Prenatal Lead Exposure, δ-Aminolevulinic Acid, and Schizophrenia

Mark G.A. Opler,[1] Alan S. Brown,[1,2] Joseph Graziano,[3] Manisha Desai,[4] Wei Zheng,[3] Catherine Schaefer,[5] Pamela Factor-Litvak,[6] and Ezra S. Susser[2,6]

[1]Department of Psychiatry, College of Physicians and Surgeons, Columbia University, New York, New York, USA; [2]New York State Psychiatric Institute, New York, New York, USA; [3]Department of Environmental Health Sciences and [4]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York, USA; [5]Division of Research, Kaiser Permanente Health Care, Oakland, California, USA; [6]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA

Schizophrenia is a severe mental disorder of unknown etiology. Recent reports suggest that a number of environmental factors during prenatal development may be associated with schizophrenia. We tested the hypothesis that environmental lead exposure may be associated with schizophrenia using archived serum samples from a cohort of live births enrolled between 1959 and 1966 in Oakland, California. Cases of schizophrenia spectrum disorder were identified and matched to controls. A biologic marker of lead exposure, δ-aminolevulinic acid (δ-ALA), was determined in second-trimester serum samples of 44 cases and 75 controls. δ-ALA was stratified into high and low categories, yielding 66 subjects in the high category, corresponding to a blood lead level (BPb) ≥ 15 μg/dL, and 53 in the low category, corresponding to BPb < 15 μg/dL. Using logistic regression, the odds ratio (OR) for schizophrenia associated with higher δ-ALA was 1.83 [95% confidence interval (CI), 0.87–3.87; $p = 0.1$]. Adjusting for covariates gave an OR of 2.43 (95% CI, 0.99–5.96; $p = 0.051$). This finding suggests that the effects of prenatal exposure to lead and/or elevated δ-ALA may extend into later life and must be further investigated as risk factors for adult psychiatric diseases. *Key words:* **δ-aminolevulinic acid, developmental, lead, Pb, prenatal, prospective, psychosis, schizophrenia.** *Environ Health Perspect* **112:548–552 (2004). doi:10.1289/ehp.6777 available via *http://dx.doi.org/* [Online 8 January 2004]**

Schizophrenia and related disorders are characterized by hallucinations, delusions, social withdrawal, and disorganized thinking. Although typically diagnosed during late adolescence and early adulthood, a growing body of evidence suggests that events during prenatal development may play a role in the etiology of these diseases. In particular, exposures to agents that may disrupt or damage the developing nervous system have been implicated. This theory, commonly referred to as the "neurodevelopmental hypothesis of schizophrenia" (Murray et al. 1992; Weinberger et al. 1996), has been supported by recent findings that suggest prenatal nutritional deprivation and infection may be risk factors for schizophrenia (Susser et al. 1999). However, few investigators have considered schizophrenia among the possible neuropsychiatric sequelae of chemical agents.

Lead, a known chemical teratogen, is capable of disrupting both behavioral and physical development (Sobotka and Rahwan 1995). Relationships between early exposure to lead and neuropsychologic abnormalities have been observed throughout the life course (Bellinger et al. 1991; Kim et al. 1995; Pocock et al. 1994). For example, the Yugoslavia Prospective Study reported that lead exposure during midpregnancy was associated with deficits in neuropsychiatric function at 24 months of age (Factor-Litvak et al. 1999; Graziano et al. 1990). Further assessments of the cohort identified persistent decrements in measures of attention, cognition, and verbal comprehension at 4, 7, 10, and 12 years of age (Wasserman et al. 2000). Needleman et al. (1979, 1990) found associations between dentine lead levels measured in deciduous teeth (6–8 years of age) and reading difficulties and failure to graduate from high school.

In a prospective study conducted in Cincinnati, Ohio, prenatal and average childhood blood lead concentrations were reported to be associated with increased delinquent behavior later in life (Dietrich et al. 2001). This suggests that prenatal lead exposure may be a risk factor for other adolescent and adult-onset outcomes, possibly psychiatric disorders. Schizophrenia is one plausible candidate because some of its premorbid features such as reduced attention, neurocognitive impairment, and diminished educational attainment (Jones et al. 1993) strongly resemble the behavioral deficits associated with lead exposure.

The present study was designed to assess the association between lead exposure in the second trimester of pregnancy and schizophrenia using prospectively collected serum samples in a nested case–control study from a birth cohort in which schizophrenia and related disorders had been diagnosed (Susser et al. 2000). Exposure to lead during the prenatal period is generally measured using whole blood because most of the lead in blood is contained in erythrocytes (Korpela et al. 1986). Only serum samples and not whole blood specimens were available for this study. Consequently, we used an indirect biologic marker of lead exposure, δ-aminolevulinic acid (δ-ALA). δ-ALA is part of the heme synthetic pathway. Under normal conditions, δ-ALA is rapidly dimerized by δ-ALA dehydratase (ALAD) to form porphobilinogen. During exposure to lead, levels of δ-ALA in serum and urine increase because lead is a potent inhibitor of the enzymatic activity of erythrocyte ALAD (Bergdahl et al. 1997).

Materials and Methods

Description of cohort. The Prenatal Determinants of Schizophrenia (PDS) study, described in detail elsewhere (Susser et al. 2000), is based on a cohort of live births collected prospectively from 1959 through 1967 at the Kaiser Foundation Health Plan clinics in Alameda County, California, as part of the Child Health and Development Study. The PDS study includes the 12,094 live-born individuals who remained in the health plan until 1981, when it became possible to use computerized records to identify potential cases of schizophrenia. In addition to detailed records on a variety of demographic characteristics of the parents and obstetric health, samples of whole blood were drawn during each prenatal visit, centrifuged, and divided into four aliquots of serum (2 cc each) (Brown et al. 2000); samples were maintained at National Institutes of Health facilities at –20°C. A prior study of this cohort indicates that the sera are in good condition, specifically that they contain concentrations of sex hormones comparable with those observed in freshly drawn sera (Udry et al. 1995) and expected quantities of antibodies to influenza (Brown et al., in press).

The available literature on the behavior of δ-ALA and porphyrin levels during pregnancy indicated that fluctuations occur in the weeks immediately preceding delivery (de Klerk et al. 1975). In contrast, blood lead (BPb) levels have been shown to change only slightly

Address correspondence to M.G.A. Opler, Department of Epidemiology, Columbia University, 722 West 168th St., New York, NY 10032 USA. Telephone: (646) 234-3607. Fax: (212) 305-9413. E-mail: mgo4@columbia.edu

Work reported in this article was supported in part by National Institutes of Health training grant 5 T32 MH 13043 (M.O.), a NARSAD (National Alliance for Research on Schizophrenia and Depression) Independent Investigator Award (A.S.B.), the Lieber Center for Schizophrenia Research, and a NARSAD Young Investigator Award (M.O.).

The authors declare they have no competing financial interests.

Received 1 October 2003; accepted 8 January 2004.

during the early stages of pregnancy and remain stable during the second and third trimesters (Baghurst et al. 1987; Graziano et al. 1990). Therefore, midpregnancy serum samples were sought for this analysis because they were thought to be a point in development when both the exposure and biomarker of interest were likely to be stable. In addition, developmental events during the second trimester of pregnancy have been previously implicated in schizophrenia, such as neuronal migration and synaptogenesis (Beckmann 1999; Bracha et al. 1992).

Case ascertainment, diagnosis, and selection of controls. Screening for potential cases of schizophrenia spectrum disorder was initiated by identifying all possible cases using computerized records from inpatient, outpatient, and pharmacy databases. Possible cases were contacted and assessed by experienced clinical interviewers with master's level training. Standardized procedures included a structured clinical interview (the Diagnostic Interview for Genetic Studies; Nurnberger et al. 1994) and a consensus diagnosis made by expert clinicians after review of the narrative, psychiatric records, and discussions with the interviewer. A complete description of the methods used has been previously published (Susser et al. 2000).

Cases identified through these methods included 43 subjects with diagnoses of schizophrenia, 17 cases of schizoaffective disorder, 5 cases of schizotypal personality disorder, 1 case of delusional disorder, and 5 cases who met criteria for nonaffective psychoses not otherwise specified. Controls were selected from the remaining subjects without diagnoses of schizophrenia spectrum disorder and were matched to cases on timing of membership in the health plan (such that controls were required to be members of the health plan during the time at which disease status was identified in the matched case), date of birth ± 28 days, sex, date of the first maternal blood draw ± 4 weeks, and equal numbers of maternal serum samples available for study. Forty-four cases and 75 controls (1–2/case) had second-trimester maternal serum available for analysis.

Laboratory protocol. A method published by Endo et al. (1993) and Oishi et al. (1996) to determine plasma levels of δ-ALA was adapted for use as a biologic marker for lead exposure (Tomokuni et al. 1993), and further adapted for use in stored serum samples in this study. Briefly, δ-ALA reacts with acetylacetone and formaldehyde to form 2,6-diactyl-1,5-dimethyl-7-(2-carboxyethyl)-3*H*-pyrrolizine (Figure 1), a derivative that can be quantified via fluorescence detection at excitation/emission wavelengths of 370 nm and 460 nm, respectively.

Frozen serum samples were identified by coded labels, rendering the analyst blind to case status. Samples were thawed in an ice bath for 1 hr and transferred to Eppendorf tubes. These tubes were placed in a 70°C water bath for 20 min and then centrifuged for 3 hr at 14,000 rpm in a Sorval microcentrifuge (Kendro Laboratory Products, Asheville, TN) at 4°C. For the derivatization reaction, an aliquot of 50 µL of supernatant was removed and added to 16 Kimax glass test tubes (125 mm; Kimble/Kontes, Vineland, NJ) containing 1.5 mL acetylacetone reagent (20% acetylacetone, 20% ethanol in deionized water, vol/vol) and 450 µL 37% formaldehyde. Tubes were loosely capped and held at 100°C for 20 min using a dual aluminum alloy block heater (VWR International, West Chester, PA).

The tubes were then cooled in an ice bath for 10 min and allowed to stand at room temperature in the dark for 1 hr. An aliquot (1 mL) of supernatant was transferred to light-proof Eppendorf tubes and centrifuged for 1 hr. The supernatant was then filtered through 3-cc disposable syringes using acrylic syringe filters with 0.45 µm pores and aspirated through 9.5 mm, 26-gauge needles (Fisher Scientific, Atlanta, GA) to a final volume of 750 µL. The filtrate was transferred to light-resistant 700 µL 8 × 30 mm crimp-top HPLC injection vials with aluminum caps (Alltech Associates, Deerfield, IL).

We used a Perkin-Elmer model LC-250 equipped with an LC-600 autosampler and an LC-40 fluorescence detector (Perkin-Elmer, Norwalk, CT) for analysis. Separation was performed at a flow rate of 1.0 mL/min using an Adsorbosphere HS C_{18} column (5 µm, 250 × 4.6 mm) attached to a Spherisorb C_{18} Guard column (5 µm, 17 × 4.6 mm; both from Alltech Associates). A CH-500 integrated heater/controller with aluminum alloy column fittings was used to maintain a temperature of 37 ± 1°C (Eppendorf/Brinkman Instruments, Westbury, NY). δ-ALA was separated using an isocratic mobile phase of methanol/water/glacial acetic acid in proportions of 500:500:10 (vol/vol/vol) that was filtered and degassed using helium.

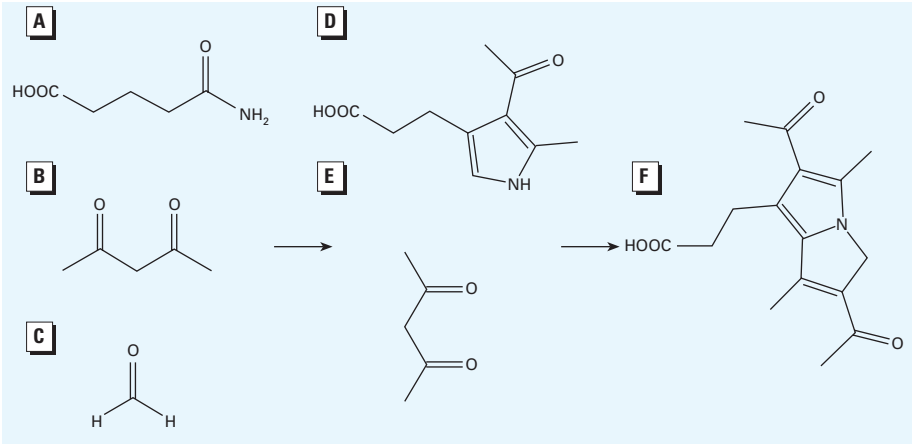

Figure 1. Derivitization of δ-ALA to 2,6-diactyl-1,5-dimethyl-7-(2-carboxyethyl)-3*H*-pyrrolizine. δ-ALA (*A*), an indicator of elevated lead exposure; acetylacetone (*B*); and formaldehyde (*C*) react at 100°C to produce several intermediate products (*D*, *E*) that combine to form 2,6-diactyl-1,5-dimethyl-7-(2-carboxyethyl)-3*H*-pyrrolizine (*F*), a fluorescent derivative.

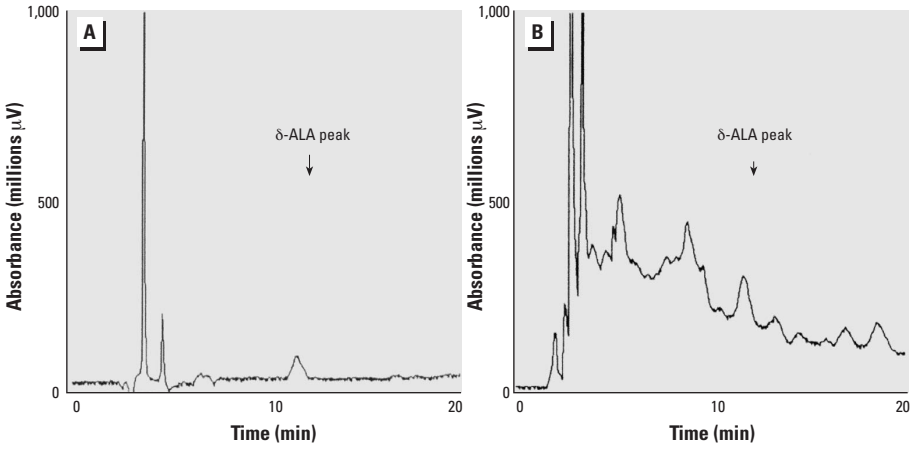

Figure 2. HPLC assay with fluorescence detection (HPLC-FD) of δ-ALA. Chromatograms from (*A*) a 10-ng/mL standard solution of δ-ALA in deionized H_2O, and (*B*) sera from the Yugoslavia study taken from a lead-exposed subject. Arrows indicate the δ-ALA peak at a retention time of approximately 11 min.

Intraday variability of 4.7% for this assay was determined by repeating the analytic procedure eight times using a standard solution of δ-ALA in deionized water at a concentration of 25 ng/mL. The interday variability by repeated injection of the same standard solution for 8 consecutive days was 7.8%. Based on the standard curve established using data from intraday analyses, the detection limit for δ-ALA was calculated to be 4.67 ng/mL (Ren et al. 1998). During the primary study, standard concentrations of 50 ng/mL δ-ALA were used for detector calibration before and after every four serum injections.

Reliability and validity studies. For the purpose of testing the reliability of the laboratory method for measurement of δ-ALA in stored maternal serum and the validity of δ-ALA as an indicator of BPb, we obtained aliquots of sera for 23 subjects (supplied by J.G.) from the Yugoslavia Study of Environmental Lead Exposure and Child Development (Graziano et al. 1990). Although these sera had been stored at –20°C for 13–15 years, whole BPb measurements had been made at Columbia University within weeks after collection of the samples. The samples selected for reliability and validity testing were drawn at random from a larger pool of subjects across a range of midpregnancy BPb levels (4.5–41.3 μg/dL). Based on literature reports of BPb levels for women living in California in the 1960s (Ludwig et al. 1965; Thomas et al. 1967), we estimated that the distribution of BPb levels in our prenatal cohort would range from 3 to 45 μg/dL and that the mean would likely fall between 10 and 20 μg/dL.

Initial assessments of δ-ALA levels from 23 Yugoslavia study subjects indicated that levels were comparable with those expected in freshly drawn sera. A subset of 18 aliquots was available in duplicate to assess the reliability of the laboratory technique for measurement of δ-ALA in stored maternal serum (Figure 2). The intraclass correlation coefficient for the 18 duplicate samples was 0.91, indicating that repeated measures of δ-ALA levels on the same sample were highly correlated. When δ-ALA levels were dichotomized at the median (9.05 ng/mL) such that subjects with levels ≥ 9.05 ng/mL were categorized as "high" and those < 9.05 ng/mL as "low," the kappa statistic for duplicate samples was 0.89 with an SE of 0.23, indicating excellent agreement between repeated measures for δ-ALA.

BPb and δ-ALA levels were compared continuously in all 23 samples (Figure 3), and the correlation coefficient was 0.64. When a regression line was drawn, points at the lower levels of BPb and δ-ALA tended to fall outside a 95% confidence interval (CI). We therefore conducted a validity study to establish a method for categorizing δ-ALA as a predictor of BPb. We chose a cutoff point of 15 μg/dL to define our exposure categories, such that subjects with BPb ≥ 15 μg/dL would be classified as "exposed" and those with levels < 15 μg/dL would be defined as "unexposed." δ-ALA levels were then dichotomized into "high" and "low" categories using the median δ-ALA value (9.05 ng/mL) as a cutoff point. Sensitivity (i.e., the proportion of subjects classified as exposed to lead and also categorized as high δ-ALA) was 91% for the first trial of the 18 samples from the Yugoslavia study analyzed in duplicate and 90% for the second trial. The positive predictive values were 89% for the first trial and 91% for the second, indicating the proportion of subjects for which an δ-ALA level ≥ 9.05 ng/mL would accurately predict a BPb level ≥ 15 μg/dL.

Statistical methods. In order to examine the relationship between δ-ALA and schizophrenia spectrum disorders, we used two approaches. The first, a Mantel-Haenszel odds ratio (MH OR), provided a summary measure that estimated the odds of having a diagnosis of schizophrenia spectrum disorder if exposed versus the odds if unexposed, after taking into account the correlation within matched sets. This approach provides a simple and readily interpretable OR that accounts for the matching variables, although it does not adjust for other covariates. Second, conditional logistic regression models were fitted (Neuhaus 1992), including δ-ALA exposure as a predictor of schizophrenia spectrum disorder, while adjusting for covariates (Greenland 2000, Greenland et al. 2000). Parameter estimates for the fitted models were calculated using the STATA statistical package (Stata Corp., College Station, TX).

Potential confounders were assessed on the basis of their known association with both lead exposure and schizophrenia, including maternal and paternal age, education, race/ethnicity, family income, father's income, maternal smoking, maternal alcohol use, hemoglobin levels, and number of previous pregnancies. After testing each for associations between serum δ-ALA and disease, addition and removal procedures were performed. During construction of the regression models, the utility of all potential covariates was assessed through sequential inclusion and exclusion. A change of ± 10% in the point estimate corresponding to δ-ALA provided justification for including a variable in the model.

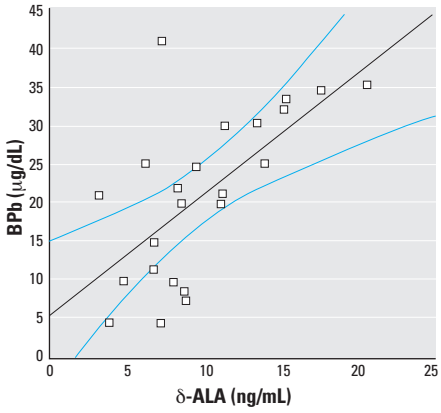

Figure 3. BPb and δ-ALA levels in midpregnancy sera, Yugoslavia study. Midpregnancy BPb levels in 23 subjects from the Yugoslavia Study of Environmental Lead Exposure and Child Development (Graziano et al. 1990) are compared with δ-ALA levels in archived serum samples stored for 13–15 years at –20°C. A regression line and 95% CIs are shown.

Results

Demographics. Thirty-one case–control sets contained 2 controls, and the remaining 13 had 1 control, for a total of 175 subjects

Table 1. Demographic characteristics of parents (%) by case status.

	Cases (n = 44)	Controls (n = 75)
Father's age at delivery (years)		
15–19	0	1
20–29	34	33
30–39	36	37
40–45	25	12
Unknown	5	16
Father's race		
White/Caucasian	43	43
African American	41	31
Mexican, other	7	12
Unknown	9	15
Father's education		
< High school diploma	20	16
High school or vocational	29	33
Some college	18	20
College graduate	20	20
Unknown	11	11
Family annual income		
< $2,500	0	3
$2,500–5,999	32	28
$6,000–9,999	33	31
$10,000–14,999	10	14
≥ $15,000	2	1
Unknown	25	21
Mother's age at delivery (years)		
15–19	9	14
20–29	50	44
30–39	38	37
40–45	2	5
Unknown	0	0
Mother's race		
White/Caucasian	45	49
African American	45	37
Mexican, other	7	12
Unknown	2	1
Mother's education		
< High school diploma	18	8
High school or vocational	38	43
Some college	18	24
College graduate	17	16
Unknown	9	8
Number of previous pregnancies		
0	23	21
1	23	25
2	16	16
3	29	27
> 3	0	2
Unknown	9	9

divided into 44 sets. Table 1 compares the demographic characteristics of the parents of cases and controls. Although the distributions for cases and controls are not significantly different for most variables examined ($p \geq 0.2$), fathers of cases were somewhat older, which is consistent with findings from a previous cohort analysis of these data (Brown et al. 2002). The difference in mean age of the father at delivery is of borderline statistical significance ($p = 0.07$). Maternal characteristics were similar, and the differences were small and not statistically significant.

δ-ALA distribution. In serum from all 119 subjects, concentrations of δ-ALA range from nondetectable to 79.5 ng/mL, with a mean concentration (± SD) of 9.0 ± 9.80 (Figure 4). Exclusion of the one control subject with a δ-ALA level of 79.5 ng/mL in the analysis did not alter the conclusions of the study.

Because the validity study described in "Materials and Methods" demonstrated that the use of a cutoff point of 9.05 ng/mL δ-ALA (corresponding to a BPb level of 15 μg/dL) yields high sensitivity and positive predictive values, this cutoff point was used to define high and low levels of serum δ-ALA. This cutoff point was used to classify exposure in subjects from the primary study. Fifty-three subjects with δ-ALA levels ≥ 9.05 ng/mL (24 cases and 29 controls) were categorized as exposed and 66 subjects with levels < 9.05 ng/mL (20 cases and 46 controls) were categorized as unexposed.

δ-ALA and schizophrenia spectrum disorder. Table 2 shows estimates of the risk of schizophrenia in individuals whose mothers had higher levels of maternal δ-ALA during the second trimester, compared with lower levels, using different statistical approaches. Using the MH method, the estimated OR was 1.83 (95% CI, 0.85–3.95). In a continuous analysis on a logarithmic scale, the effect for each unit increase in serum ALA was calculated as an OR of 1.92 (95% CI, 0.90–4.13; $p = 0.09$). One variable, mother's age at delivery, met our criteria for inclusion in a logistic regression model using the methods described above. When maternal age was categorized (15–19, 20–29, 30–39, and > 39 years) and included in the model, the estimated OR was 2.4 (95% CI, 0.99–5.96; $p = 0.051$). Controlling for additional variables such as father's age at delivery, parental race, education, family income, and number of previous pregnancies had no effect on our findings.

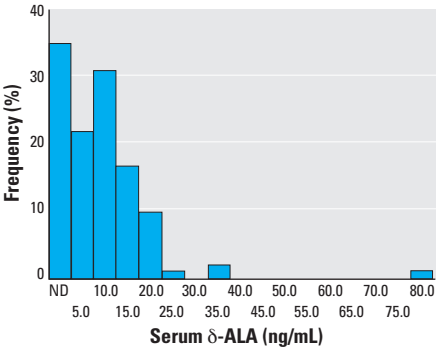

Figure 4. Distribution of second-trimester δ-ALA levels. ND, not detectable. For controls, $n = 75$; mean ± SD = 8.58 ± 10.83; and maximum = 79.54; for cases, $n = 44$; mean ± SD = 9.67 ± 7.80; and maximum = 33.27; for cases and controls combined, $n = 119$; mean ± SD = 8.99 ± 9.80; and maximum = 79.54.

Discussion

Our study represents the first report of a prospective examination of a prenatal chemical exposure as a risk factor for an adult psychiatric disease. Lead was widely distributed throughout urban areas during the era when this cohort was founded. Although BPb levels in the United States have declined, lead exposure continues to be of great concern. Despite bans on both leaded gasoline and lead-based paint that have been in effect for more than two decades, it has been estimated in national samples of children and neonates that 5% still have BPb ≥ 10 μg/dL (Satcher 2000), with regional rates as high as 29% (Vivier et al. 2001). Internationally, lead exposure continues to be a concern because use of leaded gasoline persists in many parts of the world.

On the basis of our results, we suggest that further study is required to determine whether prenatal exposure to lead and/or elevated levels of serum δ-ALA during the second trimester of pregnancy may be associated with an increased risk of schizophrenia spectrum disorder. When our finding is adjusted for covariates, the observed effect approaches statistical significance. These conclusions are subject to several limitations. First, the sample size is modest. Second, although methods for adjusting for potential confounders were used, some confounders may not have been adequately controlled for as a consequence of the matched design and sample size or because of a lack of sufficient information. For instance, data on family history of mental illness are incomplete for this cohort. Third, for similar reasons, we were unable to examine postnatal factors that might modify the effects of prenatal lead exposure. For example, childhood socioeconomic status (SES) may reverse lead-induced neuropsychologic deficits in high-SES children (Tong et al. 2000).

Although δ-ALA is a biologic indicator of lead exposure, other factors may affect δ-ALA levels. One alternative hypothesis that might explain our finding relates to the fact that ALAD is polymorphic. The most common variant, designated ALAD-1, is differentiated from its counterpart, ALAD-2, by a single locus G-to-C transversion in the coding region (Kelada et al. 2001). The carriers of the ALAD-2 allele have been shown to have higher BPb levels and lower levels of lead in bone, whereas individuals homozygous for ALAD-1 have higher levels of δ-ALA in plasma and urine (Kelada et al. 2001). A variety of findings suggest that interactions between ALAD polymorphisms and lead exposure may affect long-term outcomes, including differences in neuropsychologic effects of lead exposure (Bellinger et al. 1994). It is possible that a specific ALAD polymorphism may be a risk factor for schizophrenia, either independently or through interactions with blood lead. DNA samples were not available from the maternal cohort, although genotyping of the subjects may be possible in order to test this hypothesis in the future.

In considering lead exposure during development as a risk factor for adult mental illness, both direct and indirect mechanisms may be postulated. Direct mechanisms could involve physical interactions between lead and the developing nervous system, interfering with growth, differentiation, or structural development. Examples supported by experimental evidence include effects on molecules of neural adhesion (e.g., nerve-cell adhesion molecule, N-cadherin, L1 neural cell adhesion molecule) (Prozialeck et al. 2002) and alterations of synaptic function (e.g., *N*-methyl-D-aspartate receptor expression) (Toscano et al. 2002). Both have been implicated in the pathogenesis of schizophrenia (Olney et al. 1999; Vawter 2000).

Indirect mechanisms might include effects of lead that are not specific to the central nervous system, such as renal damage (Loghman-Adham 1997), altered transthyreitin secretion at the choroid plexus (Zheng et al. 1999), or interactions with nutrient absorption and distribution (Dawson et al. 1999). One specific indirect mechanism that must be considered is the potential toxicity of δ-ALA. δ-ALA is a known neurotoxin, and elevated levels of δ-ALA are associated with psychosis in adults, as characterized by various forms of porphyria (Estrov et al. 2000). In experimental models, δ-ALA has been shown to interfere with gamma-aminobutyric acid neurotransmission (Percy et al. 1981), a process that has also been implicated in schizophrenia (Benes 1997). Thus, it is possible that δ-ALA itself

Table 2. Estimated ORs relating δ-ALA (categorized as ≥ 9.05 and < 9.05 ng/mL) and schizophrenia spectrum disorder using three statistical methods.

Method	Estimated OR (95% CI)	*p*-Value
MH OR, unadjusted	1.83 (0.87–3.87)	0.106
Conditional logistic regression, unadjusted	1.89 (0.86–4.11)	0.109
Conditional logistic regression, adjusted for mother's age at delivery	2.43 (0.99–5.96)	0.051

elevates the risk of schizophrenia spectrum disorders, independently or as a consequence of lead exposure.

Over the past century, regulatory standards governing lead exposure during pregnancy and childhood have become less permissive, recognizing detrimental effects at progressively lower concentrations. In parallel with this trend, research has begun to focus on the effects of prenatal lead exposure at increasingly distal points along the life course as cohorts move out of infancy and childhood, through adolescence. The results of our study expand this premise to include an adult psychiatric disease, suggesting that lead-induced prenatal damage to the developing brain may manifest throughout the decades after the initial exposure.

References

Baghurst PA, McMichael AJ, Vimpani GV, Robertson EF, Clark PD, et al. 1987. Determinants of blood lead concentrations of pregnant women living in Port Pirie and surrounding areas. Med J Aust 146:69–73.

Beckmann H. 1999. Developmental malformations in cerebral structures of schizophrenic patients. Eur Arch Psychiatry Clin Neurosci 249(suppl 4):44–47.

Bellinger D, Hu H, Titlebaum L, Needleman HL. 1994. Attentional correlates of dentin and bone lead levels in adolescents. Arch Environ Health 49:98–105.

Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL, Waternaux C. 1991. Low-level lead exposure and children's cognitive function in the preschool years. Pediatrics 87:219–227.

Benes FM. 1997. The role of stress and dopamine-GABA interactions in the vulnerability for schizophrenia. J Psychiatr Res 31:257–275.

Bergdahl IA, Grubb A, Schutz A, Desnick RJ, Wetmur JG, Sassa S, et al. 1997. Lead binding to delta-aminolevulinic acid dehydratase (ALAD) in human erythrocytes. Pharmacol Toxicol 81:153–158.

Bracha HS, Torrey EF, Gottesman II, Bigelow LB, Cunniff C. 1992. Second-trimester markers of fetal size in schizophrenia: a study of monozygotic twins. Am J Psychiatry 149:1355–1361.

Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan MA. In press. Serologic evidence for prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry.

Brown AS, Schaefer CA, Wyatt RJ, Begg MD, Goetz R, Bresnahan MA, et al. 2002. Paternal age and risk of schizophrenia in adult offspring. Am J Psychiatry 159:1528–1533.

Brown AS, Schaefer CA, Wyatt RJ, Goetz R, Begg MD, Gorman JM, et al. 2000. Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. Schizophr Bull 26:287–295.

Dawson EB, Evans DR, Harris WA, Van Hook JW. 1999. Amniotic fluid B12, calcium, and lead levels associated with neural tube defects. Am J Perinatol 16:373–378.

de Klerk M, Weideman A, Malan C, Shanley BC. 1975. Urinary porphyrins and porphyrin precursors in normal pregnancy. Relationship to urinary total oestrogen excretion. S Afr Med J 49:581–583.

Dietrich KN, Ris MD, Succop PA, Berger OG, Bornschein RL. 2001. Early exposure to lead and juvenile delinquency. Neurotoxicol Teratol 23:511–518.

Endo Y, Okayama A, Endo G, Horiguchi S, Nakazono N. 1993. Improvement of urinary delta-aminolevulinic acid determination by HPLC-fluorometry using pre-column derivatization [in Japanese]. Sangyo Igaku 35:126–127.

Estrov Y, Scaglia F, Bodamer OA. 2000. Psychiatric symptoms of inherited metabolic disease. J Inherit Metab Dis 23:2–6.

Factor-Litvak P, Wasserman G, Kline JK, Graziano J. 1999. The Yugoslavia Prospective Study of Environmental Lead Exposure. Environ Health Perspect 107:9–15.

Graziano JH, Popovac D, Factor-Litvak P, Shrout P, Kline J, Murphy MJ, et al. 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environ Health Perspect 89:95–100.

Greenland S. 2000. When should epidemiologic regressions use random coefficients? Biometrics 56:915–921.

Greenland S, Schwartzbaum JA, Finkle WD. 2000. Problems due to small samples and sparse data in conditional logistic regression analysis. Am J Epidemiol 151:531–539.

Jones PB, Bebbington P, Foerster A, Lewis SW, Murray RM, Russell A, et al. 1993. Premorbid social underachievement in schizophrenia. Results from the Camberwell Collaborative Psychosis Study. Br J Psychiatry 162:65–71.

Kelada SN, Shelton E, Kaufmann RB, Khoury MJ. 2001. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: a HuGE review. Am J Epidemiol 154:1–13.

Kim R, Hu H, Rotnitzky A, Bellinger D, Needleman H. 1995. A longitudinal study of chronic lead exposure and physical growth. Environ Health Perspect 103:952–957.

Korpela H, Loueniva R, Yrjanheikki E, Kauppila A. 1986. Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes. Am J Obstet Gynecol 155:1086–1089.

Loghman-Adham M. 1997. Renal effects of environmental and occupational lead exposure. Environ Health Perspect 105:928–939.

Ludwig JH, Diggs DR, Hesselberg HE, Maga JA. 1965. Survey of lead in the atmosphere of three urban communities: a summary. Am Ind Hyg Assoc J 26:270–284.

Murray RM, Jones P, O'Callaghan E, Takei N, Sham P. 1992. Genes, viruses and neurodevelopmental schizophrenia. J Psychiatr Res 26:225–235.

Needleman HL, Gunnoe C, Leviton A, Reed R, Peresie H, Maher C, et al. 1979. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. N Engl J Med 300:689–695.

Needleman HL, Schell A, Bellinger D, Leviton A, Allred EN. 1990. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N Engl J Med 322:83–88.

Neuhaus JM. 1992. Statistical methods for longitudinal and clustered designs with binary responses. Stat Methods Med Res 1:249–273.

Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, et al. 1994. Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. Arch Gen Psychiatry 51:849–859.

Oishi H, Nomiyama H, Nomiyama K, Tomokuni K. 1996. Fluorometric HPLC determination of delta-aminolevulinic acid (ALA). J Anal Toxicol 20:106–110.

Olney JW, Newcomer JW, Farber NB. 1999. NMDA receptor hypofunction model of schizophrenia. J Psychiatr Res 33:523–533.

Percy VA, Lamm MC, Taljaard JJ. 1981. Delta-aminolaevulinic acid uptake, toxicity, and effect on [^{14}C]gamma-aminobutyric acid uptake into neurons and glia in culture. J Neurochem 36:69–76.

Pocock SJ, Smith M, Baghurst P. 1994. Environmental lead and children's intelligence: a systematic review of the epidemiological evidence. Br Med J 309:1189–1197.

Prozialeck WC, Grunwald GB, Dey PM, Reuhl KR, Parrish AR. 2002. Cadherins and NCAM as potential targets in metal toxicity. Toxicol Appl Pharmacol 182:255–265.

Ren S, Scheuer ML, Zheng W. 1998. Determination of lamotrigine in biologic materials by a simple and rapid liquid chromatographic method. Ther Drug Monit 20:209–214.

Satcher DS. 2000. The Surgeon General on the continuing tragedy of childhood lead poisoning. Public Health Rep 115:579–580.

Sobotka JM, Rahwan R. 1995. Teratogenesis induced by short- and long-term exposure of *Xenopus laevis* progeny to lead. J Toxicol Environ Health 44:469–484.

Susser EB, Brown A, Matte TD. 1999. Prenatal factors and adult mental and physical health. Can J Psychiatry 44:326–334.

Susser ES, Schaefer CA, Brown AS, Begg MD, Wyatt RJ. 2000. The design of the Prenatal Determinants of Schizophrenia Study. Schizophr Bull 26:257–273.

Thomas HV, Milmore BK, Heidbreder GA, Kogan BA. 1967. Blood lead of persons living near freeways. Arch Environ Health 15:695–702.

Tomokuni K, Ichiba M, Fujishiro K. 1993. Interrelation between urinary delta-aminolevulinic acid (ALA), serum ALA, and blood lead in workers exposed to lead. Ind Health 31:51–57.

Tong S, McMichael AJ, Baghurst PA. 2000. Interactions between environmental lead exposure and sociodemographic factors on cognitive development. Arch Environ Health 55:330–335.

Toscano CD, Hashemzadeh-Gargari H, McGlothan JL, Guilarte TR. 2002. Developmental Pb^{2+} exposure alters NMDAR subtypes and reduces CREB phosphorylation in the rat brain. Brain Res Dev Brain Res 139:217–226.

Udry JR, Morris NM, Kovenock J. 1995. Androgen effects on women's gendered behaviour. J Biosoc Sci 27:359–368.

Vawter MP. 2000. Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. Eur J Pharmacol 405:385–395.

Vivier PM, Hogan JW, Simon P, Leddy T, Dansereau LM, Alario AJ. 2001. A statewide assessment of lead screening histories of preschool children enrolled in a Medicaid managed care program. Pediatrics 108:E29. Available: http://pediatrics.aappublications.org/cgi/reprint/108/2/e29.pdf [accessed 18 February 2004].

Wasserman GA, Liu X, Popovac D, Factor-Litvak P, Kline J, Waternaux C, et al. 2000. The Yugoslavia Prospective Lead Study: contributions of prenatal and postnatal lead exposure to early intelligence. Neurotoxicol Teratol 22:811–818.

Weinberger DR. 1996. On the plausibility of "the neurodevelopmental hypothesis" of schizophrenia. Neuropsychopharmacology 14(suppl 3):1S–11S.

Zheng W, Blaner WS, Zhao Q. 1999. Inhibition by lead of production and secretion of transthyretin in the choroid plexus: its relation to thyroxine transport at blood-CSF barrier. Toxicol Appl Pharmacol 155:24–31.