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 BPbs < 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). Needelman 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.
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)-3H-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 x 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 C18 column (5 µm, 250 × 4.6 mm) attached to a Spherisorb C18 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. Derivatization of δ-ALA to 2,6-diactyl-1,5-dimethyl-7-(2-carboxyethyl)-3H-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)-3H-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 H2O, 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.
Intrada 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 measurements 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 value) 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 a variable in the model. This approach provides a summary OR that accounts for the matching variables, although it does not adjust for other covariates. Conditional logistic regression models were fitted (Neuhaus 1992), including a variable in the model.
second trimester, compared with lower levels, 29 controls) were categorized as exposed and low levels of serum δ-ALA yields high sensitivity and positive predictive 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 δ-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.

Discussion

Our study represents the first report of a prospective examination of a prenatal chemical exposure as a risk factor for an adult psychiatric disorder. 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 transthyretin 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.

<table>
<thead>
<tr>
<th>Method</th>
<th>Estimated OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MH OR, unadjusted</td>
<td>1.83 (0.87–3.87)</td>
<td>0.106</td>
</tr>
<tr>
<td>Conditional logistic regression, unadjusted</td>
<td>1.83 (0.86–4.11)</td>
<td>0.109</td>
</tr>
<tr>
<td>Conditional logistic regression, adjusted for mother’s age at delivery</td>
<td>2.43 (0.95–5.56)</td>
<td>0.051</td>
</tr>
</tbody>
</table>
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


