Autism Spectrum Disorder Phenotypes and Prenatal Exposure to Methylmercury

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Background: There continues to be public concern that mercury exposure and autism spectrum disorder (ASD) may be associated. The primary source of exposure to organic mercury in humans is to methylmercury from fish consumption. We evaluated the association between prenatal methylmercury exposure and ASD phenotype in children and adolescents in the Republic of Seychelles, where fish consumption is high.

Methods: We administered the Social Communication Questionnaire to parents of a cohort of 1784 children, adolescents, and young adults. The Social Responsiveness Scale was administered to teachers of 537 cohort subjects at about 10 years of age. Prenatal exposure to methylmercury was measured in maternal hair samples collected at or near the time of birth. Multivariable regression models evaluated the relationship between prenatal methylmercury exposure and ASD phenotypic scores, adjusting for relevant covariates.

Results: The mean prenatal methylmercury exposure for subjects in the analysis was 8.4 ppm (standard deviation [SD] = 5.7). The mean Social Communication Questionnaire score was 8.0 (SD = 4.4). The mean prenatal methylmercury exposure for subjects with Social Responsiveness Scale scores was 6.7 ppm (SD = 4.4) and the mean Social Responsiveness Scale score was 57.6 (SD = 26.8). No consistent association between prenatal methylmercury exposure and ASD screening instrument was found, using linear and nonlinear regression analyses.

Conclusions: Prenatal exposure to methylmercury was not associated with ASD phenotypic behaviors in our cohort of high fish consumers. Our findings contribute to the growing literature suggesting that exposure to methylmercury does not play an important role in the development of ASD phenotypic behavior.

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The reported global prevalence of autism spectrum disorders (ASDs) has risen dramatically in recent decades, with a current estimate of 1 per 161 children worldwide[1] and 1 per 88 children in the United States.[2] The increase in reported prevalence may be a result of broadened diagnostic criteria, better diagnostic procedures, or improved awareness of potentially affected children among families and healthcare providers. However, much of the increase in ASD prevalence remains unexplained, and environmental factors have been suggested to play a more important role than previously thought.[3]

There has been considerable speculation that prenatal or postnatal exposure to mercury plays a role in the etiology of ASD.[4] Bernard and associates[5] in 2001 hypothesized that prenatal exposure to various species of mercury might disrupt prenatal brain development or affect central nervous system development or function following birth. They also hypothesized that postnatal exposure to vaccines containing thimerosal (sodium ethylmercurithiosalicylate and its metabolite ethylmercury) might be associated with ASD symptoms.[5,6] Additional theories have suggested that genetic predisposition to the effects of mercury toxicity,[7] immune modulation by mercury,[8] or metabolic abnormalities[9] might contribute to postnatal brain injury. However, there are limited data to support these theories.[9]

In 2004, the US Institute of Medicine (IOM) reviewed the evidence linking ASD and prenatal or postnatal exposure to mercury or thimerosal. The IOM concluded that although the hypothesis that mercury and ASD prevalence are associated is biologically plausible, there is presently insufficient evidence to support or refute this hypothesis.[10] Subsequent research has focused largely on thimerosal and no association with ASD has been reported.[9] However, possible links between exposure to other mercury species such as methylmercury (which has different neurotoxic potential) and ASD prevalence have

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received less study.[11,12] Once ingested, methylmercury readily crosses the placental and blood-brain barriers and exposes the fetus' developing brain, which is known to be particularly vulnerable to methylmercury exposure.

We studied the association between prenatal methylmercury exposure and ASD behavioral phenotypes in the Republic of Seychelles. The Seychelles serves as a sentinel population because Seychellois citizens consume large amounts of ocean fish and all fish contain small amounts of methylmercury. Fish consumption is the primary human source of methylmercury exposure, and methylmercury exposures in Seychelles are between 10 and 20 times higher than in the United States or United Kingdom.[13]

METHODS

Setting and Participants

The study was conducted as an extension of the Seychelles Child Development Study, a longitudinal cohort study examining the hypothesis that prenatal exposure to methylmercury from consuming a pregnancy diet high in fish adversely affects child development. The present study participants included 1784 mother–child pairs from four study cohorts (hereafter called the "combined cohort") who met two criteria for inclusion: (1) a prenatal maternal hair sample was available, and (2) data on ASD phenotype was provided by a biological parent. The combined cohort consisted of the following: (1) 193 (23%) of the 804 subjects enrolled in 1986–1987 and termed the "Pilot cohort"[14–16]; (2) 813 (32%) of 2566 mothers who had volunteered hair samples during pregnancy between 1986 and 1990 but whose children were never evaluated; (3) 532 (70%) of 779 "main cohort" subjects enrolled in 1989–1990[16–18]; and (4) 246 (82%) of 300 "nutrition I cohort" subjects enrolled in 2001.[19] Census and maternity health records were used to successfully locate approximately 1,400 of the mothers who had voluntarily provided hair samples between 1986 and 1990. The combined cohort of 1784 subjects represents 40% of the 4449 mother–child pairs eligible for the current study. All study procedures were approved by the Institutional Review Board at the University of Rochester and the Research Review Board of the Republic of Seychelles.

In addition to information about ASD phenotype obtained in the combined cohort, we had previously collected additional information on ASD phenotypic behavior using the Social Responsiveness Scale for 537 mother–child pairs in the main cohort. Of this number, 405 participants also were later administered the Social Communication Questionnaire and were therefore also part of the combined cohort.

ASD Phenotype Survey Instruments

Social Communication Questionnaire for the Combined Cohort

For the assessment of ASD phenotypic behavior in our combined cohort, we selected The Social Communication Questionnaire[20,21] because it is based on standard DSM-IV and International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) diagnostic criteria, has been used internationally to study ASD,[22,23] and has been the screening test of choice in previous ASD prevalence studies.[24] It is based on the Autism Diagnostic Interview, the most widely accepted interview for making a research diagnosis of ASD.[25] The Social Communication Questionnaire is a 40-item parent questionnaire designed to screen for symptoms of ASD in children. It has high internal consistency.[20] Using a cutoff of 15 or greater for further evaluation of ASD in a sample of 590 clinic-referred children, the sensitivity and specificity were both estimated at 0.71 for discriminating between ASD and non-ASD groups in a sample of 590 clinic-referred children.[26] Before being administered in Seychelles, the Social Communication Questionnaire was translated to Kreol, the primary language spoken at home.

Social Communication Questionnaires were delivered to mothers or fathers who consented to participate in the study, either by members of the Seychelles Child Development Study staff based at our Child Development Center in Victoria or through a nurse based at the mother's Community Health Center. Social Communication Questionnaires were completed by the parents, either at the site where they received the questionnaire or at home with no input from study staff. Completed Social Communication Questionnaires were returned by parents or health personnel to the Child Development Center. Only questionnaires completed by a biological parent caring for the child were used in this analysis. Questionnaire scores were regarded as missing if participants did not return a questionnaire after agreeing to participate, or if the questionnaire was not completed by a biological parent.

Social Responsiveness Scale for the Main Cohort

At 10.7 years of age, children in the main cohort were administered the Social Responsiveness Scale, a 65-item rating scale that ascertains autistic symptoms across the entire range of severity occurring in ordinary social settings. Each item is rated on a 4-point Likert scale from "0" (never true) to "3" (almost always true). A total score is derived by summing the scores for each item, with a range of possible scores from 0 to 195 (higher scores indicating more autistic traits). The measure has excellent psychometric properties[27] and is intended for use in large-scale epidemiological studies to screen for symptoms across the autistic spectrum.[28] In addition to the continuous score, a total raw score cut-point of 70 or greater in males and 65 or greater in females has been recommended to indicate possible ASD.[27,29]

Symptoms are sampled across the domains of behaviors critical for the diagnosis of ASD by DSM-IV criteria.[30] The Social Responsiveness Scale has subscales for social awareness, social information processing, capacity for reciprocal social responses, social anxiety/avoidance, and characteristic autistic preoccupations/traits. In addition to the total score across all domains, we computed a total raw score for each domain.

The Social Responsiveness Scale can be completed by either parents or teachers. In our study, Seychellois teachers assigned to the children's classrooms were asked to complete the Scale.

Dosimetry

For all cohorts, methylmercury exposure was determined in the University of Rochester Mercury Analytical Chemistry Laboratory from maternal hair samples collected at or near the time of birth. Samples were analyzed by cold vapor atomic absorption as described previously.[31,32]

Covariates

Combined Cohort

Covariates included child's sex and cohort to which the child belonged. It is well known that ASD prevalence varies by sex.[2] The time windows of recruitment for the individual cohort varied. We considered the cohort variable to account for age differences between cohorts and, more importantly, differences in the intensity of follow-up across cohorts during the study. In separate models we adjusted for child's age at testing in addition to cohort, and results for methylmercury were similar. Therefore, we report only the results from models adjusting for cohort.

A limited number of covariates were used for the combined cohort because information on additional covariates was not available for children born between 1986 and 1990. They accounted for almost half the sample of the combined cohort (813 of 1784 mother–child pairs, or 46%).

Main Cohort

The influence of additional potentially relevant covariates was further investigated in the main cohort, which had a more thorough covariate assessment and which constituted about a third of the total sample. In analyses of the main cohort, we considered maternal and paternal age at the child's birth and child's sex. Maternal and paternal ages were included as covariates as increasing parental age at conception may increase a child's risk for ASD.[33,34]

Statistical Analysis

The primary endpoint for analysis of the combined cohort was the Social Communication Questionnaire total score. In descriptive analyses, prenatal methylmercury exposure was compared across the four cohorts and between those with and without total Social Communication Questionnaire scores. Exposure distributions were substantially right-skewed and were therefore log-transformed for bivariate statistics only. We tested for the presence of differences in total Social Communication Questionnaire scores across the four cohorts using one-way analysis of variance with Tukey-HSD post hoc comparisons of the pair-wise differences. We tested for differences in methylmercury exposure levels between subjects with and without useable total Social Communication Questionnaire scores, using two-sample *t* tests.

The primary analysis of the combined cohort used multivariable linear regression to examine the association between prenatal methylmercury exposure and the total Social Communication Questionnaire score adjusting for sex and cohort (primary model). Secondary analysis used binomial regression to examine the association between prenatal methylmercury exposure and the probability of having a total Social Communication Questionnaire score of 15 or higher. Because differential effects of prenatal methylmercury exposure on males and females have been reported, the models were run first with and then without a methylmercury-by-sex interaction term. Exploratory analysis using a semiparametric additive model[28] tested for nonlinear trends between prenatal methylmercury exposure and total Social Communication Questionnaire score, with adjustment for main effects of sex and cohort as in the linear regression analysis. The interaction effect between sex and methylmercury exposure was not considered in the semiparametric additive model because it was not statistically significant in the primary linear model.

Further analysis of the main cohort used multivariable linear regression to examine the association between prenatal methylmercury exposure and the autism phenotypic measure (using Social Communication Questionnaire and Social Responsiveness Scale scores in separate analyses) while controlling for covariate effects, including only sex in the primary model and sex as well as maternal and paternal age in the full model. Secondary analysis used binomial regression to examine the association between prenatal methylmercury exposure and the probability of having test score indicating possible ASD. These models were also run first with and then without a methylmercury-by-sex interaction term. Exploratory analyses using a semiparametric additive model[35] examined nonlinear trends between prenatal methylmercury exposure and each autism phenotypic measure, with adjustment for covariates. The interaction effects between sex and methylmercury exposure were not considered in the semiparametric additive models because they were not statistically significant in the primary linear models.

For the analyses of both cohorts, we performed an assessment of residuals to check the assumptions of normally distributed errors with constant variance. In addition, Cook's distances were examined to evaluate the influence of individual observations. If the assumption of normality seemed to be violated, we used a square-root transformation to stabilize the variance and produce more normally distributed errors. In all cases, the significance of results was the same; we present only the untransformed results. All analyses were done with the R system.[36]

RESULTS

Table 1 shows the average maternal hair methylmercury concentration for participants in the combined cohort for whom total Social Communication Questionnaire scores were obtained, as well as for potential participants for whom

TABLE 1. Comparison of Prenatal MeHg Exposure Levels (ppm) Between Participants and Nonparticipants: Seychelles Child Development Study

	Participants With Social Communication Questionnaire Score				Potential Participants Without Social Communication Questionnaire Score[a]			
Cohort	**No.**	**Mean**	**Min**	**Max**	**No.**	**Mean**	**Min**	**Max**
Combined cohort								
Prenatal MeHg (ppm)	1,784	8.4	0.2	56.7	2,661	9.3	0.4	68.1
Child's age at testing[b]	1,778	18.7	7.4	24.1				
Pilot cohort (1986–1987)								
Prenatal MeHg (ppm)	193	7.7	0.6	23.0	648	7.7	0.6	36.4
Child's age at testing[b]	192	22.5	19.7	24.1				
Maternal age at birth	193	25.0	15.0	43.0	647	25.2	14.0	46.0
1986–1990 cohort								
Prenatal MeHg (ppm)	813	10.4	0.5	56.7	1,753	10.3	0.7	68.1
Child's age at testing[b]	812	20.5	17.7	24.1				
Main cohort (1989–1990)								
Prenatal MeHg (ppm)	532	6.8	0.5	26.7	230	7.0	0.5	24.7
Child's age at testing[b]	532	19.6	18.7	21.1				
Maternal age at birth	532	26.2	15.0	45.0	230	25.9	14.0	44.0
Paternal age at birth	467	29.1	17.0	62.0	200	28.9	18.0	53.0
Nutrition cohort (2001)								
Prenatal MeHg (ppm)	246	5.7	0.2	22.7	30	4.9	0.4	13.3
Child's age at testing[b]	242	7.8	7.4	8.3				
Maternal age at birth	246	27.0	15.0	42.0	30	27.9	17.0	43.0
Paternal age at birth	221	31.1	17.0	51.0	25	31.7	18.0	53.0

MeHg indicates methylmercury.

[a]Missing Social Communication Questionnaire scores resulted from participants who did not return a Social Communication Questionnaire protocol after agreeing to participate, or whose Social Communication Questionnaire protocol was not completed by the biological parent.

[b]Sample size reported for child's age at testing is smaller than the sample size for all participants with a Social Communication Questionnaire score because of exclusion of children with ineligible data (ie, too many missing responses to compute a total score or questionnaire not completed by a parent).

such scores were not available. The mean prenatal methylmercury exposure for all combined cohort subjects in the analysis was 8.4 ppm (standard deviation [SD] = 5.7), which was about 1 ppm lower than methylmercury exposure among potential participants without a total Social Communication Questionnaire score. There was no difference in methylmercury exposure levels between participants with and without total Social Communication Questionnaire scores when stratified by cohort (Table 1). Methylmercury exposure values differed across cohorts, with lower exposures in the nutrition cohort enrolled in 2001. Exposure values were greatest in the cohort enrolled from 1986 to 1990 (Table 1).

The mean total Social Communication Questionnaire score in the combined cohort was 8.0 (SD = 4.4, range 0–28), and a total of 149 subjects (8%) received a total Social Communication Questionnaire score of 15 or higher. The mean total Social Communication Questionnaire scores were 7.2 (SD = 5.0), 7.7 (SD = 4.5), 8.4 (SD = 4.4), and 8.6 (SD = 3.9) in the pilot (1986–1987), 1986–1990, main (1989–1990), and nutrition I (2001) cohorts, respectively. 9.8%, 7.4%, 10.0%, and 6.9% of each cohort received a total Social Communication Questionnaire score of 15 or higher, respectively. Figure 1 shows that the preponderance of total Social Communication Questionnaire scores (both below and above 15) are associated with very low methylmercury exposure levels, a pattern that was similar for all four subcohorts (data not shown).

The average total Social Responsiveness Scale score in the main cohort was 57.6 (SD = 26.8, range 6–155). A total of 182 subjects (34%) received a total raw Social Responsiveness Scale score above the cutoff indicating possible ASD phenotypic behaviors. The average scores for Social Responsiveness Scale subscales were as follows: 8.1 (SD = 3.6) for social awareness; 11.4 (SD = 5.6) for social information processing; 19.5 (SD = 10.2) capacity for reciprocal social responses; 10.8 (SD = 4.6) for social anxiety/avoidance; and 7.8 (SD = 6.2) for characteristic autistic preoccupations/traits. Similar to the total Social Communication Questionnaire scores, the scatter plot between total Social Responsiveness Scale scores and prenatal methylmercury exposure shows no clear pattern (Fig. 2).

Results from multivariable linear and binomial regression models evaluating the association between prenatal methylmercury exposure and total Social Communication Questionnaire or Social Responsiveness Scale scores are shown in Tables 2–4. There were no influential points in the regression models, and interactions between sex and prenatal methylmercury were not statistically significant and therefore

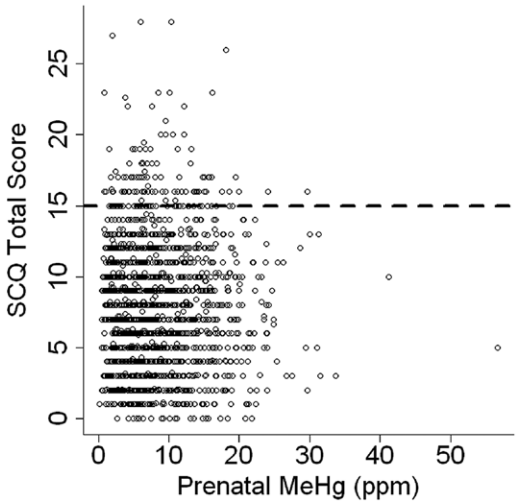

FIGURE 1. Scatter plot of total Social Communication Questionnaire (SCQ) scores and prenatal methylmercury (MeHg) exposure levels in the combined cohort. The dashed line indicates the screening criterion score of 15.

not further reported. Prenatal methylmercury exposure was not associated with total Social Communication Questionnaire scores in the combined cohort in either linear or binomial regression models (Table 2). Linear regression findings were similar when Social Communication Questionnaire analyses were restricted to the main cohort and adjusted for additional covariates (resulting in a smaller sample size of participants with complete covariate data). In binomial regression analyses, prenatal methylmercury showed an adverse effect on total Social Communication Questionnaire scores in a model with additional covariates and reduced sample size (Table 2, Fully Adjusted Model, Binomial Regression). Further evaluation

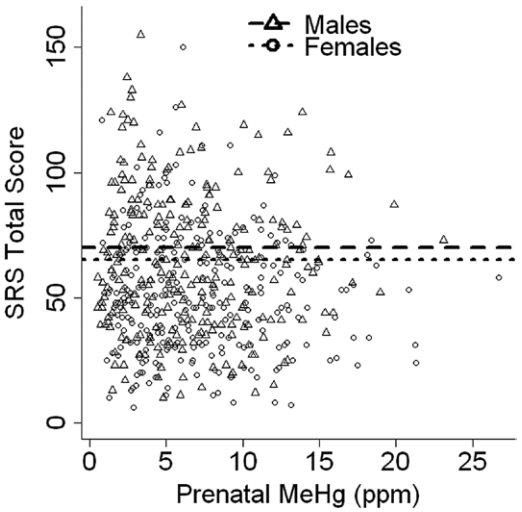

FIGURE 2. Scatter plot of total Social Responsiveness Scale (SRS) scores and prenatal methylmercury (MeHg) exposure levels in the main cohort. The dashed lines indicate the screening criterion score of 70 for males and 65 for females.

of the association in this smaller sample without additional covariates yielded similar results (Table 2, Restricted Primary Model, Binomial Regression) suggesting that the associations with prenatal methylmercury in this subgroup were due to the exclusion of participants with missing data on the additional covariates rather than to residual confounding by these covariates. No covariates other than possibly subcohort (in the combined cohort analyses) and maternal age (showing an inverse, beneficial association) were predictive of total Social Communication Questionnaire scores.

In multivariable regression analyses, there was no association between prenatal methylmercury exposure and the total Social Responsiveness Scale score in the main cohort, regardless of the sample size or inclusion of additional covariates. In these models, the only consistent predictor of total Social Responsiveness Scale scores was child's sex, with males having about a 12-point greater score (Table 3).

Multivariable linear regression analyses of Social Responsiveness Scale subscales suggest an inverse, beneficial association between the social awareness subscale and prenatal methylmercury exposure. There were associations when analyses adjusted for child's sex and a similar magnitude of association (but a slightly increased *P* value) when analyses controlled for additional covariates. Interestingly, three of the four other Social Responsiveness Scale subscales showed associations in the same beneficial direction.

In semiparametric additive models, there was no evidence for a nonlinear relationship between prenatal methylmercury levels and total Social Communication Questionnaire or Social Responsiveness Scale scores.

DISCUSSION

This is the first study to examine the association between ASD phenotypic behavior and prenatal exposure to methylmercury from consumption of a pregnancy diet high in fish. We found no consistent evidence for an association between prenatal methylmercury levels and total Social Communication Questionnaire or Social Responsiveness Scale scores in the Republic of Seychelles. Our findings are consistent with increasing evidence of the lack of an association between methylmercury exposure and ASD. Prior studies have focused largely on vaccines and exposure to thimerisol.[10]

We did observe an adverse association between prenatal methylmercury exposure and being at risk for ASD as defined by a total Social Communication Questionnaire score ≥15 in our main cohort after adjustment for maternal and paternal age and child's sex. This is unlikely to represent a meaningful association, however, for several reasons. First, the magnitude of association was relatively small (with a slope of 0.07), corresponding to a relative risk increase of 1.07 for each 1 ppm increase in maternal hair methylmercury level. Second, the observed association was most likely due to exclusion of participants, in that adjustment for additional covariates resulted in only a slight increase in the magnitude of association. The

TABLE 2. Multivariable Regression Models Evaluating the Association Between Prenatal MeHg Exposure and Total Social Communication Questionnaire Scores in the Seychelles Child Development Study

		Main Cohort		
	Combined Cohort (N = 1,784)	Primary Model (N = 532)[a]	Fully Adjusted Model (N = 467)[b]	Restricted Primary Model (N = 467)[a]
Covariate	Slope (95% CI)	Slope (95% CI)	Slope (95% CI)	Slope (95% CI)
Linear regression				
Prenatal MeHg	0.00 (−0.04 to 0.04)	0.03 (−0.05 to 0.12)	0.06 (−0.03 to 0.14)	0.05 (−0.03 to 0.14)
Cohort[c]				
Pilot (1986–1987)	−1.26 (−1.99 to −0.53)	—	—	—
1986–1990	−0.68 (−1.19 to −0.18)	—	—	—
Nutrition (2001)	0.16 (−0.51 to 0.83)	—	—	—
Male child	0.02 (−0.39 to 0.43)	−0.37 (−1.12 to 0.38)	−0.25 (−1.06 to 0.56)	−0.27 (−1.08 to 0.53)
Maternal age	—	—	−0.02 (−0.11 to 0.07)	—
Paternal age	—	—	0.01 (−0.07 to 0.09)	—
Model R^2 (*P* value)	0.0105 (<0.01)	0.0029 (0.46)	0.0047 (0.70)	0.0042 (0.38)
Binomial regression				
Prenatal MeHg	0.01 (−0.02 to 0.04)	0.05 (−0.01 to 0.11)	0.07 (0.00 to 0.13)	0.06 (0.00 to 0.12)
Cohort[c]				
Pilot (1986–1987)	−0.02 (−0.60 to 0.51)	—	—	—
1986–1990	−0.36 (−0.77 to 0.04)	—	—	—
Nutrition (2001)	−0.39 (−0.99 to 0.16)	—	—	—
Male child	0.02 (−0.32 to 0.35)	−0.06 (−0.63 to 0.51)	−0.00 (−0.63 to 0.61)	−0.09 (−0.70 to 0.52)
Maternal age	—	—	−0.08 (−0.15 to −0.01)	—
Paternal age	—	—	0.05 (−0.00 to 0.11)	—
Model *P* value	0.50	0.27	0.04	0.12

CI indicates confidence interval; MeHg, methylmercury; —, variables that are not included in the multivariable regression model.
[a]Adjusted for the effects of child sex.
[b]Adjusted for the effects of child sex and maternal and paternal age at the child's birth.
[c]The Pilot, 1986–1990, and Nutrition cohorts were compared with the Main cohort (1989–1990).

association was seen only in the smaller main cohort subsample with complete information on covariates and not in the main or combined cohorts with fewer covariates but a substantially larger sample size.

Although prenatal methylmercury exposure was not associated with the total Social Responsiveness Scale score in the main cohort, we did observe an inverse, beneficial association with the social awareness subscale. There is no reason to believe that low levels of exposure to methylmercury would improve scores on the total Social Responsiveness Scale or any of the other subscales. In previous studies of the main cohort, we have also observed beneficial associations of prenatal methylmercury exposure with developmental outcomes on several occasions,[37] which may be due to unmeasured confounding effects of beneficial nutrients found in ocean fish. Recent data suggest that these findings may indeed be due to the beneficial effects of nutrients such as long-chain polyunsaturated fatty acids.[38,39] Our findings on the social awareness subscale may also be due to chance. On the other hand, the associations for three other Social Responsiveness Scale subscales are in the same direction, suggesting that chance occurrence may not be the correct interpretation. It should be kept in mind, however, that scores for the five Social Responsiveness Scale subscales are moderately to strongly correlated, with correlation coefficients ranging from 0.46 to 0.82. Nevertheless, these patterns of association with Social Responsiveness Scale subscales will deserve evaluation in a larger cohort in which both toxicants and nutrients are assessed.

Our findings should be interpreted in light of some limitations. First, the Social Communication Questionnaire and Social Responsiveness Scale are screening instruments that can be used only to identify persons who have the characteristics of ASD and require closer evaluation. They are not diagnostic instruments and further expert clinical evaluation is necessary to confirm a diagnosis of ASD, especially considering the lack of correlation between Social Communication Questionnaire and Social Responsiveness Scale scores (r = 0.04) in the main cohort. Therefore, our analyses of dichotomized Social Communication Questionnaire and Social Responsiveness Scale scores should not be interpreted as addressing ASD per se. Also, interpretation of the

TABLE 3. Multivariable Regression Models Evaluating the Association Between Prenatal MeHg Exposure and Total Social Responsiveness Scale Scores in the Seychelles Child Development Study Main Cohort

	Primary Model (N = 537)[a]	Fully Adjusted Model (N = 474)[b]	Restricted Primary Model (N = 474)[a]
Covariate	**Slope (95% CI)**	**Slope (95% CI)**	**Slope (95% CI)**
Linear regression			
Prenatal MeHg	−0.33 (−0.83 to 0.18)	−0.33 (−0.88 to 0.21)	−0.40 (−0.94 to 0.15)
Male child	11.88 (7.44 to 16.31)	13.72 (8.97 to 18.46)	13.33 (8.58 to 18.09)
Maternal age	—	−0.49 (−1.03 to 0.05)	—
Paternal age	—	−0.03 (−0.51 to 0.44)	—
Model R^2 (*P* value)	0.0534 (<0.01)	0.0785 (<0.01)	0.0665 (<0.01)
Binomial regression			
Prenatal MeHg	−0.02 (−0.06 to 0.02)	−0.03 (−0.08 to 0.01)	−0.04 (−0.08 to 0.01)
Male child	0.36 (0.00 to 0.73)	0.49 (0.10 to 0.88)	0.46 (0.07 to 0.85)
Maternal age	—	−0.02 (−0.06 to 0.03)	—
Paternal age	—	−0.01 (−0.05 to 0.03)	—
Model *P* value	0.08	0.02	0.01

CI indicates confidence interval; MeHg, methylmercury; —, variables that are not included in the multivariable regression model.
[a]Adjusted for the effects of child sex.
[b]Adjusted for the effects of child sex and maternal and paternal age at the child's birth.

screening tests may have been complicated by translation to another language and application in a culture that may have different norms for social interaction.

Misclassification of ASD phenotypic behavior may be of concern in particular for the Social Communication Questionnaire. For example, Social Communication Questionnaire scores in this study were not related to the child's sex. Although there is some evidence that Social Communication Questionnaire scores and child's sex may not be related,[26] males are reported to be five times more likely than females to have an ASD diagnosis.[2] Indeed, child's sex in this study was strongly predictive of total and subscale Social Responsiveness Scale scores. Also, mean Social Communication Questionnaire scores were slightly higher for the main and nutrition cohorts, which have been part of the study for longer than the other subcohorts. These children's parents have on numerous occasions answered survey questions related to their child's history and behavior. Their experience may have affected their sensitivity to their children's behavior and their diligence in completing the Social Communication Questionnaire, resulting in a higher score. Even so, limiting our Social Communication Questionnaire analysis to the Main cohort did not greatly affect our findings.

We had limited covariate information on subjects. Exposure to prenatal mercury vapor from maternal amalgams was not considered because data were available only for the main and nutrition cohorts.[40,41] However, the correlation between prenatal methylmercury and prenatal amalgam surfaces in those two cohorts was very close to zero (−0.003), indicating that exposure to prenatal mercury vapor is unlikely to be a

TABLE 4. Parameter Estimates (Slope) for Prenatal MeHg from Multivariable Regression Models Evaluating the Association Between Prenatal MeHg Exposure and Social Responsiveness Scale Subscale Scores: Seychelles Child Development Study Main Cohort

	Primary Model (N = 537)[a]	Full Model (N = 474)[b]	Restricted Primary Model (N = 474)[a]
Subscale	**Slope (95% CI)**	**Slope (95% CI)**	**Slope (95% CI)**
Social awareness	−0.07 (−0.14 to 0.01)	−0.07 (−0.14 to 0.01)	−0.07 (−0.15 to −0.00)
Social information processing	−0.04 (−0.15 to 0.07)	−0.05 (−0.16 to 0.07)	−0.06 (−0.17 to 0.06)
Capacity for reciprocal social responses	−0.15 (−0.35 to 0.04)	−0.16 (−0.37 to 0.04)	−0.18 (−0.39 to 0.02)
Social anxiety/avoidance	0.03 (−0.06 to 0.12)	0.04 (−0.06 to 0.13)	0.03 (−0.07 to 0.12)
Characteristic autistic preoccupation/traits	−0.09 (−0.21 to 0.03)	−0.09 (−0.22 to 0.04)	−0.11 (−0.24 to 0.02)

CI indicates confidence interval; MeHg, methylmercury.
[a]Adjusted for the effects of child sex.
[b]Adjusted for the effects of child sex and maternal and paternal age at the child's birth.

confounder. Also, we did not measure exposure to thimerosal. However, for over 25 years the Seychelles has routinely achieved nearly 100% immunization rates using vaccines that contain thimerosal. Thus, thimerosal exposure was assumed universal among study participants.

Finally, there was a small difference in prenatal methylmercury exposure between participants with and without Social Communication Questionnaire scores. This was probably due to the different representation of the four cohorts in the missing and nonmissing Social Communication Questionnaire groups. Prenatal methylmercury exposure values were lower in the nutrition cohort as compared with the earlier cohorts (possibly due to a decline in fish consumption) and greatest in the 1986–1990 cohort. A greater proportion of Social Communication Questionnaire scores were missing in the older cohort subjects with the higher methylmercury levels, resulting in a lower methylmercury level in the combined cohort relative to the larger group of eligible participants. Otherwise, the characteristics of participants in the study did not differ substantially from those who either declined or whose Social Communication Questionnaire data were incomplete, suggesting that there was little participation bias.

Our study had several strengths. All combined cohort members had their prenatal methylmercury exposure accurately measured, and levels were approximately 10 times that of mothers in the United States. The cohort size was large, and our study design was longitudinal. There were also a number of covariates available for all subjects, and one cohort had additional covariates that could be considered.

In conclusion, we found no association between prenatal methylmercury exposure and phenotypic ASD behaviors. Our findings contribute to the growing literature suggesting that exposure to organic forms of mercury does not play an important role in the development of ASD phenotypic behavior.

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