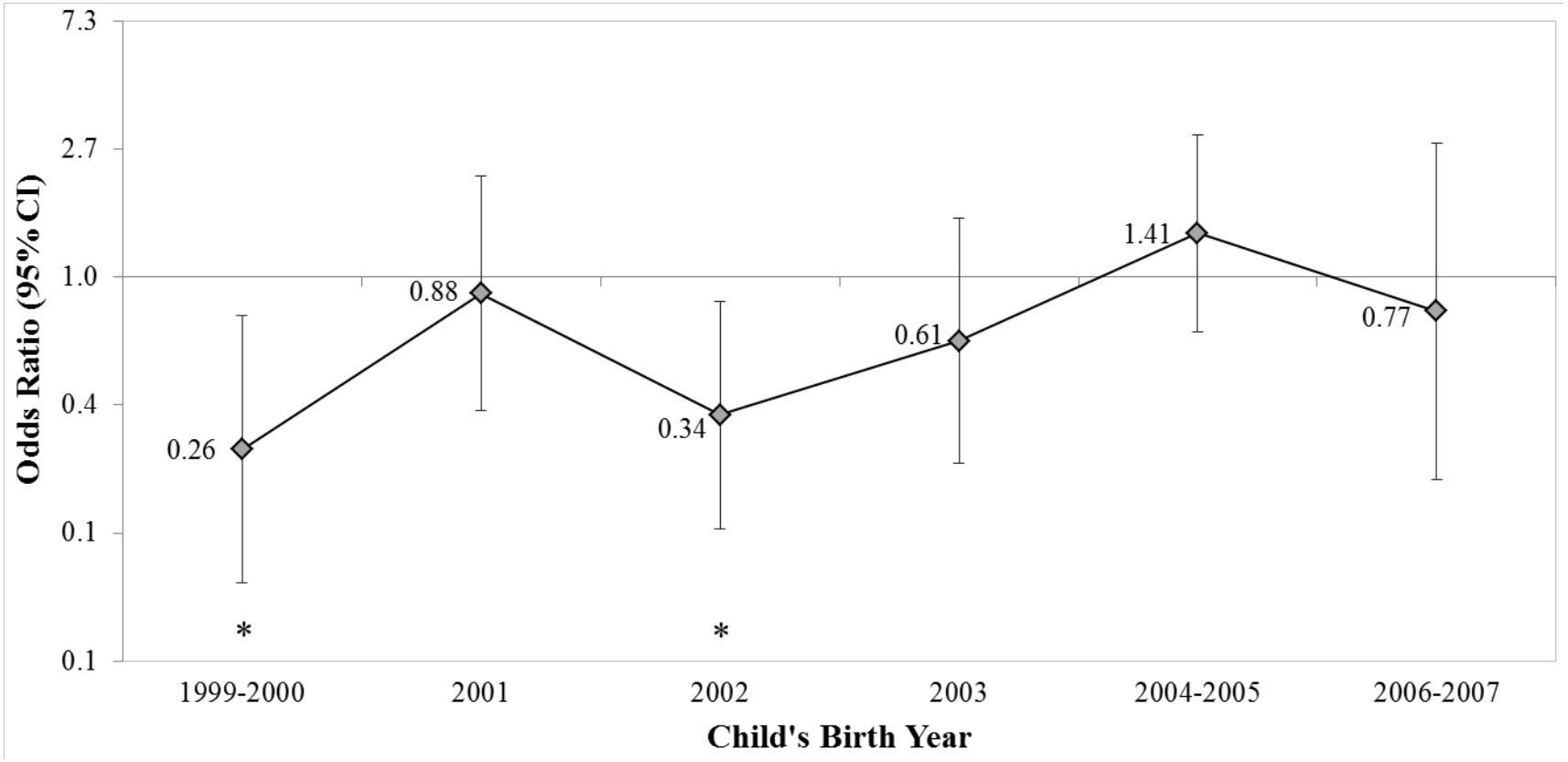
Time Period	Month	OR[a] (95% CI)	*P* Value	OR[b] (95% CI)	*P* Value
Prepregnancy	–3	0.98 (0.32, 3.02)	0.97	0.89 (0.19, 4.13)	0.88
	–2	0.98 (0.32, 3.02)	0.97	0.89 (0.19, 4.13)	0.88
	–1	0.88 (0.32, 2.46)	0.81	0.78 (0.21, 2.84)	0.70
Pregnancy	1	0.71 (0.36, 1.40)	0.32	0.67 (0.29, 1.55)	0.35
	2	0.75 (0.43, 1.30)	0.30	0.72 (0.37, 1.39)	0.33
	3	0.74 (0.45, 1.20)	0.22	0.71 (0.40, 1.27)	0.25
	4	0.64 (0.41, 0.98)	0.04	0.64 (0.39, 1.04)	0.07
	5	0.67 (0.44, 1.01)	0.06	0.65 (0.41, 1.04)	0.07
	6	0.67 (0.45, 0.99)	0.04	0.66 (0.43, 1.02)	0.06
	7	0.76 (0.52, 1.12)	0.16	0.73 (0.48, 1.13)	0.16
	8	0.79 (0.53, 1.17)	0.24	0.77 (0.49, 1.20)	0.24
	9	0.68 (0.45, 1.04)	0.08	0.64 (0.39, 1.03)	0.06
Breastfeeding	BF	0.57 (0.34, 0.95)	0.03	0.51 (0.28, 0.93)	0.03

[a] Adjusted for child's birth year and home ownership.

[b] Adjusted for folic acid intake during the first month (<600 μg/day, ≥600 μg/day), child's birth year, and home ownership.

Web Table 2. Frequency of Cases and Controls with Information on Maternal Iron Supplement Intake During the Index Period and Confounders By Child's Year of Birth

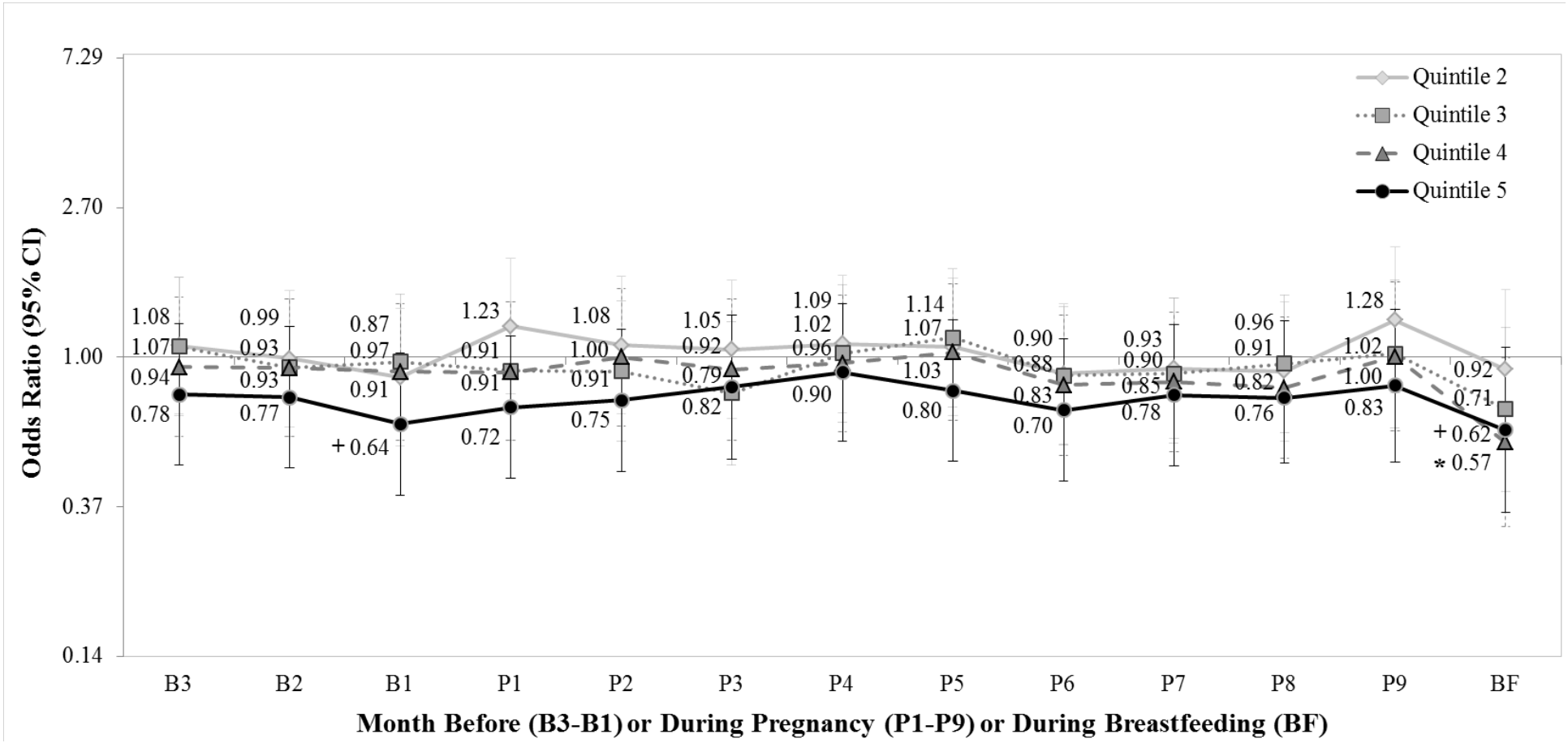
Took	1998		1999		2000		2001		2002		2003		2004		2005		2006		2007		2008	
Iron	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD
No	6	0	24	1	49	7	60	23	54	31	33	41	36	31	36	24	20	19	7	16	0	2
Yes	1	0	5	1	21	11	23	10	12	19	9	18	13	13	14	4	3	5	2	4	0	0



Web Figure 1. Adjusted odds ratios (95% CI) for the association between maternal iron supplement intake during the index period and autism spectrum disorder stratified by the child's year of birth in the CHARGE Study, California, United States, 2003–2009. Years having cells containing less than 5 individuals were combined. Odds ratios were adjusted for maternal folic acid intake during the first month of pregnancy, child's year of birth, and home ownership. *P* values for multiplicative interaction between child's birth year and iron supplement intake were: 0.47, 0.13, 0.42, 0.02, and 0.44, for years 1999–2000, 2001, 2003, 2004–2005, and 2006–2007, respectively (2002 was the reference year). Significant associations with ASD are signified by an asterisk (*) at $P < 0.05$ and by a plus sign (+) at $P < 0.10$.

Web Table 3. Adjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) for Associations Between Quintiles of Mean Maternal Iron Intake and Child's Risk of ASD in the CHARGE Study, California, United States, 2003–2009

Time Period	Month	Quintile 2		Quintile 3		Quintile 4		Quintile 5	
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Prepregnancy	–3	1.13 (0.69, 1.87)	0.62	1.02 (0.62, 1.69)	0.93	1.11 (0.66, 1.88)	0.69	1.13 (0.65, 1.98)	0.67
	–2	1.07 (0.65, 1.75)	0.79	0.92 (0.56, 1.52)	0.75	1.21 (0.72, 2.05)	0.47	1.24 (0.70, 2.19)	0.46
	–1	0.92 (0.57, 1.51)	0.75	0.98 (0.59, 1.61)	0.93	1.27 (0.76, 2.15)	0.37	1.07 (0.61, 1.91)	0.81
Pregnancy	1	1.36 (0.83, 2.23)	0.22	1.63 (0.76, 3.50)	0.21	1.63 (0.72, 3.71)	0.24	1.30 (0.57, 2.98)	0.53
	2	1.27 (0.77, 2.09)	0.36	1.88 (0.90, 3.97)	0.10	2.22 (1.04, 4.75)	0.04	1.30 (0.63, 2.71)	0.48
	3	1.17 (0.70, 1.97)	0.54	1.01 (0.59, 1.72)	0.98	1.07 (0.61, 1.85)	0.82	0.76 (0.44, 1.29)	0.30
	4	1.16 (0.71, 1.90)	0.55	1.37 (0.83, 2.27)	0.21	1.11 (0.66, 1.87)	0.69	0.86 (0.52, 1.41)	0.55
	5	1.18 (0.72, 1.93)	0.51	1.47 (0.89, 2.41)	0.13	1.08 (0.65, 1.79)	0.78	0.73 (0.44, 1.23)	0.24
	6	0.98 (0.59, 1.62)	0.94	1.09 (0.66, 1.80)	0.75	0.81 (0.48, 1.36)	0.43	0.65 (0.39, 1.09)	0.10
	7	0.95 (0.58, 1.56)	0.84	1.09 (0.65, 1.81)	0.75	0.78 (0.47, 1.31)	0.35	0.69 (0.41, 1.17)	0.17
	8	0.90 (0.55, 1.49)	0.69	1.12 (0.68, 1.86)	0.65	0.73 (0.44, 1.23)	0.23	0.68 (0.40, 1.15)	0.15
	9	1.32 (0.78, 2.23)	0.30	1.19 (0.70, 2.04)	0.52	0.88 (0.50, 1.54)	0.65	0.67 (0.38, 1.17)	0.16
Breastfeeding	BF	0.83 (0.45, 1.52)	0.54	0.80 (0.43, 1.48)	0.47	0.53 (0.28, 1.03)	0.06	0.46 (0.24, 0.88)	0.02



Web Figure 2. Unadjusted odds ratios (95% CI) for associations between mean iron intake during the index period and autism spectrum disorder in the CHARGE Study, California, United States, 2003–2009. Significant associations with ASD are signified by an asterisk (*) at $P < 0.05$ and by a plus sign (+) at $P < 0.10$.

Web Table 4. Adjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) for Associations Between Taking an Iron-Specific Supplement and Quintiles of Mean Maternal Iron Intake During Breastfeeding for those who Breastfed and Child's Risk of ASD in the CHARGE Study, California, United States, 2003–2009

		TD	ASD	OR[a]	95 % CI	*P* Value	OR[b]	95 % CI	*P* Value
Took iron-specific supplement	No	147	239	Reference			Reference		
	Yes	28	24	0.51	0.28, 0.93	**0.03**	0.41	0.21, 0.83	**0.01**
Quintiles of mean iron intake (mg/day)	<21	35	69	Reference			Reference		
	21–< 30.7	33	62	0.83	0.45, 1.52	0.54	0.75	0.28, 1.98	0.56
	30.7–< 36.5	31	50	0.80	0.43, 1.48	0.47	0.73	0.26, 2.06	0.55
	36.5–< 62.1	36	40	0.53	0.28, 1.03	0.06	0.45	0.16, 1.28	0.13
	≥62.1	38	41	0.46	0.24, 0.88	**0.02**	0.32	0.11, 0.93	**0.04**
	2-sided *P* for trend					**0.01**			**0.003**

[a] Adjusted for folic acid intake during the first month (<600 µg/day, ≥600 µg/day), child's birth year, and home ownership.
[b] Adjusted for folic acid intake during breastfeeding (<800 µg/day, ≥800 µg/day), child's birth year, and home ownership.

Web Table 5. Mean Maternal Iron Intake and Odds Ratios (OR) and 95% Confidence Intervals (95% CI) for Autism Spectrum Disorder (ASD) With or Without ASD-Related Characteristics in the CHARGE Study, California, United States, 2003–2009

ASD-Related Characteristic	ASD With Condition							ASD Without Condition							
	n	Mean	SD	*P* [1]	OR [2]	(95% CI)	*P*	*n*	Mean	SD	*P* [1]	OR [2]	(95% CI)	*P*	*P* [3]
Regression [4]	212	53.4	34.6	0.20	0.60	(0.32, 1.13)	0.11	242	50.2	33.5	**0.02**	0.39	(0.21, 0.74)	**0.004**	0.29
Delayed or atypical development [5,6]	383	50.3	33.0	**0.01**	0.49	(0.26, 0.79)	**0.005**	63	57.7	37.7	0.89	0.73	(0.39, 1.85)	0.51	0.23
Experienced seizures [7]	28	49.4	35.4	0.24	0.36	(0.10, 1.29)	0.12	384	50.9	33.8	**0.01**	0.49	(0.28, 0.84)	**0.01**	0.78
Verbal status [8]	303	52.2	34.8	0.06	0.46	(0.25, 0.82)	**0.009**	150	50.9	32.5	0.07	0.50	(0.25, 1.03)	0.06	0.77

[1] Two-sided *P* values derived from Wilcoxon 2-sample test comparing iron intake between this ASD diagnostic subgroup and the TD group; mean iron intake = 57.1 (SD, 36.6), *n* = 307.

[2] Highest quintile of iron intake (≥86 mg/day) compared with the lowest quintile of iron intake (<30 mg/day), ASD versus TD, adjusted for maternal folic acid intake during the first month of pregnancy, child's year of birth, and home ownership.

[3] Two-sided *P* values derived from Wilcoxon 2-sample test comparing iron between the ASD diagnostic subgroups (with and without the condition).

[4] Regression was defined as present if the child experienced loss of language skills after acquisition and/or social engagement/responsiveness, derived from questions on the Autism Diagnostic Interview–Revised (1) and the Early Development Questionnaire (2). More specifically, the child was considered to have regressed if question 11 [language loss] or 25 [possible or definite social engagement/responsiveness loss] was coded as 1 for "yes" or 3 or more communication or social skills losses were reported on questions A3-4, B1-5 [gestures and imitative behaviors, understanding spoken language, interest in family or peers, responsive smiling, reaching for caregiver, direct eye contact during communication, interest in interactive games] in Part 3 of the Early Development Questionnaire.

[5] Atypical development/mild delays was defined as having either Mullen Scales of Early Learning (MSEL) <70 or Vineland Adaptive Behavior Scales (VABS) <70, but not both <70, and the assessment with a score above the cutpoint is more than 0.5 SD above the cutpoint (a score of 78 points or more) for children recruited as DD or TD (but not meeting criteria for either) and scoring 14 or lower on the Social Communication Questionnaire.

[6] Delayed development was defined as MSEL composite of less than 70 and VABS composite of less than 70, or below 70 on one assessment and within 0.5 standard deviation of 70 (±7 points) on the other assessment.

[7] Seizures reported by parents on a physician-administered child medical history.

[8] Verbal/nonverbal status was defined as in Gotham et al. (3): a child was considered verbal if he/she received a code of 0, 1 or 2 on item A1 of the Autism Diagnostic Observation Schedule–Generic (ADOS) module 1 (for children using single words), or used ADOS Module 2 or 3 (for children speaking in phrases or speaking fluently); a child was considered nonverbal if he/she received a code of 3 or 8 on item A1 of the ADOS module 1.

Web Table 6. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Associations Between Maternal Iron Intake During the Index Period and Child's Risk of ASD Stratified by Child's Age at the Time of Interview as a Proxy for the Length of Maternal Recall

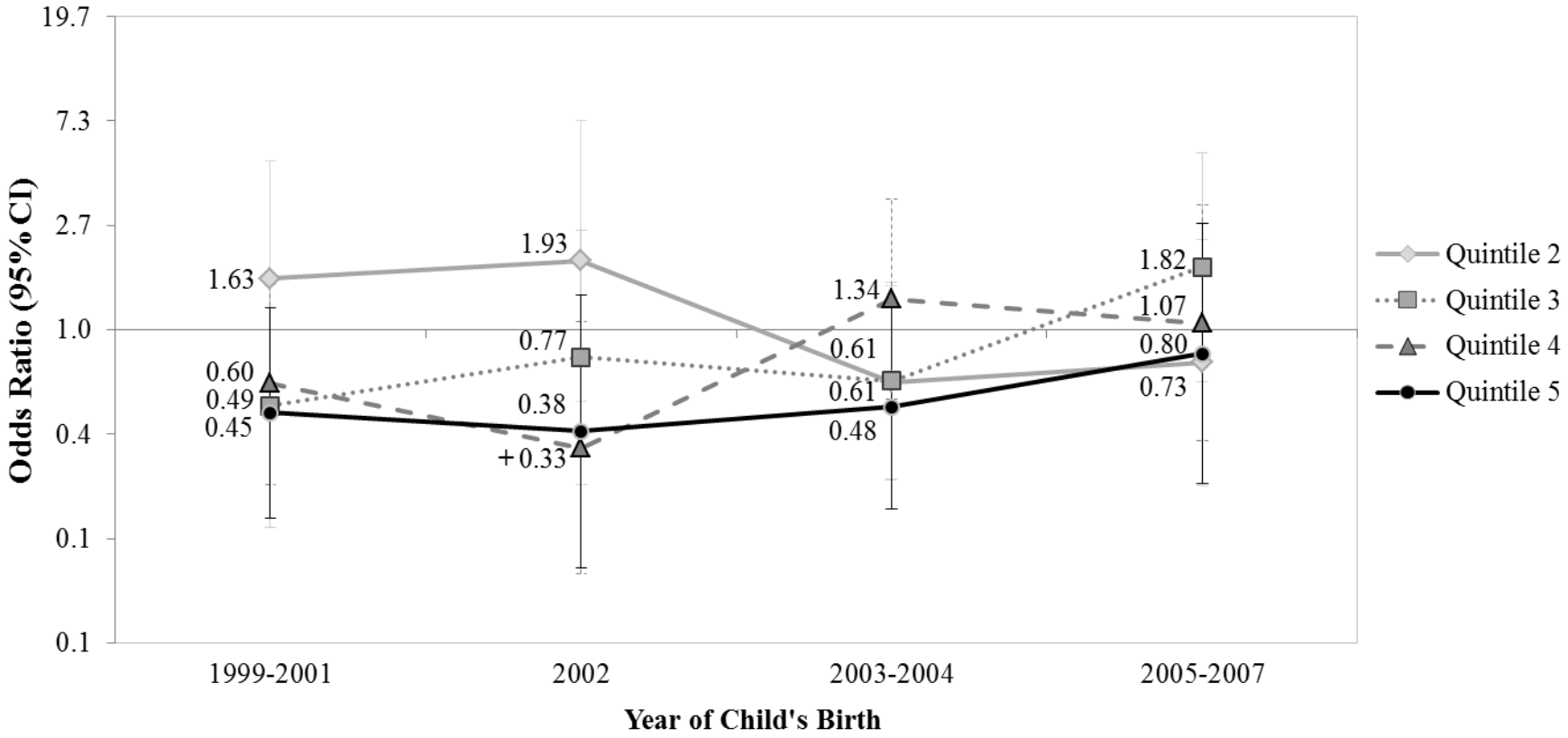
	Child Aged <3.6 Years[a]					Child Aged ≥3.6 Years				
	TD	ASD	OR[b]	95 % CI	*P*	TD	ASD	OR[b]	95 % CI	*P*
Iron-specific vitamin										
No	118	164		Reference		116	216		Reference	
Yes	52	50	0.52	0.30, 0.90	**0.02**	55	80	0.74	0.45, 1.20	0.22
Quintiles of mean iron intake (mg/day)										
<30	28	45		Reference		32	71		Reference	
30–<36	33	47	1.00	0.48, 2.07	0.997	28	45	0.89	0.45, 1.76	0.74
36–<52	31	42	1.12	0.52, 2.41	0.78	32	46	0.60	0.30, 1.19	0.14
52–<86	21	28	1.05	0.46, 2.39	0.90	39	64	0.63	0.33, 1.20	0.16
≥86	41	27	0.38	0.18, 0.83	**0.01**	22	39	0.65	0.31, 1.37	0.26
2-sided *P* for trend					**0.03**					0.11

[a] 3.6 years was the median age at the start of the study interview for children with typical development.

[b] Adjusted for folic acid, child's birth year, and home ownership.

Web Table 7. Frequency of Cases and Controls with Information on Maternal Quantitative Iron Intake During the Index Period and Confounders by Child's Year of Birth

	1998		1999		2000		2001		2002		2003		2004		2005		2006		2007		2008	
	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD	ASD	TD
Quintile 1	0	0	9	0	19	4	18	6	17	9	12	7	10	11	13	6	5	5	2	5	0	1
Quintile 2	1	0	4	0	10	0	23	6	17	5	6	15	10	8	8	10	5	6	2	3	0	0
Quintile 3	2	0	4	1	5	3	11	5	14	9	9	12	9	13	13	5	9	5	2	3	0	1
Quintile 4	2	0	6	1	17	5	16	8	10	15	10	10	11	3	11	4	3	5	0	2	0	0
Quintile 5	1	0	5	0	14	5	8	7	8	10	5	14	8	9	5	2	1	2	2	6	0	0



Web Figure 3. Adjusted odds ratios (95% CI) for the association between the highest quintile compared to the lowest quintile of maternal mean iron intake during the index period and autism spectrum disorder stratified by the child's year of birth in the CHARGE Study, California, United States, 2003–2009. Years having cells containing less than 5 individuals were combined. Odds ratios were adjusted for maternal folic acid intake during the first month of pregnancy, child's year of birth, and home ownership. *P* values for multiplicative interaction between child's birth year and the highest quintile of iron intake are: 0.89, 0.93, and 0.62 for years 1999–2001, 2003, 2003–2004, and 2005–2007, respectively (2002 was the reference year). Significant associations with ASD are signified by an asterisk (*) at $P < 0.05$ and by a plus sign (+) at $P < 0.10$.

References

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2. Ozonoff S, Williams BJ, Landa R. Parental report of the early development of children with regressive autism: the delays-plus-regression phenotype. *Autism.* Dec 2005;9(5):461–486.

3. Gotham K, Pickles A, Lord C. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord.* 2009;39(5):693–705.